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Feasibility of Mainstream Nitrite Oxidizing Bacteria Out-Selection and Anammox Polishing for Enhanced Nitrogen Removal

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**FEASIBILITY OF MAINSTREAM NITRITE OXIDIZING
BACTERIA OUT-SELECTION AND ANAMMOX POLISHING FOR
ENHANCED NITROGEN REMOVAL**

by

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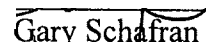
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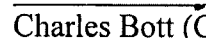
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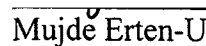
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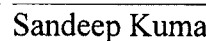
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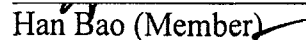
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ABSTRACT

FEASIBILITY OF MAINSTREAM NITRITE OXIDIZING BACTERIA OUT-SELECTION AND ANAMMOX POLISHING FOR ENHANCED NITROGEN REMOVAL

Pusker Raj Regmi

Old Dominion University, 2014

Co-Director: Dr. Gary Schafran

Dr. Charles Bott

Short-cut nitrogen removal avoids nitrite oxidation to nitrate by nitrite oxidizing bacteria (NOB) and allows a) reduction of formed nitrite to nitrogen gas via heterotrophic denitrification and/or b) oxidation of remaining ammonia with formed nitrite to nitrogen gas via anaerobic ammonia oxidation (anammox). The precondition for achieving short-cut nitrogen removal is suppression of NOB, which is favored by warm and high ammonia strength conditions found in internally generated ammonia-rich waste streams through anaerobic digestion of waste solids referred to as sidestreams or reject water. The discovery of anammox bacteria in the mid-1990s, which are capable of transforming NH_4^+ to nitrogen gas utilizing NO_2^- as a substrate, has made suppression of NOB even more critical for nitrogen removal processes that take advantage of the lower energy and cost requirements of this nitrogen conversion compared to traditional nitrogen removal processes. Deammonification relies on ammonia oxidizing bacteria (AOB) to partially convert NH_4^+ to NO_2^- and anammox bacteria (AMX) to convert the remaining NH_4^+ and NO_2^- to nitrogen gas. The challenges of retaining slow growing AMX initially limited the expansion of benefits from autotrophic nitrogen removal; however, granular sludge and attached growth systems have proven highly effective in achieving deammonification in sidestream processes. Owing to the benefits that include energy and chemical savings, short-cut nitrogen removal has emerged as a viable technology for sidestream treatment. Consequently, mechanisms of NOB suppression to perform short-cut nitrogen removal are generally quite well understood for sidestream applications, which has allowed for the development of robust process control strategies. To date, the concept of deammonification has successfully been implemented in 100 full-scale treatment

facilities treating high ammonia strength waste streams around the world.

Due to the success of sidestream short-cut nitrogen removal systems, there is great interest in applying this form of nitrogen removal to mainstream processes. Since the dilute and cold conditions of mainstream are not well-suited for suppression of NOB, short-cut nitrogen removal, in particular deammonification, has yet to be implemented in full-scale. The successful implementation of mainstream deammonification would revolutionize and disrupt the way in which biological nitrogen removal is achieved at wastewater treatment facilities. It represents a paradigm shift for the industry, offering the opportunity for sustainable wastewater treatment, energy neutral or even energy positive facilities and dramatic reductions in treatment costs, which has widespread environmental, economic and societal benefits.

This dissertation deals with the pilot-scale investigation of short-cut nitrogen removal in low ammonia strength wastewater with temperatures $<25\text{ }^{\circ}\text{C}$. An A-B process pilot-scale system was operated over a two year period. The A-stage was a high-rate activated sludge system for carbon removal and the B-stage consisted of an activated sludge system that targeted NOB out-selection which was followed by a fully anoxic anammox MBBR. In this study, by employing a combination of intermittent aeration, high DO ($>1.5\text{ mg/L}$), residual effluent NH_4^+ ($>2\text{ mg/L}$), and aggressive SRT ($<5\text{ days}$ at $23\text{--}25\text{ }^{\circ}\text{C}$) and HRT ($<4\text{ hr}$), NOB out-selection was achieved in the continuous-flow activated sludge process. The development of novel aeration and SRT control strategies based on advanced instrumentation, control, and automation for achieving NOB out-selection in an activated sludge process and nitrogen polishing in subsequent anammox MBBR was shown. A very fast startup time (less than 2 weeks) for anammox MBBR was achieved by seeding anammox granules obtained from a full-scale, sidestream anammox treatment process. Anammox MBBR proved highly stable during the study and a very high maximum nitrogen conversion rate ($>1\text{ gN/m}^2/\text{d}$) was demonstrated. Therefore, this study shows carbon re-direction (potentially for energy production) in a high rate A-stage does not cause carbon limitation in the B-stage for nitrogen removal if control strategies and anammox-based nitrogen polishing is used as investigated in this study.

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This dissertation is dedicated to my parents and my wife, Pragya Rasmi Shrestha.

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NOMENCLATURE

A-B process	2-stage activated sludge system
AMX	anaerobic ammonia oxidizing bacteria
ABAC	ammonia based aeration control
Afc	AvN (NH ₄ -NO _x) aeration controlled aerobic fraction
AMO	ammonia monooxygenase
Anammox	anaerobic ammonia oxidation process
AOB	ammonia oxidizing bacteria
AS	activated sludge
AvN	AOB versus NOB process
AvN+	AvN with nitrogen polish add-on
BNR	biological nutrient (nitrogen) removal
BOD	biochemical oxygen demand
CAS	conventional activated sludge
cBOD	carbonaceous BOD
cCOD	colloidal COD
CEL	central environmental laboratory
CEPT	chemically enhanced primary treatment
CETP	Chesapeake-Elizabeth treatment plant
COD	chemical oxygen demand
CSTR	continuous stirred tank reactor
Deammonification	combined nitrification and anammox process
DEMON	pH controlled aeration sidestream deammonification
DO	dissolved oxygen
EPS	extracellular polymeric substances
ESS	effluent suspended solids
F:M	food-to-microorganisms
FA	free ammonia
FISH	fluorescence <i>in-situ</i> hybridization

FNA	free nitrous acid
GHG	greenhouse gas
HAO	hydroxylamine oxidoreductase
HRAS	high rate activated sludge
HRT	hydraulic residence time
IC	inorganic carbon
ICA	instrumentation, control, and automation
IMLR	internal mixed liquor recycle
MBBR	moving bed biofilm reactor
MBR	membrane bioreactor
MCRT	mean cell residence time
MLSS	mixed liquor suspended solids
MLVSS	mixed liquor volatile suspended solids
MOV	mechanically operated valve
NAR	nitrite accumulation ratio
NOB	nitrite oxidizing bacteria
NOR	nitrite oxidoreductase
NXR	nitrite oxidoreductase
OHO	ordinary heterotrophic organisms
OUR	oxygen uptake rate
PAO	polyphosphate accumulating organisms
pCOD	particulate COD
PE	population equivalent
PID	proportional-integral-derivative
PLC	programmable logic controller
PTF	preliminary treatment facility
PVC	polyvinylchloride
qPCR	quantitative polymerase chain reaction
RAS	returned activated sludge

RBC	rotating biological contactor
RWI	raw wastewater influent
SBR	sequencing batch reactor
sCOD	soluble COD
SLR	solids loading rate
SND	simultaneous nitrification and denitrification
SOR	surface overflow rate
SRT	solids retention time
SVI	sludge volume index
TIN	total inorganic nitrogen
TKN	total Kjeldahl nitrogen
TN	total nitrogen
TP	total phosphorus
TSS	total suspended solids
UASB	upflow anaerobic sludge blanket
VFA	volatile fatty acid
VSS	volatile suspended solids
WAS	waste activated sludge
WWTP	wastewater treatment plant

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CHAPTER 1

INTRODUCTION

1.1 Nitrogen Pollution

Fresh water accounts for less than 1% of the total biosphere, which is increasingly stressed by a growing population on the global level. Although water is considered to be a renewable resource, widespread pollution is limiting its availability and transforming it into a non-renewable resource at the local level. In 1908, the German scientist Fritz Haber filed the patent for a novel process that was capable of mass-scale fixation of atmospheric N_2 as ammonia. Later this discovery was further improved into the Haber-Bosch process. Nitrogen fixation through the Haber-Bosch process accounted for $120 \text{ Tg N year}^{-1}$ in 2005 (Galloway et al., 2008), which has been projected to increase to $165 \text{ Tg N year}^{-1}$ by 2050 (Galloway et al., 2004). The inexpensive and ubiquitous Haber-Bosch nitrogen has allowed intensification of agricultural production that, among other factors, is the primary driver for unprecedented growth in human population (7 times more people than it was in 1900). Unfortunately, this growth has not been sustainable and has imbalanced biogeochemical cycles both at the local as well as the global levels. As large amounts of nitrogen accumulates, undesirable species which cause destruction of the ozone layer (N_2O , NO), global warming (N_2O), acid rain (NO_x), pollution of aquifers and water bodies (NH_4^+ , NO_2^- , NO_3^-) are on the rise.

Although nitrogen is a building block that creates amino acids and proteins of living organisms and a limiting nutrient for the crops grown by humans, excessive deposition of nitrogen species such as ammonium and nitrate in the terrestrial and marine ecosystems triggers wide-scale problems such as eutrophication and ground water pollution. Eutrophication and aquatic toxicity can be caused by flow of nitrogen-laden water in sensitive water bodies. In this context, removal of nitrogen species from wastewater before being discharged is desired. Nitrogenous compounds with oxidation states in the range of -3 to +5 are of concern when the topic is nitrogen pollution. These compounds include

ammonia-nitrogen (NH_4^+ , -3), dinitrogen gas (N_2 , 0), nitrite-nitrogen (NO_2^- , +3), and nitrate-nitrogen (NO_3^- , +5). In nature, nitrogen is constantly transformed between various species, comprising the basis of the nitrogen cycle. As a result of organic nitrogen hydrolysis, nitrogen in wastewater is mostly present as ammonium (Barnes et al., 1983).

In each oxidation state, the nitrogen atom combines with atoms of hydrogen, oxygen and nitrogen resulting in the formation of unique inorganic molecules (Figure 1). Since kinetics control the oxidation state rather than the thermodynamic equilibrium, all oxidation states are viable in an aqueous systems. However, some of these molecules are not highly stable in terms of thermodynamics (Williams et al., 1996).

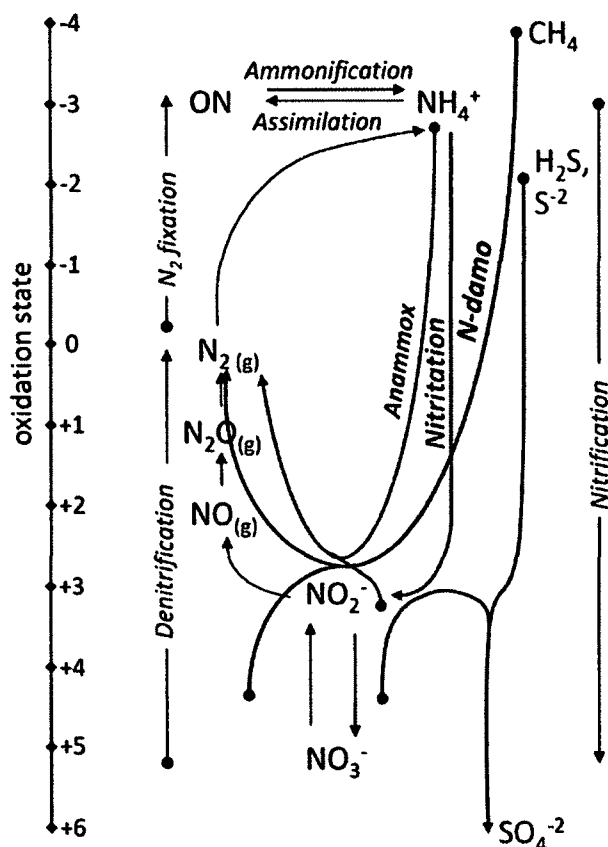


Figure 1. Nitrogen cycles (based on Grady et al., 2011).

The Chesapeake Bay

The Chesapeake Bay is the largest estuary in North America and the third largest in the world. The watershed that drains into the Chesapeake Bay covers a region of 165,800 km² that includes parts of six states: Delaware, Maryland, New York, Pennsylvania, Virginia, and West Virginia. The Bay covers an area of approximately 11,400 km² as it stretches 332 km from Virginia Beach, Virginia, to Havre de Grace, Maryland, at the mouth of the Susquehanna River. The Bay watershed consists of about 150 major rivers and streams. The Bay and its tidal tributaries have around 18,804 km of shoreline and 11,613 km² of surface area. The Bay watershed has a 14:1 land to water ratio (Land Area: Water Area), accompanied by an average depth of 21 feet. This shallow depth and large watershed make the Bay very susceptible to land use practices.

The Chesapeake Bay is home to a wide variety of plant species, sea life and waterfowl. The Bay supports more than 3,600 species of plants, fish and animals. The combination of fresh water and salt water in the Bay promotes a fertile place for organisms to grow. The Bay provides commercial and recreational resources for more than 16 million residents in its watershed. It also supplies about 500 million pounds of seafood per year (Chesapeake Bay Program, 2012).

In 1975, the United States Congress invoked the United States Environmental Protection Agency (US EPA) to study the deteriorating water quality and declining health of the Chesapeake Bay as authorized by the Clean Water Act of 1972. The study concluded that nutrient enrichment within the Bay resulted from increased agricultural development, population growth and sewage treatment plant discharges was having an adverse impact on water quality. Nitrogen, phosphorous, and sediment overloading was identified as the major cause of the declining water quality in the Bay. Algal blooms caused by excess nutrients result in the depletion of dissolved oxygen from water. This process, referred to as eutrophication, results from the growth and decay of algae. The density of algal blooms reduces sunlight penetration through the water column which is needed to support growth of submerged aquatic vegetation. In addition, decomposition of algae further deplete the

water of oxygen. Hypoxic conditions and lack of aquatic plants negatively impacts aquatic life.

In 1998, the Bay was listed as impaired in the states of Virginia, Maryland and the District of Columbia under the Clean Water Act (Chesapeake Bay Program, 2012). Nutrients from airborne contaminants (e.g., automobile emissions), nonpoint sources (e.g., runoff) and point sources (e.g., municipal wastewater treatment plant discharge) enter the Bay on a daily basis. The Chesapeake Bay Foundation reports nitrogen pollution is the most significant problem facing the Bay. It also states that wastewater treatment plants are the second largest source of nitrogen pollution. The Bay's largest recorded "dead zones" (hypoxic areas) occurred in 2003.

To protect and restore the Chesapeake Bay's ecosystem, state governments, the District of Columbia and the US EPA signed various agreements in 1983, 1987, and 2000. With the goal of removing the Bay from the list of impaired waters and improving water quality by 2010, the Bay partners signed an agreement in 2000. These goals were supported by Delaware, New York and West Virginia, the non-signatory Chesapeake Bay watershed states.

Despite many substantial efforts by the Chesapeake Bay partners for more than 25 years, Chesapeake Bay water quality has not improved significantly. On May 12, 2009, President Obama signed an executive order empowering the USEPA to set a demanding timetable for Bay restoration efforts. The order also granted the EPA the ability to penalize states failing to meet the outlined goals. Further, in order to fast-track efforts to significantly reduce nitrogen and phosphorus pollution, the Chesapeake Bay partners have been required to implement two-year goals called "milestones", with an ultimate goal to restore the health of the Bay by 2025. In December 2011, the six states and the District of Columbia began meeting every two years for discussion and implementation of these "milestones".

1.2 Project Background

On December 29, 2010, the USEPA established the Chesapeake Bay Total Maximum Daily Load (TMDL) to restore clean water in the Chesapeake Bay and the region's streams, creeks and rivers. Under this mandate, each state that discharges into the Chesapeake Bay has prepared a Watershed Implementation Plan (WIP) designed to accomplish a set of allocation goals identified in the USEPA Chesapeake Bay TMDL. In Virginia's WIP, the Hampton Roads Sanitation District (HRSD) is required to meet the 2021 nutrient allocation of 3.4 million pounds per year (current nutrient allocation is 6 million pounds per year) for the seven wastewater treatment plants (cumulatively) that discharge into the James River basin:

Since the limits are based on the total mass discharged from all seven of the aforementioned HRSD-operated plants discharging to the James River basin, HRSD has the flexibility to make the most cost-effective plant modifications collectively to meet the nutrient allocations. In 2005, HRSD determined that upgrading the 24 MGD Chesapeake-Elizabeth Treatment Plant (CETP), which primarily serves Virginia Beach and Norfolk.

CETP is a conventional high-rate activated sludge (HRAS) process without primary clarifiers operated at a 1.5-2.5 day solids retention time (SRT). The plant discharges into the Chesapeake Bay, however, it does not perform nitrogen removal. In order to meet future limits, HRSD anticipates reducing effluent nitrogen concentrations at CETP to within a range of 5-8 mg/L TN. To meet this objective, construction of a conventional 3-stage BNR process with an effluent goal of 8-12 mg/L TN would be followed by construction of second anoxic zones or denitrification filters that would reduce the effluent TN to approximately 5 mg/L was considered. Three conventional BNR processes, a VIP process followed by denitrification filters, 5-stage Bardenpho process, and a step-feed BNR were reviewed. Process simulation using the Biowin (EnviroSim, Ontario, Canada) model indicated that while all three processes would be able to reliably meet a total nitrogen effluent limit of 5 mg/L, significant reactor volume upgrades would be required (Hazen and Sawyer, PBSJ, McKim & Creed, 2002). These upgrades were estimated at \$125 – 150 million, and a significant increase over current operating cost would be expected.

A proposed alternative is to install a European-style Adsorption-Biooxidation (A-B) process. The A-B process is a two-sludge system that utilizes an adsorption-style high-rate activated sludge A-stage for COD removal followed by a B-stage for biological nitrogen removal (BNR). With the high soluble COD (sCOD) fraction typical of HRSD wastewater, the reduced organic load from the A-stage would allow the B-stage to fit into the existing aeration basin volume and would greatly reduce the capital cost of an upgrade, as compared to traditional primary clarification or chemically enhanced primary treatment (CEPT). Because denitrification requires an organic carbon source as an electron donor, the reduction in influent carbon can limit the denitrification capacity of the B-stage. To overcome this carbon limitation, the B-stage intermittent aeration control strategy based on effluent NH_4^+ and/or NO_2^- and NO_3^- and a short-cut nitrogen removal through nitrification-denitrification can be integrated into the B-stage process. This operating condition requires high intensity monitoring and automated controls to maintain the desired operating conditions. Additional nitrogen removal can be achieved by integration anammox bacteria into the system.

1.3 Motivation of the Research

Biological nitrogen removal is considered economical compared to physicochemical methods of nitrogen recovery for wastewater containing less than 5 gN/L (Mulder, 2003). The low volumetric carbon and nitrogen loading rates (~ 1 gCOD/L/d and 0.08 gN/L/d) render conventional activated sludge (CAS) systems inefficient in terms of energy utilization. Typically 60-70% energy consumption of wastewater treatment is associated with aeration required for carbon and nitrogen removal (Zessner et al., 2010). When enhanced primary settling (to increase physicochemical sludge production) is employed in conjunction with a separate nitrogen removal step for the digestate of primary and secondary sludge, a 25% decrease of aeration requirements can be realized (Siegrist et al., 2008). Further, a highly-loaded activated sludge step targeting conversion of influent carbon to biomass at maximal yield and an anaerobic digestion to produce electricity can result in energy-neutral wastewater treatment (Wett et al., 2007).

It is generally understood that the energy content of raw wastewater is more than the energy requirements of the treatment (reference). The organic compounds present in wastewater contain approximately 14 MJ/kg COD of energy; mostly lost as metabolic heat when aerobically oxidized (Jetten et al., 1997). The basis of energy-neutral wastewater treatment requiring nitrogen removal is energy recovery from concentrated organic carbon and subsequent minimization of energy requirement for biological nitrogen removal (McCarty et al., 2011). Many studies report the possibility of energy-positive wastewater treatment (Siegrist et al., 2008, Verstraete et al., 2009, Kartal et al., 2010), however, plants without anaerobic digestion can still benefit from carbon concentration followed by short-cut nitrogen removal.

For wastewater containing low COD/N ratio (typically $\leq 2-3$), short-cut nitrogen removal with partial nitrification and anammox results in 60% less aeration, 90% less sludge production and 100% reduction of organic carbon addition compared to conventional nitrification-denitrification (Mulder, 2003), hence, 30-40% reduction in nitrogen removal cost can be expected (Fux et al., 2004). Short-cut nitrogen removal with nitrification and denitrification also results in savings of 40% carbon requirement in the denitrification step and 25% oxygen consumption in the nitrification step (Turk et al., 1986).

The high rate operation (<1 day SRT, 30 min HRT, <1 mg/L DO) of the carbon concentration step [A-stage; Böhnke et al., 1980] results in the removal of influent particulate, colloidal, and soluble COD with minimal energy input in a small footprint by maximizing sludge production (i.e., yield), bacterial storage, and bioflocculation. The positive side-effect of operating with high yield is high nitrogen and phosphorus assimilation (Jetten et al., 1997). The A-stage biomass has better digestion characteristics compared to secondary sludge, which results in lower overall sludge production where sludge digestion is connected to a wastewater treatment plant (van Loosdrecht et al., 1997). A-stage also offers 57-68% reduction in specific aeration requirements compared to single-step CAS (Müller-Rechberger et al., 2001).

The domestic wastewater is characterized by carbon and nitrogen concentrations of 450-1000 mgCOD/L and 30-100 mgN/L (Tchobanoglous et al., 2003; Henze et al., 2008). A highly loaded A-stage is capable of removing 50-80% and 10-15% of influent COD and

nitrogen, respectively, reducing the COD/N ratio to less than what is required for conventional nitrification-denitrification in the nitrogen removal step (B-stage). To achieve the same level of nitrogen removal efficiency through the A/B process external carbon must be added undermining the advantages of an A-stage. However, if the B-stage is optimized for short-cut nitrogen removal, external carbon may not be required and benefits of efficient carbon removal by an A-stage treatment can be realized. Suppression of nitrification (conversion of NO_2^- to NO_3^-) through out-selection of nitrite oxidizing bacteria (NOB out-selection hereafter) is a prerequisite for short-cut nitrogen removal. NOB out-selection (where NOB populations are maintained at low levels) in a highly loaded nitrogen stream ($>250 \text{ mgN/L}$) and high temperatures ($>30^\circ\text{C}$) has made short-cut nitrogen removal possible for treating digestate of primary and secondary sludge. The characteristics of domestic wastewater (nitrogen: $30\text{-}100 \text{ mgN/L}$, temperature: $8\text{-}30^\circ\text{C}$) has rendered NOB out-selection difficult to achieve for mainstream wastewater treatment.

The main objective of this study is to develop technologies based on control strategies to treat domestic wastewater efficiently in a mainstream application by harnessing carbon concentration and diversion and short-cut nitrogen removal for stringent nitrogen limits. The goal is to optimize the influent carbon utilization such that energy (aeration), volume/footprint (capital investment), and chemical (external carbon and alkalinity) savings for carbon and nitrogen removal is achieved, while using existing infrastructure and meeting specific effluent criteria.

1.4 Research Objectives

Partial nitritation and anammox (deammonification) for mainstream wastewater treatment represents a more efficient biological pathway of removing nitrogen than currently employed processes, however, the out-selection of NOB and retention of anammox bacteria (AMX) are the main challenges for mainstream deammonification. For plants treating their primary and secondary sludge using anaerobic digesters there is an opportunity for sidestream deammonification. One possible way of mainstream deammonification in such a scenario can be bioaugmentation of sidestream generated AOB (system can be operated at an SRT below AOB washout for NOB out-selection) and

anammox. However, for the plants without anaerobic digesters such as CETP, which relies on incineration for biosolids management without energy recovery, mainstream deammonification will have to rely on a different approach of NOB out-selection and AMX retention.

For plants with anaerobic digestion there is an incentive of diverting carbon for energy recovery, while non-digester plants could use the influent carbon for denitrification. If short-cut nitrogen removal with nitrification-denitrification is promoted to the extent possible, taking advantage of heterotrophic denitrification to assist with NOB out-selection, the use of anammox to polish residual ammonia and nitrite can be considered (Figure 2). The benefits of this strategy may include minimization of aeration energy usage and elimination of supplemental carbon. However, it is important to note that if carbon is not diverted using a carbon concentration step (e.g., HRAS) in the mainstream, surplus COD as a result of 40% reduction of COD demand due to nitrification-denitrification gets oxidized aerobically. In fact, O_2 demand to oxidize this surplus COD equals the 25% reduction in O_2 demand that results from nitrification-denitrification pathway (simple electron balance). Therefore, it is very important to recognize the purported aeration energy savings is only possible if the influent wastewater is diverted prior to the nitrogen removal step in mainstream systems.

When wastewater carbon is diverted the nitrogen removal step aeration tank and/or secondary clarifier volume requirement can also be decreased, particularly for high carbon strength wastewater. Further, the diverted carbon can also be converted to energy or some other valuable commodity (e.g. chemicals), for example in an incinerator plant with energy recovery; the value of the redirected carbon improves the overall benefit of the A-B process configuration.

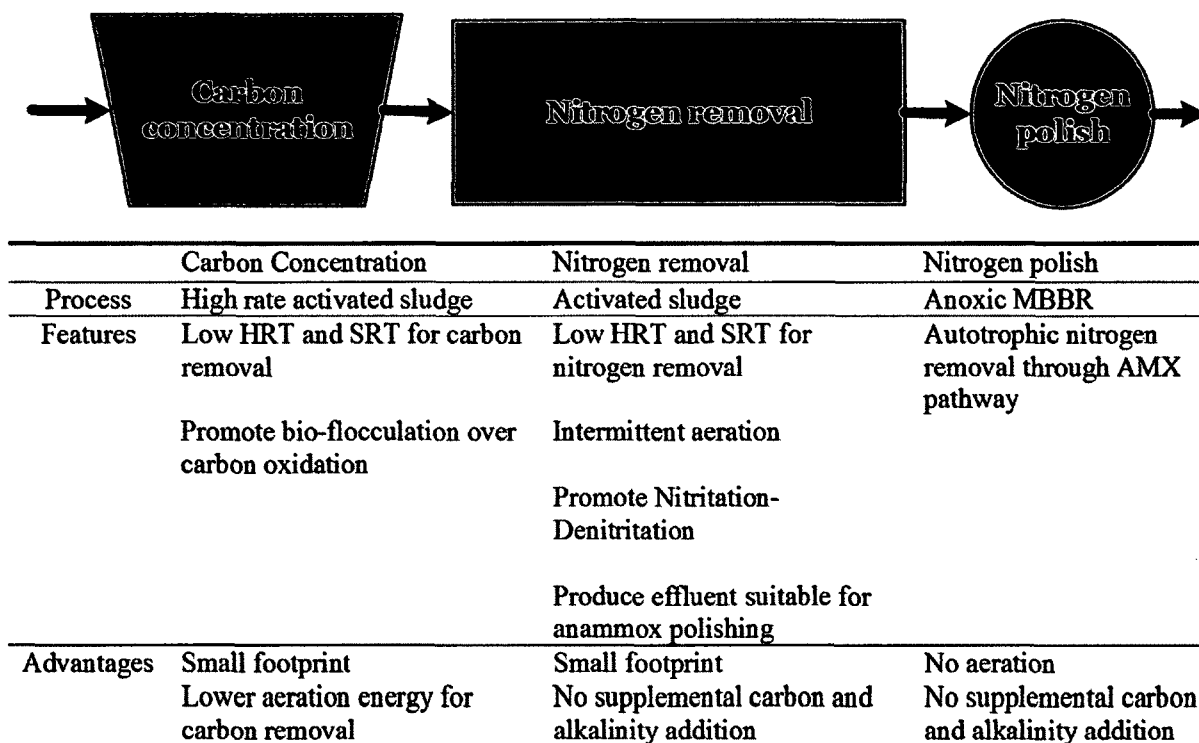


Figure 2. Description of A-B process with nitrogen polish step.

The principal goal of this research is to carry out a comprehensive study on the feasibility of nitrification-denitrification and anammox in a mainstream wastewater treatment process using optimized reactor configurations and control strategies at ambient wastewater temperatures without relying on bioaugmentation. The new insights should help to understand how the relatively broad knowledge and experience from sidestream deammonification systems can be transferred to successful mainstream nitrification-denitrification and anammox applications. To accomplish that goal, the following objectives are outlined:

HYPOTHESIS 1: NOB out-selection in warmer, high NH_4^+ strength wastewater is aided by free ammonia (FA) and/or free nitrous acid (FNA) inhibition. There is a need to approach NOB out-selection in low NH_4^+ strength wastewater at ambient temperature differently such that mainstream nitrification-denitrification, mainstream deammonification, or some combination thereof could be effectively deployed.

OBJECTIVE 1: *Develop control strategies unique to mainstream activated sludge application for reliable NOB out-selection without relying on sidestream deammonification processes.*

HYPOTHESIS 2: NOB out-selection in mainstream would require completely different control strategies compared to sidestream strategies, therefore mechanisms for NOB out-selection are also expected to be different.

OBJECTIVE 2: *Understand underlying mechanisms to increase the reliability of NOB out-selection in mainstream activated sludge.*

HYPOTHESIS 3: The concentration and diversion of carbon prior to nitrogen removal step allows for energy recovery and has the added advantage of smaller aeration volume particularly for high carbon strength wastewater.

OBJECTIVE 3: *Develop criteria and strategies for controlling carbon concentration step such that optimum influent carbon to nitrogen ratio (C/N ratio) is provided for nitrogen removal step. (This was an important part of the overall effort but is not covered in this study).*

HYPOTHESIS 4: Nitrogen removal performance step may not be satisfactory for meeting low nitrogen limits. The effluent of nitrogen removal step is likely to contain NO_2^- -N and NH_4^+ -N in the effluent due to NOB out-selection.

OBJECTIVE 4: *Develop control strategies that allow the use of anammox for nitrogen polishing.*

HYPOTHESIS 4: Sidestream anammox processes have shown slow startup times due to challenges associated with retention of slow growing and low yield AMX organisms.

OBJECTIVE 5: *Evaluate the feasibility of AMX retention in an MBBR to meet low effluent TN objectives.*

1.5 Dissertation Organization

A comprehensive overview of BNR technologies and recent developments are covered in **Chapter 2**. It focuses on fundamental science to application of the novel short-cut nitrogen removal technologies, which rely on out-selection of NOB. This chapter is intended to provide extensive background on the topic of short-cut nitrogen removal based on the latest literature review from a wide range of sources.

Chapter 3 deals with materials and methods used in this study. Many important aspects of the pilot-plant that was conducted for this study is covered. The novel process control strategies that were developed based on latest sensor technology and advanced instruments, control and automation is presented in this chapter. The cutting-edge assessment techniques of microbial population is also presented.

The novel nitrogen removal technologies that are possible based on the findings of this study (that includes four full non-provisional patents application) is presented in **Chapter 4**. The technologies proposed in this chapter are further supported by references to other chapters in this dissertation.

The major findings of pilot process train (plug-flow) focused on the upgrade Chesapeake-Elizabeth wastewater treatment plant is presented in **Chapter 5**. The emphasis is on the novel ammonia-based aeration control and operational strategies that proved highly effective for achieving efficient nitrogen removal at limited influent C/N ratio.

The novel NOB out-selection strategies in mainstream wastewater conditions were explored in another experimental pilot process train (CSTR). The possible mechanisms of NOB out-selection in mainstream conditions and operational strategies is presented in **Chapter 6**.

The findings of first ever mainstream anammox nitrogen polishing MBBR receiving effluent of a CSTR optimized for nitrification-denitrification is presented in **Chapter 7**. This chapter also includes the start-up of fully anoxic anammox MBBR and long-term stability.

To leverage the findings from Chapters 5, 6, and 7 and expand the boundaries of application scenarios the pilot-plant was further upgraded. **Chapter 8** presents the major outcomes of 1) plug-flow reactor optimized for NOB out-selection to achieve nitrification-denitrification at a wide range of nitrogen loadings and influent C/N ratio 2) anammox MBBR optimized for removal of $\text{NH}_4^+\text{-N}$, $\text{NO}_2^-\text{-N}$, and $\text{NO}_3^-\text{-N}$ through limited addition of acetate.

A brief summary of the results of this study and the wider implications on the sustainability of advanced nitrogen removal systems is presented in **Chapter 9**. It also highlights future needs for the widespread implementation of technological solutions proposed in this study.

CHAPTER 2

LITERATURE REVIEW

2.1 Nitrification and Denitrification

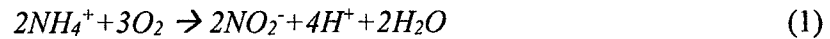
Conventional nitrification and denitrification is the most common biological method of nitrogen removal from municipal wastewater with typical characteristics summarized in Table 1. This two-step process involving different groups of organisms is often achieved through alternating wastewater between aerobic/anoxic reactors, typically in an activated sludge system. However, biological nitrification alone is sufficient where NH_4^+ removal is the only requirement. The conversion of NH_4^+ is carried out by a group of specific bacteria referred to as nitrifiers while heterotrophic organisms that denitrify NO_3^- to N_2 gas are termed denitrifiers as a whole. The specific substrate affinities and environmental niche of these bacteria are considered when designing biological nitrogen removal processes.

Table 1. Typical composition of raw municipal wastewater with minor contribution of industrial wastewater (Henze et al., 2008).

Parameter	Concentration (mg/L)
COD total	500-1200
COD soluble	200-480
BOD	230-560
N total	30-100
Ammonia-N	20-75
P total	6-25
Ortho-P	4-15
TSS	250-600

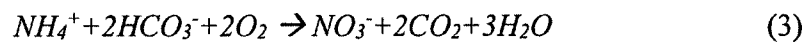
Nitrification

Nitrification is the first step in the biological nitrogen removal processes, therefore optimizing nitrogen conversion by ammonia oxidizing bacteria (AOB) and nitrite-oxidizing bacteria (NOB) has been the focus. The reactions involved in nitrification by AOB and NOB are provided in Equations 1 and 2 respectively.



In the first step of nitrification, AOB oxidize NH_4^+ to NO_2^- and then NOB oxidize NO_2^- to NO_3^- . The stoichiometric oxygen demand for complete NH_4^+ oxidation is 4.57 g O_2 /g N, where 3.43 g O_2 /g N is used by AOB to produce NO_2^- while 1.14 g O_2 /g N is used by NOB to produce NO_3^- .

The stoichiometric consumption of alkalinity by nitrification is 7.14 g $CaCO_3$ /g NH_4^+ -N (Equation 3).



The stoichiometric demand of oxygen and alkalinity is used to estimate the cost of operating a conventional nitrification process (Tchobanoglous et al., 2003) and provides a baseline for comparing alternative ammonia oxidation processes.

Nitrification is sensitive to environmental and operating conditions such as pH, temperature, DO, and substrate loading. The optimal pH range for nitrification is between 7.5 and 8; as pH drops below 6.8, nitrification rates decrease. In order to maintain optimum pH, supplemental alkalinity is added to wastewater streams with low indigenous alkalinity. As NH_4^+ is consumed by nitrification, the pH of the wastewater decreases due to free H^+ release (Tchobanoglous et al., 2003). Both free ammonia NH_3 and un-ionized nitrous acid HNO_2 have been shown to inhibit nitrification. The extent of inhibition

depends on total nitrogen species concentrations, bacteria taxonomic group, pH, and temperature. However, NOB are prone to inhibition at lower concentrations of free ammonia and free nitrous acid compared to AOB (Vadivelu et al., 2007). Since nitrifiers are slow growing organisms, solids retention time (SRT) is a key parameter for nitrification process. Nitrification processes operated below 28°C are typically rate limited by the ammonia oxidation step (Tchobanoglous et al., 2003), as such the design of these systems are based on the Monod kinetics for ammonia oxidation (Equations 4 and 5)

$$1/SRT_{min} = \mu_n \quad (4)$$

$$\mu_n = (\mu_{nm}/K_n + N)(DO/K_o + DO) - K_{dn} \quad (5)$$

where SRT_{min} is the critical or wash-out SRT of nitrifying bacteria, μ_n is the specific growth rate of the nitrifying bacteria, μ_{nm} is the maximum specific growth rate, N is the target NH_4^+ -N concentration of the effluent, K_n is the nitrifier Monod half-saturation coefficient for ammonia, K_o is the half-saturation coefficient for DO, DO is the dissolved oxygen concentration in the basin, and K_{dn} is the endogenous decay rate (Tchobanoglous et al., 2003).

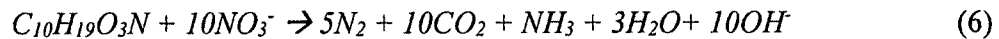
Typical operating SRT values vary depending on temperature and wastewater characteristics but may be between 10-20 days at 10°C or 4-7 days at 20°C. The SRT values given above are design considerations for conventional nitrification, however, they provide a good baseline comparison when considering SRTs for innovative ammonia oxidation processes.

Denitrification

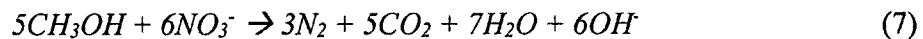
Biological denitrification is the reduction of NO_3^- to NO_2^- and then further to dinitrogen (N_2) gas by a group of heterotrophic bacteria (also known as denitrifiers) in anoxic conditions. The release of N_2 gas through denitrification completes the total nitrogen removal process. Denitrifiers are heterotrophic, facultative, anaerobic organisms which, in the absence of oxygen, activate the nitrate reductase enzyme, allowing oxidized nitrogen species to be used as an electron acceptor. The stoichiometry of the reaction depends on the nature of the organic carbon source. The complete denitrification reactions with typical

wastewater as the carbon source and methanol as a carbon source are shown in Equations 6 and 7 (Tchobanoglous et al., 2003).

Wastewater:



Methanol:



Each g of NO_3^- -N has the electron accepting capacity of 2.86 g O_2 . Biological denitrification produces 3.57 g of alkalinity as $CaCO_3$ per g N reduced, so by combining it with nitrification (-7.14 g of alkalinity as $CaCO_3$) the alkalinity requirement of nitrogen removal can be reduced by approximately 50% (Grady et al., 2011). The reduction capacity of denitrification depends on the length of time that the biomass is allowed to grow using nitrate as a substrate. Additionally, sufficient COD must also be present to act as an electron donor. The need for an exogenous carbon source is dependent both on where along the treatment train denitrification takes place and the wastewater characteristics. To use the influent COD contained in the wastewater pre-anoxic denitrification preceding nitrification is often used, however it requires cycling of nitrified sludge in order to provide NO_3^- for denitrification. In a post-anoxic approach, the denitrification process follows nitrification, which requires the addition of an external carbon donor to increase the reaction rate of the process as COD of the influent wastewater is aerobically oxidized by heterotrophs during nitrification. The carbon required as bsCOD for denitrification can be determined from Equation 8.

$$g \text{ bsCOD}/g \text{ } NO_3\text{-N} = 2.86/(1-1.42Y_n) \quad (8)$$

Where: Y_n is the net anoxic biomass yield as g VSS/g bsCOD, bsCOD = biodegradable soluble COD (Tchobanoglous et al., 2003).

Denitrification as a part of conventional nitrification-denitrification scheme is not ideal to remove nitrogen from sidestream wastewater with typical characteristics as shown in Table 2 since it requires high COD loadings that is not typically present in sidestream wastewaters.

Table 2. Typical municipal sidestream centrate characteristics.

Parameter	Typical Range
sCOD	200-1,000 (mg/L)
COD	500-3,000 (mg/L)
TSS	50-3,000 (mg/L)
T	30-40 °C
NH ₄ ⁺	500-1500 (mgN/L)
Alkalinity	3.5 x NH ₄ ⁺ -N (mg/L as CaCO ₃)

Typical COD requirements for conventional nitrification/denitrification, based on the - 2.86g COD/g N would be higher thus supplemental carbon (typically methanol is used) would be required (Hellings et al. 1998). Additionally, sidestream centrate typically contains enough alkalinity for partial nitrification but not full nitrification. Therefore supplemental alkalinity is also required for conventional nitrification/denitrification sidestream treatment processes.

2.2 Conventional Biological Nitrogen Removal (BNR)

Because biological nitrogen removal consists of an aerobic step as well as an anoxic step, conventional BNR treatment processes often contain separate aerobic and anoxic tanks/reactors though this is not required. The processes are grouped into two basic flow configurations, pre-anoxic and post-anoxic denitrification.

Post-anoxic Denitrification

The basic post-anoxic configuration has an anoxic zone after the aerobic zone (Figure 3). Nitrification occurs in the aerobic zone and then subsequent denitrification in the anoxic zone. During nitrification in the aerobic zone simultaneous heterotrophic carbon oxidation also occurs lowering the amount of organic substrate available for post denitrification. Therefore, post-anoxic denitrification process relies on the organic matter released through endogenous decay, resulting in very long reaction times and consequently requiring very large reactor volumes, or the addition of an external carbon source such as methanol or acetate. External carbon addition can be very expensive and may require special care for safe storage, handling and application.

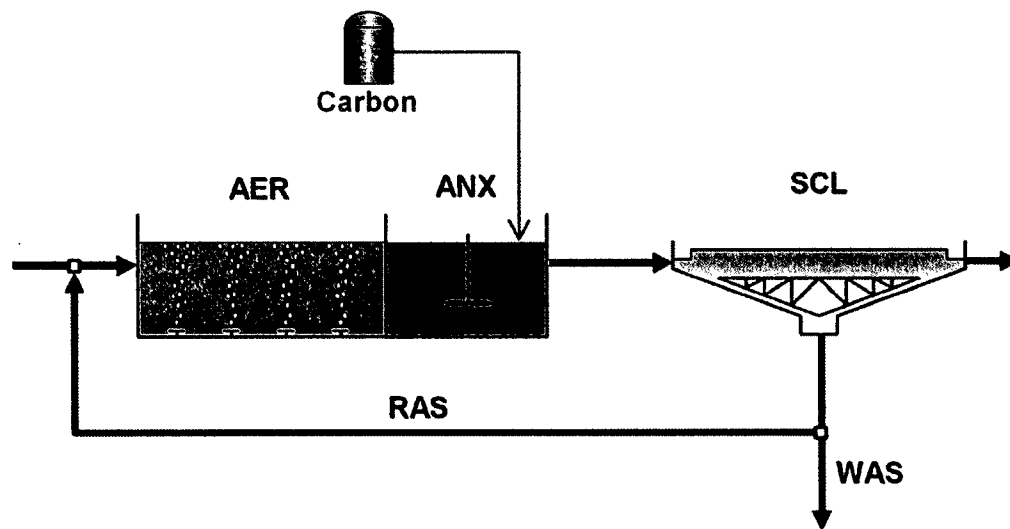


Figure 3. Basic post-anoxic BNR configuration with supplemental carbon addition

Pre-anoxic Denitrification

Pre-anoxic denitrification processes have the anoxic zone ahead of the aerobic zone (Figure 4). Since the denitrification zone occurs ahead of the aeration zone, pre-anoxic processes are able to utilize the organic carbon present in the influent wastewater for denitrification. In addition to eliminating the need for external carbon addition, this also reduces the organic load to the aeration zone as well. The overall denitrification in this system is limited by the amount of NO_3^- -N that is recycled by the RAS line to the anoxic zone.

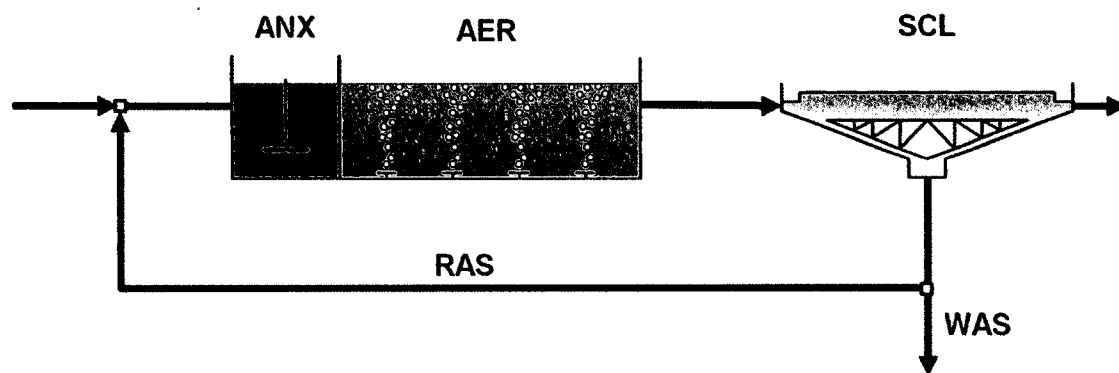


Figure 4. Basic pre-anoxic BNR configuration.

The MLE Process

To improve upon the benefits of the pre-anoxic process and alleviate limitations, it was modified to include an internal mixed liquor recycle (IMLR) stream from the aerobic zone back to the anoxic zone (Figure 5). This process, termed Modified Ludzak-Ettinger (MLE), is the most common process used for domestic wastewater treatment requiring moderate N removal performance and is the core for many of the more complex BNR processes that have been developed since (Tchobanoglous et al., 2003). The MLE process

offers simple control, good nitrogen removal, and moderate volume requirements, but cannot achieve the high levels of nitrogen removal that may be required of modern plants. Nitrogen removal in an MLE process is dependent on flow rate of the IMLR. The alkalinity generated during denitrification becomes available to maintain proper pH for nitrification. The recycle of nitrified mixed liquor (nitrate) to a denitrifying zone is the primary driver for nitrogen removal; the recycle ratio determines the percentage of achievable nitrogen removal. Since this approach relies on simultaneous growth of autotrophic nitrifiers and heterotrophic denitrifiers, nitrification rates remains relatively low for a given treatment volume.

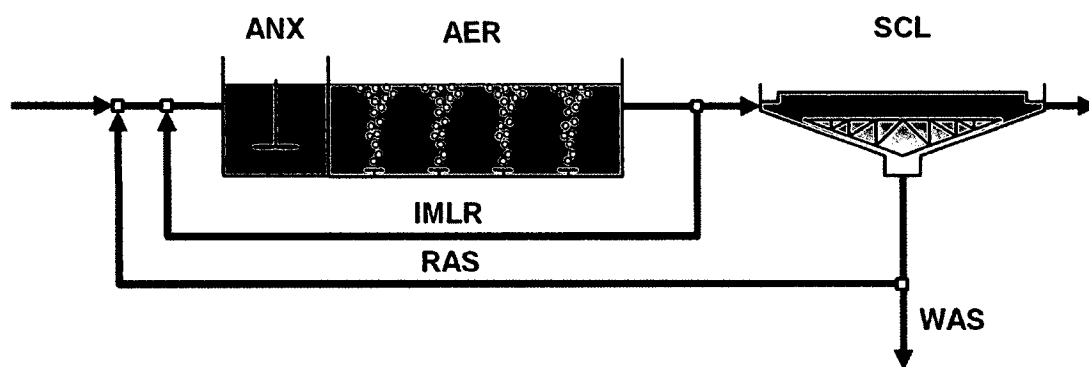


Figure 5. Modified Ludzack-Ettinger process.

4-Stage Bardenpho

The four-stage Bardenpho process is an MLE with an additional anoxic zone added after the aerobic zone followed by a small aerobic zone preceding the clarifier (Figure 6). This additional anoxic zone allows for greater denitrification capacity, capable of producing effluent in the range of 1.5 – 4 mg N/L total nitrogen. This comes at the cost of increased reactor volume and may require the addition of external carbon to the second anoxic zone (Grady et al., 2011).

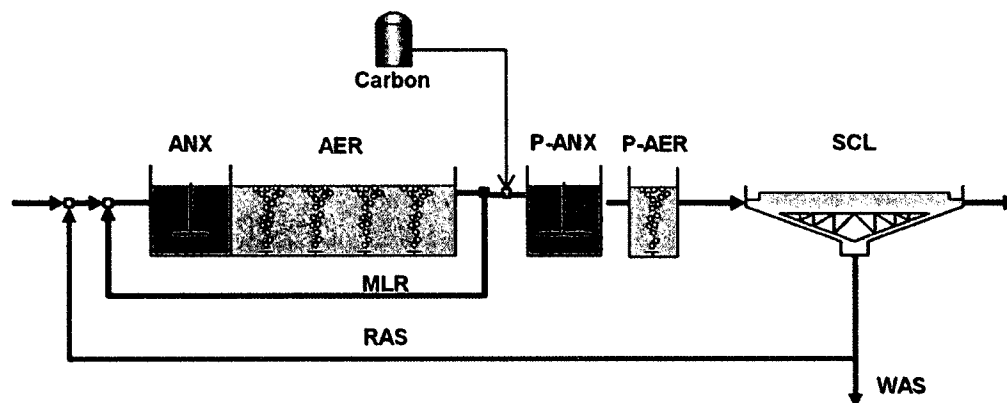


Figure 6. 4-stage Bardenpho process.

Step-Feed BNR With Internal Recycle

In a step-feed BNR process the influent is split between a series of anoxic/aerobic reactor combinations (Figure 7). This allows the influent organic substrate to be distributed throughout the process. Returned activated sludge (RAS) dilution in a step-feed process which creates a condition where the mixed liquor is not evenly distributed throughout, and the MLSS is greatest at the front of the process. This enables the system to operate at a higher MLSS without increasing volume and can be advantageous during heavy loading conditions. Many non-BNR plants are already set-up in a step feed (or with an optional step-feed) configuration, making the upgrade to BNR relatively easy. Step-feed BNR plants can typically achieve effluent TN goals of <10 mg N/L. With the addition of an IMLR at the last application point, effluent TN levels as low as 3 – 5 mg N/L may be achieved. The disadvantage of a step-feed system is that process control is often complicated as precise DO control is required in all of the aeration tanks in order to prevent the bleeding of DO into the following anoxic zone. Determining the best distribution of influent flow requires successive iterations in a modeling program. The uneven distribution of the MLSS complicates the SRT calculation and may not be suitable if tight SRT control is desired (Tchobanoglous et al., 2003).

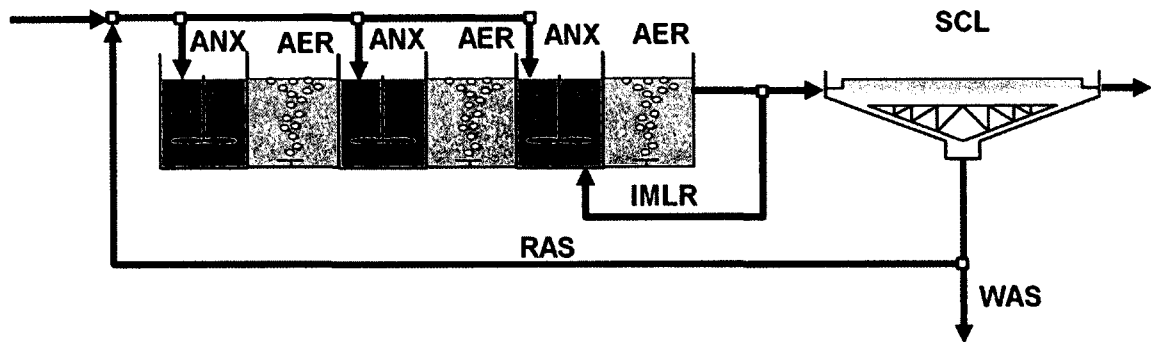


Figure 7. Step-feed BNR with internal recycle.

2.3 Multi-stage Carbon and Nitrogen Removal

In single sludge systems, multiple microbial groups (e.g., heterotrophs, AOB, NOB, PAO) co-exist across a range of redox environments. Therefore, single-sludge systems do not favor a particular group of organisms and changes in activities of each group depends on reactor configuration used. This provides operational simplicity as only one biomass is managed through a single set of secondary clarifiers or membranes. Further, this configuration ensures inherent COD content of wastewater is available for denitrification and biological phosphorus removal. The disadvantage, however, is that every microbial group is functioning outside of its optimal growth conditions inhibiting overall performance. Furthermore, to protect the most vulnerable among the microbial groups (e.g., AOB), designs have large safety factors which are more than adequate to another group of microorganisms (e.g., heterotrophs).

The main benefit of multi-stage systems is that the separation of carbon removal from nitrogen removal allows each process to be optimized independently. Two-sludge systems decrease the infrastructure required for adequate nitrification since autotrophic doubling times are approximately twice that of heterotrophs. However, if a carbon removal or nitrification plant receives a total nitrogen limit, expansion of existing tankage and addition

of post-denitrification process with supplemental carbon is required (Figure 8). Although it is possible to include denitrification in two-stage plants, the carbon removal in the first stage and nitrification in the second stage reduces the availability of carbon for the nitrate reduction in the second stage (Matsché et al., 1993). Therefore, supplemental carbon is required to achieve adequate denitrification. The Blue Plains advanced wastewater treatment plant in Washington, DC, USA is an example of a two-sludge system for nitrogen removal.

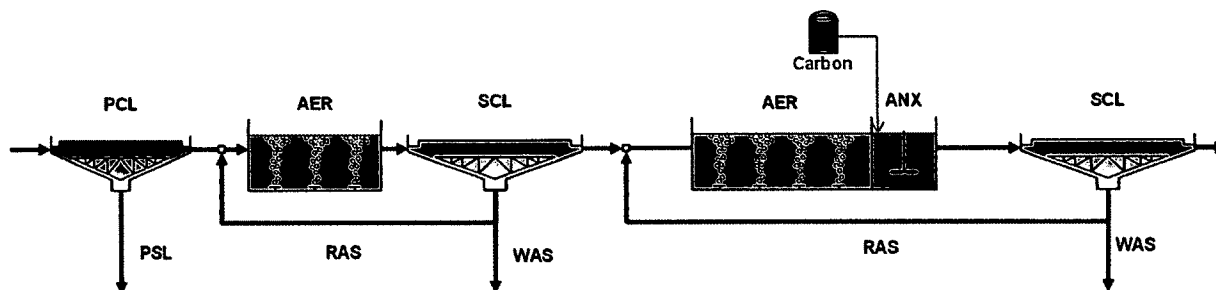


Figure 8. A high-rate activated sludge process for carbon removal followed a low-rate nitrogen removal process with external carbon addition.

The low cost of supplemental carbon in the past made such approach common to meet nitrogen limits, however, the sustainability of these practices are being questioned now. Hence, the emerging short-cut nitrogen removal technologies that do not require supplemental carbon are capable of preserving the benefits of two sludge systems.

Adsorption-Bio-oxidation (A-B) Process

The A-B process consists of a very high-rate activated sludge (HRAS) A-stage for COD removal followed by another activated sludge process, a B-stage for nitrogen removal (Figure 9). The perceived advantage of two biological ecosystems is that each can be operated optimally to achieve the goal of the overall system. The A-stage typically operates at a very short SRT (0.25-0.5 days) and a high food to microorganism (F:M) ratio

similar to conditions of sewer lines, which contain a significant amount of bacteria (Böhnke et al., 1997). Therefore, the microbial population selected by a very high rate operation is similar to the indigenous bacteria in influent wastewater (Böhnke et al., 1997). The A-stage bacterial population not only oxidizes organic carbon to CO_2 but also removes both soluble and particulate COD through bioflocculation and enmeshment in bacterial extracellular polymeric substances (EPS). Consequently, low cost COD removal at reduced volumetric requirements is achieved by the A-stage. A-stage processes are minimally aerated to avoid anaerobic conditions, but bulk DO levels are usually lower than the detection limit of a commercially available on-line DO sensor. Böhnke et al. (1997) showed a linear increase in COD removal efficiencies with increasing COD load. As a result an A-stage is known to buffer and protect the B-stage during shock loading periods. A stable A-stage provides relatively constant COD loading to the B-Stage in spite of fluctuations in the influent wastewater. Further, A-stage operation breaks down complex organic molecules into readily biodegradable substrate which could be used for denitrification in the B-stage (Böhnke et al., 1997).

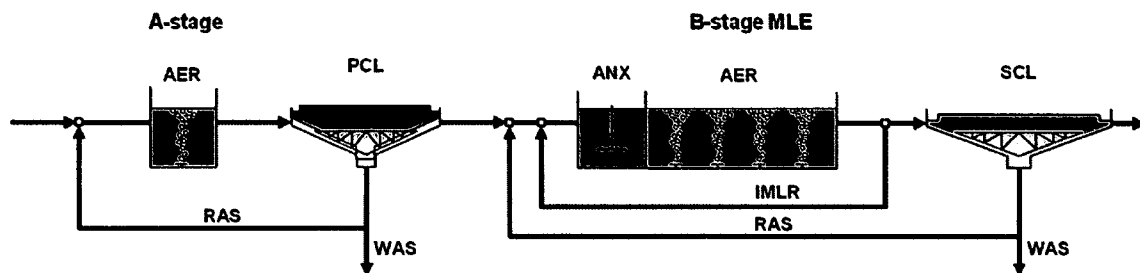


Figure 9. Adsorption-Bio-oxidation (A-B) process configuration.

A multi-stage A-B process was developed at the Krefeld treatment plant in Germany specifically to tackle nitrification toxicity where industrial sources comprised of ~50% of the influent load. Before the upgrade, the treatment plant employed primary clarification followed by a combined carbon and nutrient removal that experienced periodic inhibition of nitrification. A very-high-rate adsorption or A-stage was installed and the existing primary was converted to intermediate clarification. The A-B process upgrade at the

Krefeld plant achieved complete nitrification with average effluent NH_4^+ concentrations of 0.14 mg N/L, and effluent TP and TN concentrations of 0.19 mgP/L and 5.45 mgN/L, respectively. Further, the volumetric requirement for the A-B process was considerably lower when compared to a single-stage nitrification plant with primaries (Böhnke 1983).

The compact A-B process could reduce the required specific aeration tank volume to as low as 65 L/PE (population equivalent), compared to 150-200 L/PE for single-stage processes (Müller-Rechberger et al., 2001). Another example of an A-B process is the main treatment plant of Vienna, Austria (MTPV), which previously was an HRAS process operated with a 1.2 day SRT and 1.5 hour HRT but now operating as an A-B process (von der Emde, 1982). The new A-B process compared to the previous two-stage treatment at MTPV meets the treatment goals with a low specific aeration tank volume (including volume of intermediate clarifiers) of only 70 L/PE or 320,000 m³ for the entire plant (Wandl et al., 2006). To achieve comparable treatment efficiency with the single-stage process, the aeration tank volume of approximately 150 L/PE was required. By design, MTPV can operate in either of two modes to best achieve nitrogen removal given the influent conditions. In Bypass mode, a fraction of the influent can be fed directly to the B-stage, while excess sludge from the B-stage can be returned to the A-stage. The sludge wasting is only performed in the A-stage. In Hybrid mode, wet-weather conditions trigger shifting to the Bypass mode. In dry weather, B-stage nitrifying sludge is transferred to the A-stage for nitrification while A-stage sludge is transferred to the B-stage providing a carbon source for denitrification. The effluent from the B-stage clarifier is returned to the A-stage for denitrification in all operational modes (Emde et al., 1992, Wandl et al., 2002). Increased SVI and temporary loss of nitrification was reported when the system was operated in Bypass mode (Wandl et al., 2002).

During pilot testing at the Innsbruck treatment plant in Austria, the nitrification rates in the B-stage were found to be 1.5-2 times more than that of the single-stage process (Winkler et al., 1994). The nitrogen removal performance of the A-B process was also comparable to a single-sludge system because of nitrogen assimilation in the A-stage biomass.

The main treatment plant in Vienna is an example of an A-B process with a unique method of operation. The effluent of the A-stage typically contains COD/N ratios of 5-7, which is

often not sufficient to meet the carbon requirement for the nitrogen removal in the B-stage. Therefore, operating the B-stage under carbon-limited conditions may require the use of ammonia-based aeration, simultaneous nitrification-denitrification (SND), and nitrite shunt (proposed here) to meet effluent nitrogen limits and to avoid B-stage alkalinity limitations.

2.4 Simultaneous Nitrification-Denitrification

Simultaneous nitrification-denitrification (SND) is defined as the NH_4^+ oxidation and subsequent reduction to dinitrogen gas, occurring at the same time in the same reactor without clearly defined aerobic and anoxic zones independent of bulk DO levels. Nitrification and denitrification in the same tank is highly desirable compared to the conventional systems, since separate tanks and recycling of mixed liquor containing NO_3^- from the aerobic nitrifying zone to the anoxic denitrifying zone is not required. It also results in more complete nitrogen removal and reduced aeration requirements (Rittmann et al., 1985). The reactor microenvironments (aerobic and anoxic zones developing within a reactor due to a combination of poor mixing and reactor design) and the floc microenvironments (anoxic zones developing within the activated sludge flocs) have been postulated as possible mechanisms for SND (Daigger et al., 2007). It is difficult to incorporate control strategies in the above-mentioned mechanisms to achieve stable SND performance and nitrite-shunt. The occurrence of SND is reported in staged, closed loop reactors (such as oxidation ditch, orbal) (Daigger et al., 2000) that typically employ long hydraulic residence time (HRT), solids retention time (SRT), and continuous low dissolved oxygen (DO).

The factors that influence the occurrence of SND have been reported as floc size, bulk DO concentrations, and carbon supply (Pochana et al., 1999). To optimize SND the reactor DO should be targeted to provide adequate oxygen to allow for nitrification while maintaining low enough levels to ensure sufficient denitrification. However, at this DO level, nitrification and denitrification rates will be lower than maximum. Optimum DO levels for SND are reported to range from 0.5 – 1.0 mg/L, and are highly dependent on specific operating conditions and reactor design (Liu et al., 2010, Münch et al., 1996, Pochana et al., 1999). The influent COD/N ratio is crucial in determining the success of a

SND process much like conventional nitrification-denitrification. SND is favored by higher COD/N ratios (Chiu et al., 2007, Xia et al., 2008), however, SND results in better nitrogen removal performance for a given COD/N ratio compared to nitrification-denitrification processes (Bratby et al., 2012). This attribute of SND is ideally suited for a nitrogen removing B-stage (of A-B process) operating under carbon-limited conditions.

2.5 Alternative Biological Nitrogen Removal for High Nitrogen Sidestreams

The conventional nitrification-denitrification process for nitrogen removal is used for treating wastewater with relatively low nitrogen concentrations (<100 mgN/L). Wastewater streams such as anaerobic digester effluent typically contain high concentrations of nitrogen (>250 mgN/L) usually in the form of NH_4^+ . These waste streams increase the ammonia loading to the main plant if not treated separately. The volumetric flow of sidestreams (e.g., effluent from dewatering of digested sludge) is small compared to the total inflow (< 5%), however, it may account for up to 30% of the total nitrogen load to the WWTP (Henze et al., 2008).

The treatment of these high ammonia strength streams with warm temperatures (20-35 °C) can sustain high bacterial activity. Therefore, it is possible to operate sidestream treatment with small tanks operating at aggressive short SRTs. The ammonia load reduction with separate treatment can improve the final effluent quality significantly (Henze et al., 2008). The low COD/N ratio and limited alkalinity makes conventional nitrification-denitrification extremely inefficient for sidestream application. Recently, several cost-effective alternatives have emerged to treat these low COD/N waste streams.

AOB-NOB Bioaugmentation

In the context of biological nitrogen removal, bioaugmentation refers to a transfer of sidestream generated nitrifying organisms (AOB and NOB) to increase nitrification capacity of the mainstream treatment. Typically, BNR plants are operated at longer SRTs to account for the slower growth rates of nitrifiers during winter months. The addition of

biomass enriched with nitrifiers can increase the nitrification capacity of the mainstream activated sludge process without extending the SRT (Head et al., 2004, Salem et al. 2004, Lifang et al. 2012).

The transfer of nitrifying biomass from a high-strength sidestream reactor to a low strength mainstream reactor, such that the solids retention time required to perform nitrification is decreased in the mainstream process was reported by Bailey et al. (2008). When the system is operated at longer SRTs, the mixed liquor suspended solids concentration increases. To deal with the increased solids, inventory plants are required to increase their aeration tank and/or clarifier volume. Therefore, BNR plants incur risk of failure or a large capital cost for extra capacity to perform nitrification reliably during winter months. Bioaugmentation offers a cost-effective alternative for winter nitrification and improved nitrogen removal performance.

To facilitate bioaugmentation of nitrifiers, a conventional nitrification/denitrification process is operated as a side-stream treatment process, typically with input of alkalinity and supplemental carbon. However, the temperature difference between side-stream and mainstream can exceed 20°C. Therefore, loss of activity of bioaugmented nitrifiers due to temperature shock is an important consideration (Head et al., 2004).

The optimal SRT of the bio-augmentation processes is critical for maximizing the overall nitrogen removal efficiency. Longer SRT results in a higher nitrifying biomass enrichment and greater nitrification efficiency of the side-stream process. Conversely, biomass decay also increases with increasing SRT. This could result in less active nitrifying biomass, decreasing the desired bioaugmentation effect on the main activated sludge line (Berends et al., 2005).

InNitri®

The aim of the InNitri® process is to reduce mainstream aerobic SRT for cold weather nitrification via bioaugmentation. The InNitri® process consists of a sidestream nitrification system for treating high ammonia strength reject water for the enrichment of nitrifiers. It can use supplemental commercial ammonia to augment the reject water to

ensure nitrifying biomass for bioaugmentation. It is typically operated at high ammonia loading (NH_4^+ concentration: 300-900 mgN/L) and temperatures around 30-35°C. Primary effluent is fed as a carbon source. Activated sludge from the InNitri® process is transferred to the mainstream aeration tank as a part of nitrifier bioaugmentation. Plants without anaerobic digestion can still use this process with commercially available ammonia. Since the aim is to sustain full nitrification, external alkalinity is required.

Kos (1998) proposed a short-SRT nitrification process/flowsheet as an alternative for plants in cold regions needing an upgrade for year-round nitrification and nitrogen removal (Figure 10). The modeling results of this process indicated that the aerobic SRT needed for nitrification in the mainstream could be lowered to ~5 days from 13-18 operating at 10°C with an effluent ammonia of 2 mgN/L.

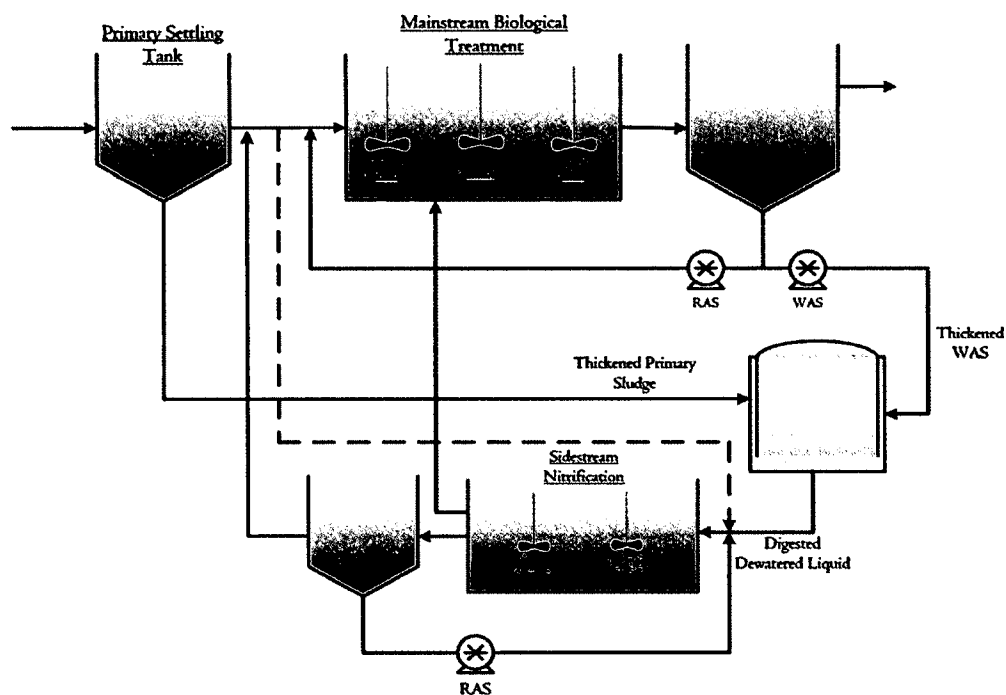


Figure 10. Bioaugmentation of sidestream generated nitrifiers to mainstream for low temperature nitrification [After Kos (1998)]

BABE®

BABE® or bioaugmentation batch enhanced process was developed in The Netherlands by collaborators including the Technical University in Delft, DHV-Water consultants, and STOWA (Zilverentant, 1999). A unique feature of BABE® is that the mainstream RAS is diverted to the sidestream activated sludge system for reject water treatment where the endogenous nitrifiers are enriched at ~25°C and a high ammonia load. The RAS lowers the temperature of the BABE® reactor such that adaptability and survivability of the bioaugmented nitrifying biomass is increased in the mainstream (Bouchez et al. 2000).

The enriched nitrifiers are transferred to the mainstream process for enhanced nitrification without extending SRT. The BABE® reactor is operated for conventional nitrification which requires external carbon and/or alkalinity depending on the reject water characteristics.

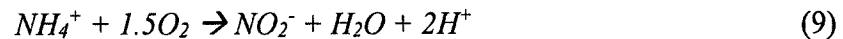
Salem et al. (2004) reported 74% ammonia removal at ~16-20 °C for the first installation of BABE® at Garmerwolde WWTP, The Netherlands. A process train without bioaugmentation removed only half of the ammonia that the process train with the BABE® was able to remove at the same plant. The process train with the BABE® process was operated at a significantly lower SRT than would be required even during colder months.

There are other process based on the idea of nitrifiers bioaugmentation from the sidestream to the mainstream such as MAUREEN (Constantine et al., 2005), BAR (Parker et al., 2007), and AT-3 (Katehis et al., 2002).

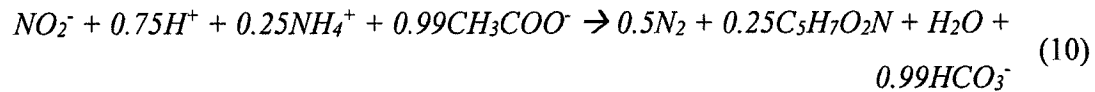
Nitrification-Denitrification

Nitrification is the oxidation of NH_4^+ to NO_2^- by AOBs primarily *Nitrosomonas* and *Nitrosospira* while nitrification is the oxidation of NO_2^- to NO_3^- by NOBs primarily *Nitrobacter*, *Nitrococcus*, *Nitrospina*, *Nitrospira*, *Nitrotoga* (Ward et al. 2011). The principal reactions are presented in Equations 9 and 10.

Nitrification:



Denitrification:



Based on these reactions the stoichiometric oxygen and COD demand for nitrification-denitrification is 3.43 gO₂/g NH₄⁺-N oxidized and 4.45 g acetate as COD/g NO₂⁻-N oxidized respectively.

The benefits of nitrification-denitrification over conventional nitrification/denitrification includes 25% reduction in O₂ demand, 40% reduction in carbon demand, and a reduction in biomass. The biomass production is reduced by avoiding oxidation of NO₂⁻ to NO₃⁻ and the reduction of NO₃⁻ back to NO₂⁻. Nitrification-denitrification requires the addition of an exogenous carbon source or step feeding of a high COD waste stream to provide a carbon source (e.g. methanol) for denitrification. The alkalinity to maintain stable pH is typically not sufficient, however, the recovery of alkalinity during methanol-driven denitrification usually provides the needed pH buffer.

A number of nitrification-denitrification processes have been developed for treating high ammonia strength waste streams. These processes aim to stop nitrification at NO₂⁻ and then reduce NO₂⁻ to N₂ via denitrification thus avoiding nitrification (NO₂⁻ oxidation to NO₃⁻) and denitrification (NO₃⁻ reduction to NO₂⁻). One of the most common processes is the SHARON® (Single reactor system for High-activity Ammonia Removal over Nitrite) process developed by Hellinga et al. (1998). It takes place in a continuous stirred tank reactor (CSTR) with suspended biomass. The reactor is operated at temperatures between 30 and 40 °C and low sludge retention time (~HRT= SRT). In these conditions, AOB are selectively retained while the slower growing NOB are washed out. The low SRT (typically < 1.5 d) and high temperatures select AOB while causing NOB to wash out (Egli, 2003). Both nitrification and denitrification may take place in the same stirred reactor using intermittent aeration. Nitrification-denitrification similar to SHARON® has been

successfully demonstrated in sequencing batch reactors (SBR) with sludge retention time (SRT) control (Fux et al., 2002). Although nitrification-denitrification is an improvement over the conventional pathway, the discovery of anammox provides even more opportunities to reduce resources for biological nitrogen removal.

The STRASS process uses a high-sludge sequencing batch reactor to oxidize NH_4^+ to NO_2^- followed by reduction of the produced NO_2^- to N_2 gas (Wett et al., 1998). A supplemental carbon, such as primary sludge is needed for the denitrification process. The key feature of the STRASS process is the highly effective intermittent aeration control systems that are based on pH control mechanisms. The nitrification step consumes alkalinity which causes pH to drop during aeration. As the pH drops to the low set-point, the aeration stops and alkalinity recovers. When the pH rises to the upper pH setpoint, aeration is switched on again resulting in a characteristic sawtooth pH profile. The frequency and length of aeration intervals adjusts to the loading rate and concentrations of sidestreams. NOB out-selection is achieved by selection of proper pH set-points, which also helps to avoid inorganic carbon limitation.

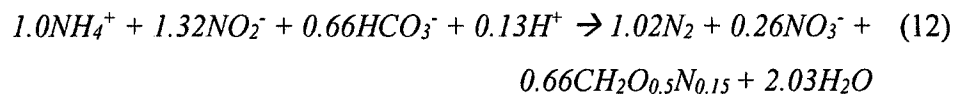
Both the STRASS process and the SHARON process were developed around same time and are considered to be an equivalent technology. The key difference is that the STRASS process is operated in an SBR with sludge retention whereas the SHARON process is operated in a chemostat without sludge retention. The SHARON process relies on a short SRT to out-select NOB, while the STRASS process depends on pH-controlled intermittent aeration for NOB out-selection.

Partial Nitrification and Anammox

Since its discovery in the mid-1990s (Mulder et al., 1995), anammox process has been successfully applied to sidestream treatment. Partial nitrification plus anammox (also known as deammonification) is a two-step process, which utilizes the symbiotic relationship of two genera of bacteria, ammonia oxidizing bacteria (AOB) and anaerobic ammonia oxidizers (AMX).

In the nitrification step, approximately half of the NH_4^+ load is oxidized to NO_2^- by AOB while the remaining NH_4^+ and the produced NO_2^- is then reduced to N_2 during the second

step by AMX. The basic energy reaction and complete reaction for cell synthesis (11) was first postulated by van de Graaf et al. (1996).



These stoichiometric ratios (12) determined by Strous et al. (1998) are widely accepted in literature to indicate anammox activity. For every mole of NH_4^+ consumed by anammox 1.32 moles of NO_2^- are consumed and 0.26 moles of NO_3^- are produced. Additionally, the anammox reaction also provides alkalinity as 0.13 moles of H^+ are consumed.

Deammonification is mediated by AOB and AMX populations, which ideally requires complete NOB inhibition and heterotrophic denitrification is not needed. Therefore, it theoretically can result in approximately 63% reduction in required O_2 , nearly 100% less carbon, and 80% less biomass. Since only about half of the influent NH_4^+ is oxidized to NO_2^- , deammonification in sidestream may not require supplemental alkalinity. The stoichiometric alkalinity demand of deammonification is 1.95 g of alkalinity as $CaCO_3$ per g NH_4^+-N oxidized to $NO_2^- -N$ versus 3.9 g of alkalinity as $CaCO_3$ per g NH_4-N oxidized to $NO_2^- -N$ for complete nitrification. With typical centrate characteristics as shown in Table 2 the alkalinity is sufficient for this reaction.

2.6 Implementation of Deammonification in Sidestream Treatment

The principal considerations for deammonification are providing the different redox environments for both groups of organisms (i.e., AOB and AMX) and decoupling the solids retention time of AMX from the AOB. In order to provide conditions which will facilitate deammonification a variety of reactor configurations and biomass separation techniques have emerged. One-step (single sludge: all biological reactions take place in one reactor) and two-step processes (two sludge: AMX and AOB reactions take place in separate reactors) are two major alternatives. Further, AMX retention techniques and operational strategies may differ within these reactor configurations.

Two-Step Deammonification Processes

Typically a two-step process consists of an aerobic reactor for AOB reaction (i.e., nitrification) which is followed by a second reactor for anammox. The most well-known example of two-step process is the patented SHARON®-ANAMMOX®, where the aerated SHARON® reactor is optimized for partial nitrification (i.e., inhibition of NO_2^- oxidation) that is followed by a fully anoxic reactor for anammox. A two-stage OLAND® (oxygen-limited autotrophic nitrification-denitrification) process is another example of two-step configuration. A summary of processes using the two-step approach can be seen in Table 3. The first reactor in a two-step process is typically operated with the goal to achieve 55% conversion of NH_4^+ to NO_2^- and the consumption of the remaining NH_4^+ and NO_2^- is targeted in the subsequent anammox reactor. Ideally, the goal of the nitrification reactor is to provide a stoichiometric ratio of NO_2^- : NH_4^+ of 1.3 for the anammox reaction without producing any NO_3^- . Therefore, it is crucial to inhibit NO_2^- oxidation by suppressing NOB activity in the nitrification reactor. The most common measures of NOB suppression are DO manipulation, inhibitory chemicals, and short SRTs; however, when all these are used in conjunction the relative influence of one particular factor it is difficult to distinguish and remains a topic of research.

The AMX process requires a ratio of NO_2^- : NH_4^+ of 1.3. Providing more NO_2^- to the system results in NO_2^- accumulation which may inhibit AMX. Therefore, it is critical to maintain the proper ratio of the AMX substrates in the two-step processes. Further,

sufficient mixing conditions should be maintained to prevent localized elevated concentrations of substrates especially at the influent to the anammox reactor which could potentially be inhibitory. Waste stream characteristics dictate the techniques to ensure proper ratio of NO_2^- : NH_4^+ . For example, typical centrate stream contains the alkalinity: NH_4^+ ratio that is only sufficient to support ~50% of the nitrification process, which roughly maintains the desired NO_2^- : NH_4^+ ratio. Other waste streams rely on bypassing influent flow to the anammox reactor to maintain the proper ratio.

Sufficient AMX biomass retention is the most important criteria to sustain deammonification in the two-step process. Recently, to achieve the retention of AMX biomass, techniques involving wasting through hydrocyclone, fixed film media, and other selective biomass retention devices have been used. The fixed film media and granular sludge in an upflow reactor are commonly used for the final anammox step in a two-step process (Table 3).

Table 3. World-wide full-scale two-step deammonification installations for reject water treatment (after Lackner et al., 2014)

Location	Year	Size m^3	N load		Process
			kgN/d	kgN/ m^3 /d	
Fulda Gläserzell, Germany	2008		150-200		Terrana®
Landshut, Germany	2010	210/210	340		Terrana®
Rheda Wiedenbrück, Germany	2007	3700	1400		PANDA+
Rotterdam, The Netherlands	2002	1500/72	500	0.3(10)	SHARON®/ ANAMMOX®
Hattingen, Germany	2003	104/2×67			MBBR
Oslo/Bekkelaget, Norway	2011	2×328/328	710		MBBR

One Step Deammonification Process

The challenges of the one-step approach for deammonification is relatively different from two-step processes. Combining the steps in a one-reactor system significantly lowers the investment costs and avoids the difficulty to control two reactors. Further, preliminary full scale investigations showed that one-stage autotrophic nitrogen removal emits less of the environmentally harmful nitric and nitrous oxides than the two-stage process (Kampschreur et al., 2008). Since, AMX and AOB grow in the same reactor continuous or sometimes intermittent aeration is required.

The reversible inhibition of AMX at high DO concentrations (>0.4 mg/L) has been well documented in literature. Therefore, the key is to provide sufficient DO concentrations to allow AOB reaction while avoiding DO inhibition of AMX. The AMX stoichiometric ratio of NO_2^- : NH_4^+ of 1.3, which also requires inhibition of NO_2^- oxidation, is maintained in the one-step process through strategies which control air flow, pH, and SRT. However, more importantly, since both the AMX and AOB biomass are located in the same reactor, decoupling of the two populations' SRT becomes the major challenge for the success of the one step process. To address challenges associated with separating SRTs, three configurations have been developed to achieve one-step deammonification.

SBR with Hydrocyclone

Although there are a number of SBR based technologies for one step deammonification, the DEMON® system is the only one to use hydrocyclones for selective wasting and retention of AMX biomass. The world-wide installations of DEMON® in the capacity of sidestream treatment for reject water can be seen in Table 4. In an SBR, slow growing AMX forms granules and faster growing AOB inhabit the flocs. The aeration to provide appropriate DO levels in the reactor is controlled to produce sufficient NO_2^- by AOB, while aeration is terminated to provide anoxic environment for AMX. The control of aeration duration and DO levels is important to ensure proper AOB and anammox activity. DEMON® systems are operated at DO concentrations of 0.2-0.3 mg O_2 /L during aerated periods (Wett, 2007). To prevent excessive inhibition of AMX activity, low DO levels are

preferred. This also ensures quicker transitions to anoxia at the end of aerobic periods thus providing a truly anoxic environment for optimum AMX activity.

Successful operation of DEMON® depends on the aeration level, DO control, and decoupling of AOB and AMX SRTs. The target SRT for AMX is greater than 30 days whereas AOB SRT is maintained at 5-6 days. This is made possible by the use of hydrocyclones which promote granulation of AMX and achieve biomass separation. The heavier granules with AMX are retained in the underflow of the cyclone and returned back to the process, while the lighter floc containing AOB, NOB, heterotrophs, and debris are wasted in the overflow. The SRT of the floc phase is maintained with the consideration of selecting AOB while washing out the undesired NOB. Therefore, proper SRT control plays an important role in preventing NOB growth as well as determining overall nitrogen removal performance of the DEMON® process. NOB suppression in the sidestream DEMON® process is also favored by the high temperatures, typically around 30-35°C. At these temperatures AOB are postulated to grow faster than the NOB (Hellings et al., 1998). Further, AMX are also close to their optimum activity which provides NO_2^- competition for NOB.

Table 4. Word-wide full-scale DEMON® installations for reject water treatment (modified after Lackner et al., 2014)

Location	Year	Size m ³	N load kgN/d	KgN/m ³ /d
Alexandria, USA	in construction	3000	1282	0.42
Amersfoort, Netherlands			600	
Apeldoorn, Netherlands			1690	0.66
Bad Sobernheim, Germany		2x180	200	
Balingen, Germany	2009	705	120	
Bickenbach, Germany			100	
Bilten, Switzerland				
Blue Plains, USA	in design	22000	9072	0.58
Breda, Netherlands	2013	1000	990	0.99
Dietikon, Switzerland				
Eisenhüttenstadt, Germany	2013			
Erpfendorf, Germany			120	
Gengenbach, Germany	2008		50	
Glarnerland, Switzerland	2006	400	250	0.4
Guelph, Canada	in design			
Heidelberg, Switzerland	2008	2x550	330 (480)	
Helsinki, Finland			250	
Hema, Germany	2014		110	
Kaster, Germany	2013	2x150	100	
Kecskemet, Turkey			1300	
Kokkola, Finland			600	
Lahr, Germany	2011	400	235	150
Limmattal, Switzerland	2010		250	
Neumarkt, Germany	2011			0.92
Nieuwegein, Netherlands			450	
Michelstadt, Germany				
Philidelphia, USA	in design			
Pierce County, USA	in design			
Plettenberg, Germany	2008	134	100	0.5

Pustertal, Italy	2012		500	
Strass, Austria	2004	500	300	0.6
Thun, Switzerland	2008	606	400	0.67
York River, USA	2012		1000	
Zalaegerszeg, Hungary	2010		160	

Moving-Bed Biofilm Reactor (MBBR)

Moving-bed biofilm reactors are continuous operation systems, which provide no control of biomass wasting. In an MBBR system, carrier media provides the surface area for slow-growing organisms (e.g., AMX) to adhere. The bacterial growth takes place in the form of a biofilm which attaches to the support media. The biofilm after sufficient growth provides substrate gradients for the organism residing at different layers within the biofilm. AMX, which grow slower and are inhibited by high levels of DO, are found in the deeper anoxic layers of the biofilm while the aerobic AOB are found on the outer layers. Since NO_2^- is produced by the AOB in the biofilm, it can penetrate the biofilm deeper than oxygen thus providing substrate for AMX.

The support media also provides a zone of growth competition between AOB and NOB and is considered a major concern in MBBR configurations (Pellicer-Nacher et al., 2010). The aeration and DO control play an important role in preventing NOB activity in MBBRs. Currently there are two major MBBR deammonification systems; Veolia-AnoxKaldnes ANITAMoxTM, and DeAmmon® process. Their status in terms of world-wide installations to treat reject water can be seen in Table 5.

Table 5. Installation of MBBR based deammonification systems world-wide for reject water treatment (modified after Lackner et al., 2014)

Location	Year	Size m ³	N load kgN/d	kg N /m ³ / d
Malmö, Sweden*	2011	4 x 50	200	
Växjö, Sweden*	2011	300	320 / 430	
James River, USA*	2013	393	253	0.63
South Durham, USA*	in construction	318	303	0.95
Holbæk, Denmark*	2012	600	120	
Grindsted, Denmark*	2013	140	100	
Hattingen, Germany**	2003	230	120 (180)	0.5
Dalian XiaJiaHe, China**	2009		2200	
Stockholm, SE**	2007	1400	600	0.3

* ANITA MoX™

**DeAmmon®

Granular Sludge

Like the SBRs, there are other granular sludge systems that rely on the formation of AMX granules as the main separation mechanism for AMX retention. Completely autotrophic nitrogen-removal over nitrite (CANON®) (Strous 2000, Third et al., 2001, Sliekers et al. 2002) and ANAMMOX® are two schemes based on upflow reactor technology for granulation. These systems are continuously fed from the bottom of the reactor. Biomass retention occurs as the effluent is drawn from the top while the reactor is continuously aerated. The world-wide installations of ANAMMOX®, which is marketed by Paques of The Netherlands to treat reject water, is shown in Table 6.

Table 6. Installation of ANAMMOX® systems world-wide for reject water treatment (after Lackner et al., 2014)

Location	Year	Size m ³	N load kgN/d	kgN/m ³ / d
Minworth, UK	2011	1760	4000	
Stoke Bardolph, UK	in construction		3000	2
Tilburg, Netherlands	in construction		2000	2
Zwolle, Netherlands	2010	425	600	

2.7 Worldwide Status of Sidestream Deammonification

Historically, the low-risk wastewater industry has been very slow to implement innovative technologies at the full-scale application (Parker, 2000). In context the development of deammonification based technologies since the discovery of anammox in mid-1990s and the worldwide adoption at the full-scale level has been a notable trend. Culmination of better research/tools, faster dissemination of findings, perception of investment in research and development as profitable in a long-term, better sensor and operational controls make innovative technologies such as deammonification feasible. Further, increasingly stringent nitrogen limits and the cost of energy for wastewater treatment might be responsible for faster adoption rates. This trend can be seen in (Figure 11), which clearly shows the rapid rate of publication and full-scale implementation of anammox based technologies after discovery of the bacteria. However, in United States the rate of scientific research and full-scale installations have been much slower (Figure 12). In fact, the first installation was in 2012 at HRSD's York River WWTP, which was almost a decade after the first publication related to the use of anammox bacteria for wastewater treatment. It highlights that the US wastewater industry tends to be even more conservative when it comes to implementation of novel technologies. However, there were an additional 8 sidestream deammonification installations planned in 2013, which highlights the recent sense of urgency among utilities to adopt energy efficient technologies to meet their nitrogen removal goals.

Worldwide, the treatment of high-ammonia strength waste streams with deammonification is gaining popularity with close to 100 full-scale installations planned for 2014 (Lackner et al., 2014). The findings of a recent survey by Lackner shows that 50% of all installations were based on SBRs and 88% of them were operated as single-stage systems, while the majority (~75%) were being operated at municipal wastewater treatment plants.

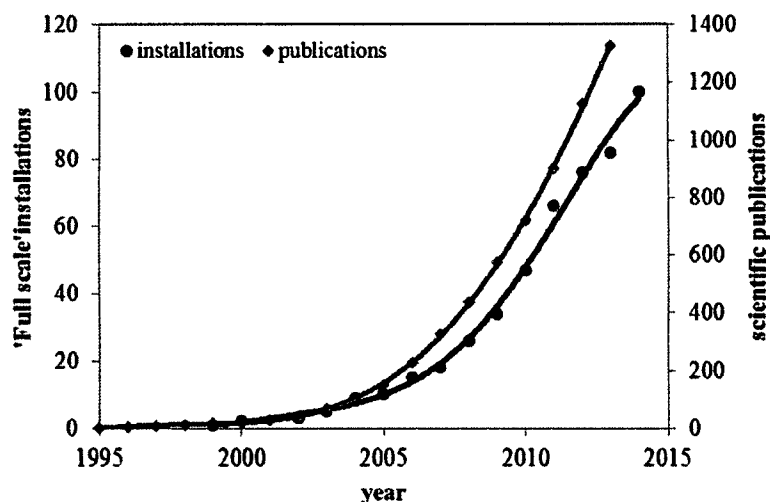


Figure 11. Cumulative full-scale installations of deammonification based technologies (including plants under design/construction) and the number of scientific publications on the topic of anammox/deammonification (based on the results returned by Web of Science and Scopus repositories on 10/24/2013). Adopted directly from Lackner et al. (2014).

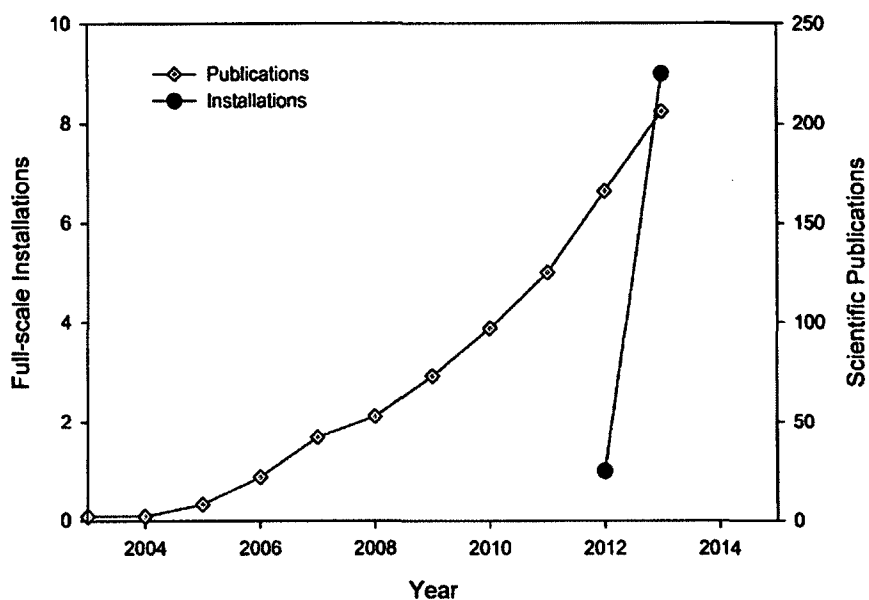


Figure 12. Cumulative full-scale installations of deammonification based technologies (7 plants under design/construction and 2 operational) and the number of scientific publications on the topic of anammox/deammonification (based on the results returned by Web of Science from 2003-2013 on 2/27/2014) in the United States.

2.8 Comparison of Alternative Pathways of Biological Nitrogen Removal

The high cost of the conventional nitrification-denitrification pathway for nitrogen removal has resulted in alternatives being explored. These alternatives are the nascent stage of development, however, promise efficiency and low environmental impact. The summary of these alternatives compared to the conventional systems is presented in Table 7.

Table 7. Important considerations of conventional and alternative nitrogen removal processes.

	Nitrification-Denitrification	Nitritation-Denitrification	Partial nitritation-Anammox
Target organisms	AOB, NOB, denitrifiers	AOB, denitrifiers	AOB, AMX
Oxygen demand (gO_2/gN)*	4.18	3.16	1.94
COD needed without assimilation (gCOD/gN)**	2.86	1.72	0
COD needed with assimilation (gCOD/gN)**	4.0	2.4	0
Sludge production (gVSS/gN ***)	1	N/R	0.1
Alkalinity consumption (gCaCO_3/gN)*	7.07/-3.57	7.07/-3.57	3.68
Application status	Established	Full-scale plants	Full-scale sidestream only

*Ahn et al. (2006)

**van Hulle et al. (2010)

***Mulder et al. (2003)

N/R: Not reported

The resource savings (i.e., oxygen and carbon) associated with nitrification-denitrification and anammox over conventional nitrification/denitrification are provided in Table 8.

Table 8. Relative oxygen demand, COD demand, alkalinity consumption and biomass production of nitrification-denitrification, partial nitrification-anammox compared to conventional nitrification-denitrification

Process	Oxygen demand	COD demand	Alkalinity consumption	Biomass production
Conventional Nitrification-Denitrification	100%	100%	100%	100%
Nitrification-Denitrification*	75%	40%	100%	60%
Partial (50%) nitrification-anammox**	37%	~0	~0	20%

*Turk et al. (1986)

**Mulder et al. (2003)

The implementation of partial deammonification for mainstream treatment could potentially turn the energy consuming wastewater treatment plant into energy producing one as seen in Table 9.

Table 9. Oxygen consumption and energy balances for selected wastewater treatment variations. Case A: Conventional treatment; Case B: Conventional treatment with anammox used for treatment of digester effluent; Case C: Optimized treatment, with anammox for mainstream treatment (Kartal et al., 2010).

Oxygen and energy need	Mass Flux ($\text{g p}^{-1}\text{d}^{-1}$)			Energy ($\text{Wh p}^{-1}\text{d}^{-1}$)		
	Case A	Case B	Case C	Case A	Case B	Case C
Aeration for COD removal	40	30	15	-40	-30	-15
Aeration for nitrogen removal*	22	22	16	-22	-22	-16
Pumping and mixing energy	-	-	-	-20	-20	-15**
Methane-COD and electrical energy production from biogas	30	40	55	+38	+51	+70
Net energy	-	-	-	-44	-21	+24

*Nitrate effluent for cases A and B: $2.5 \text{ g p}^{-1}\text{d}^{-1}$; for case C: $1.1 \text{ g p}^{-1}\text{d}^{-1}$

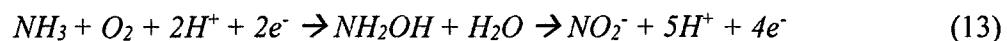
**Lower because of recirculating flows

2.9 Microbial Protagonists of Biological Nitrogen Removal

In biological nitrogen removal, four important functions are linked to four groups of bacteria which gain energy from oxidation and/or reduction of a specific nitrogen compound. Nitrification is performed by autotrophic aerobic ammonia oxidizing bacteria (AOB), and nitrification by autotrophic nitrite oxidizing bacteria (NOB). Further, heterotrophic denitrification (reduction of NO_3^- to NO_2^-) and subsequently denitrification (reduction of NO_2^- to N_2 gas) is performed by denitrifiers. The anoxic ammonium oxidation or anammox (Mulder et al., 1995) is performed by autotrophic, anoxic ammonium-oxidizing bacteria (AMX) (van de Graaf et al., 1996). AMX rely on AOB to oxidize NH_4^+ to produce NO_2^- which is their other substrate. In this study we will focus more on the NOB and AMX as they are the organisms of interest. The former for out-selection and latter for retention.

AOB

AOB catalyze the conversion of ammonia into nitrite (nitrification). The catabolic reaction ($\Delta G^\circ = -235 \text{ kJ/mol}$) consists of two sequential oxidation steps (Kowalchuk et al., 2001, Bock et al., 2006): First ammonia (NH_3) is oxidized to hydroxylamine (NH_2OH) with the membrane bound enzyme ammonia monooxygenase (AMO). The second step involves the oxidation of NH_2OH to NO_2^- with the periplasmic enzyme hydroxylamine oxidoreductase (HAO), and provides the two reducing equivalents needed for the first step (Equation 13). The two other produced electrons are passed via an electron transport chain to the terminal oxidase, thereby generating a proton motive force.



The taxonomy of AOB is based on ribosomal sequencing and comparative genomics (Hörn et al. 1997). *Nitrosomonas* are commonly found in wastewater treatment plants and as a result have been widely studied. *Nitrospira* and *Nitrosococcus* are mostly found in soils as well as marine and freshwater systems. *Nitrospira* have been identified in colder wastewater temperatures with higher DO saturation (Ward, 2011).

AOB are primarily obligate aerobic chemoautotrophs. AOB exclusively use NH_3 as the sole electron donor for energy and inorganic carbon (IC) as their carbon source and are unable to oxidize NH_4^+ (pK_a of 9.25 at 25°C). At lower pH conditions the protonated form is the dominant species. Further, NH_4^+ oxidation causes pH to decrease due to the release of H^+ which results in a reduction of NH_3 for the AMO enzyme driven reaction (Ward et al., 2011, Lauchnor 2011). The reduction in pH has been linked to partial inhibition due to limited availability of substrate. Furthermore, low pH is also inhibitory due to other physiological factors.

The optimum pH range for nitrification is perceived to be between 7 and 8 (Suzuki 1974, Tchobanoglous et al., 2003). However, more recent studies have shown that certain species of AOB may maintain higher activity rates in more acidic environments (Tarre et al., 2004).

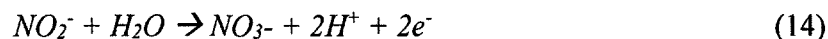
Even the fastest growing AOB (*Nitrosomonas europaea/eutropha*) have a doubling time of approximately 8 hours or a maximum specific growth rate (μ_{max}) of 2 day^{-1} (Siripong et al., 2007) which is significantly slower than heterotrophs that dominate the bacteria population in activated sludge. *Nitrosospira* have a μ_{max} ranging from $0.70\text{--}0.9 \text{ day}^{-1}$ (Siripong et al., 2007). Temperature plays a vital role in selection of AOB species with different growth rates. At high temperatures *Nitrosomonas* could double twice as fast as *Nitrosospira* which results in *Nitrosomonas* being more dominant. At low temperatures, however, growth rates of these species are closer which prevents out-selection of *Nitrosospira* by *Nitrosomonas*.

The nitrifier biomass fraction, in particular AOB, is a relatively small portion of the total biomass population in aerobic reactors (Dytchak et al. 2008). This is a result of slow growth rates and the ratio of COD/NH_4^+ in the wastewater, which is typically greater than 10. In addition, AOB are prone to washout caused by seasonal changes in temperature, inhibitory compounds in wastewater, and shifts in pH. Traditionally, AOB are considered the most sensitive bacteria in activated sludge systems for biological nitrogen removal. Therefore, these nitrogen removal systems are typically designed with large factors of safety to account for the sensitivity of this important but fragile microbial population.

NOB

Although AOB and NOB share a synergistic relationship, NOB have not been as extensively studied as AOB (Ward et al., 2011). Historically, *Nitrobacter* have been considered the model NOB which might be due to the relative ease to culture and study it over other species in the laboratory. Recent studies indicate *Nitrospira* as the dominant NOB species in wastewater systems (Siripong et al., 2007, Starkenburg, 2007, Ward et al. 2011).

Nitrite oxidation ($\Delta G^{\circ} = -54$ kJ/mol) involves the enzyme nitrite oxidoreductase (NOR) that delivers two electrons to the NOB which are transferred to oxygen with a terminal oxidase (Bock et al., 2006). NO_2^- is oxidized to NO_3^- by nitrite oxidoreductase (NXR) according to the reaction in Equation 14 (Starkenburg, 2007).



NOB are considered chemolithoautotrophic bacteria, which oxidize NO_2^- for growth and energy and fix CO_2 through the Calvin cycle for biosynthesis. However, there are some NOB species capable of mixotrophic growth on both NO_2^- and organic energy donors.

NO_2^- rarely accumulates in the natural environment as result of a symbiotic relationship between AOB and NOB, which is the basis for biological nitrification-denitrification processes. AOB produces NO_2^- , a substrate for NOB and by consuming this substrate, NOB prevents buildup of NO_2^- which could potentially be inhibitory to AOB. NOB are present in variety of natural environments ranging from fresh water, marine environments, and soils although dominant species may differ.

NOB are reported to grow optimally at temperatures between 25 and 30 °C. Usually, NOB diversity increases at low wastewater temperatures. During the winter, AOB growth rates decrease more than NOB growth rates which increases the NOB's ability to compete for DO against AOB. *Nitrobacter* and *Nitrospira* grow optimally at pH of 7.8 and 8, respectively (Blackburne et al. 2007).

The two most abundant genera of NOB, *Nitrobacter* and *Nitrospira*, exhibit different affinities for dissolved oxygen and NO_2^- concentrations as well as preference of wastewater temperatures. These differences are often the focus of the research to better understand how to control NOB population in different operating and environmental conditions.

Nitrous acid (HNO_2) is inhibitory to NOB. The concentration of HNO_2 increases as pH falls ($\text{pK}_a = 3.398$ at 20°C) value and it decreases as the pH rises. Therefore, NO_2^- accumulation at lower pH creates toxic conditions for NOB.

NOB Population Dynamics

The K/r classification is often used to describe competitive abilities of microorganisms. K-strategists are able to survive longer periods of starvation since they are slower growing and have high affinity for substrates. K-strategist organisms tend to survive better at high temperatures than their r-strategist counterparts. Alternatively r-strategist micro-organisms thrive only when high concentrations of substrate are available (low affinity to the substrate) but are relatively fast growers.

Presently, NOB are classified into four lineages: *Nitrobacter*, *Nitrospina*, *Nitrococcus* and *Nitrospira* that are members of the Alpha-, Delta- and Gammaproteobacteria (Teske et al., 1994) and the phylum *Nitrospireae*, respectively. *Nitrobacter* is easy to culture and is the most common NOB to be isolated, which made it the primary model organism for studying NO_2^- oxidation and established *Nitrobacter* as the key NOB in wastewater treatment plants (Grady et al., 1980). However, over the last decade, this notion has been challenged when no *Nitrobacter* related organisms were detected in nitrifying activated sludge samples by fluorescence *in-situ* hybridization (FISH) with rRNA-targeted oligonucleotide probes specific for the genus *Nitrobacter* (Wagner et al., 1996). In a non-culture based approach, it was further indicated that *Nitrospira*, not *Nitrobacter*, was the numerically dominant lineage in many wastewater treatment systems (Juretschko et al., 1998, Schramm et al., 1998, Daims et al., 2000, Daims et al., 2001). Despite the numerical dominance of *Nitrospira* in many ecosystems, *Nitrobacter* has subsequently been detected and isolated in wastewater (Bartosch et al., 2002, Moussa et al., 2006, Matsumoto et al., 2007) and recent investigations have indicated that *Nitrobacter* may have a selective advantage over

Nitrospira under high NO_2^- loads (Daims et al., 2001, Bartosch et al., 2002), high oxygen tension (Matsumoto et al., 2007) and salt stress (Moussa et al., 2006).

Experimental determination of substrate affinities and growth rates of NOB are shown in Table 10.

Table 10. Substrate affinities reported for *Nitrospira* and *Nitrobacter* in literature.

NOB	Strategy	K_s , mg NO_2^- -N/L	K_o , mg $\text{O}_2/\text{LO}_2/\text{L}$	μ_{\max} , h^{-1}	b_{\max} , d^{-1}
<i>Nitrospira</i>	K (slow)	0.14 ¹ 0.11-0.55 ³ 0.25 ⁶	0.13 ³ 0.47 ³ 0.43±0.08 ⁶	N.R.	0.14 – 0.15 ⁴
<i>Nitrobacter</i>	r (fast)	7.0 ¹ 9.88-17.36 ² 1.49 ⁵ 0.39 ⁶	1.98 ¹ 0.17 – 4.32 ² 0.54 ± 0.14 ⁶	0.02 ⁵	0.07 ⁵

¹(Schramm et al., 1999)

²(Laanbroek et al. 1994)

³(Manser et al. 2005)

⁴(Manser et al. 2006)

⁵(Vadivelu, 2006)

⁶(Blackburne et al. 2007)

N.R.:Not reported

In a study, Kim et al. (2006) demonstrated that in a continuous biofilm airlift reactor when NO_2^- concentration was maintained very low, *Nitrospira* accounted as a dominant NOB while *Nitrobacter* was merely a small fraction of total bacteria. In contrast, in a sequencing batch reactor (SBR) where nitrite concentration was relatively high, it was *Nitrobacter* that was dominant NOB while *Nitrospira* was less prevalent. Further, the specific activity of *Nitrobacter* (93.8 mgN/gNOB.hr) was found to be greater than that of *Nitrospira* (10.5 mgN/gNOB.hr), thus strengthening the K/r hypothesis that *Nitrospira* is a K-strategist while *Nitrobacter* is an r-strategist. Fundamental characteristics for each type of nitrifying

bacteria lineage are not completely understood, even though these differences can have a significant impact on the success or failure of nitrifying systems.

Anammox Bacteria

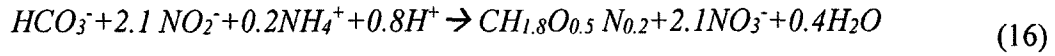
In 1977, the possibility of NH_4^+ oxidation with NO_2^- or NO_3^- as an electron acceptor was predicted by Engelbert Broda on thermodynamic and evolutionary grounds (van der Star, 2008). This prediction was turned into reality as Strous et al. (1999) discovered a bacteria of the order *Planctomycetales* which are capable of anaerobic ammonium oxidation, now referred to as anammox bacteria (AMX). With the discovery of anammox bacteria, several wastewater treatment processes have been developed to exploit this unique and efficient pathway for nitrogen removal. So far, these new technologies have been applied to high-ammonia strength streams often as sidestream treatment processes (Horn et al. 1997, Strous et al. 1997, Toh et al. 2002, Schmidt et al. 2003, Wett 2007, Van der Star et al. 2007).

AMX catabolism ($\Delta G^\circ = -358 \text{ kJ/mol}$) has not been fully revealed yet (Equation 15). Previously, hydroxylamine (NH_2OH) and hydrazine (N_2H_4) were hypothesized to be intermediates (Jetten et al., 2001), but more recently the involvement of nitric oxide (NO) and hydrazine has been put forward (Strous et al., 2006, Kartal, 2008a). The autotrophic anammox process uses HCO_3^- as the carbon source for anabolism (Equation 16). The oxidation of NO_2^- to NO_3^- generates the electron that is required for the HCO_3^- reduction process (van de Graff et al., 1996). When catabolism and anabolism are combined using a yield of carbonate on NH_4^+ ($0.066 \text{ mol C/mol NH}_4^+$, Strous et al., 1998) Equation 17 can be derived. The experimentally-found stoichiometry by Strous et al. (1998) (in Equation 18) is in close agreement with stoichiometry of Equation 17.

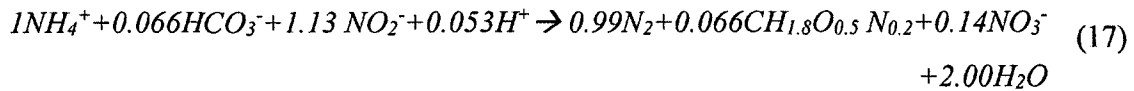
Catabolism:



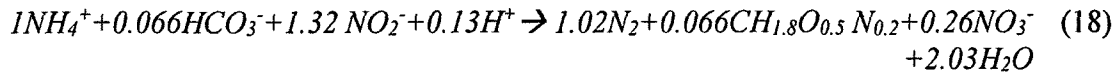
Anabolism:



Combined:



Experimental Stoichiometry by Strous et al. (1998):



The growth as well as decay rates associated with anammox metabolism are significantly lower than nitrifying bacteria, which are historically known for slower growth rates. The energy gained during anammox catabolism (calculated per mole of electrons) is similar to the nitrification, however AMX has extremely slower growth rates. Suboptimal growth conditions during enrichment in the cultivation systems and/or intrinsically low conversion rate of NH_4^+ and NO_2^- have been put forward as an explanation for slower AMX growth rates (van der Star, 2008).

The majority of AMX research has been conducted at 30 to 35 °C. Typically, AMX requires 5-10 times the SRT that is required by AOB in wastewater treatment systems. The doubling times for AMX have been reported between 11 and 19 days (Mulder et al., 1995, Strous et al., 1998, Schmidt et al., 2003, Dapena-Mora et al., 2007). The slower growth of AMX compared to AOB requires separation of SRTs for these populations in deammonification systems. On average AMX maximum specific growth rates (0.05-0.09 day⁻¹: Strous et al., 1999, van der Star et al., 2008, Ward et al., 2011) are 10 times slower than that of AOB (0.9 to 1.2 day⁻¹: day⁻¹ Sin et al., 2008). The anoxic decay rates of AMX

(0.008 day⁻¹) are 25 times slower than aerobic decay rates of AOB (0.2 day⁻¹) at 20°C (Udert et al., 2008).

AMX have been identified to grow in different environmental niches. A summary of genus and species of AMX that have been reported in the literature is presented in Table 11. The characteristics of each of these groups is still unclear. It has been found that specific genera have dominated the community in each environment that they have been identified, which is an indication that these genera have adapted to distinct environmental niches (Boumann et al., 2009).

Table 11. Species of AMX discovered to date (Kumar et al., 2010).

Genus	Species	Origin	Reference
Scalindua	<i>Candidatus</i> "Scalindua sorokinii"	Seawater (Black Sea)	Kuypers et al. (2003)
	<i>Candidatus</i> "Scalindua brodae"	Wastewater (landfill leachate)	Schmid et al. (2003)
	<i>Candidatus</i> "Scalindua wagneri"	Wastewater (landfill leachate)	Schmid et al. (2003)
	<i>Candidatus</i> "Scalindua Arabica"	Seawater (Black sea, Namibia, Peru and Arabian sea)	Woebken et al. (2008)
Brocadia	<i>Candidatus</i> Brocadia anammoxidans	Wastewater (Denitrifying pilot- plant)	Strous et al. (1999)
	<i>Candidatus</i> "Brocadia fulgida"	Wastewater (anammox reactor)	Kartal et al. (2004)
Kuenenia	<i>Candidatus</i> "Kuenenia stuttgartiensis"	Wastewater (nitrifying plant)	Schmid et al. (2000)
Jettenia	<i>Candidatus</i> "Jettenia asiatica"	Wastewater (granular anammox reactor)	Quan et al. (2008)
Anammoxoglobus	<i>Candidatus</i> "Anammoxoglobus propionicus"	Wastewater (Dokhaven WWTP, Rotterdam, NL)	Kartal et al. (2007)

NO₂⁻ Inhibition

The unionized HNO₂ has been shown to cause inhibition of AOB and NOB above certain concentrations causes. However, high levels of anion NO₂⁻ has been shown to be inhibitory to AMX (Strous et al., 1999, Lotti et al., 2012), which is also the primary substrate for AMX metabolism. Reports on the level of NO₂⁻ that is inhibitory to AMX vary over a wide range. Further, the exposure time and inhibitory effects also vary for different biomass niches such as granules, biofilm, and suspended flocs.

Strous et al. (1999) found that the anammox process was completely inhibited at concentrations more than 100 mgNO₂⁻-N/L. Similarly, a long term exposure at 40 mgNO₂⁻-N over several days led to inactivation of AMX (Fux, 2003). Egli et al. (2001) showed that the anammox process was only inhibited at concentrations higher than 182 mgNO₂⁻-N/L. These experiments were conducted with *Candidatus* “Kuenenia stuttgartiensis” whereas inhibition experiments performed by Strous et al. (1999) were with *Candidatus* “Brocadia anammoxidans”. This illustrates a notable difference in tolerance for NO₂⁻ among different AMX genera. Strous also showed that increasing the NO₂⁻ concentration changed the stoichiometry ratio of NH₄⁺ consumption: NO₂⁻ consumption from 1.3 gNO₂⁻-N/gNH₄⁺-N at 0.14 gNO₂⁻-N/L to almost 4 gNO₂⁻-N/gNH₄⁺-N at 0.7 gNO₂⁻-N/L. The change in the stoichiometric ratio was attributed to generation internal electron donor to reduce NO₂⁻ by AMX under these conditions.

To elucidate inhibitory effects of NO₂⁻ on AMX, Lotti et al. (2012) conducted a study with biomass consisting of a “Brocadia” enrichment which showed a total recovery from AMX inhibition after exposure at 400 mgNO₂⁻-N/L. It was also shown that longer exposure times and high NO₂⁻ concentrations increased the degree of inhibition. Further, it was argued that reports of NO₂⁻ accumulation could be the result of loss of activity rather than the cause. This study showed that the anammox process can be resilient against temporary adverse effects of NO₂⁻ accumulation. Strous et al. (1999) demonstrated recovery from complete inhibition with the addition of hydrazine and/or hydroxylamine. The exact mechanism of NO₂⁻ inhibition on AMX still remains unclear.

Dissolved Oxygen

In spite of having catalase and siperoxide dismutase (Strous, 2000), AMX exhibit inhibition at DO concentration above 0.01 mg/L (van de Graaf et al., 1996). From the intermittent oxygen supply experiments conducted by Strous et al. (1997) it was made clear that DO inhibition on AMX is reversible. The reversible nature of DO inhibition on AMX makes the partial nitrification-anammox in a single reactor possible. However, the operating DO should be maintained to allow both AOB and AMX to grow optimally. Higher DO favors NOB in a single stage deammonification process (Henze, 2000, Starkenburg, 2007, De Clippeleir et al., 2009, Huang et al., 2010) which could compete with AMX for NO_2^- . This could be another reason to keep the DO at the lowest possible level for AOB growth.

Operational Temperatures of Anammox

AMX has been identified within wide temperature ranges in the natural environment [-2 to 30 °C, (Dalsgaard et al., 2002, Rysgaard et al., 2004)]. In each of the microbial communities studied different optimum temperatures and different tolerances in different temperature ranges was observed. However, the accepted optimum temperature for sidestream AMX is between 30-40°C (van Dongen et al., 2001, Guo et al. 2010).

Vlaeminck et al. (2009) showed successful AMX operation at 25°C in high-strength bench-scale reactors. Additionally batch reactors have been successfully operated at sustained low temperatures of 18°C and 20-22°C (Isaka et al., 2007, Cema et al., 2007, Guo et al., 2010, Hendrickx 2012).

Temperatures above 45° are detrimental to AMX activity (Dosta et al. 2008). It has also been verified that extreme temperature shifts negatively impact AMX activity (Szatkowska, 2006, Dosta et al. 2008). Considering that Strous et al. (1997) reported optimum temperatures of 43°C, there is a small window between optimum and detrimental temperatures. AMX has not only been found in low temperature marine sediments but found to thrive at these low temperatures; therefore, it may be possible to successfully treat low-temperature wastewaters with AMX. If AMX can be harnessed at low temperatures in

wastewater treatment, then mainstream AMX can be a reality. Recently, partial nitrification-anammox was demonstrated in a RBC at 15 °C (De Clippeleir et al., 2013) and in an SBR at 12 °C (Hu et al., 2013). However, both studies relied on prior enrichment of AMX with high-strength ammonia wastewater at high temperature.

Alternate metabolic pathways

In addition to the conversion of NH_4^+ and NO_2^- , the anammox genera “*Brocadia*”, “*Anammoxoglobus*” and “*Kuenenia*” are also capable of co-metabolizing the fatty acids such as propionate, acetate and formate (Güven et al., 2005, Kartal et al., 2007, Kartal et al., 2008b). The reduction of NO_3^- via NO_2^- to NH_4^+ is coupled with the oxidation of fatty acids to CO_2 (Kartal et al., 2007). Therefore, AMX are capable of producing their own substrate to perform the catabolism reaction. However, whether the amount of energy from the conversion of NO_3^- to NH_4^+ that would be available for additional catabolism is unknown. The fatty acids are completely converted to CO_2 without being incorporated into biomass (Kartal et al., 2007, Kartal et al., 2008b). Considering AMX perform the energy-expensive CO_2 -fixation via acetate, this is a puzzling characteristic of AMX still to be understood. Furthermore, *Candidatus* “*Kuenenia stuttgartiensis*” has demonstrated a capability to oxidize Fe^{2+} to Fe^{3+} with NO_3^- as electron acceptor and the ability to reduce Fe^{3+} to Fe^{2+} as well as Mn^{4+} to Mn^{2+} with formate as the electron donor (Strous et al. 2006). The metabolic function and actual growth of AMX on these substrates remains unknown.

2.10 Suppression of Nitrite Oxidation for Shortcut Biological Nitrogen Removal

The suppression of nitrite oxidation by out-selection of nitrite oxidizing bacteria (NOB out-selection) is a precondition for the implementation of shortcut biological nitrogen removal processes such as nitrification-denitrification (Yoo et al., 1999, Yu et al., 2000, Ciudad et al., 2005, Gee et al., 2004, Ju et al., 2007, Zeng et al., 2008) and partial nitrification-anammox (Hippen et al., 1997, van Dongen et al., 2000, Fux et al., 2002, Wett, 2006, Wett, 2007, Wett et al., 2010). Successful suppression of nitrite oxidation by controlling nitrite oxidizing bacteria (NOB) saves 25% oxygen and 40% organic carbon compared to conventional nitrification-denitrification (Turk et al., 1986, Abeling et al., 1992).

In deammonification processes, the control of NOB results in added benefits of further reductions in aeration energy required, and reduced costs of electron donor and solids handling. In view of the high cost of biological nitrogen removal to meet increasingly stringent effluent standards, shortcut nitrogen removal through suppression of NOB is a topic of interest. Efforts to understand NOB out-selection have been discussed in many publications including those that are more specific to the use of high temperature (Hellinga et al., 1998), high levels of free ammonia inhibition, low DO concentration (Blackburne et al., 2008a), and transient anoxia (Kornaros et al., 2010). Particularly, all of these conditions are used in part or as a whole in various approaches with success in controlling NOB in systems treating 'high strength' (high free ammonia) waste streams such as anaerobic digester dewatering liquor (also usually at high temperature) and landfill leachate. NOB out-selection in low-strength waste streams such as domestic wastewater remains a challenge.

Temperature and SRT

Microorganisms typically operate within a narrow range of temperatures. Their optimum temperature usually falls within this range. Temperature affects specific growth rate and substrate utilization due to the changes in enzymatically catalyzed reactions and substrate diffusion (Grady et al., 2011). Reaction rate coefficients typically increase with an increase

in temperature until the critical temperature, at which cellular deterioration takes place, is reached.

Further, high temperature is known to favor growth of AOB over NOB (Kim et al., 2008). In the literature, activation energies of AOB and NOB are reported between 72 to 60 KJ/mol and 43 to 47 KJ/mol respectively within 7°C to 30°C (Knowles et al., 1965, Stratton et al., 1967, Helder et al., 1983, Jetten et al., 1999). Therefore, the theoretical rate of increase in AOB activity is higher than NOB with increasing temperature. In fact, the SHARON process relies on this phenomenon to achieve stable partial nitrification (Hellenga et al., 1998). The specific ammonia and nitrite utilization rates increased by 5.3 times and 2.6 times at 10°C to 30°C (Figure 13). Further, the higher activity of AOB at higher temperature allows the SHARON reactor to be operated at low SRT (1 to 1.5 days) which results in the enrichment of AOB and selective wash out of NOB (Figure 14).

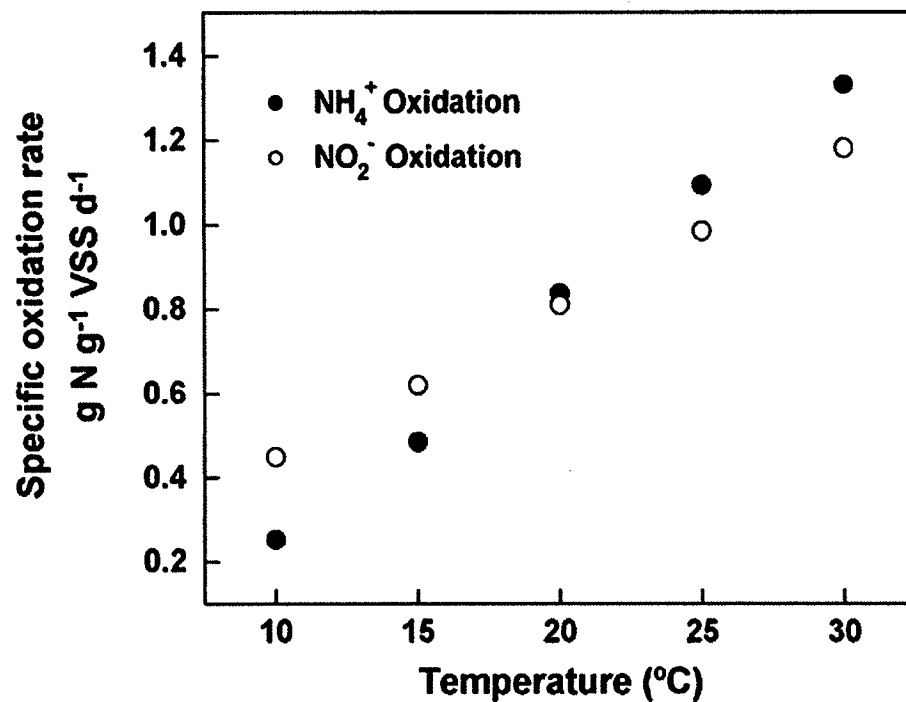


Figure 13. Specific ammonia and nitrite oxidation within 10°C to 30°C [After Kim et al. (2008)]

The increased activity of AOB compared to NOB at higher temperatures, greater disassociation of total ammonia to free ammonia and resulting NOB inhibition at higher temperatures, combined with low DO operation often conducted using intermittent aeration and with managed aerobic SRT, results in enrichment of AOB and selective wash out of NOB. These methods either use suspended growth (Katsogiannis et al., 2003), attached growth on support media (Christenson et al., 2013), or granular sludge (Wett, 2007) to accomplish shortcut nitrogen removal. In spite of being effective, the role of elevated temperatures to increase activity of AOB while controlling NOB growth is not feasible in low strength mainstream processes operating under a wide range of temperatures. Consequently, NOB control in low strength wastewater remains intractable and could require careful manipulation of factors other than temperature or free ammonia. The shift in focus from a more typical use of temperature and free ammonia to achieve NOB out-selection seems forthcoming. Unlike in sidestream systems, the use of temperature to control relative growth rates of AOB and NOB may not be possible within mainstream processes.

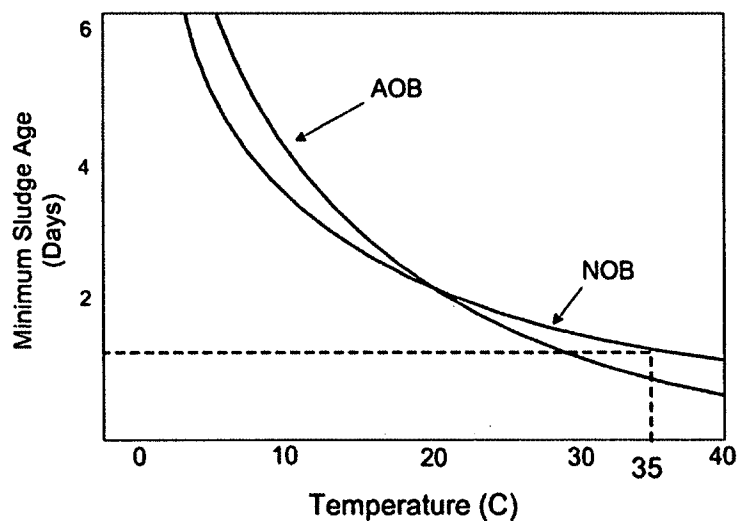


Figure 14. Minimum sludge age for AOB and NOB as a function of temperature (based on temperature coefficients found by Hunik, 1993 and Hellinga et al., 1998)

Dissolved Oxygen

The parameter that can play a significant role in control of NOB in low strength wastewater is DO. Sustained nitrification with the use of low DO concentrations has been observed in a variety of reactor configurations (Wyffels et al., 2004, Sliekers et al., 2005, Blackburne et al., 2008a). All of these reports lack account of underlying mechanisms, however, they resort to a hypothesis of higher oxygen affinity of AOB compared to NOB (Hanaki et al., 1990, Laanbroek et al., 1993, Bernet et al., 2001) as an explanation for the observed phenomenon (Yoo et al., 1999, Peng et al., 2007, Lemaire et al., 2008, Gao et al., 2009, Zeng et al., 2009). In a study, Hanaki et al. (1990) demonstrated that nitrite oxidation was repressed by low DO (<0.5 mg/L) in a suspended growth reactor at 25°C. Although the hypothesis that low DO operation favors AOB versus NOB is very widespread (see review of oxygen half-saturation parameters in Sin et al., 2008), some research results point in the opposite direction (Daebel et al., 2007, Manser et al., 2005). These research efforts indicate stronger adaptation to low DO concentration for NOB compared to AOB. In literature, considerable variation exists with respect to reported AOB and NOB oxygen affinity coefficient (K_o). It is argued that DO concentration within the flocs is highly affected by the size of the floccular aggregates due to the oxygen mass transfer resistance. DO concentration inside a floc or biofilm is possibly different than the bulk DO. It is likely that high K_o values are the result of oxygen mass transfer limitations, and therefore, do not represent intrinsic biological characteristics of the AOB or NOB (Blackburne et al., 2008a). Consequently, the DO half saturation constant is dependent on the floc size, biomass density, the mixing intensity and the rate of DO diffusion in the floc (Münch et al., 1996).

To eliminate the effects of floc size, Blackburne et al (2008a) determined K_o of enriched AOB and NOB cultures with negligible oxygen mass transfer resistances. They reported K_o values of 0.033 ± 0.003 mg/L and 0.43 ± 0.08 mg/L for AOB and NOB, respectively. These values are in agreement with previously believed DO half saturation constants for AOB compared to NOB. Manser et al (2005) determined the AOB and NOB K_o in conventional activated sludge (CAS) and membrane bioreactor (MBR). In both systems they found K_o was higher for AOB (MBR: 0.18 ± 0.04 mg/L, CAS: 0.79 ± 0.08 mg/L)

compared to NOB (MBR: 0.13 ± 0.04 mg/L, CAS: 0.47 ± 0.08 mg/L). In another study by Daebel et al (2007), very similar results were replicated (AOB (MBR: 0.31 ± 0.09 mg/L, CAS: 0.51 ± 0.07 mg/L), NOB (MBR: 0.14 ± 0.09 mg/L, CAS: 0.19 ± 0.03 mg/L)). Blackburne et al. (2008a) used DO concentration as the only selection factor to suppress nitrite oxidation in a continuous system at ambient temperature. It was perceived that differences in oxygen affinities of AOB and NOB favored suppression of nitrite oxidation. There are processes that promote simultaneous oxidation of ammonia and reduction of thus produced oxidized nitrogen to nitrogen gas, possibly through nitrite, at low DO conditions. These include simultaneous nitrification and denitrification (Pochana et al., 1999), OLAND (Kuai et al., 1998), completely autotrophic nitrogen Removal over nitrite (CANON) (Third et al., 2001), and nitrifier denitrification (Kampschreur et al., 2006). However, in these types of processes, other nitrite reducers such as heterotrophic denitrifiers and anammox compete for nitrite against NOB (Hanaki et al., 1990, Kuai et al., 1998, Pochana et al., 1999, Third et al., 2001, Wyffels et al., 2004).

Recently, low DO operation has proven completely incapable of NOB suppression in laboratory-scale SBRs operated at the Blue Plains WWTP (Al-Omari et al., 2012) and a full-scale pilot process at Strass WWTP (Wett et al., 2012a). As per Table 12, AOB (or AOB+NOB) had higher K_o values compared to the NOBs (Al-Omari et al., 2012). Similar observation was made during similar tests at the full-scale pilot at the Strass WWTP where average K_o for AOBs was 0.37 mg/L and K_o for NOBs was 0.16 mg/L (Wett et al., 2012).

Table 12. DO half-saturation constant average values for low DO operation (After Al-Omari et al., 2012).

Respiration Method	Unit	Half saturation constant at Constant Low DO		Half saturation constant at Intermittent aeration – Low DO	
		<u>AOB</u>	<u>NOB</u>	<u>AOB</u>	<u>NOB</u>
Declining DO	(mg/L)	0.31	0.14	0.22	0.12
Constant DO	(mg/L)	0.60	0.05	0.20	0.08

Free Ammonia and Free Nitrous Acid Inhibition

Measured values of nitrite and ammonia include both protonated and ionized forms, and the nitrous acid and ammonium concentrations can be determined using temperature, pH, and the acid's dissociation constant (K_a) or pK_a . The pK_a for the ammonia/ammonium acid base pair is 9.25 and for nitrite/nitrous acid is 3.398 respectively at 20 °C. The ammonia and ammonium species are present in equal concentrations at pH of 9.25 at 20 °C. As the pH falls below the pK_a value of 9.25, the ammonia (FA) becomes less prevalent as compared to ammonium and vice versa as pH increases above the pK_a value. Additionally, temperature affects the pK_a value; as temperature increases the pK_a value decreases.

The unionized nitrogen forms are considered to be the actual substrate/inhibitor for ammonium and nitrite oxidation. Free ammonia (FA) inhibition of NOB has been well documented in the literature ever since it was considered by Anthonisen et al. (1976). AOB and NOB have a different sensitivity towards the uncharged form of ammonium and nitrite (Table 13). At high nitrite concentrations and low pH it is possible to maintain high enough concentrations of nitrous acid to inhibit NOB (0.02 mg HNO_2 -N/L) as compared to AOB inhibition at 0.4 mg HNO_2 -N/L (Vadivelu 2007).

Table 13. Free ammonia and free nitrous acid concentrations inhibitory to AOB and NOB (Anthonisen et al., 1976).

	AOB	NOB
NH ₃ (mgN/L)	8-120	0.08-0.82
HNO ₂ (mgN/L)	0.2-2.8	0.06-0.83

Therefore, higher inhibition sensitivity of free ammonia on NOB has been used to suppress NOB in high ammonia and temperature waste streams. However, the controlling FA inhibition to obtain stable nitrification can be limited since NOB adaptation has been reported (Turk et al., 1989, Wong-Chong et al., 1978).

Bioaugmentation

Mainstream bioaugmentation of AMX is an emerging concept, however, successful AOB/NOB bioaugmentation is well documented. The physical separation of a more dense biomass fraction containing predominantly anammox organisms and recycling this heavier fraction by the use of the hydrocyclone in order to enrich this very slowly growing biomass has also been implemented (Nyhuis, 2009). Different from this approach, in order to select the lighter biomass fraction (the overflow and not the underflow of the hydrocyclone) containing predominantly AOB in order to bioaugment the relatively fast growing AOB from the sidestream reactor to the mainstream without uncontrolled loss of anammox activity in the sidestream reactor is implemented in the Strass WWTP for mainstream deammonification (Figure 15). The selection of the light biomass fraction using a cyclone or sieve, or the separation of unattached biomass from a biomass carrier media, allows a maximum seeding rate which helps to repress NOB in both the high-strength sidestream reactor (selectively decreasing SRT) and the low-strength system (transfer of AOB but almost no NOB).

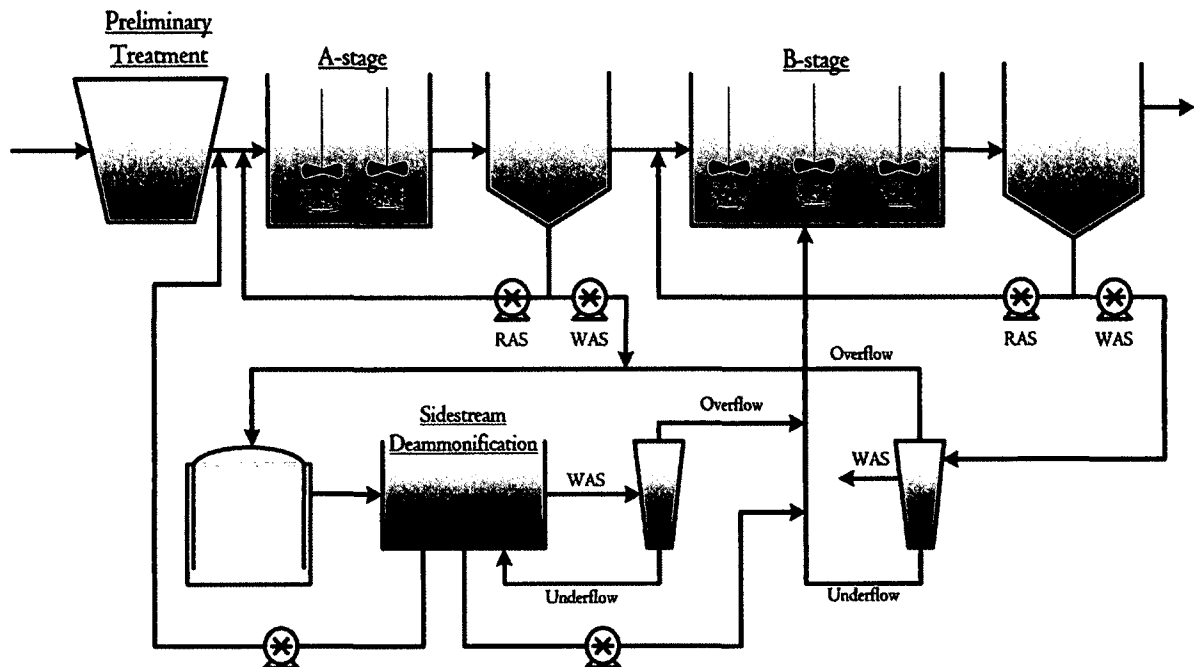


Figure 15 Mainstream deammonification at Strass WWTP with bioaugmentation from sidestream deammonification (Wett et al., 2012)

Transient Anoxia

The use of transient anoxia (alternating aerobic and anoxic conditions) has been a common approach to achieve NOB out-selection (Pollice et al., 2002, Rosenwinkel et al., 2005, Ling, 2009, Li et al., 2012, Zekker et al., 2012). Transient anoxia allows for a measured approach to control the aerobic SRT as well as introduce a lag-time for NOB to transition from the anoxic to aerobic environment. Kornaros et al. (2010) showed a delay in NOB recovery and NOB lag adaptation compared to AOB in aerobic conditions following transient anoxia, thus confirming the observations of the usefulness of transient anoxia by many others (Allenman et al., 1980, Katsogiannis et al., 2003, Sedlak, 1991, Sliverstein et al., 1983, Yang et al., 2011, Yoo et al., 1999). Transient anoxia has been used successfully to control NOB in high strength wastes (Wett, 2007) and the ability to use it in low strength wastes has been suggested (Peng et al., 2004).

Gilbert et al. (2014) presented a NOB suppression approach which involved exploiting a lag phase after the transition from anoxic to aerobic conditions. This study concluded that intermittent aeration with short anoxic periods of 15-20 min at a minimum followed by aerobic periods that are smaller than the specific lag phase might be sufficient to suppress NOB. The lag phase lasted 5-15 min and was dependent on prior biomass acclimatization (i.e., substrate and temperature conditions for growth). This study also claims that the lag phases of NOB adapted to high operational DO were typically longer than at a lower operational DO. The prior adaptation to continuous or intermittent aeration was not a significant factor. The length of the lag phase was not dependent on the temperature as long as the duration of anoxic periods were greater than 15-20 min. It was indicated that transient anoxia to suppress NOB is more suitable for NOB species that are enriched at a high operational DO.

NO and N₂O Emissions

The sustainability of wastewater treatment in view of gaseous emissions invariably relies on minimal emissions of gases such as nitric oxide (NO), which is known to deplete ozone, and nitrous oxide (N₂O), which is a potent greenhouse gas. In monitored full-scale short-cut nitrogen removal systems, 0.4-1.3% of the nitrogen load was emitted as N₂O (Joss et al., 2009, Kampschreur et al., 2009a, Weissenbacher et al., 2010), which is close to acceptable N₂O emissions from nitrification/denitrification systems (Kampschreur et al., 2009a). However, NO emissions generally below 0.01% of N load were reported (Joss et al., 2009, Kampschreur et al., 2009a, Weissenbacher et al., 2010), which can be attributed to the low water solubility of NO that causes NO to be emitted easily when it is formed.

AOB are the major players for N₂O/NO emissions in short-cut nitrogen removal systems as they are purported to perform the so-called 'nitrifier denitrification'. AOB gain their energy primarily through aerobic metabolic pathways (Chain et al., 2003). However, AOB (including *Nitrosomonas europaea* and *N. eutropha*) can use NO₂⁻ or N₂O₄ as electron acceptors and NH₃ or H₂ as electron donors to produce NO and N₂O under oxygen limited and anoxic conditions (Ritchie et al., 1972, Poth et al., 1985, Schmidt et al., 2004). Goreau et al. (1980) reported N₂O emissions up to 10% of the nitrogen load at a DO below 1 mg

O₂/L. NO emissions have been observed under both aerobic and completely anoxic conditions (Ritchie et al., 1972, Yu et al., 2010), however, N₂O production by AOB is limited to aerobic or microaerophilic conditions. Transition from anoxic to aerobic conditions in the presence of ammonia has shown AOB to produce N₂O (Yu et al., 2010). Further, nitrite accumulation also plays an important role in AOB NO and AOB N₂O emissions (Kampschreur et al., 2009b). Nitrite accumulation is unavoidable in short-cut nitrogen removal systems and considerably increases AOB N₂O emissions (Colliver et al., 2000). The periods of high nitrogen loads trigger high specific activity of AOB, which is linked to high N₂O production (Yu et al., 2010). AOB populations with lower substrate affinities could result in an imbalance in enzyme expression while close to their maximum specific activity (Yu et al., 2010). Therefore, according to Monod kinetics AOB is prone to N₂O emission at lower substrate levels. Consequently, systems operated under constant specific activity values in terms of DO and ammonia levels, are less likely to produce high N₂O. Therefore, discontinuous systems such as SBRs are more vulnerable to produce N₂O due to inevitable transitions. It is desired to maintain stable nitrogen concentrations by employing prolonged feeding during the reaction phase in SBRs (Wett, 2006). This could possibly lead to lower N₂O production. NO and N₂O are also produced through abiotic reactions, which could be important in other instances. Hydroxylamine, an AOB intermediate, can react either biochemically (Yu et al., 2010) or chemically (van Cleemput, 1998) with nitrite to form NO and N₂O.

NO and N₂O emissions may increase with the airflow rate being used since their concentrations remain constant in the gas phase. The stripping of NO and N₂O can also be decreased to lower their emissions. Thus, NO and N₂O emissions can be controlled by lowering the airflow rate within optimal conditions (Kampschreur et al., 2008), and in a membrane-aerated biofilm reactor (MABR) using bubble less aeration (Pellicer-Nacher et al., 2010). Denitrification rates are known to decrease due to high nitrite concentration, which leads to NO and N₂O formation (von Schulthess et al., 1995). Similarly, COD limited denitrification results in NO or N₂O emissions (von Schulthess et al., 1996, Chung et al., 2000). Additionally, denitrifying enzymes and N₂O reductase (Otte et al., 1996) are inhibited by DO. A low DO in short-cut nitrogen removal systems could also trigger N₂O emission by denitrifiers.

Based on the finding noted above, it could be inferred that stable conditions which allow constant specific microbial activities and control the accumulation of nitrite and ammonia are key to lowering NO and N₂O emissions from short-cut nitrogen removal systems. However, transiently oxygen-limited conditions are unavoidable in short-cut nitrogen removal systems to apply pressure on NOB. Also, the continuous aeration was not shown to lower N₂O emission compared to intermittent aeration (Joss et al., 2009). Therefore, there exists a need to explore DO levels and aeration patterns in order to mitigate NO/N₂O emissions.

Inorganic Carbon Limitation Impact on AOB versus NOB

In a study with pure AOB cultures, increased growth rates and yields were demonstrated when cells were grown under CO₂ rich conditions (i.e. greater than 0.03% CO₂) (Jahnke, 1984). The effects of IC limitation on ammonia oxidation have been reported through modeling and experimental efforts (Wett, 2003, Guisasola et al., 2007) that consider partial nitrification of high N streams. IC limitation results in decreased ammonia removal efficiency which might be the result of slowed AOB growth causing biomass washout when a sufficiently long SRT is not provided (Wett, 2003, Khunjar et al., 2011). Under moderate IC limitation, AOB activity recovered; however, no recovery was observed under severe IC limitation (Khunjar et al., 2011). Nitric and nitrous oxide emissions also increased when cells were subjected to inorganic carbon limitation (Khunjar et al., 2011).

Moreover, it was observed that IC limitation had a stronger effect on the ammonia oxidation than on the nitrite oxidation (Guisasola et al., 2007). It was suggested that AOB were limited by inorganic carbon availability at concentrations as low as 3 mmol/L, while the NOB were not limited even at concentrations below 0.1 mmol/L. This is potentially problematic, particularly in high N streams where transient IC limitation could be common, which may potentially interfere with autotrophic N-removal processes such as anammox, by providing selective advantage to the growth of NOB over AOB and anammox. In contrary to these concerns, IC limitation has been demonstrated to adversely impact NOB growth, leading to NOB washout due to decreased cell synthesis rates or cell

yields (Kim et al., 2012). Further, adaptation to IC limitation was not observed and nitrite oxidation recovered only after re-supplying CO₂ (Kim et al., 2012).

2.11 Mainstream NOB Out-selection

Short-cut nitrogen removal over NO₂⁻ has been proven difficult in treating low nitrogen wastewater (Guo et al., 2009a) especially in continuous processes (Ma et al., 2009, Peng et al., 2012). Ammonia oxidation to nitrite causes the pH to drop due to alkalinity consumption, whereas nitrite oxidation to nitrate does not significantly change the pH. However, if the aeration is continued pH ascends due to CO₂ stripping. Therefore, a bending point on the pH profile at the end of ammonia oxidation termed the “ammonia valley” is observed (Alghusian et al., 1995).

There are many publications that claim that achieving nitrification in SBRs can be achieved by controlling aeration duration based on ammonia valley or other similar indicators (Peng et al., 2007, Yang et al., 2007, Blackburne et al., 2008b, Guo et al., 2009a, Ye et al., 2009). Guo et al. (2009a) demonstrated nitrite accumulation (> 90% NO₂-N/NO_x-N) in a SBR (10 L, 8-10 hr HRT, 30 d SRT) treating domestic wastewater (influent C/N= 3.7, < 26°C) using real-time aeration duration control. In this study, DO was maintained at 2.5 mg/L, which contradicts the purported role of low DO in suppressing NOB. In fact, high DO (≥2.5 mg O₂/L) was also used to achieve partial nitrification in a SBR treating municipal wastewater at ambient temperature (Yang et al., 2007). It was worth noting that finite residual ammonia (> 1 mg/L) was maintained throughout the study, while during start-up the effluent ammonia was around 2 mg/L. The high effluent nitrite (>25 mg/L) indicated that NOB suppression was possible in spite of abundant substrate availability to the NOB. However, the role of HNO₂ itself or NO (nitrite generated) that could potentially cause inhibition to NOB was not discussed. It was believed that the NOB growth could be suppressed through aeration duration control by providing no extra time for NOB to utilize the accumulated nitrite (Guo et al., 2009b). Blackburne et al. (2008b) investigated the aerobic duration control to achieve NOB suppression in a laboratory-scale SBR treating domestic wastewater. After initially inducing 40% nitrite accumulation with formic acid

addition, the process proved effective in achieving a steady state whereby over 80% nitrification was sustained.

Considering the difficulty of suppression of NOB in continuous mainstream processes, reports of successful short-cut nitrogen removal are rare. Recently, in a continuous plug-flow step-feed BNR pilot-scale study, Ge et al. (2014) reported high degree of NOB out selection (effluent $\text{NO}_2^-/\text{NO}_x\text{-N} = 0.82$) and a high nitrogen removal efficiency of 85% with moderate influent $\text{COD}/\text{NH}_4^+\text{-N}$ ratio of 5 at 16-28 °C. The successful NOB out-selection was attributed to transient anoxic conditions and step feed in the anoxic zone to remove accumulated NO_2^- following the aerobic zone. In a pilot-scale continuous predenitrification system (9-10 hr HRT, 15 d SRT, 300-4000 mg/L MLSS), Ma et al. (2009) demonstrated the nitrite pathway (established within 2-3 SRT) treating domestic wastewater (influent C/N = 3) at ambient temperature (21 °C). The successful NOB out-selection was attributed to low operational DO (0.4-0.7 mg O_2/L) due to hypothesized lower DO affinity of NOB compared to AOB. It was shown that by operating at high DO (2-3 mg O_2/L) nitrification was destroyed. It was also reported that maintaining high residual ammonia was beneficial for establishing nitrification, although the exact reason was not provided.

Similarly, in a continuous anaerobic-anoxic-aerobic (A^2O) process (15-20 d SRT, 2000-3000 mg/L MLSS) treating domestic wastewater (influent C/N = 2.5) at ambient temperature (22 °C), nitrification was achieved using a combination of short aerobic HRT and low DO levels (0.3-0.5 mg O_2/L). In this study, during the start-up period of nitrification in the A^2O process, a short aerobic HRT (4.97 h) was applied to avoid excessive aeration and inhibit NOB growth, which also coincides with high effluent ammonia. In contradiction to the previous study by Ma et al (2009), low DO sludge bulking was not observed in spite of the high nitrite accumulation.

2.12 Worldwide Status of Mainstream Deammonification Research

The stringent nitrogen limits and rising energy cost of wastewater treatment is the primary driver for alternative technologies for nitrogen removal. Mainstream deammonification represents the enhanced efficiency for biological nitrogen removal. It is potentially a possible way to achieve energy positive wastewater treatment for plants requiring a high degree of nitrogen removal. The high ammonia strength waste stream treatment via deammonification has almost state-of-the-art status with close to 100 installations worldwide. These facilities are reporting enhanced nitrogen removal performance at reduced energy and resources consumption. Therefore, mainstream deammonification has become the next frontier in nitrogen removal and is being pursued by several research groups the world over. Table 14 summarizes ongoing research projects on mainstream deammonification that are known to the author as of April, 2014 (excluding this study).

Table 14. Mainstream deammonification research status as of April, 2014.

Location	Scale	Process type	Research groups	Source
Rotterdam, The Netherlands	Pilot-scale	Up flow Granular biomass	TU-Delft/Paques	Lotti et al. (2013)
Malmo, Sweden	Pilot-scale	MBBR and IFAS	Veolia	Lemaire et al. (2013)
Santiago de Compostela, Spain	Pilot-scale	Up flow Granular biomass	Aqualia/university of Santiago de Compostela	Padin et al. (2013)
Nieuwveer, The Netherlands	Pilot and Lab scale	Up flow Granular biomass	Colsen/Gent University	Vlaeminck 2014*
Strass, Austria	Full-scale test	Sidestream Bioaugmentation	ARA consult /DC Water	Wett et al. (2013)
Changi, Singapore	Full-scale test	Step-feed BNR	PUB Singapore	Cao et al. (2013)

*Personal communication

In a full-scale trial at the Strass WWTP (Austria), Wett et al. (2013) demonstrated anammox implementation by bioaugmentation of sidestream generated AMX. The results of this study showed that it was possible to maintain AOB activity in the reactor higher than the activity of NOB. However, the exact contribution of anammox and nitrification-denitrification in terms of nitrogen removal efficiency were not elucidated. In another full-scale trial at the Changi WWTP (Singapore), at high wastewater temperatures of 28-32 °C (year around), it was possible to repress NO_2^- oxidation (Cao et al., 2013). Nitrogen removal efficiency of greater than 85% was demonstrated at a moderate influent $\text{COD}/\text{NH}_4^+\text{-N}$ of around 10. Further, in the study a high percentage of the nitrogen removal was speculated due to AMX activity. However, at such a high COD/N ratio and high

temperatures in a step-feed BNR the nitrogen removal through nitrification-denitrification could also result in a similar efficiency.

It is important to note that anoxic and autotrophic nitrogen removal by AMX provides aeration and carbon savings only if the influent carbon is captured prior to the nitrogen removal step, which was not the case in this study. Furthermore, NOB out-selection relied on high temperature which is not available in temperate and cold climate areas, where wastewater temperatures are much lower. In a pilot-scale study of one stage partial nitrification/anammox in upflow reactor with granular biomass, Lotti et al. (2013) reported nitrogen removal efficiencies of 29% and 49% during stable and best performance periods, respectively.

Therefore, the implementation of mainstream deammonification has yet to be demonstrated at low temperatures ($<25\text{ }^{\circ}\text{C}$) with high nitrogen removal efficiencies ($>50\%$) in a long-term study.

CHAPTER 3

MATERIAL AND METHODS

3.1 Pilot Study

The two major objectives of the pilot study at HRSD's Chesapeake Elizabeth Treatment Plant (CETP hereafter) were: 1) To study the feasibility of a biological nitrogen removal upgrade of CETP at a reduced capital and operating costs 2) To explore possibilities for the implementation of new short-cut nitrogen removal through repression of NO_2^- oxidation and polishing using anammox, which could be beyond the CETP upgrade. The A-B pilot study consisted of a common A-stage feeding two parallel B-stages. The A-stage was a high-rate activated sludge (HRAS) process. The B-stage intended for the first objective was a plug-flow activated sludge process and was named AOB versus NOB (AvN). The second B-stage was a continuous stirred tank reactor (CSTR) activated sludge process (AvN CSTR hereafter) followed by an anammox moving bed biofilm reactor (MBBR) for nitrogen polishing, the combined process was named AvN+. The process flow diagram presented in Figure 16 gives an overview of the pilot study setup. A more detailed version of the pilot process flow diagram including preliminary treatment is presented in Figure A1.

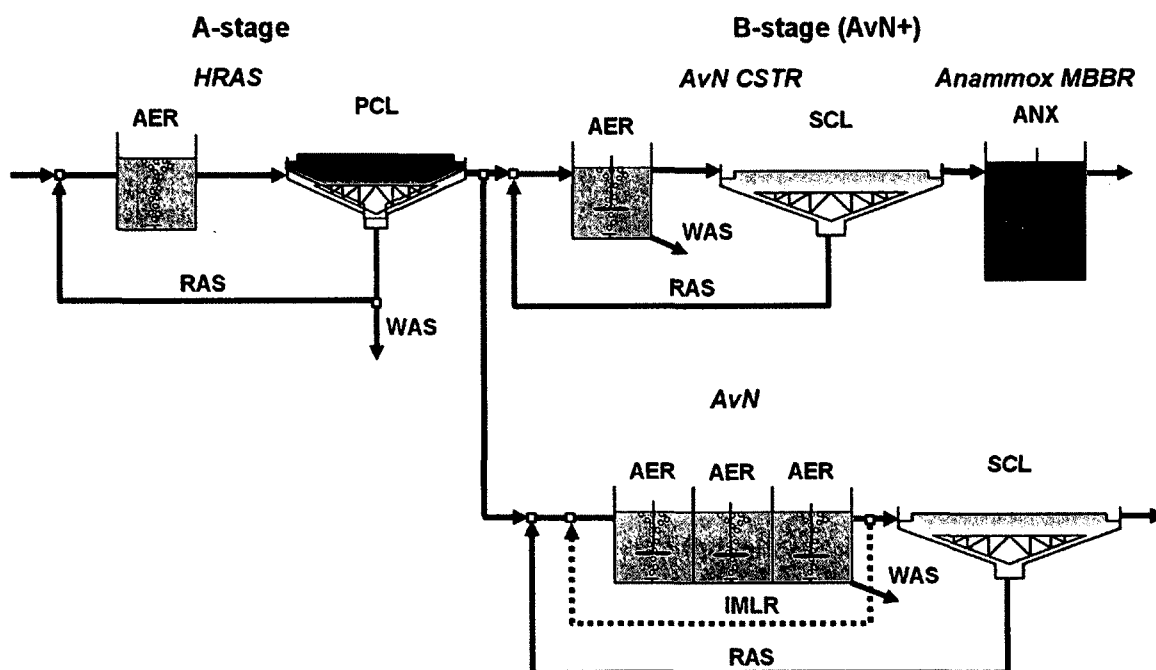


Figure 16. A-B pilot process flow diagram (Pilot 1.0)

The majority of the results and discussion provided in this dissertation originates from the pilot of Figure 16. This pilot was later modified significantly, which included two parallel A-stages and one plug-flow AvN that was followed by a MBBR with anammox. Only one A-stage (HRAS-control) was connected to the B-stage, while the other A-stage served as an experimental train (HRAS-experimental) to understand the mechanisms of carbon removal in high-rate processes (Table 15). The second iteration of the pilot will be presented in greater detail in the following sections.

Table 15. Pilot process trains before and after the upgrade.

Generation	A-stage	B-stage
Pilot 1.0	HRAS	AvN
		AvN+ (AvN CSTR with Anammox MBBR)
Pilot 2.0	HRAS-control	AvN+ (AvN with Anammox MBBR)
	HRAS-experimental	

3.2 Pilot 1.0 Setup

Preliminary Treatment

Using a chopper pump, raw wastewater influent (RWI) for the pilot process was pumped from the effluent channel of the preliminary treatment facility (PTF) at the CETP. The PTF includes fine screens and forced vortex grit removal. Due to the inefficiencies of the PTF, the pumped RWI first passed through a 208 L drum equipped with a variable speed mixer that was operated at a speed that allowed grit to settle but kept particulate and colloidal organic matter in suspension. Accumulated grit was periodically removed by draining and cleaning out the tank. Floatable material, such as oil and grease, was continuously removed by allowing the tank to overflow to a floor drain. From the grit and scum removal tank, the RWI was pumped by a peristaltic pump through basket screens with 2.4 mm openings into a temperature control tank. This tank contained a submersible heater and a finned-tube coil. Coolant was circulated through the coil and a water-cooled water chiller.

A programmable logic controller (PLC) controlled power to the heater and chiller based on a signal from a thermocouple in the temperature control tank and a user set-point. This setup provided the capability to provide a constant influent wastewater temperature to the biological processes anywhere from 15 to 25°C. The temperature control tank also contained a constant speed mixer. These processes were only necessary for the pilot and not intended for the full-scale process.

High-rate Activated Sludge Process (HRAS)

The reactor was constructed from clear polyvinylchloride (PVC) pipe supported vertically on one end with an operating volume of 170 L, a HRT of 30 minutes, and a side water depth of 3.4 meters. Aeration was provided using compressed air and a 17.7 cm membrane disc diffuser with the DO monitored by a DO sensor (Table 16). The desired DO set-point was maintained using a single-loop proportional-integral-derivative (PID) controller controlling a mechanically operated valve (MOV) on the compressed air line. Since the HRAS reactor was mixed only by aeration, a minimum MOV closure was set to ensure continuous airflow.

The reactor overflowed by gravity to a steep cone-bottom clarifier. The clarifier had a submerged vertical inlet inside of a center well. This configuration helped dissipate the influent hydraulic energy and allowed additional bioflocculation to occur before solids separation. The clarifier was fitted with a scraper mechanism that rotated at 0.25 rpm and directed settled solids to the bottom of the clarifier cone. A peristaltic pump returned settled biomass in the clarifier to the aeration tank. The surface overflow rate (SOR) was $0.7 \text{ m}^3/\text{m}^2 \cdot \text{hr}$ and a solids loading rate (SLR) of $1.4 \text{ kg}/\text{m}^2 \cdot \text{hr}$ at 100% RAS and 3000 mg/L MLSS. The RAS flow was monitored using a magnetic flow meter. The SRT of the HRAS process was controlled by wasting solids from the underflow of the clarifier using a programmable digital peristaltic pump. Effluent from the clarifier overflowed to a 208 L drum that served as a flow through feed storage tank for the B-stages. Effluent suspended solids (ESS) and pH were monitored in this tank (Table 16). Mixing was maintained by a constant speed mixer. A-stage effluent was pumped from the feed storage tank to the B-stages with a programmable digital peristaltic pump.

Table 16. List of sensors used in A-stage monitoring.

Sensor Name	Measurement	Type	Location	Manufacturer	Origin
Foxboro pH	pH	ISE	Effluent	Invensys	London, UK
LDO	DO	Optical	Aeration tank	HACH	Loveland, CO, USA
VisoTurb	TSS	Optical	Effluent	WTW	Weilheim, Germany

*Ion selective electrode

B-stage AvN

The B-stage-AvN consisted of three equal volume tanks in series, each 151 L for a total operating volume of 454 L, and a cone-bottom clarifier. The clarifier had a submerged vertical inlet inside of a center well. This configuration helped dissipate the influent hydraulic energy and allowed additional bioflocculation to occur before solids separation. The clarifier was fitted with a scraper mechanism that rotated at 0.25 rpm and directed settled solids to the bottom of the clarifier cone. A peristaltic pump returned settled biomass in the clarifier to the aeration tank. The SOR was $0.1 \text{ m}^3/\text{m}^2 \cdot \text{hr}$ and a SLR of $0.3 \text{ kg}/\text{m}^2 \cdot \text{hr}$ at 100% RAS and 3000 mg/L MLSS. All three biological reactors were equipped with a variable speed mixer (Caframo: Georgian Bluffs, Ontario, CA) at $G = 106 \text{ s}^{-1}$ to maintain complete-mix conditions. Aeration was provided using compressed air and a 22.9 cm membrane disc diffuser with the DO monitored by a DO sensor (Table 17). The desired DO set-point was maintained using ON/OFF switching of solenoid valves on the compressed air line. Tanks were intermittently aerated and the aeration pattern was controlled based on the effluent $\text{NH}_4^+\text{-N}$ set-point (Table 17). The aeration capacity allowed all 3 tanks to be intermittently aerated without a defined anoxic zone. The AvN had a total HRT of 4 hours, with the influent set at a constant flow of 1.9 L/min. This HRT represents the existing HRT of CETP's aeration tanks when operating at design flow.

There was a provision for an internal mixed liquor recycle (IMLR) line to return nitrified mixed liquor from the last aerobic reactor to the first reactor using a peristaltic pump at a

rate between 100-400% of the influent flow. When IMLR was used the first tank was not aerated. RAS from the clarifier was returned to the first reactor at 100% of the influent flow. SRT was controlled by wasting solids from the last aerobic tank (Garrett configuration). The SRT was maintained between 5-10 days based on operation performance and MLSS concentration. pH was monitored using a pH probe in the last aerobic reactor (Table 17). Although there was a provision to control pH using a proportional controller with sodium hydroxide solution addition to the final aerobic reactor, it was rarely used.

Table 17. List of sensors used in AvN monitoring and process control.

Sensor Name	Measurement	Type	Main Function	Location	Manufacturer	Origin
Foxboro pH	pH	ISE	Monitoring	Last aerobic reactor	Invensys	London, UK
LDO	DO	Optical	Process control	All aerobic reactors	HACH	Loveland, CO, USA
NH4D sc	NH ₄ ⁺ -N	ISE	Process control	Last aerobic reactor	HACH	Loveland, CO, USA

B-stage AvN CSTR

The AvN CSTR included a single 340 L aeration tank and a cone-bottom clarifier. While it is recognized that a more plug-flow reactor configuration would be expected for full-scale implementation, a single CSTR was used for this study for simplicity associated with the development and testing of the aeration control schemes. The clarifier had a submerged vertical inlet inside of a center well. This configuration helped dissipate the influent hydraulic energy and allowed additional bioflocculation to occur before solids separation. The clarifier was fitted with a scraper mechanism that rotated at 0.25 rpm and directed settled solids to the bottom of the clarifier cone.

A peristaltic pump returned settled biomass in the clarifier to the aeration tank. The SOR was $0.1 \text{ m}^3/\text{m}^2 \cdot \text{hr}$ and a SLR of $0.5 \text{ kg}/\text{m}^2 \cdot \text{hr}$ at 100% RAS and 3000 mg/L MLSS. This tank was equipped with a variable speed mixer (Caframo: Georgian Bluffs, Ontario, CA) at $G = 175 \text{ s}^{-1}$ in order to maintain complete-mix conditions. Aeration was provided using compressed air and a 23 cm membrane disc diffuser with the DO monitored by a DO sensor (Table 18). The desired DO set-point was maintained using a single-loop PID controlling a MOV on the compressed air line. RAS from the clarifier was returned to the AvN CSTR with a peristaltic pump at 100% of the influent flow. SRT was controlled by wasting solids from the bioreactor (Garrett configuration) with a programmable digital peristaltic pump. The AvN CSTR was equipped with sensors to monitor $\text{NO}_3^- \text{-N}$, $\text{NO}_2^- \text{-N}$ and $\text{NH}_4^+ \text{-N}$ (Table 18). These signals were used to control the intermittent aeration pattern of the AvN CSTR.

Table 18. List of sensors used in AvN reactor monitoring and process control.

Sensor Name	Measurement	Type	Main Function	Manufacturer	Origin
Foxboro pH	pH	ISE	Monitoring	Invensys	London, UK
LDO	DO	Optical	Process control	HACH	Loveland, CO, USA
Spectro::lyser	$\text{NO}_2^- \text{-N}$, $\text{NO}_3^- \text{-N}$	Optical	Process control	S::CAN	Vienna, Austria
VARiON	$\text{NH}_4^+ \text{-N}$	ISE	Process control	WTW	Weilheim, Germany

Anammox MBBR

The anammox MBBR had a volume of 454 L where 50% of the volume was filled with K3 biofilm carriers (AnoxKaldnes: Lund, SE). The effective surface area of the carriers was $500 \text{ m}^2/\text{m}^3$. Mechanical mixing of the carriers was achieved by a variable speed mixer (Caframo: Georgian Bluffs, Ontario, CA) at $G = 14 \text{ s}^{-1}$. The pH was recorded continuously by an online pH probe and the reactor was covered with Styrofoam to avoid oxygen transfer from the atmosphere. During startup, the anammox MBBR was operated with a

temporary clarifier to recycle sludge back to the MBBR. The anammox MBBR did not rely on any sensor-based process control.

B-stage AvN: AvN (NH₄) aeration control

Under this control strategy, a fixed total cycle time (in minutes) was defined by the user. Each cycle consisted of an aerobic period followed by an anoxic period, each of which would vary based on effluent NH₄⁺-N. The desired range of effluent NH₄⁺-N concentration was user-selected. For example, the user selects an effluent NH₄⁺-N range of 2-4 mg N/L, a total cycle time of 14 minutes, a DO level of 1.5 mg O₂/L (these were typical values used throughout the experiments), and assume the initial aerobic/anoxic fraction is 7 minutes aerobic and 7 minutes anoxic. If the effluent NH₄⁺-N increased above 4 mg/L, the aerobic fraction was increased by 1 minute and the anoxic fraction was decreased by 1 minute, so the new ratio was 8 minutes aerobic/6 minutes anoxic. This continued until the effluent NH₄⁺-N was within the desired range, at which point the controller did not change the time periods (Figure 17a). When the effluent NH₄⁺-N level dropped below 2 mg/L, the length of the aerobic period was decreased and the length of the anoxic period increased, until the effluent NH₄⁺-N level returned to within the desired range. To prevent over or under-aeration, the system contained a user-defined maximum and minimum aeration period duration. Because air flow was controlled by solenoid valves, to achieve an average DO of 1.5 mg O₂/L, the solenoids were set to open at 1.2 mg O₂/L (Low DO set-point) and close at 1.7 mg O₂/L (High DO set-point). The ON/OFF DO controller graphic representation can be seen in Figure 17b.

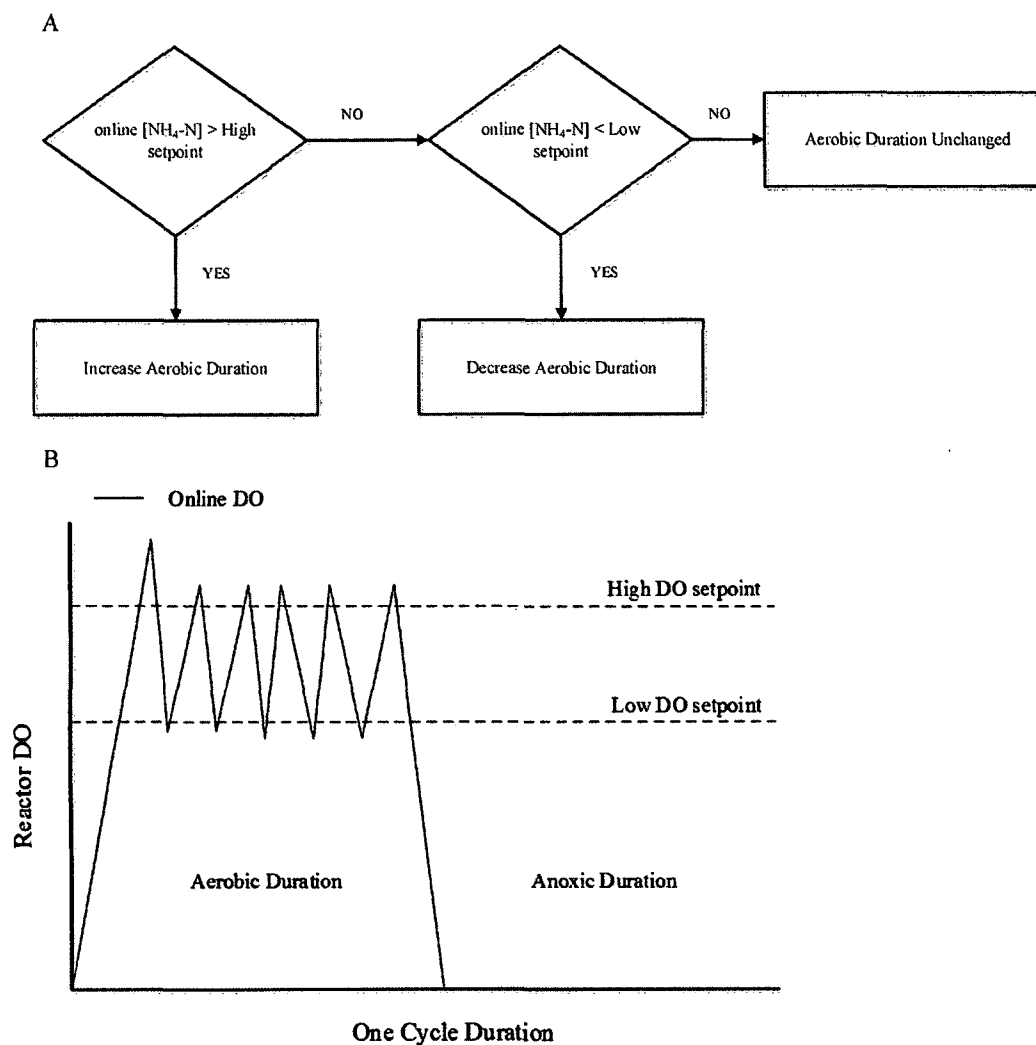


Figure 17. A) Graphic representation of the control logic of ammonia-based intermittent aeration control. B) Graphic representation of ON/OFF DO controller during one cycle.

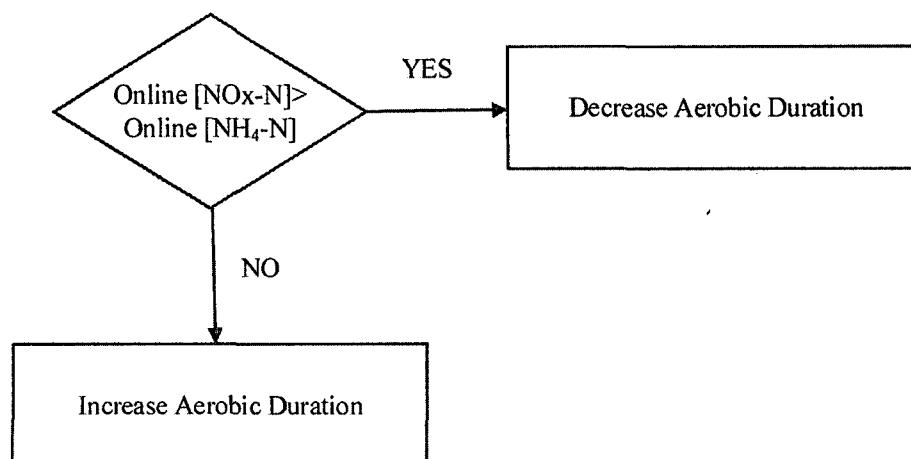
B-stage AvN CSTR: AvN ($\text{NH}_4\text{-NO}_x$) aeration control

To impose conditions favorable for NOB out-selection and to provide effluent suitable for anaerobic ammonia oxidation (AMX) polishing, an aeration controller was developed which uses online *in-situ* DO, NH_4^+ , NO_2^- and NO_3^- sensors. The first component of AvN control was the aerobic duration controller with the goal of maintaining equal effluent $\text{NH}_4^+\text{-N}$ and $\text{NO}_x\text{-N}$ ($\text{NO}_x\text{-N}/\text{NH}_4^+\text{-N} = 1$) in the AvN CSTR at all times (Figure 18a). The

latter would guarantee a treatable effluent for the final polishing step with AMX. The other component of the AvN control was the DO controller, which maintains the DO at a desired set-point during the aerated period (Figure 18b).

Under the AvN strategy, $\text{NH}_4^+\text{-N}$ was compared to the sum of $\text{NO}_2^-\text{-N}$ and $\text{NO}_3^-\text{-N}$ concentrations ($\text{NO}_x\text{-N}$). First, the cycle duration (aerobic time + anoxic time) had a defined minimum and maximum aerobic time. The cycle duration was kept constant at 12 minutes and minimum and maximum aeration times were set at 4 and 10 minutes, respectively. These set-points were selected to avoid $\text{NH}_4^+\text{-N}$ concentrations below 1.5 mg-N/L. As the AvN controller aimed at maintaining $\text{NH}_4^+\text{-N}$ concentrations equal to $\text{NO}_x\text{-N}$. When the $\text{NH}_4^+\text{-N}$ concentration was greater than $\text{NO}_x\text{-N}$ concentration, the aerobic time was increased and the aerobic time was decreased when the $\text{NO}_x\text{-N}$ concentration was greater than $\text{NH}_4^+\text{-N}$ concentration, while maintaining the cycle duration constant. The aerobic time was allowed to fluctuate between the minimum and maximum set-points by a PID controller. When aerated, a PID controller controlled a MOV to maintain the target DO set-point of 1.6 mg $\text{O}_2\text{/L}$.

A



B

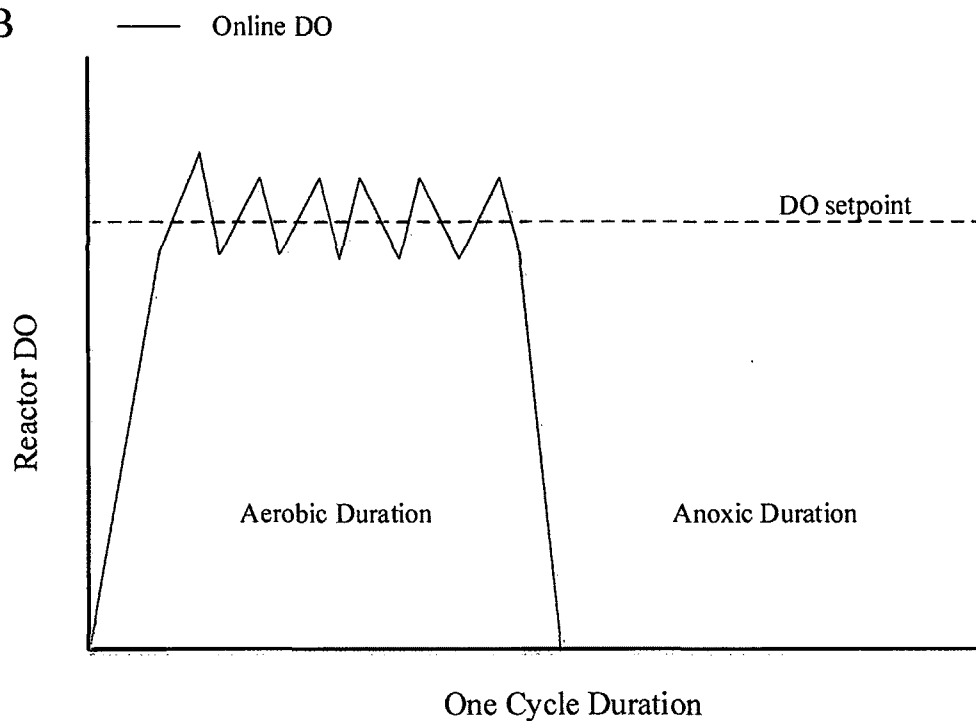


Figure 18. A) Graphic representation of the logic of AvN aeration control. B) Graphic representation of ON/OFF control during one cycle and PID DO control during aerobic duration.

The aeration control strategies used in this study are compared with traditional ammonia-based aeration control in Table 19.

Table 19. Comparison of main features of ammonia-based aeration control, AvN (NH₄) control and AvN (NH₄-NO_x) aeration control

	ABAC*	AvN (NH ₄) Control	AvN (NH ₄ -NO _x) Control
Control set-point	Effluent NH ₄ ⁺ -N	Effluent NH ₄ ⁺ -N	Effluent NH ₄ ⁺ -N set-point = Effluent NO _x -N
Control variable	DO intensity	Aerobic Fraction	Aerobic Fraction
DO	Variable DO set-point	Constant DO	Constant DO
Aeration Pattern	Continuous aeration	Intermittent aeration	Intermittent aeration
Sensors	NH ₄ ⁺ -N and DO	NH ₄ ⁺ -N and DO	NH ₄ ⁺ -N, NO ₂ ⁻ -N, NO ₃ ⁻ -N and DO

*Most commonly used feed-back ammonia-based aeration control (ABAC)

3.3 Pilot 2.0 Setup

The second generation pilot was shaped by the knowledge and insights gained during the operation of the first generation pilot. Based on the results and keeping full-scale implementation in mind, AvN aeration control with the plug flow configuration and anammox polishing was chosen for a consolidated B-stage. The major issues of the A-stage in the first generation pilot were filamentous bulking, aeration control, and process stability. Therefore, the new A-stage consisted of a more plug-flow configuration with 3 reactors in series. In addition, because the A-stage precedes the B-stage, the extent of experimentation was limited, therefore, an identical A-stage train was added to serve as an

experimental train. The detailed schematic of Pilot 2.0 is presented in Figure A2. The process flow diagram excluding the experimental A-stage train is presented in Figure 19.

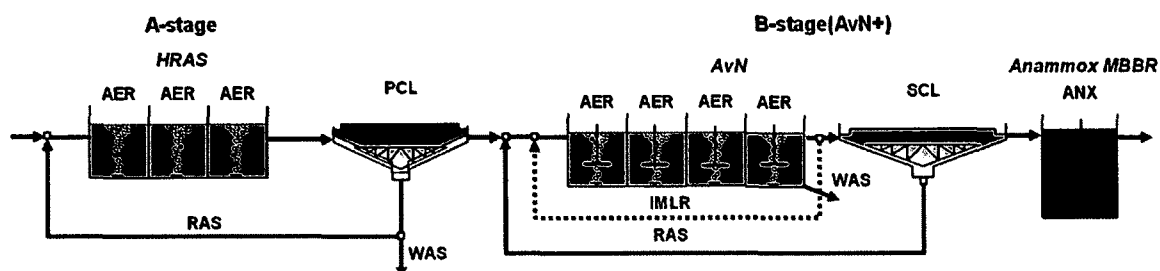


Figure 19. A-B pilot process flow diagram (Pilot 2.0).

Preliminary Treatment

Using a chopper pump, RWI for the pilot process was pumped from the effluent channel of the PTF at CETP. The PTF includes fine screens and forced vortex grit removal. Due to the inefficiencies of the PTF, the pumped RWI first passed through a 568 L drum equipped with a variable speed mixer that was operated at a speed that allowed grit to settle but kept particulate and colloidal organic matter in suspension. Accumulated grit was periodically removed by draining and cleaning out the tank. Floatable material, such as oil and grease, was continuously removed by allowing the tank to overflow to a floor drain. From the grit and scum removal tank, the RWI was pumped by a progressive cavity pump through basket screens with 2.4 mm openings into a temperature control tank. This tank contained submersible heaters and a finned-tube coil. Coolant was circulated through the coil and a water-cooled water chiller. A PLC controlled power to the heater and chiller based on a signal from a thermocouple in the temperature control tank and a user set-point. This setup provided the capability to provide a constant influent wastewater temperature to the biological processes anywhere from 15 to 25°C. The temperature control tank also

contained a constant speed mixer. These processes were only necessary for the pilot and not intended for the full-scale process.

High-rate Activated Sludge Process (HRAS-control)

The three reactors were constructed from PVC pipe supported vertically on one end with a total operating volume of 511 L, a HRT of 30 minutes, and a side water depth of 3.4 meters. Aeration was provided using compressed air and a 17.7 cm membrane disc diffuser in each reactor with the DO monitored by a DO sensor in the last reactor (Table 20). The desired airflow set-point was maintained using a single-loop PID controller controlling a MOV on the compressed air line. Additional mixing was provided by large bubble mixing every two minutes. The last reactor overflowed by gravity to a steep cone-bottom clarifier. The clarifier had a submerged vertical inlet inside of a center well. This configuration helped dissipate the influent hydraulic energy and allowed additional bioflocculation to occur before solids separation. The clarifier was fitted with a scraper mechanism that rotated at 0.25 rpm and directed settled solids to the bottom of the clarifier cone.

A progressive cavity pump returned settled biomass in the clarifier to the first aeration tank. The SOR was $0.7 \text{ m}^3/\text{m}^2 \cdot \text{hr}$ and a SLR of $4.5 \text{ kg}/\text{m}^2 \cdot \text{hr}$ at 100% RAS and 3000 mg/L MLSS. The influent and RAS flows were monitored using magnetic flow meters. The SRT of the HRAS process was controlled by wasting solids from the underflow of the clarifier using a programmable digital peristaltic pump. Effluent from the clarifier overflowed to a 208 L drum that serves as a flow through feed storage tank for the B-stage. ESS, COD, and sCOD were monitored in this tank (Table 20). Mixing was maintained by a constant speed mixer. A-stage effluent was pumped from the feed storage tank to the B-stage with a progressive cavity pump.

Table 20. List of sensors used in A-stage monitoring.

Sensor Name	Measurement	Type	Location	Manufacturer	Origin
Foxboro pH	pH	ISE	Effluent	Invensys	London, UK
LDO	DO	Optical	Aeration tank	HACH	Loveland, CO, USA
Carbo::lyser	COD, sCOD, TSS	Optical	Effluent	S::CAN	Vienna, Austria

B-stage AvN

The B-stage-AvN consisted of four equal volume tanks in series, each 151 L for a total operating volume of 606 L, and a cone-bottom clarifier. The clarifier had a submerged vertical inlet inside of a center well. This configuration helped dissipate the influent hydraulic energy and allowed additional bioflocculation to occur before solids separation. The clarifier was fitted with a scraper mechanism that rotated at 0.25 rpm and directed settled solids to the bottom of the clarifier cone. A peristaltic pump returned settled biomass in the clarifier to the first aeration tank. The SOR was $0.1 \text{ m}^3/\text{m}^2\cdot\text{hr}$ and a SLR of $0.8 \text{ kg}/\text{m}^2\cdot\text{hr}$ at 100% RAS and 3000 mg/L MLSS. All four biological reactors were equipped with a variable speed mixer (Caframo: Georgian Bluffs, Ontario, CA) at $G = 163 \text{ s}^{-1}$ to maintain complete-mix conditions.

Aeration was provided using compressed air and a 17.7 cm membrane disc diffuser with the DO monitored in each reactor by a DO sensor (Table 21). The desired DO set-point was maintained using a single-loop PID controlling a MOV on the compressed air line. The aeration capacity allowed all 4 tanks to be intermittently aerated without a defined anoxic zone. There was a provision for an IMLR line to return nitrified mixed liquor from the last aerobic reactor to the first reactor using a peristaltic pump at a rate between 100-400% of the influent flow. When IMLR was used the first tank was not aerated. RAS from the clarifier was returned to the anoxic zone at 100% of the influent flow. SRT was controlled by wasting solids from the last aerobic tank. The wasting was automated to

maintained desired SRT. The AvN process was equipped with sensors to monitor NO_3^- -N, NO_2^- -N and NH_4^+ -N (Table 21). These signals were used to control intermittent aeration pattern of the AvN process. pH was monitored using a pH probe in the last aerobic reactor.

Table 21. List of sensors used in AvN monitoring and process control.

Sensor Name	Measurement	Type	Main Function	Location	Manufacturer	Origin
Foxboro pH	pH	ISE	Monitoring	Last reactor	Invensys	London, UK
LDO	DO	Optical	Process control	All reactors	HACH	Loveland, USA
Spectro::lyser	NO_2^- -N, NO_3^- -N	Optical	Process control	Last reactor	S::CAN	Vienna, Austria
VARiON	NH_4^+ -N	ISE*	Process control	First and last reactor	WTW	Weilheim, Germany

*Ion selective electrode

Anammox MBBR

The anammox MBBR had a volume of 340 L where 50% of the volume was filled with K3 biofilm carriers (AnoxKaldnes: Lund, SE). The effective surface area of the carriers was $500 \text{ m}^2/\text{m}^3$. Mechanical mixing of carriers was achieved by a variable speed mixer (Caframo: Georgian Bluffs, Ontario, CA) at $G = 8 \text{ s}^{-1}$. The pH was recorded continuously by an online pH probe and the reactor was covered by Styrofoam to avoid oxygen transfer from the atmosphere. The concentration of nitrate was measured by a HACH Nitratax sensor (HACH: Lovelond, CO, USA). A micro peristaltic pump was used to feed acetate to the anammox reactor to maintain desired influent COD: NO_3^- -N ratio. The anammox MBBR did not rely on any sensor based process control.

AvN Aeration Control

The AvN aeration control remained unchanged from the previous pilot (AvN CSTR).

AvN SRT Control

The oxidation of NH_4^+ is the first step of biological nitrogen removal. Ammonia oxidizing bacteria (AOB) oxidize NH_4^+ to NO_2^- and then nitrite oxidizing bacteria (NOB) oxidize NO_2^- to NO_3^- to complete full nitrification. Although the second step is not needed for nitrogen removal, since AOB and NOB co-exists closely in environment; elimination of NOB has been proven difficult in biological wastewater treatment processes. The higher rates associated with AOB results in better conversion of NH_4^+ to NO_2^- which is critical for achieving higher nitrogen removal rates. Since, AOB grow slower than the heterotrophs that convert NO_2^- and NO_3^- to N_2 , AOB population and NH_4^+ oxidation rates usually dictate the solids retention time (SRT) of the biological nitrogen removal system. The AvN aeration control maintains the proper aerobic fraction to achieve the effluent $\text{NO}_x\text{-N}:\text{NH}_4^+\text{-N}$ ratio of 1. The aerobic fraction required to maintain effluent $\text{NO}_x\text{-N}:\text{NH}_4^+\text{-N}$ ratio of 1 is a function of $\text{NH}_4^+\text{-N}$ oxidation rate or AOB rate. Further, AOB rate is related to system SRT. Moreover, denitrification rates are much higher than $\text{NH}_4^+\text{-N}$ oxidation rates in systems similar to AvN with influent COD/N ratio >5 .

In intermittent aeration systems oxidation of COD occurs during the aerobic and anoxic periods. COD oxidation during the aerobic period uses DO as an electron acceptor while $\text{NO}_x\text{-N}$ is used as the electron acceptor during anoxic COD oxidation. More aerobic oxidation of COD limits the electron donor for denitrification during the anoxic period. Heterotrophic consumption of NO_2^- is key to out-selection of NOB. In an AvN aeration controlled system an aerobic fraction (AFc hereafter) of 0.5 represents that the $\text{NH}_4^+\text{-N}$ removal rate is lower than the $\text{NO}_x\text{-N}$ removal rate, which is the result of lower AOB rates. To boost AOB activity and decrease the AFc to 0.5 or lower, the system SRT has to be increased. At an AFc below 0.5, the system can be operated at the lowest SRT (dependent on temperature) possible. This aggressive SRT allows washout of the NOB without affecting the AOB. The objective of operating at an aggressive SRT stems from the fact that NOB washout has been proven difficult at longer SRTs due to their ability to supplement growth with certain organic substrate (Ward et al., 2008). To maintain an aggressive SRT for AOB and NOB, wasting was performed based on the goal of maintaining $\text{AFc} \leq 0.5$. A program was devised with predefined minimum and maximum wasting rate, which results in the desired range of SRTs. If the AFc was below 0.5 the

wasting rate was increased and if it was greater than 0.5 the wasting rate was decreased (Figure 20). This was done by a PID controller. The system can operate at an AFc much lower than 0.5, if AOB enrichment occurs at the lowest predefined SRT. It is important to note that AFc set-point of 0.5 is specific to the system of this study and could be different for other systems with different influent characteristics. Also, if anammox is the primary mechanism for nitrogen removal, this could be different.

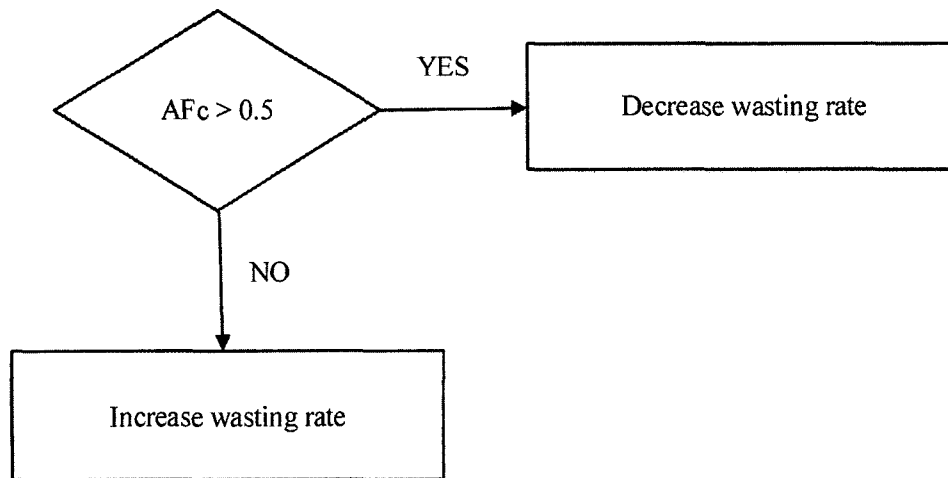


Figure 20. Graphic representation of PID logic of AvN SRT control. AFc is AvN aeration controlled aerobic fraction.

3.4 *In-situ* and *Ex-situ* Measurements for Assessing Nitrogen Removal Performance

AOB-NOB Maximum Activity Measurement

To measure AOB and NOB activity, 4 L samples were collected and dispensed into 4L vessels from the AvN process and aerated for 30 minutes to oxidize excess COD, spiked with 20-30 mg/L $\text{NH}_4^+\text{-N}$ (as ammonium chloride) and 2-4 mg/L $\text{NO}_2^-\text{-N}$ (as sodium

nitrite), respectively and sampled continuously for 1 hour at 20-minute intervals. All collected samples were analyzed for $\text{NH}_4^+\text{-N}$, $\text{NO}_2^-\text{-N}$, and $\text{NO}_3^-\text{-N}$. Mixing was provided by a magnetic stir bar. The dissolved oxygen was maintained between 2.5 and 4 mg O_2/L . pH was maintained between 7-7.5 by adding sodium bicarbonate. The AOB and NOB rates were calculated as the slope of $\text{NO}_x\text{-N}$ produced and $\text{NO}_3^-\text{-N}$ produced respectively.

AMX Maximum Activity Measurement

To measure Anammox activity, the Anammox MBBR Reactor was isolated from the system. Approximately 15 minutes of mixing was performed to allow the consumption of excess COD. A sample was taken at time 0 for sCOD, $\text{NH}_4^+\text{-N}$, $\text{NO}_2^-\text{-N}$, and $\text{NO}_3^-\text{-N}$. The MBBR was then spiked with 10 mg/L $\text{NH}_4^+\text{-N}$ (as ammonium chloride) and 8 mg/L $\text{NO}_2^-\text{-N}$ (as sodium nitrite) and sampled continuously at 20-minute intervals until the $\text{NO}_2^-\text{-N}$ was less than 1.5 mg/L $\text{NO}_2^-\text{-N}$. On the last sample of the activity measurement, a sCOD sample was taken along with $\text{NH}_4^+\text{-N}$, $\text{NO}_2^-\text{-N}$, and $\text{NO}_3^-\text{-N}$. The dissolved oxygen was maintained less than 0.01 mg O_2/L and was recorded at 20 minute intervals. The pH was recorded at 20 minute intervals as well. Ammonia uptake and nitrite uptake rates were calculated as the slope of the $\text{NH}_4^+\text{-N}$ and $\text{NO}_2^-\text{-N}$ values taken during the activity test. Nitrate production rates were calculated as the slope of the $\text{NO}_3^-\text{-N}$ production.

Biomass Density

Biomass density measurements of the anammox media were conducted on an approximately bi-weekly basis. Total biofilm solids on the media were measured by collecting media samples from the top of the completely mixed MBBR reactor. Aluminum pans were weighed and the initial weight was recorded. Pans were filled with six pieces of media and dried in an oven at 105 °C for ≥ 1.5 hr. After drying, the pans were moved to a desiccator to cool to room temperature for ≥ 30 minutes. The pans plus media were then weighed and recorded. The media was then removed from aluminum pans and placed into a 500 mL Erlenmeyer flask containing 200 mL of concentrated 1N H_2SO_4 , which was capped and shaken vigorously for approximately 30 seconds. The flask was then placed on

a stir plate for a minimum of two hours. After two hours, the media was cleaned off by running tap water through the media pieces and scrubbed with a small pipe cleaner. The empty pans were reweighed and their values were recorded. The media was placed into the pans and placed into the oven at 105 °C for ≥ 1.5 hr. After drying, the pans were moved to a desiccator to cool to room temperature for ≥ 30 minutes. The pans with the media were then weighed and recorded. The difference in initial and final weight was used to calculate the biomass on the carriers. To calculate the unit biomass (biomass per m^2 of surface area available) the biomass weight was divided by the number of elements sampled and standard conversions for the number of elements per cubic meter and square meters of surface area per cubic meter is used as shown in equation 19.

$$\text{Unit Biomass (g/m}^2\text{)} = \text{Weight of attached biomass (g)} \times (114,000 \text{ elements/m}^3) / \text{(Number of elements samples} \times 500 \text{ m}^2/\text{m}^3\text{)} \quad (19)$$

Nitrifier Rate Measurements as a Function of DO

To determine AOB and NOB rates as a function of DO, an isolated reactor batch test was performed. The AvN CSTR was isolated and aerated for > 1 hour to remove all COD present. The reactor was analyzed for $\text{NH}_4^+\text{-N}$, $\text{NO}_2^-\text{-N}$ and $\text{NO}_3^-\text{-N}$, and then spiked with a solution of NH_4^+ and NO_2^- . The reactor was aerated for one hour at a DO level of 0.1 mg O_2/L . Samples were taken every 15 minutes and analyzed for $\text{NH}_4^+\text{-N}$, $\text{NO}_2^-\text{-N}$ and $\text{NO}_3^-\text{-N}$. AOB rates were calculated as production of $\text{NO}_x\text{-N/L/d}$. NOB rates were calculated as production of $\text{NO}_3^-\text{-N/L/d}$. This was repeated at DO levels of 0.3, 0.6, 1.2, and 2.0 mg O_2/L . The entire experiment was performed 3 times, with the results averaged and plotted.

Sample Collection and Analysis

Performance was monitored by collecting 24-hour flow-weighted composite samples from the influent and effluent. The samples were analyzed for COD, sCOD, TSS, TVSS, $\text{NH}_3^+\text{-N}$,

N, NO_2^- -N, NO_3^- -N, TP, OP, total Kjeldahl nitrogen (TKN), and alkalinity. TSS, TVSS, TP, and TKN were analyzed by the HRSD's central environmental laboratory (CEL) using Standard Methods. Composite samples were also analyzed on-site at the pilot, all on-site analysis was done with Hach test kits, except TSS which was done by Standard Methods. Grab samples were routinely taken from each tank and analyzed for NH_3^+ -N, NO_2^- -N, NO_3^- -N, OP using Hach kits; and SVI by Standard Methods. The list methods used in the study with references can be found in Table A1 and Table A2.

Analytical Methods

Ammonia HACH Test Kit

The HACH Test kit used for ammonia analysis was the HACH Test N' Tube Plus (TNT Plus) 832, high range (2 to 47 mg/L NH_3 -N). This test kit uses the Salicylate Method 10205 in which ammonium ions react with hypochlorite ions and salicylate ions in the presence of sodium nitroprusside as a catalyst to form indophenol. The amount of color formed is directly proportional to the ammonia nitrogen present when measured at 690 nm.

Nitrate HACH Test Kit

Nitrate analysis was conducted using HACH Test N' Tube Plus (TNT Plus) 835 (low range, 0.23 to 13.50 mg/L NO_3 -N) and 836 (high range, 5 to 35 mg/L NO_3 -N). Both kits use the Dimethylphenol Method 10206 in which nitrate ions in solutions containing sulfuric and phosphoric acids react with 2,6-dimethylphenol to form 4-nitro-2, 6-dimethylphenol. Tests were measured at 345 nm. Prior to performing nitrate analysis, nitrite concentrations were verified. If the sample contained over 2.0 mg/L NO_2 -N, 50 mg of sulfamic acid (amidosulfonic acid) were added to 5.0 mL of sample, dissolved, and allowed to wait for 10 minutes to prevent interference.

Nitrite HACH Test Kit

For samples with nitrite concentrations ranging from 0.003 to 0.500 mg/L NO₂-N HACH TNT Plus LR (low range) was used. Method 10019 is a diazotization method, in which the nitrite in the sample reacts with sulfanilic acid to form an intermediate diazonium salt. This couples with chromotropic acid to produce a pink colored complex directly proportional to the amount of nitrite present when measured at 507 nm.

For samples with nitrite concentrations ranging from 0.6 to 6.0 mg/L NO₂-N the HACH TNT Plus 840 (high range) Method 10237 was used. Nitrite in the sample reacts with a primary aromatic amine in acidic solution to form a diazonium salt. This couples with an aromatic compound to form a colored complex that is directly proportional to the amount of nitrite present. Test results were measured at 515 nm.

Ortho-Phosphate HACH Test Kit

Orthophosphate was measured with HACH TNT PhosVer 3 Method (Method 8048), ranges from 0.06 to 5.00 mg/L PO₄³⁻. Orthophosphate reacts with molybdate in an acid medium to produce a mixed phosphate/molybdate complex. Ascorbic acid then reduces the complex, giving an intense molybdenum blue color, measure at 880 nm.

COD HACH Test Kit

COD was measured using COD TNT plus HR, LR and ULR (Method 8000). In this procedure, the sample is heated for two hours with a strong oxidizing agent, potassium dichromate. Oxidizable organic compounds react, reducing the dichromate ion (Cr₂O₇²⁻) to green chromic ion (Cr³⁺). When the 0-150 mg/L colorimetric method is used, the amount of Cr⁶⁺ remaining is determined. When the 0-1,500 mg/L or 0-15,000 mg/L colorimetric method is used, the amount of Cr³⁺ produced is determined. The COD reagent also contained silver and mercury ions. Silver is a catalyst, and mercury is used to complex the chloride interference.

Molecular Analysis

The methods of molecular sample collection and preparation for AOB, NOB and AMX is presented in A1 and A2. DNA and RNA extraction was conducted using the DNeasy and RNeasy mini kits (Qiagen, CA). Resulting DNA and RNA concentrations and quality were initially checked by UV spectrophotometry (Varian, CA). The abundance of AOB and NOB was quantified via SYBR® Green chemistry quantitative polymerase chain reaction (qPCR) assays, NH_4^+ monooxygenase subunit A (*amoA*) gene (Rotthauwe et al., 1997), *Nitrobacter* 16S rRNA gene (Graham et al., 2007) and *Nitrospira* 16S rRNA gene (Kindaichi et al., 2007), respectively. Total bacterial abundance was quantified using eubacterial 16S rRNA gene targeted primers (Ferris et al., 1996). qPCR assays were conducted on a iQ5 real-time PCR thermal cycler (BioRad Laboratories, Hercules, CA). Standard curves for qPCR were generated via serial decimal dilutions of plasmid DNA containing specific target gene inserts. qPCR for standard plasmid DNA and sample DNA were conducted with duplication and triplication, respectively. DNA grade deionized distilled water (Fisher Scientific, MA) was used for non-template control. Primer specificity and the absence of primer-dimers were confirmed via melt curve analysis of each and every qPCR profile.

DNA extraction was conducted using the DNeasy mini kit (Qiagen, CA). Resulting DNA concentrations and quality were measured by Nanodrop Lite UV spectrophotometry (ThermoFisher, MA). The abundance of AMX was quantified via SYBR® Green chemistry quantitative PCR (qPCR) assays targeting AMX 16S rRNA gene (van der Star et al., 2007). *C. "Brocadia fulgida"* specific qPCR assay was applied based on the highly variable region of the *hzsA* gene (Park et al., in submission). qPCR primers were used with TaqMan chemistry (forward, 5'-AGT TAG TGA GTG TGG ATG GCG TGT-3'; reverse, 5'-TCA TCC TGC GTG AGG AAC TTG TCA-3'; probe, 5'-/56-FAM/AT TCA GCC G/Zen/T GCG TAC ACC AGC TTG CTT /3IABkFQ/-3') (IDTDNA, IA).

qPCR assays were conducted on a iQ5 real-time PCR thermal cycler (BioRad Laboratories, CA). Standard curves for qPCR were generated via serial decimal dilutions of plasmid DNA containing specific target gene inserts. qPCR for standard plasmid DNA and sample DNA were conducted with duplication and triplication, respectively. DNA

grade ddH₂O (Fisher Scientific, MA) was used for non-template control. Primer specificity and the absence of primer-dimers were confirmed via melt curve analysis.

3.5 Measurement of NO₂⁻ Using Spectral Sensor

The ability to measure nitrite with an insitu sensor is critical because nitrite accumulation is desired to control the nitrite-shunt mechanism. An optical *in situ* ultra violet light sensor (Spectro::lyser, scan Messtechnik GmbH, Vienna, Austria) was used to measure nitrite and nitrate in the AvN CSTR because of its ability to discriminate nitrite when high background nitrate concentrations are present. Reference samples for analyses in the laboratory were taken three times a week. The sensor was calibrated as necessary (3-4 times) during the study and cleaned at least once a week with DI water. The performance of the Spectro::lyser was compared over 8 months against those of grab samples analyzed for nitrite using the Hach colorimetric method. These data are shown in Figure 21.

The 95 percent prediction interval for nitrite was ± 1.9 mg NO₂⁻-N/L at a mean laboratory measured value of 3.9 mg NO₂⁻-N/L. Considering the relatively high TSS concentrations in the AvN CSTR (3-4 g/L) and the difficulty of obtaining representative grab samples in the intermittently aerated reactor in which nitrite concentrations are constantly changing, the results represent usable Spectro::lyser performance for the purposes of process control. The sensor also has the advantages of low maintenance, no consumables and reliable self-cleaning with pressurized air.

To investigate further the impact of TSS and nitrate on the nitrite measurement several experiments were performed. In one experiment the Spectro::lyser was placed in a continuously stirred 15 L bucket containing anammox effluent (TSS = 20 mg/L) representing the same dissolved components matrix as in the AvN reactor. Then nitrite was added to the matrix while Spectro::lyser nitrite readings were recorded as well as grab samples were analyzed for every corresponding measurement.

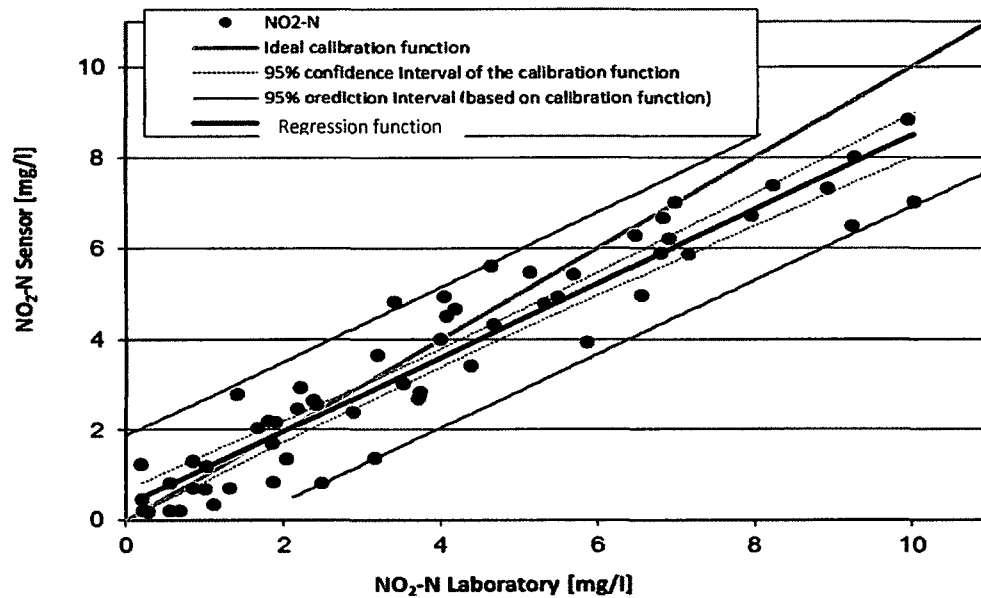


Figure 21. Comparison of nitrite measurements determined by Spectro::lyser sensor versus grab samples from the AvN reactor.

The background nitrate concentration was kept constant at 2 mg NO₃⁻-N/L. In the next experiment nitrite addition was repeated with MLSS from AvN CSTR (TSS= 3.6 g/L). In Figure 22, it can be seen that at low TSS concentrations correlation coefficients between the sensor measurement and laboratory values were better than at high TSS concentrations. However, the high TSS data suggest that these readings will suffice to determine trends in relative differences in nitrite concentrations and therefore provide continuous tracking of reactor performance.

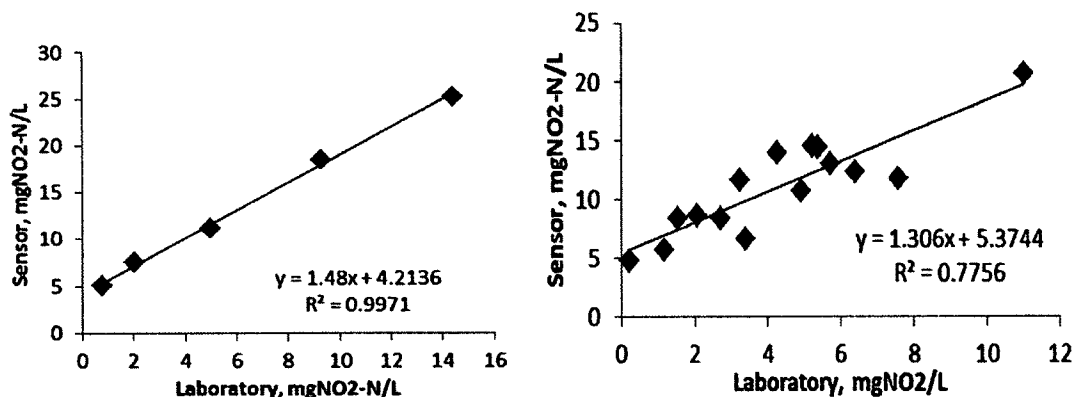


Figure 22. Spectro::lyser sensor readings vs. laboratory nitrite measurements (left: with TSS = 20 mg/L, right: with TSS = 3.6 g/L).

To test the Spectro::lyser's ability to differentiate between nitrite and nitrate, a 15L bucket containing anammox effluent (TSS= 20 mg/L) was spiked with a range of nitrate concentrations (1.8, 4.6, 7.3, 9.6, 13.4 mg NO₃⁻-N/L) while maintaining nitrite at 0.8 mg NO₂⁻-N/L. Then, the bucket was spiked with a range of nitrite concentrations (0.8, 2, 5, 9.3, 14.4 mg NO₂⁻-N/L) while maintaining nitrate at 2 mg NO₃⁻-N/L. Figure 23 shows that discrimination between nitrite and nitrate was acceptable to the extent that it did not affect the measurements in the nitrate and nitrite concentrations range encountered during this study.

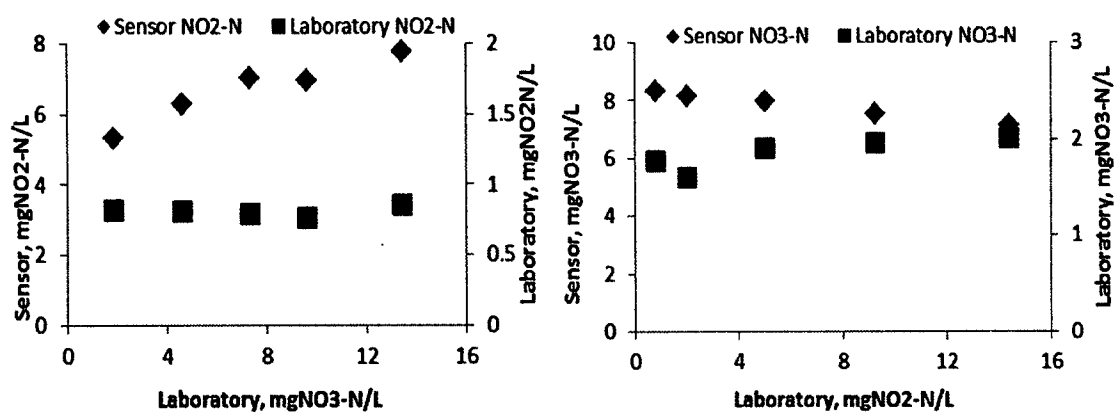


Figure 23. Spectro::lyser sensor discrimination between NO₂⁻-N and NO₃⁻-N.

CHAPTER 4

DEVELOPMENT OF NOVEL NITROGEN REMOVAL TECHNOLOGIES – AvN

4.1 Background

Conventional nitrogen removal technologies evolved from conventional activated sludge (CAS) systems for carbon removal. When a CAS plant is required to comply with effluent nitrogen standards, a huge capital and operating cost is incurred. Typically, the plant footprint for biological nitrogen removal (BNR) is increased with the addition of aeration tank capacity and subsequent electricity consumption from aeration, pumping, and mixing is also increased. Also larger quantities of chemicals for supplemental carbon and alkalinity must be provided. Further, the greenhouse gas (GHG) emissions associated with the latter renders the carbon footprint of the plant unsustainable. The raw wastewater contains energy in the form of indigenous carbon which is more than the energy needed for the treatment. However, conventional combined carbon and nitrogen systems are woefully inefficient in using the inherent wastewater carbon. In fact, a large portion of influent carbon is mineralized aerobically at the expense of aeration energy and to achieve sufficient denitrification external carbon is added. Furthermore, the added complexity with nitrogen removal exerts a huge burden on operators. To cope with this while concurrently meeting stringent nitrogen discharge permits, conventional BNR plants are designed with a large factor of safety.

The primary driver for BNR innovations has been changes in regulations. So far these innovations are based on incremental changes to the original concept and can be qualified as evolutionary rather than revolutionary. Consequently, the status quo of nitrogen removal technologies from plant-wide sustainability perspective has plagued widespread application of BNR. This chapter focuses on innovative BNR technologies that are the subject of full non-provisional patent applications that were developed as part of this project (US20140069863A1, US20140069864A1, US20140091035A1, and WO2014043547A1). These patents support a host of highly sustainable BNR technologies that are compatible with existing and new infrastructure.

4.2 Main Foundation for AvN BNR Technology Development

The main focus of innovation is based on the selection of specialist organisms, which provide a more sustainable pathway for biological nitrogen removal, using reliable process control strategies that leverage modern advances in instrumentation, control and automation (ICA) (Figure 24). These technologies also make use of existing facilities and can be tailored to different influent wastewater characteristics and effluent discharge permits.

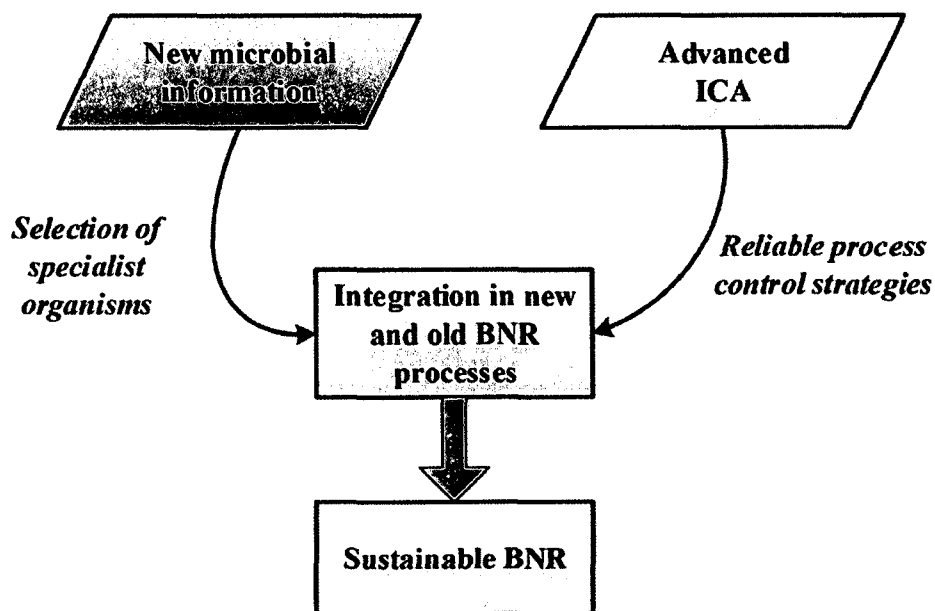
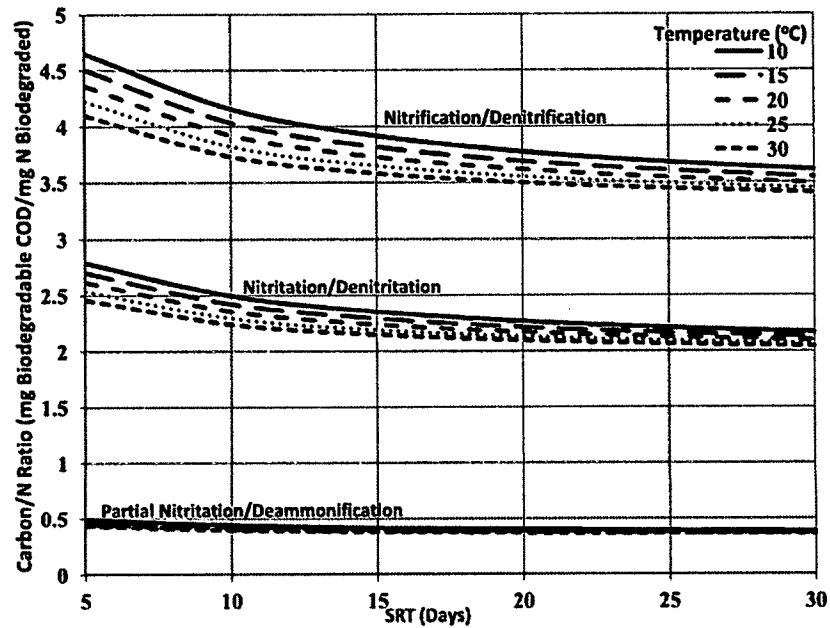


Figure 24. Main features of innovative AvN BNR technologies.

The innovative solutions target a wide-range of applications from improved performance of conventional nitrification-denitrification to systems performing combinations of nitrification-denitrification and anammox. The main benefit of short-cut nitrogen removal processes lies in carbon savings for denitrification (Figure 25). Therefore, short-cut nitrogen removal reduces the carbon requirement and offers opportunity to capture carbon upstream. The upstream carbon concentration process can use a combination of highly efficient (small foot-print, low energy consumption) aerobic and anaerobic processes to produce electricity. The efficiency of carbon removal technologies can range from 20 - 80%, while combination of these technologies can result up to 90% removal efficiency. When a high rate activated sludge process ($HRT = 30$ mins, $SRT < 0.5$ d, $DO = 0 - 0.5$ mg O_2/L) is used to concentrate carbon, a carbon removal efficiency of 30-75% can be expected. Selection and optimization of carbon removal technologies mostly depends on plant facilities and effluent nitrogen discharge permits.



	Process Option		
	Nitrification-denitrification	Nitritation-denitrification	Partial nitritation-deammonification
C/N ratio for nitrogen removal (mg biodegradable COD/mg N biodegraded)	3.5 - 4.0	2.0 - 2.5	0.5
Minimum influent C/N ratio for nitrogen removal (mgCOD/mgTKN)*	7 - 10	4 - 6	1 - 1.25
Net oxygen requirement (mgO ₂ /mgN converted to N ₂)	1.71	1.71	1.71

*Based on Assumed Efficiency of Use of Influent Biodegradable Carbon for Denitrification of 40 to

50%.

Figure 25. C/N ratio requirements for nitrogen removal for conventional and short-cut nitrogen removal pathways (Daigger et al., 2014).

4.3 AvN Technology

AvN technologies include operational and process control strategies (AvN strategies hereafter) as well as reactor configurations for novel short-cut nitrogen removal schemes. NOB out-selection under mainstream conditions is the core of the technology. AvN

strategies for NOB out-selection when typical sidestream conditions, such as high temperature and high free ammonia, are not available include the following:

1. Maintain residual effluent ammonia.
2. Operate at high DO (typically greater than 1.2 mg O₂/L)
3. Rapid transition between anoxic and aerobic conditions
4. Aggressive SRT (i.e., short SRT)
5. Bioaugmentation of AOB and AMX

The results of AvN strategies for NOB out-selection and underlying mechanisms are presented and discussed in great depth in Chapters 6 and 7. The first three strategies are very important and are executed by AvN aeration control (see Chapter 3). AvN SRT control is a manual or automated strategy of wasting such that SRT is close to AOB washout SRT. The main principle of AvN SRT control is that maintaining aggressive SRT would out-select NOB that are stressed by strategies 1-3. The description and results associated with automated AvN SRT control can be found in Chapter 3 and Chapter 8. The fifth strategy is to bioaugment sidestream generated AOB and AMX to the mainstream. AOB bioaugmentation allows shorter SRTs for NOB out-selection and AMX bioaugmentation and subsequent retention in mainstream offers deammonification pathway for nitrogen removal. Effluent quality of AvN systems depends on the extent of NOB out-selection, influent C/N ratio, and the main pathway of biological nitrogen removal. The effluent of AvN systems are expected to contain varying concentrations of NH₄⁺-N, NO₂⁻-N, and NO₃⁻-N and may require further nitrogen polishing to meet very low effluent nitrogen discharge permits. When AvN systems are coupled with add-ons for nitrogen polishing they are referred to as AvN+. The use of an anammox MBBR to polish nitrogen from the effluent of nitrification-denitrification system is presented in Chapters 7 and 8.

Selection of AvN Technologies for Nitrogen Removal

There are several factors that play a role in determining the most viable AvN technology for nitrogen removal, including, but not limited to, existing infrastructure (e.g. primaries, anaerobic digesters, effluent filters, disinfection process etc.), level of nitrogen removal

required, and influent wastewater characteristics- most notably the influent C/N ratio. The two major considerations that determine the carbon management and ultimately the C/N ratio available for nitrogen removal are 1) solids management technology and 2) effluent quality standards. When wastewater treatment plants use incinerators or any other technologies to manage solids with no heat or energy recovery (incinerator plants hereafter), there is no incentive to capture more carbon and also there is no opportunity to employ sidestream deammonification. However, for wastewater treatment plants with anaerobic digesters (digester plants hereafter) more carbon re-direction could increase the capacity for energy production. The reject waste stream from the anaerobic digester presents an opportunity for sidestream deammonification to reduce the nitrogen load to the main plant. It is important to note that sidestream deammonification offers the potential to bioaugment AOB and AMX to mainstream to facilitate nitrification-denitrification and deammonification, which is not available in an incinerator plant.

Depending on geographical location and type of receiving water body, wastewater treatment plants are required to meet different effluent nitrogen discharge permits. For example, many of HRSD's wastewater treatment plants discharging into Chesapeake Bay are required to remove total nitrogen but there is no ammonia discharge limit. However, there are many other wastewater treatment plants imposed with very low effluent ammonia discharge permits. Consequently, these requirements dictate the selection of AvN process control strategies and nitrogen polishing add-ons. The influent C/N ratio to the nitrogen removal step can also be used as a general guideline for selecting AvN process control and reactor configurations as seen in Table 22. Although process-specific, efficiency of the use of influent biodegradable carbon for nitrogen removal is suggested to be around 50% (Grady et al., 2011). AvN aeration control strategies, which dynamically manage aerobic and anoxic volumes according to influent load for nitrification and denitrification, improve the efficiency of influent carbon utilization for nitrogen removal. Further enhancement in nitrogen removal is realized by leveraging nitrification-denitrification and deammonification pathways. Flowsheets and schemes that are possible with AvN technologies will be explored in the following sections.

Table 22. Implementation of AvN technology for wide-scale sustainable BNR solutions.

Biological process	Nitrification-Denitrification	Nitritation-Denitrification*	Nitritation-Denitrification + Nitrogen Polish/anammox*
Influent C/N ratio	>10	5 to 10	3 to 8
Main action	Dynamically optimize anoxic/aerobic volume	Dynamically optimize anoxic/aerobic volume NOB out-selection	Dynamically optimize anoxic/aerobic volume NOB out-selection Nitrogen polish, AMX and/or AOB bioaugmentation
Benefits	Improvement of efficiency of influent carbon utilization for denitrification	Low carbon required for nitrogen removal	Very low carbon required for nitrogen removal
	Reduction in supplemental carbon	Opportunity for upstream carbon capture and electricity production	Opportunity for upstream carbon capture and electricity production
	Tighter design criteria	Small tank volume	Small tank volume
	Reduced supplemental alkalinity	Reduced supplemental alkalinity	Reduced supplemental alkalinity
Control	AvN aeration control and AvN SRT control (optimized for effluent ammonia limit if needed)	AvN aeration control and AvN SRT control optimized for NOB out-selection and/or effluent ammonia limit	AvN aeration control and AvN SRT control optimized for NOB out-selection AOB and AMX bioaugmentation from sidestream deammonification

*Depending on extent of NOB out-selection, nitrogen removal *via* nitrification-denitrification can occur.

Incinerator Plant with Moderate TN limit and No or Low Ammonia Limit

In this case, upstream carbon removal can save volume for nitrogen removal. The lower C/N ratio for nitrogen removal resulting from carbon removal can be alleviated by using AvN ($\text{NH}_4\text{-NO}_x$) aeration control to meet an effluent TN limit if there is no ammonia limit. AvN (NH_4) control can be used to meet low effluent ammonia limit. The process flow-sheet for this scenario is presented in Figure 26.

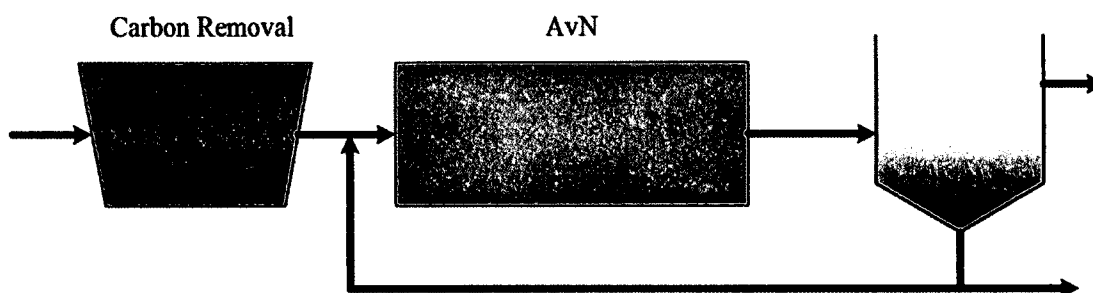


Figure 26. AvN flowsheet for incinerator plant with moderate TN limit and no or low ammonia limit.

Incinerator Plant with Low TN limit and No or low Ammonia Limit

In this case, upstream carbon removal can save volume for nitrogen removal. The lower C/N ratio for nitrogen removal resulting from carbon removal will require short-cut nitrogen removal to meet effluent TN limits. A final nitrogen polishing step may be necessary to meet low TN and low ammonia limits. An anammox polishing process is possible after AvN that uses optimized AvN ($\text{NH}_4\text{-NO}_x$) aeration control to produce an amenable effluent for anammox metabolism. The ratio of $\text{NO}_x\text{-N}/\text{NH}_4^+\text{-N}$ can be maintained such that $\text{NH}_4^+\text{-N}$ is removed to very low levels by the anammox polishing step. The AvN+ process flow-sheet for this scenario is presented in Figure 27.

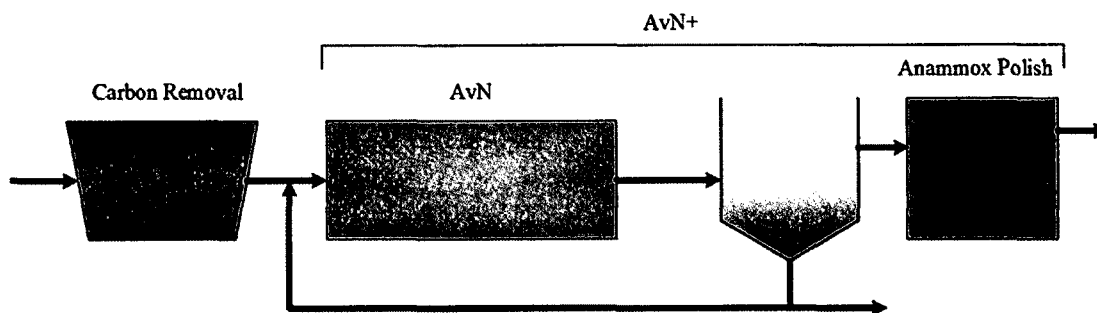


Figure 27. AvN+ flowsheet for incinerator plant with low TN limit and no or low ammonia limit.

Digester Plant with Moderate TN limit and No or Low Ammonia Limit

For digester plants there is an incentive to redirect more carbon for energy generation, thus the C/N ratio to achieve even moderate TN limits can be too low. Therefore, short-cut nitrogen removal is required. The process scheme described in Figure 27 is applicable in such a scenario as well.

Digester Plant with Low TN limit and No or Low Ammonia Limit

To meet low TN limits digester plants can employ sidestream deammonification. In such a scenario there is incentive to redirect maximum carbon for energy recovery. Sidestream deammonification provides an opportunity to bioaugment AOB and AMX to the mainstream system. AOB bioaugmentation allows mainstream AvN to be operated at very short SRT for NOB out-selection. Due to limited C/N ratio, nitrogen removal in AvN can be low, and additional nitrogen removal may be required. When anammox is used for nitrogen polishing, NO_3^- production can limit the nitrogen removal. Nitrogen removal in the anammox step can be further boosted by the addition of limited amounts of carbon. Limited acetate addition ($\text{COD}/\text{NO}_3^- \text{-N} < 1.5$) to the anammox step has shown

to result in removal of NO_3^- -N and corresponding NH_4^+ -N *via* anammox pathway (Chapter 8). The mainstream process can also be equipped with an AMX retention mechanism to facilitate deammonification. In such case, AvN (NH_4 - NO_x) aeration control needs to be optimized to allow the anammox reaction to take place and further polish the nitrogen. AvN+ process scheme with bioaugmentation from sidestream is shown in Figure 28. It is not mandatory to use anammox as the nitrogen polishing step; heterotrophs with carbon addition or other microbial groups can be employed in anoxic conditions.

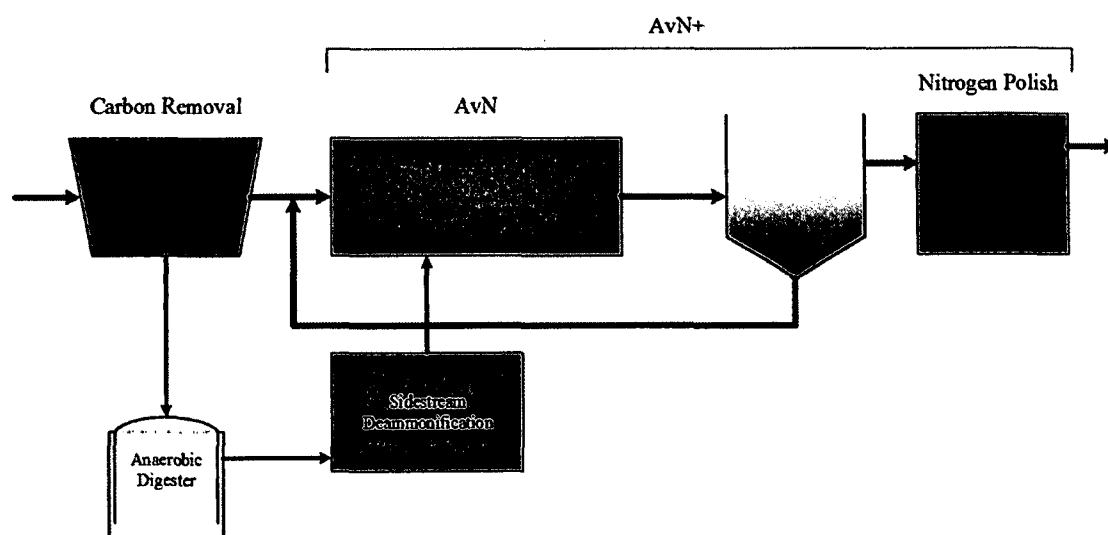


Figure 28. AvN+ flowsheet with bioaugmentation for digester plant with low TN limit and no or low ammonia limit.

4.4 Conclusion

AvN and AvN+ systems represent a new paradigm in sustainable biological nitrogen removal. These systems are compatible with existing plant facilities and can be customized to take advantages of energy and cost saving opportunities. Intensification of treatment is also possible and particularly attractive for urban wastewater treatment plants constrained for space.

CHAPTER 5

AMMONIA-BASED INTERMITTENT AERATION CONTROL OPTIMIZED FOR EFFICIENT NITROGEN REMOVAL

Note: The contents of this chapter have been submitted to Biotechnology and Bioengineering. Regmi, P., Ryder Bunce, R., Miller M., Park, H., Chandran, K., Wett, B., Murthy, S., Bot, C., Ammonia-based intermittent aeration control optimized for efficient nitrogen removal.

5.1 Introduction

Recently, ammonia-based aeration control using *in-situ* analyzers and controls has gained popularity for significant aeration energy savings and performance improvements of activated sludge processes (Rieger et al., 2014). The ammonia-based aeration control has demonstrated that aerating to nitrify just enough ammonia to meet regulations not only saves aeration energy, but also increases capacity for denitrification and provides alkalinity and supplemental carbon savings (Åmand et al., 2013). The commonly used ammonia-based aeration control uses 1) Feedback control with effluent $\text{NH}_4^+\text{-N}$ concentration to adjust DO set-point 2) Feedforward control with a model prediction based on influent and effluent $\text{NH}_4^+\text{-N}$ concentration to adjust both volume and DO set-point 3) Feedback+Feedforward control (Åmand et al., 2013). Moreover, there has been renewed interest in intermittent aeration control from the perspective of nitrite oxidizing bacteria (NOB) out-selection (Kornaros et al., 2010, Gilbert et al., 2014) that allows for the efficient short-cut nitrogen removal *via* nitrite, also referred to as “nitrification-denitrification”. Recently, alternating anoxic and aerobic conditions with step feeding was demonstrated as successful strategy to out-select NOB in a mainstream plug-flow process (Ge et al., 2014). The benefits of nitrification-denitrification include decreased aeration demand (if carbon is diverted) for nitrification and decreased carbon demand for denitrification allowing efficient nitrogen removal.

Effluent total nitrogen discharge standards are becoming increasingly stringent and widespread. Therefore, there is a need for efficient and inexpensive nitrogen removal technologies. Nitrification and denitrification in a single bioreactor with intermittent aeration has long been recognized as an efficient scheme for nitrogen removal (Batchelor et al., 1983). However, intermittent aeration systems that perform efficient nitrogen removal require advanced instruments and controls that were not available in the past. Many biological nitrogen removal (BNR) plants have strict regulations to meet, and improved efficiency at the cost of process stability is not justified. Historically, BNR processes have been prone to internal and external disturbances, and a limited understanding of the microbial protagonists performing the desired biochemical reactions has impeded selection of specialist microorganisms. In recent years, BNR optimization has been greatly aided by the new knowledge from molecular science and advancements in instrumentation, control and automation (ICA). However, there is a huge void in holistic integration of these discrete optimizations to develop highly efficient BNR systems. Hence, the sustainability of BNR systems in the future relies greatly on the integration of new microbial knowledge and robust process control strategies.

Although ICA was considered in wastewater treatment from the early 1970s (Olsson et al., 2012), ubiquitous implementation of ICA has been late in coming to wastewater treatment plants. The initial implementation of ICA involved aeration control using DO sensors and programmable logic controllers (PLC), resulting in improved biological processes and the efficiency of the overall system (Briggs et al., 1973). Therefore, when online ammonia sensors became more reliable and affordable, control of aeration based on ammonia set-points related to the level of ammonia removal required, burgeoned as an effective alternative to meet increasingly stringent ammonia and nitrogen limits (Åmand et al., 2013).

In this paper, the attempt is to explore the efficacy, simplicity, and savings related to a novel ammonia-based intermittent aeration control for efficient nitrogen removal. It is also intended to summarize findings of a pilot study with intermittent aeration that was operated at an aggressive SRT for mainstream nitrification-denitrification. The goal is also to explore benefits and limitation of repression of nitrite oxidation or nitrite oxidizing bacteria (NOB) out-selection in mainstream wastewater after partial carbon removal by a high rate activated sludge process.

5.2 Material and Methods

AOB vs NOB (AvN) pilot

The pilot process described in this study was part of a larger configuration including an adsorption-style high rate activated sludge A-stage (HRT= 30 minutes, $0.1 > \text{SRT} < 0.25$ days) for chemical oxygen demand (COD) removal providing the influent for the B-stage AvN (Miller et al., 2012, Figure 29). The AvN pilot consisted of two parallel trains 1. AvN CSTR (Regmi et al., 2014) followed by anammox MBBR polishing (Chapter 7) 2. AvN 3 CSTRs in series (Figure 16). In this study, the focus is on the performance of the second AvN train without anammox polishing. AvN (three equal sized tanks in series) was operated at a 4 h HRT at the flow rate of 1.9 L/min. The total SRT was targeted to be around 6-8 days and temperature was maintained at 25 °C during the entire study. The aerobic SRT was controlled to achieve the desired effluent $\text{NH}_4^+\text{-N}$ concentration.

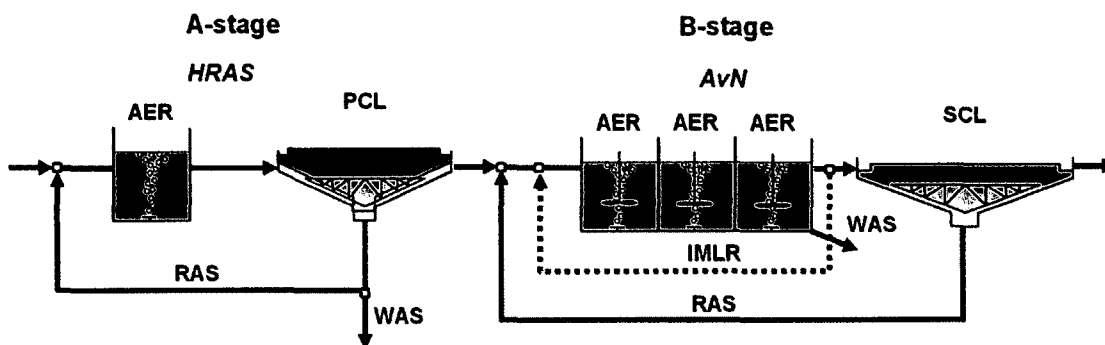


Figure 29. Process flow diagram of the A-B pilot with AvN.

The AvN consisted of three tanks with a combined volume of 0.45 m^3 followed by a clarifier. The individual tanks were mechanically mixed using variable speed mixers ($G = 106 \text{ s}^{-1}$) in order to maintain completely-mix conditions. Return activated sludge (RAS) from the clarifier was returned to the first AvN reactor with a peristaltic pump at 100% of the influent flow rate. SRT was controlled by wasting solids from the last reactor with a programmable digital peristaltic pump. The pH was recorded continuously by an online pH probe in the last reactor. The AvN reactors were equipped with sensors to monitor DO (Hach LDO, CO, USA), and $\text{NH}_4^+\text{-N}$ (WTW, Germany). $\text{NH}_4^+\text{-N}$, and DO signals were used to control aeration (Figure 2). There was a provision for internal mixed liquor recycle (IMLR) from the last tank to the first AvN tank. The use of IMLR during the study period is documented in Table 23.

Table 23. The use of IMLR during the pilot study.

IMLR	Fixed anoxic volume	Intermittent aeration volume	Days
0	0	100%	87
100-300%	33%	67%	114
400%	33%	67%	165

Startup

The AvN was seeded with nitrifying sludge from the Virginia Initiative Plant (VIP) in Norfolk, Virginia, USA. The AvN received effluent from the high-rate A-stage (Miller et al., 2012). Typical A-stage effluent was characterized by $\text{pH} = 7.05 \pm 0.14$, $\text{COD} = 306 \pm 87.3 \text{ mg/L}$, $\text{NH}_4^+ = 29.7 \pm 3.9 \text{ mg N/L}$, $\text{COD/TKN} = 6.7 \pm 1.4$, $\text{Ortho-P} = 3 \pm 1.2 \text{ mg P/L}$, and $\text{Alkalinity} = 159.7 \pm 17.1 \text{ mgCaCO}_3/\text{L}$ (Table 24). The AvN was operated at a 4 h HRT at the flow rate of 1.9 L/min. The total SRT was targeted to be around 6 days and temperature was maintained at 25°C. The aerobic SRT was controlled by an online aeration controller (by changing the aerated fraction of the volume) to achieve the desired effluent $\text{NH}_4^+\text{-N}$ concentration.

Table 24. Average characteristics of AvN influent (A-stage effluent) and effluent over the entire experimental period.

Parameter	Influent	Effluent
pH	7.05±0.14	6.8±0.2
COD (mg/L)	306±87.3	56.6±22.4
sCOD (mg/L)	128±41.9	29.1±5.9
NH ₄ ⁺ (mgN/L)	29.7±3.9	3.6±2.5
TKN (mgN/L)	38.5±4.6	-
COD/TKN	6.7±1.4	-
Ortho-P (mgP/L)	3±1.2	2.4±1.1
Alkalinity (mgCaCO ₃ /L)	159.7±17.1	69.3±14.7

AvN (NH₄) aeration control

The first component of AvN (NH₄) aeration control was the aerobic and anoxic duration control based on the goal of maintaining a desired effluent NH₄⁺-N set-point. The second component was the DO control, which maintains the DO set-point as desired during the aerated period (Figure 30).

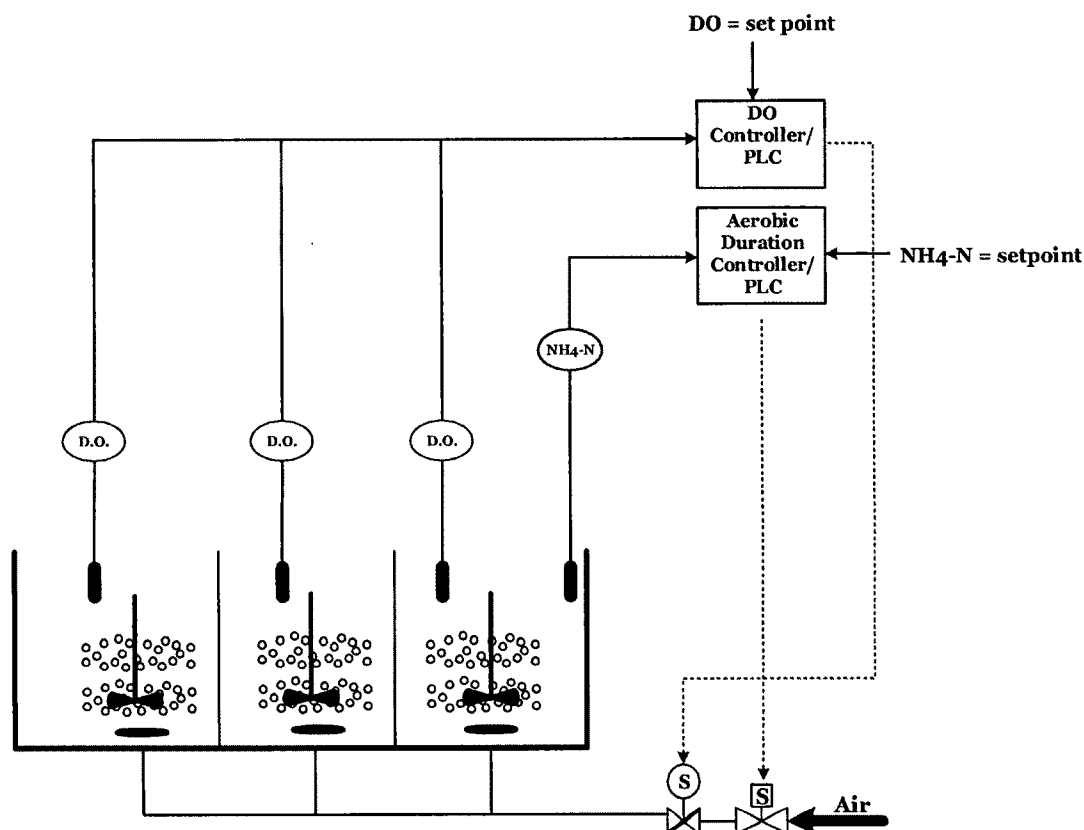


Figure 30. AvN (NH_4) aeration control depicting aerobic duration controller receiving NH_4^+ (WTW NH_4^+ ISE, Germany) signal and DO controller receiving dissolved oxygen (Hach LDO, USA) signal. The solenoid valves (S) were used to control the length of aeration duration and the DO set-point.

First, the cycle duration (aerobic duration + anoxic duration), minimum aerobic duration and maximum aerobic duration were defined such that anoxic time was greater than or equal to 25% of the total cycle time. Under this strategy, the user defined high and low $\text{NH}_4^+\text{-N}$ set-points which triggered changes in the duration of aerobic time. When the reactor $\text{NH}_4^+\text{-N}$ was greater than the high $\text{NH}_4^+\text{-N}$ set-point, the aerobic duration was increased with a delay of 1-2 minutes until it reached the maximum aerobic duration. Similarly, when the reactor $\text{NH}_4^+\text{-N}$ was below the low $\text{NH}_4^+\text{-N}$ set-point, the aerobic duration was decreased with a delay until it reached the minimum aerobic duration. When

the $\text{NH}_4^+\text{-N}$ was between the high and low set-points, the aerobic duration remained unchanged. When aerated, the DO cycled between the high and low DO set-points through switching the air ON and OFF by a solenoid valve on the air supply to achieve an average target DO ($1.6 \text{ mg O}_2/\text{L}$). The working of this controller can be seen in Figure 31. The oscillations in $\text{NH}_4^+\text{-N}$ and DO concentrations is the result of the ON/OFF controller. A PID controller would ideally be used for this type of application.

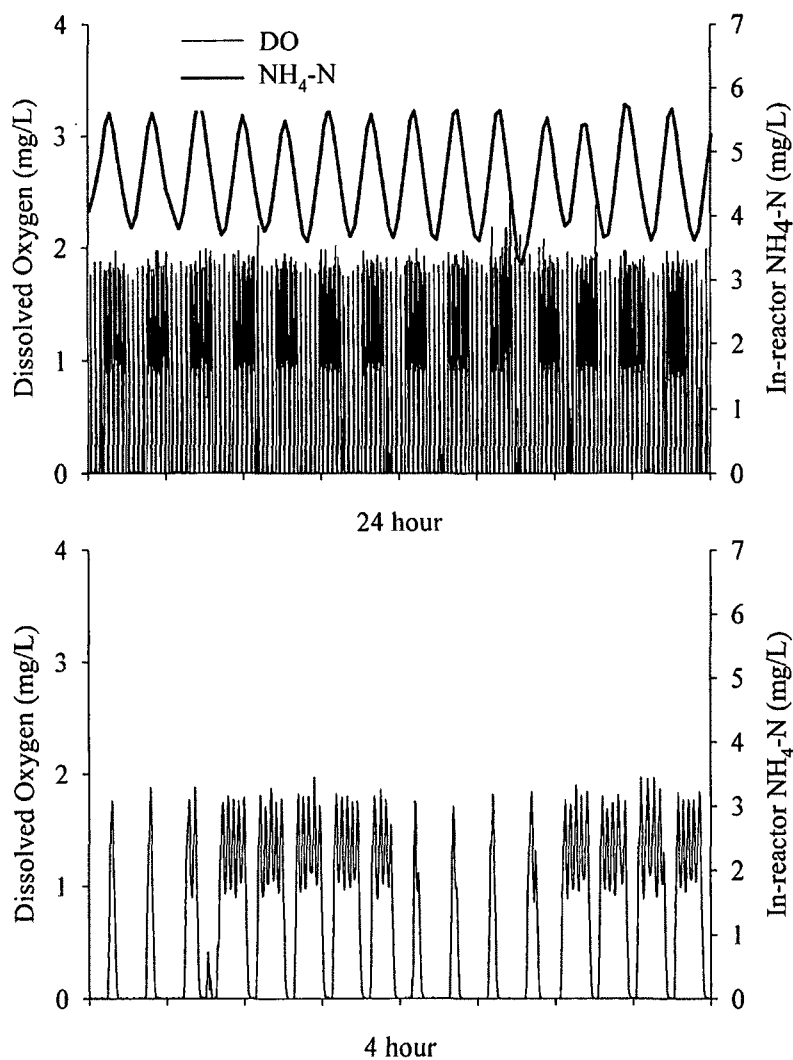


Figure 31. The working of AvN (NH_4) aeration control ($\text{NH}_4^+\text{-N}$ set-point = $4\text{-}5 \text{ mg/L}$, DO set-point = $1.2\text{-}1.7 \text{ mg O}_2/\text{L}$).

Influent/Effluent monitoring

Performance of the AvN pilot was monitored by collecting 24-hr flow-weighted composite samples from the influent and effluent. Samples were analyzed for TSS, VSS, total and soluble COD, TKN, TP, OP, NO_3^- -N, NO_2^- -N, NH_4^+ -N and alkalinity. All relevant analytical methods for solids and liquids are presented in Table A1 and Table A2.

Microbial activity measurements

To measure AOB and NOB activity, 4 L samples were collected and dispensed into 4L vessels from the AvN process and aerated for 30 minutes to oxidize excess COD and spiked with 20-30 mg/L NH_4^+ -N (as ammonium chloride) and 2-4 mg/L NO_2^- -N (as sodium nitrite), respectively and sampled continuously for 1 hour at 20-minute intervals. All collected samples were analyzed for NH_4^+ -N, NO_2^- -N, and NO_3^- -N. Mixing was provided by a magnetic stir bar. The dissolved oxygen was maintained between 2.5 and 4 mg O_2 /L. pH was maintained between 7-7.5 by adding sodium bicarbonate. The AOB rates were calculated as the slope of NO_x -N production and the NOB rates were calculated as the slope of NO_3^- -N production.

Molecular methods for microbial quantification

DNA and RNA extraction was conducted using the DNeasy and RNeasy mini kits (Qiagen, CA). Resulting DNA and RNA concentrations and quality were initially checked by UV spectrophotometry (Varian, CA). The abundance of AOB and NOB was quantified via SYBR® Green chemistry quantitative PCR (qPCR) assays NH_4^+ monooxygenase subunit A (*amoA*) gene (Rotthauwe et al., 1997), *Nitrobacter* 16S rRNA gene (Graham et al., 2007) and *Nitrospira* 16S rRNA gene (Kindaichi et al., 2007), respectively. Total bacterial abundance was quantified using eubacterial 16S rRNA gene targeted primers (Ferris et al., 1996). qPCR assays were conducted on a iQ5 real-time PCR thermal cycler (BioRad Laboratories, Hercules, CA). Standard curves for qPCR were generated via serial decimal dilutions of plasmid DNA containing specific target

gene inserts. qPCR for standard plasmid DNA and sample DNA were conducted with duplication and triplication, respectively. DNA grade deionized distilled water (Fisher Scientific, MA) was used for non-template control. Primer specificity and the absence of primer-dimers were confirmed via melt curve analysis of each and every qPCR profile.

Statistical analysis

Statistical comparison between variables was performed using the t-test (for a normally distributed data set) and Mann-Whitney rank sum test (for not a normally distributed data set) on Sigma Plot (Systat Software, San Jose, CA). Shapiro-Wilk test was used to determine the normality of the data set. A *p*-value of 0.05 or lower indicates that variables being compared are statistically different at the 95% confidence level.

5.3 Results

Long-term operation

There were many operational variables that had an effect on the performance of the B-stage; however, A-stage performance influenced the operation and performance of AvN. The goal of the A-stage operation was 50% - 60% influent COD removal, but optimal reactor design and A-stage control strategy proved to be very much a trial-and-error process. As a result, the influent COD to the B-stage varied and was difficult to control for much of the pilot operation. Therefore, during the course of the study period AvN showed variable performance in terms of N removal (Figure 32a).

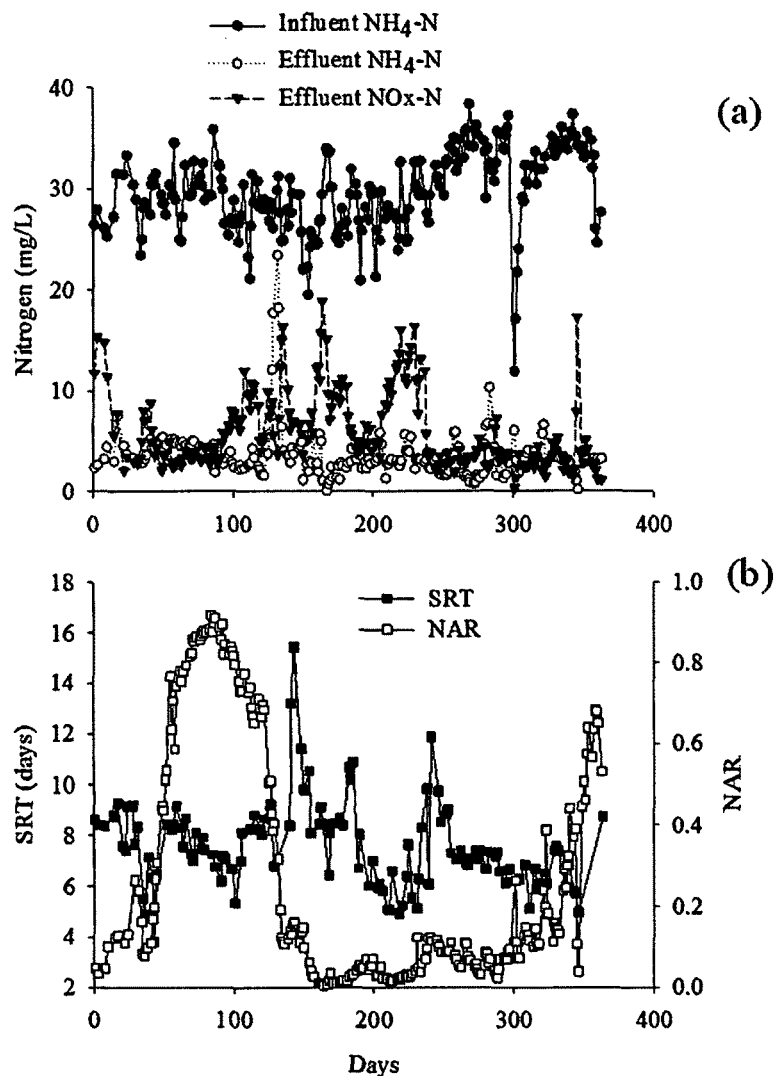


Figure 32. Trends of a) influent $\text{NH}_4^+\text{-N}$, effluent $\text{NH}_4^+\text{-N}$ and $\text{NO}_x\text{-N}$ b) Nitrite accumulation ratio (NAR) and total SRT.

The influent $\text{NH}_4^+\text{-N}$ concentration and $\text{COD}/\text{NH}_4^+\text{-N}$ ratio fluctuated due to changes in A-stage operations as a part of fine tuning control over COD removal (Figure 32a, Figure 33a). The effluent $\text{NH}_4^+\text{-N}$ and $\text{NO}_x\text{-N}$ variability can be seen in Figure 32a, while the fluctuations in nitrite accumulation ratio [$\text{NAR} = \text{NO}_2^+\text{-N} / (\text{NO}_2^+\text{-N} + \text{NO}_3^-\text{-N})$] can be seen in Figure 32b. The changes to the SRT were made constantly to keep up with the changing influent load (Figure 32b). The TIN removal rate was 95 ± 30 mgN/L/d for the influent $\text{COD}/\text{NH}_4^+\text{-N}$ ratio of 10.2 ± 2.2 at a 4 hr HRT during the study. Trends of TIN

removal rates and influent COD/NH₄⁺-N ratios followed each other as expected (Figure 33a). The mixed liquor suspended solids (MLSS) was quite variable and followed the trends of the COD removal rate (Figure 33b). The variability in MLSS can be attributed to changes in SRT and influent COD/NH₄⁺-N during the study period.

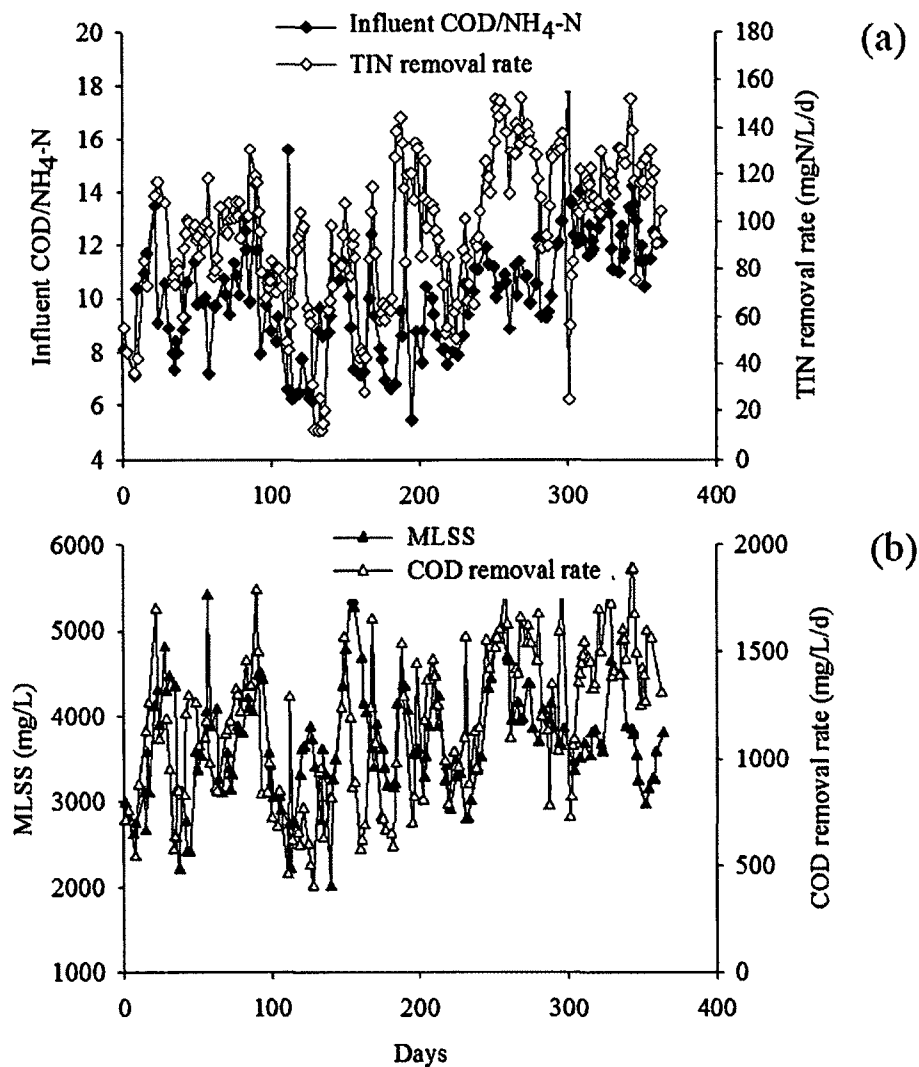


Figure 33. Trends of a) influent COD/NH₄⁺-N ratio and TIN removal rate b) MLSS and COD removal rate.

TIN removal efficiency

The influent COD/NH₄⁺-N ratio impacted TIN removal efficiency which is clearly seen from the positive correlation between these parameters (Figure 34a). The TIN removal efficiency of the AvN process was 66±17% during the study. Efforts were made to explore other factors that might influence TIN removal efficiency beyond the obvious effect of influent COD/NH₄⁺-N ratio. The results presented in Figure 34b, show that there is a positive correlation between *ex-situ* maximum AOB rates and TIN removal efficiency. The correlation is even stronger between moderate influent COD/NH₄⁺-N ratios of 8-11. Since the anoxic and aerobic times were controlled based on the target residual NH₄⁺-N, higher AOB rates allowed more anoxic time for denitrification, improving the overall TIN removal performance. Contrary to expectation, there was no correlation between the extents of NOB out-selection represented by the ratio of maximum AOB rate: NOB rate and TIN removal efficiency (Figure 34c). In fact, this was true within the full spectrum of influent COD/NH₄⁺-N ratios.

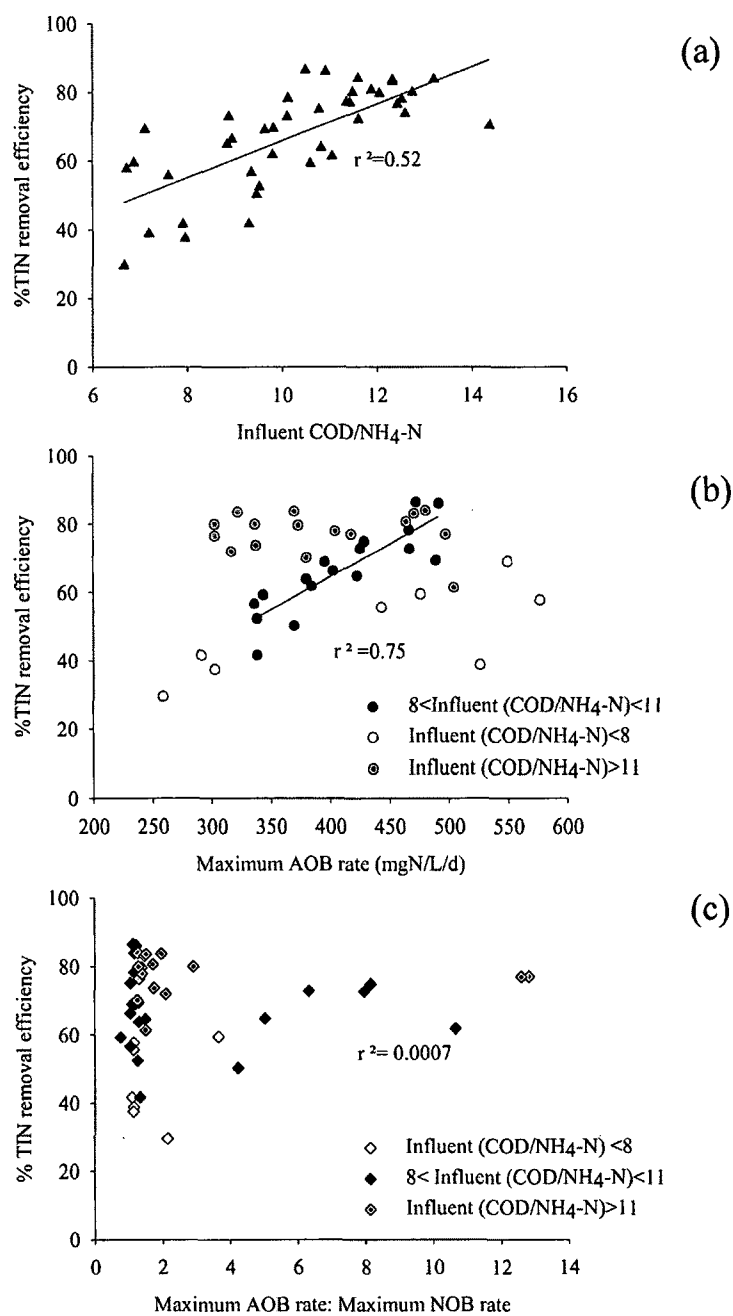


Figure 34. Correlation between TIN removal efficiency and influent COD/NH₄⁺-N (a), maximum AOB rates (b), Maximum AOB/NOB rates ratio (c).

The impact of IMLR on TIN removal efficiency can be seen in Figure 35. The TIN removal efficiency and influent COD/NH₄⁺-N ratio were not statistically different with 100-300 % IMLR and no recycle (0% IMLR: 60±18%, 100-300% IMLR: 64±17%, $p=0.091$, 0% IMLR: 10.07±2.17, 100-300% IMLR: 9.67±2.15, $p=0.356$). The TIN removal efficiency and influent COD/NH₄⁺-N ratio were greater with 400% IMLR compared to 100-300% IMLR (400% IMLR: 74±13%, 100-300% IMLR: 64±17%, $p<0.001$, and 400% IMLR: 10.87±2.0%, 100-300% IMLR: 9.67±2.15%, $p=0.003$). Therefore, it can be concluded that IMLR did not significantly improve the overall TIN removal efficiency compared to operation where all reactors were intermittently aerated. Although the NAR was greater when an IMLR of 100-300% was employed, there was no improvement in the TIN removal efficiency for similar influent COD/NH₄⁺-N ratios compared to operation without IMLR (Figure 35).

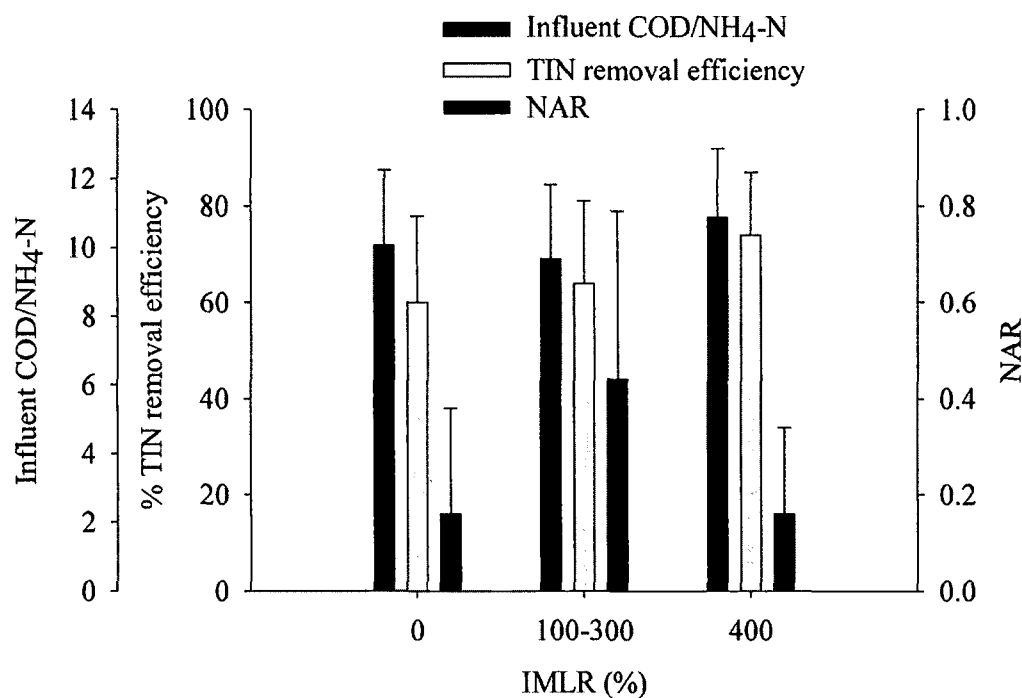


Figure 35. Comparison of TIN removal efficiency with influent COD/NH₄⁺-N and NAR at IMLR 0% (n=87), IMLR 100-300% (n=114), IMLR 400% (n=165).

NOB out-selection in AvN

NOB out-selection was inferred through *ex-situ* AOB and NOB maximum activity measurements, NAR, and targeted molecular analysis for bacterial populations. The AOB activity was greater than NOB activity (AOB: 400 ± 79 mgN/L/d, NOB: 257 ± 133 mgN/L/d, $p < 0.001$) during the entire study. The results of targeted molecular analysis for AOB, NOB (*Nitrobacter sp.* and *Nitrospira sp.*) and total bacterial population clearly showed the declining trend for NOB population during the period of low *ex-situ* NOB activity (Figure 36). The trends of NAR can be seen in Figure 32c and averages in Figure 35.

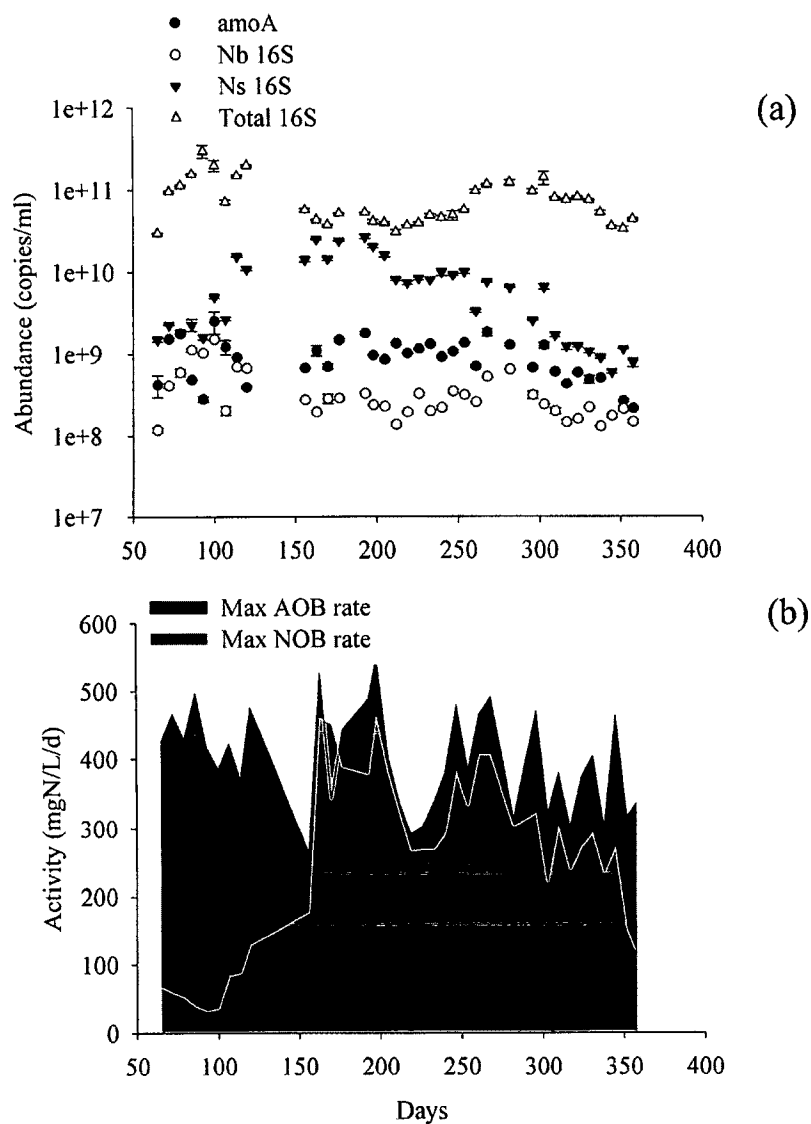


Figure 36. Trends of microbial populations (AOB, NOB and total bacteria) presented as copies of DNA per mL of sample from targeted qPCR (a) and weekly AOB and NOB activities (b).

5.4 Discussion

Single-tank nitrogen removal

It was showed that internal recycle was not necessary for improved performance in terms of nitrogen removal within the AVN concept of intermittent aeration. It was believed that an intermittently aerated single-stage nitrification/denitrification system can be an efficient way to maximize nitrogen removal while minimizing aeration volume, aeration demand, supplemental carbon and alkalinity addition (Bishop et al., 1976, Batchelor, 1983, Hao et al., 1996). However, due to the complexity associated with controlling nitrification and denitrification in the same reactor, application of such a system was limited to simulation studies (Batchelor, 1982). Further, the need for sophisticated ICA prevented single-stage nitrogen removal from being implemented at any significant scale in the past. In this study, through the use of accurate sensors and process control strategies single-stage nitrogen removal was shown possible. In fact, it was also demonstrated that managing aerobic and anoxic volumes (aeration duration control) dynamically to deal with non-steady influent results in efficient nitrogen removal.

One such example is the optimum aerobic volume control concept (OAV-control concept), which is a control strategy that is capable of adjusting the aerobic and anoxic volume required for complete nitrification and subsequent maximization of denitrification (Svardal et al., 2003). It uses the measured airflow rate and DO concentration to change the aerobic volume to the $\text{NH}_4^+\text{-N}$ load. Therefore, the anoxic volume is maximized with the goal of complete nitrification. The OAV-control concept was implemented successfully at the Linz-Asten WWTP (Austria), where effluent $\text{NH}_4^+\text{-N}$ of <1 mg NL and 70% to 80% total nitrogen removal was achieved. This strategy also demonstrated the capacity to deal with $\text{NH}_4^+\text{-N}$ peak loads. Another example is the BioDenitro (nitrification-denitrification) system that cycles the tank volumes through aerobic and anoxic conditions, utilizing the full reactor volume for nitrification and denitrification (Ingildsen, 2002). The Himmark WWTP (Denmark) was able to increase the treatment capacity by 33% with the BioDenitro concept that involved a control scheme based on on-line $\text{NH}_4^+\text{-N}$ sensors to adjust the relative length of the aerobic and anoxic phases, and improvements to the aeration and SCADA systems (Ingildsen, 2002).

The Marselisborg WWTP (Denmark) is an A/B system, where B-Stage is operated as BioDenitro. The Marselisborg plant, operating under COD-limited conditions, has a similar aeration control scheme and was able to reduce average effluent total nitrogen from 7.8 mgN/L to 5.1 mgN/L (Sorensen et al., 1994).

Aeration schemes for optimized N removal

The ammonia-based strategy in AvN is based on volume control as opposed to the more commonly seen aeration intensity control with DO set-points. Although the two controllers are different because of their different goals, they both involve limiting complete nitrification for increased denitrification and aeration energy savings. $\text{NH}_4^+\text{-N}$ and $\text{NO}_2^-\text{-N}$ oxidation follows a typical Monod curve, which suggests a linear increase in nitrifier activity with increasing DO to a certain point (e.g., 2 mg $\text{O}_2\text{/L}$) and increasing DO beyond this point has no added benefit since the nitrification rate is kinetically limited by the nitrifier concentration. The nitrifier concentration in a system is determined by the average influent $\text{NH}_4^+\text{-N}$ load and can only change in a matter of days. Therefore, the $\text{NH}_4^+\text{-N}$ control based on increasing the aeration intensity is limited by the aerated fraction of nitrifiers in a system. However, this limitation can be alleviated by increasing the aerated volume such that more nitrifiers are active.

The use of volume control (e.g., switching swing zones) is often based on the influent $\text{NH}_4^+\text{-N}$ load, also known as feedforward control. Feedforward volume control could be a robust tool to provide protection against the influent peak $\text{NH}_4^+\text{-N}$ loads compared to feedback control which might be slower to react in such situations. The aeration strategy of cycling the reactor through controlled aerated and un-aerated periods based on effluent $\text{NH}_4^+\text{-N}$ provides the similar control authority as volume control. Further, the DO was set at 1.6 mg $\text{O}_2\text{/L}$ based on the finding that the AOB rates were higher than NOB rates at this DO (Regmi et al., 2014); similar observations were made in a bench-scale study at the DCWater Blue Plains WWTP (Al-Omari et al., 2012) and at the full-scale pilot at the Strass WWTP (Wett et al., 2012). Therefore, in this study's strategy, aeration intensity was not changed by changing DO set-points, and rather the aerobic volume was changed while the DO set-point was a constant (1.5 mg $\text{O}_2\text{/L}$). Furthermore, low DO operation has

been linked with high emissions of N_2O (Kampschreur et al. 2009) and favoring filamentous microorganisms, which could adversely impact sludge settleability (Martins et al. 2004).

The primary advantages of volume control are the ability to provide control authority during the high $\text{NH}_4^+\text{-N}$ loads by increasing the active nitrifiers in the systems and to provide denitrification and aeration savings during low $\text{NH}_4^+\text{-N}$ loads. Therefore, volume control can play an important role in balancing total N removal by better utilizing the plant capacity for both nitrification and denitrification. This flexibility and optimization is not available in conventional systems where nitrification and denitrification volumes are fixed regardless of the influent loads and operating conditions.

NOB out-selection and TIN removal efficiency

Nitrification-denitrification achieved through out-selection of NOB has been associated with a reduction in the amount of internal and supplemental carbon and energy required for nitrogen removal. However, internal carbon and energy savings can only be realized if the excess influent carbon is diverted away from the nitrogen removal step through the use of a carbon redirection step. If not, redirected carbon will be oxidized aerobically, which precludes the benefits of nitrification-denitrification. We showed that the use of on-line controllers (developed in this study) allows measured control over the aerobic SRT to meet the desired effluent $\text{NH}_4^+\text{-N}$ set-point. The implication of controlling aerobic SRT to the minimum that is required to meet the target effluent $\text{NH}_4^+\text{-N}$ quality allows the overall system to be operated at an aggressive total SRT. It is clear that in an intermittent aeration system maintaining an AOB/NOB rate differential causes $\text{NO}_2\text{-N}$ to accumulate during the aerated period which is consumed by heterotrophs during the subsequent un-aerated period. As a result, NOB growth is limited due to the $\text{NO}_2^- \text{-N}$ consumption by heterotrophs. Therefore, operating at an aggressive SRT could cause a slight AOB wash-out, however, it eliminates NOB that were already limited in terms of their preferred substrate.

In this study, NOB out-selection did not result in higher nitrogen removal efficiencies for a similar influent $\text{COD}/\text{NH}_4^+\text{-N}$ ratio in an intermittently aerated system. This could

result from the fact that longer periods with a high degree of NOB out-selection and concurrent high AOB rates were not sustained. However, NOB out-selection and nitrite accumulation allows for downstream anammox polishing, which can provide additional nitrogen removal without aeration and supplemental carbon addition (Chapter 7).

5.5 Conclusion

Aeration control based on direct measurement of $\text{NH}_4^+\text{-N}$ in the bioreactor has proven to be highly effective to reduce aeration energy and increase denitrification capacity of BNR systems. In this study, a novel aeration strategy that controlled the aerobic time (while maintaining anoxic time $\geq 25\%$ total cycle time) based on the *in-situ* effluent $\text{NH}_4^+\text{-N}$ set-point was implemented. The success of this aeration strategy led to realized NOB out-selection and nitrogen removal through the nitrite pathway at ambient temperature. NOB out-selection did not improve the TIN removal efficiency, as was expected. However, the advantages of NOB out-selection can be capitalized by employing downstream anammox polishing, which offers efficient nitrogen removal without oxygen and supplemental carbon.

The benefits of volume control includes 1) control authority at high loads to attenuate effluent $\text{NH}_4^+\text{-N}$ peaks 2) instant increase in nitrification capacity (not possible with increasing DO set-point) 3) increased denitrification capacity and reduced aeration for nitrification at low loads. The model based feedforward control is mostly used to achieve volume control, which adds extra sensors and complexity. The aeration strategy used in this study achieved the benefits of volume control without the drawback of feedforward control.

Therefore, this study demonstrates a novel aeration strategy that expands the benefits of long established ammonia-based aeration control.

CHAPTER 6

CONTROL OF AERATION, AEROBIC SRT AND COD INPUT FOR MAINSTREAM NITRITATION/DENITRITATION

Note: The contents of this chapter have been published in Water Research. Regmi, P., Miller, M.W., Holgate, B., Bunce, R., Park, H., Chandran, K., Wett,B., Murthy, S., Bott, C.B., Control of aeration, aerobic SRT and COD input for mainstream nitritation/denitritation, Water Research (2014), 57, 162-71.

6.1 Introduction

Biological nitrification and denitrification are commonly used to remove nitrogen from wastewater (Grady et al., 1999). Since the inherent organic carbon present in wastewater is not always enough for complete nitrogen removal through nitrification and denitrification, supplemental organic carbon is used ubiquitously (USEPA, 2013). Nitritation-denitritation, which avoids the oxidation of nitrite to nitrate by nitrite oxidizing bacteria (NOB) and allows for the reduction of the formed nitrite to dinitrogen gas by heterotrophic denitrification, could decrease the organic carbon demand for total nitrogen removal by 40%. Additionally 25% of the aeration costs can be saved by avoiding nitrite oxidation (Turk et al., 1986).

The implementation of nitritation/denitritation is successfully applied in highly loaded sidestream processes (van Kempen et al., 2001). High removal efficiencies and efficient NOB out-selection is achieved by high temperature, low DO, low solids retention time (SRT) and free ammonia (FA) inhibition (Anthonisen et al., 1976, Hellinga et al., 1998, Joss et al., 2009, Van Dongen et al., 2001). Ahn et al. (2008) showed that sidestream out-selection of NOB is possible at lower temperatures (20°C), primarily based on a combination of FA inhibition and limiting overall SRT. However, since the above mentioned factors are not available for typical wastewater streams (hereafter termed mainstream), NOB out-selection becomes challenging. The use of approaches such as

online aeration control that terminates aeration close to the completion of ammonium oxidation has been shown to be effective for NOB out-selection in batch processes (Peng et al., 2007, Lemaire et al., 2008, Gao et al., 2009, Zeng et al., 2009). Detailed modeling of an intermittent aeration profile for nitrification in SBRs is presented by Bournazou et al. (2013). However, the usefulness of this strategy for achieving nitrite accumulation remains unknown (Peng et al., 2012), and is considered difficult for continuous processes (Ma et al., 2009, Zeng et al., 2010) because of challenges associated with systems with process control compared to transiently loaded systems.

To obtain successful nitrification/denitrification, a differential between ammonia oxidizing bacteria (AOB) and NOB rates should be obtained to be able to wash out NOB based on SRT control (Table 26). When no inhibition factors are available to out-select NOB, the AOB-NOB differential can only be obtained by optimal oxygen and nitrogen substrate levels, as based on their individual Monod kinetics. Chandran et al. (2000) showed that NOB have a higher affinity for nitrogen substrates than AOB. Additionally, in mainstream conditions *Nitrospira* sp., which have higher affinities for NO_2^- and DO, are more abundant than the *Nitrobacter* sp. (Juretschko et al., 1998, Schramm et al., 1998, Daims et al., 2001). This abundance of *Nitrospira* sp. implies that one way to achieve this differential is by simultaneously imposing non-limiting NO_2^- and DO concentrations to out-select *Nitrospira* sp. and non-limiting NH_4^+ concentrations to selectively enrich for AOB. Indeed, operation at a DO concentration of 1.5 mg O_2/L was shown to be successful for mainstream deammonification, which is also based on efficient NOB out-selection (Wett et al., 2012, De Clippeleir et al., 2013).

Building upon these past observations, this study proposes a novel online control strategy maintaining an ammonium residual and high DO concentration to allow for minimum aerobic SRT control. The latter is inferred to be the key in obtaining successful nitrification/denitrification under mainstream conditions. As an ammonium residual is considered essential for NOB out-selection (Knowles et al., 1965, Chandran et al., 2000), a final polishing step by anammox for residual ammonia removal is targeted. Therefore, an effluent quality with an ammonium to NO_x ratio of 1:1 is preferred and included in the online control. Maintaining ammonium to NO_x ratio of 1:1 provides optimum alkalinity conditions for higher AOB rates (Wett et al., 2003), and therefore optimum nitrification and

NO_x reduction, which results in higher N-removal for a given influent COD/N ratio and total SRT.

6.2 Material and Methods

AOB versus NOB (AvN) pilot

The pilot process described in this study was part of a larger configuration including a high rate activated sludge A-stage (HRT= 30 minutes, SRT = 0.25 days) for COD removal providing the influent for the AvN reactor (Miller et al., 2012) and a post anoxic anammox moving bed bioreactor after the AvN reactor (Figure 37) allowing for a final polishing of the treated sewage. In this study, we will focus only on the performance and operation of the AvN pilot.

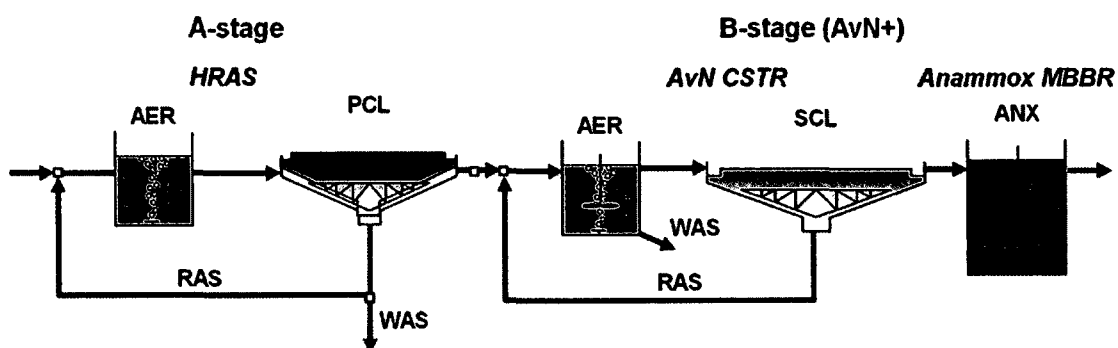


Figure 37. Process flow diagram of the A-B process pilot.

The AvN process included a single 340 L aeration tank operated as a continuously-stirred tank reactor (CSTR) followed by a clarifier. This tank was equipped with a variable speed mixer (300 rpm) in order to maintain complete-mix conditions. Return activated sludge (RAS) from the clarifier was returned to the AvN CSTR with a peristaltic pump at 100% of the influent flow rate. SRT was controlled by wasting solids from the bioreactor

with a programmable digital peristaltic pump. The AvN CSTR was equipped with sensors to monitor NO_3^- , NO_2^- (s::can Spectro::lyser, Austria), DO (Hach LDO, CO, USA), and NH_4^+ (WTW VARiON, Germany). NH_4^+ , NO_3^- , NO_2^- signals were used to control aeration (Figure 38).

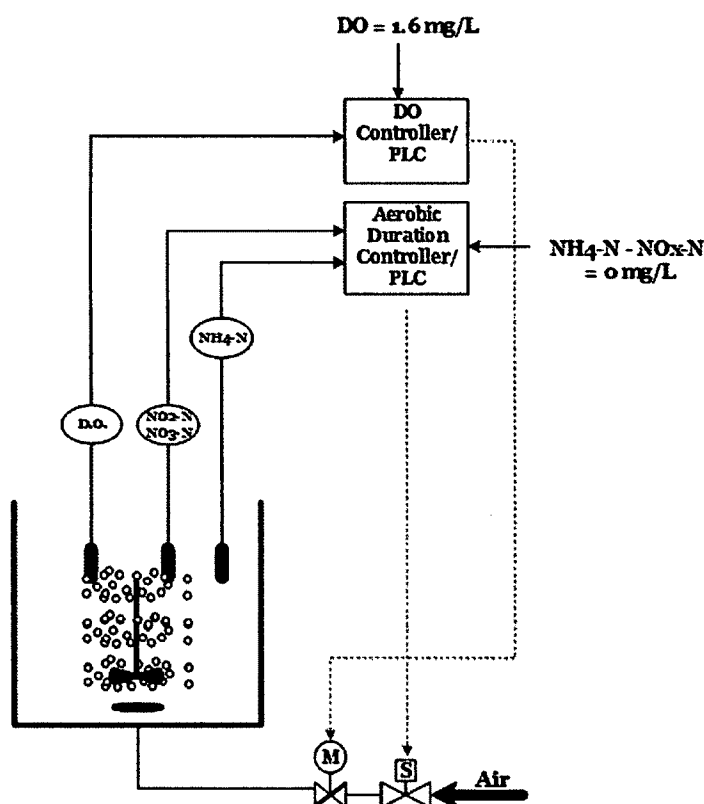


Figure 38. AvN controller depicting aerobic duration controller receiving NH_4^+ (WTW VARiON, Germany), NO_2^- and NO_3^- (s::can Spectro::lyser, Austria) signals and DO controller receiving dissolved oxygen (Hach LDO, USA) signal.

Start-up and long term operation

The AvN CSTR reactor was seeded from the parallel process pilot train, which was nitrifying/denitrifying stably at that time and fed A-stage effluent (Miller et al., 2012). Typical A-stage effluent was characterized by $\text{pH} = 7.05 \pm 0.14$, $\text{COD} = 306 \pm 87 \text{ mg/L}$,

$\text{NH}_4^+ = 29.7 \pm 3.9$ mg N/L, COD/TKN = 6.7 ± 1.4 , Ortho-P = 3 ± 1.2 mg P/L, and Alkalinity = 159.7 ± 17.1 mgCaCO₃/L (Table 25). The AvN CSTR was operated at a 3 h HRT with a flow rate of 1.9 L/min. The total SRT was targeted at around 6 days and temperature was maintained at 25°C during the entire study. The aerobic SRT was controlled by an on-line aeration controller to achieve the desired $\text{NH}_4^+:\text{NO}_2^-$ ratio. The nitrogen removal performance was evaluated by dividing the study period in five phases based on the degree of NOB out-selection that was achieved during the study. Unlike other studies, the phases were not distinct operating conditions, since the pilot was intended for an upgrade and was dynamically operated to account the seasonal variations.

Table 25. Average characteristics of AvN CSTR influent (A-stage effluent) and effluent over the entire experimental period.

Parameter	Influent	Effluent
pH	7.05 ± 0.14	6.88 ± 0.12
COD (mg/L)	306 ± 87.3	66 ± 22.5
sCOD (mg/L)	128 ± 41.9	33 ± 9.8
NH_4^+ (mgN/L)	29.7 ± 3.9	7.3 ± 4.4
TKN (mgN/L)	38.5 ± 4.6	-
COD/TKN	6.7 ± 1.4	-
Ortho-P (mgP/L)	3 ± 1.2	2.7 ± 0.7
Alkalinity (mgCaCO ₃ /L)	159.7 ± 17.1	85.3 ± 23.3

AvN ($\text{NH}_4\text{-NO}_x$) aeration control

To impose conditions favorable for NOB out-selection and to provide effluent suitable for anaerobic ammonia oxidation (AMX) polishing, an aeration controller was developed which uses online *in-situ* DO, NH_4^+ , NO_2^- and NO_3^- sensors. The first component of AvN control was the aerobic duration controller with the goal of maintaining equal effluent $\text{NH}_4^+\text{-N}$ and $\text{NO}_x\text{-N}$ ($\text{NH}_4^+\text{-N} - \text{NO}_x\text{-N} = 0$) in the AvN CSTR at all times. The latter would guarantee a treatable effluent for the final polishing step with AMX. The other

component of the AvN control was the DO controller, which maintains the DO at a desired set-point during the aerated period (Figure 2).

Under the AvN strategy, $\text{NH}_4^+\text{-N}$ was compared to the sum of $\text{NO}_2^-\text{-N}$ and $\text{NO}_3^-\text{-N}$ ($\text{NO}_x\text{-N}$). First, the cycle duration (aerobic duration + anoxic duration) had a defined minimum and maximum aerobic duration. The cycle duration was kept constant at 12 minutes during the entire experiment and minimum and maximum aeration times were set at 4 and 10 minutes, respectively. These set points were selected to avoid the $\text{NH}_4^+\text{-N}$ below 1.5 mg N/L. As the AvN controller aimed at ammonium concentrations equal to NO_x concentrations, aerobic duration was increased up to a predetermined maximum aeration time set-point, while maintaining the cycle duration constant at $\text{NH}_4^+\text{-N}$ over $\text{NO}_x\text{-N}$ ratios greater than 1. When $\text{NH}_4^+\text{-N}$ was less than $\text{NO}_x\text{-N}$, aerobic duration was decreased until it reached the minimum aeration time. When aerated, the proportional-integral-derivative (PID) controlled a mechanically operated valve (MOV) to maintain the target DO set-point of 1.6 mg $\text{O}_2\text{/L}$.

Influent/Effluent monitoring

Performance of the AvN pilot was monitored by collecting 24-hr flow-weighted composite samples from the influent and effluent. Samples were analyzed for TSS, VSS, total and soluble COD, TKN, TP, OP, $\text{NO}_3^-\text{-N}$, $\text{NO}_2^-\text{-N}$, $\text{NH}_4^+\text{-N}$ and alkalinity. All relevant analytical methods for solids and liquids are presented in Table A1 and Table A2

Microbial activity measurements

To measure maximum AOB and NOB activity, 4 L samples were collected and dispensed into 4L vessels from the AvN CSTR and aerated for 30 minutes to oxidize excess COD, and spiked with 20-30 mg/L $\text{NH}_4^+\text{-N}$ (as ammonium chloride) and 2-4 mg/L $\text{NO}_2^-\text{-N}$ (as sodium nitrite), respectively, and sampled continuously for 1 hour at 20-minute intervals. All collected samples were analyzed for $\text{NH}_4^+\text{-N}$, $\text{NO}_2^-\text{-N}$, and $\text{NO}_3^-\text{-N}$. Mixing was provided by a magnetic stir bar. The dissolved oxygen was maintained between 2.5 and 4 mg $\text{O}_2\text{/L}$. pH was maintained between 7-7.5 by adding sodium bicarbonate. The AOB

rates were calculated as the slope of the $\text{NO}_x\text{-N}$ production and NOB rates were calculated as the slope of the $\text{NO}_3^-\text{-N}$ production.

Half-Saturation Coefficient Evaluation

Batch experiments were conducted in the AvN CSTR itself by temporarily stopping feed flow and maintaining a constant DO at concentrations of 0.1, 0.3, 0.6, 1.2, 2 mg $\text{O}_2\text{/L}$ and mixing continuously. A PID controller was used to maintain the desired DO set-point. The DO concentrations were recorded and logged during each test. An identical protocol to the microbial activity measurements described in the previous section was followed to calculate AOB and NOB rates. The recorded results from these tests were analyzed and fitted to a modeled Monod curve based on the Monod kinetic expression and a spreadsheet (Excel, Microsoft Inc.) to calculate half saturation coefficients using five different DO concentrations. The entire experiment was performed 3 times, with the results averaged and plotted. These experiments were not conducted during a period of NOB out-selection.

Molecular methods for microbial quantification

DNA and RNA extraction was conducted using the DNeasy and RNeasy mini kits (Qiagen, CA). Resulting DNA and RNA concentrations and quality were initially checked by UV spectrophotometry (Varian, CA). The abundance of AOB and NOB was quantified via SYBR® Green chemistry quantitative polymerase chain reaction (qPCR) assays, NH_4^+ monooxygenase subunit A (*amoA*) gene (Rotthauwe et al., 1997), *Nitrobacter* 16S rRNA gene (Graham et al., 2007) and *Nitrospira* 16S rRNA gene (Kindaichi et al., 2007), respectively. Total bacterial abundance was quantified using eubacterial 16S rRNA gene targeted primers (Ferris et al., 1996). qPCR assays were conducted on a iQ5 real-time PCR thermal cycler (BioRad Laboratories, Hercules, CA). Standard curves for qPCR were generated via serial decimal dilutions of plasmid DNA containing specific target gene inserts. qPCR for standard plasmid DNA and sample DNA were conducted with duplication and triplication, respectively. DNA grade deionized distilled water (Fisher Scientific, MA) was used for non-template control.

Primer specificity and the absence of primer-dimers were confirmed via melt curve analysis of each and every qPCR profile.

Statistical analysis

Statistical comparison between variables was performed using the t-test (for a normally distributed data set) and Mann-Whitney rank sum test (for not a normally distributed data set) on Sigma Plot (Systat Software, San Jose, CA). Shapiro-Wilk test was used to determine the normality of the data set. A *p*-value of 0.05 or lower indicates that variables being compared are statistically different at the 95% confidence level.

6.3 Results

AvN CSTR performance

The key characteristics of the AvN CSTR effluent are summarized in Table 25. The trends of influent $\text{NH}_4^+\text{-N}$ and effluent $\text{NH}_4^+\text{-N}$ and $\text{NO}_x\text{-N}$ are presented in Figure 3a, which also demonstrates the effectiveness of AvN control in maintaining equal $\text{NH}_4^+\text{-N}$ and $\text{NO}_x\text{-N}$ in the effluent. The $\text{NH}_4^+\text{-N}$ loading rate and COD removal rate can be compared with TIN removal rate during the entire study in Figure 39b. The trend of the nitrite accumulation ratio [$\text{NAR} = \text{NO}_2^-\text{-N} / (\text{NO}_2^-\text{-N} + \text{NO}_3^-\text{-N})$], which is an indicator of the extent of NOB out-selection, and the aerobic fraction (aerobic time: cycle time), is presented in Figure 39c. It can be seen that the aerobic fraction follows the trends of the NAR during Phase III-IV, when aeration controller was able to achieve equal effluent $\text{NH}_4^+\text{-N}$ and effluent $\text{NO}_x\text{-N}$ concentrations in the effluent (Figure 39a). The total SRT and aerobic fraction presented in Figure 39, gives a measure of the aerobic SRT of the AvN CSTR during the study. Since, the AvN aeration controller and nutrient sensors were still being fine-tuned, the ratio of effluent $\text{NH}_4^+\text{-N}$ and $\text{NO}_x\text{-N}$ remained variable in Phase I (Figure 39a). In Figure 40, a 24-hr profile of $\text{NH}_4^+\text{-N}$, $\text{NO}_3^-\text{-N}$, $\text{NO}_2^-\text{-N}$, $\text{NO}_x\text{-N}$ and DO, as controlled by the AvN strategy, is presented. The functioning of the AvN control can be seen in this figure.

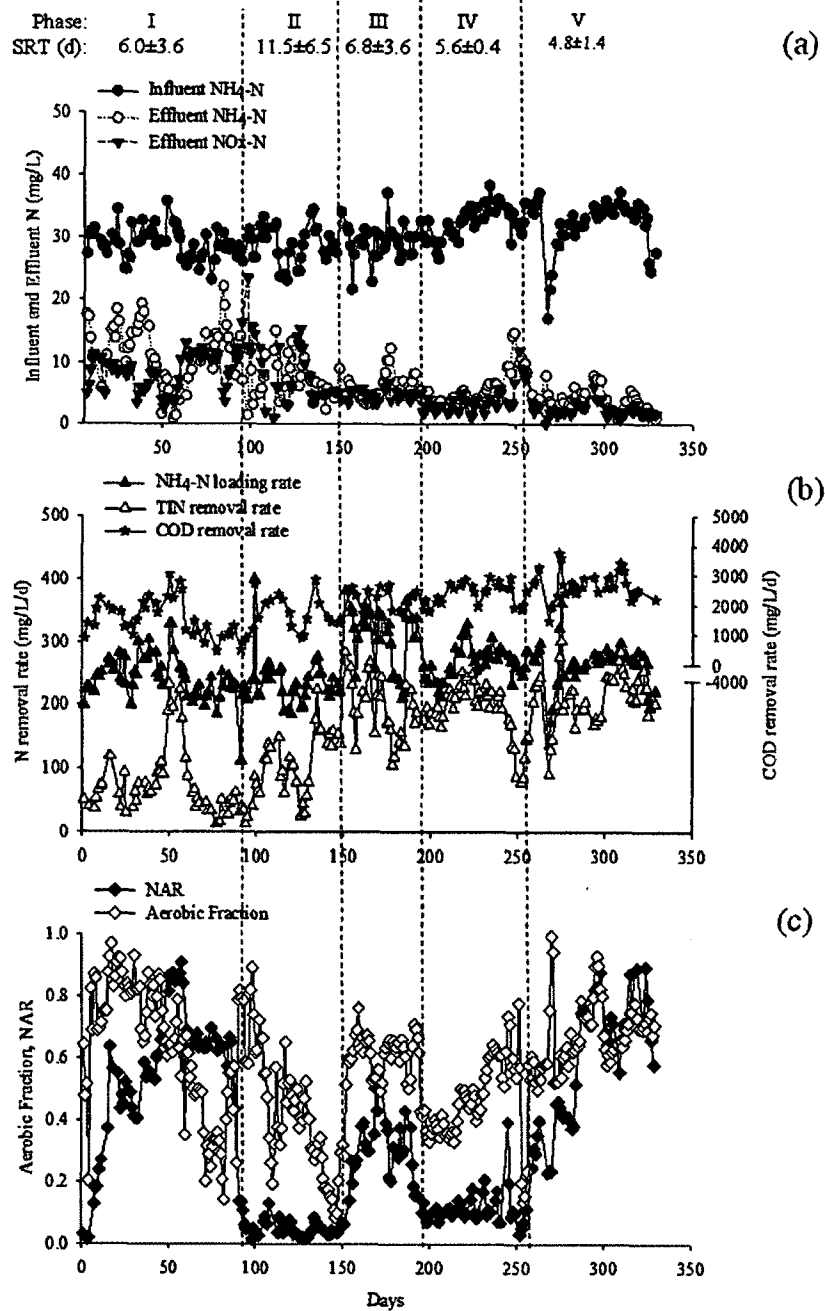


Figure 39. AvN CSTR a) influent $\text{NH}_4^+\text{-N}$, effluent $\text{NH}_4^+\text{-N}$ and $\text{NO}_x\text{-N}$ b) Influent $\text{NH}_4^+\text{-N}$ loading, COD removal rate and TIN removal rate c) NAR and Aerobic Fraction.

During Phase I, the TIN removal rate, the efficiency and the ratio of TIN removal rate: COD removal rate was the lowest among all phases (Figure 41). In general, the ratio of TIN/COD removal rate is an indicator of the efficiency of the TIN removal in terms of influent COD/N. The very low TIN/COD removal rate (0.05 ± 0.021) and TIN removal efficiency ($30 \pm 18\%$) during Phase I suggests more aerobic oxidation of COD was occurring than anoxic oxidation of COD using NO_x as the electron acceptor (Figure 41). This is in line with the fact that the aerobic SRT fraction during Phase I was 0.65 ± 0.21 , while the total SRT was 6 ± 3.6 d (Figure 39). Further, the TIN removal rate was lower during Phase I compared to other phases in relation to the COD removal rates, as seen in Figure 41.

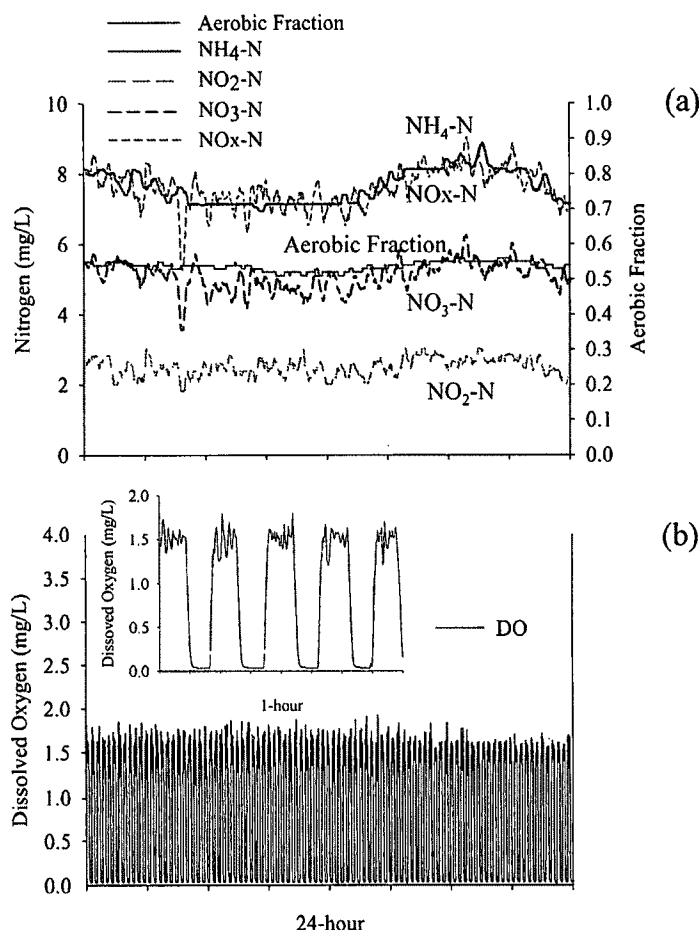


Figure 40. AvN controller performance a) 24-hour (12 AM to 11:59 PM) trends of reactor $\text{NH}_4^+\text{-N}$, $\text{NO}_2^-\text{-N}$, $\text{NO}_3^-\text{-N}$ and aerobic fraction (ratio of aerobic time: total cycle time) b) 24-hour DO profile and an insert showing DO profile for 1 hour. The aerobic fraction was allowed to fluctuate between 0.33 to 0.83.

From Figure 39b, Figure 41 and Figure 43c, the following can be observed. In Phase II, there was an overall improvement in the TIN removal rate ($p=0.002$), and efficiency ($p=0.018$), however the NAR was lower ($p<0.001$) and the influent COD/ $\text{NH}_4^+\text{-N}$ was not statistically different ($p=0.55$). The ratio of TIN removal rate: COD removal rate in both phases were not statistically different ($p=0.075$). In Phase III, the TIN removal rate ($p=0.001$), efficiency ($p=0.004$) and ratio of TIN removal rate: COD removal rate were

higher than Phase II ($p=0.003$) for the similar influent COD/NH₄⁺-N ($p=0.99$). In fact, the ratio of TIN removal rate: COD removal rate in Phase III was similar to Phase IV ($p=0.25$) and the TIN removal efficiency was slightly higher in Phase IV compared to Phase III ($p=0.001$) for a higher influent COD/ NH₄⁺-N ($p=0.002$). The increased NAR during Phase III (0.3 ± 0.11) compared to Phase II (0.05 ± 0.025) and Phase IV (0.11 ± 0.06) could explain the improvement of the TIN removal rate for the influent COD/NH₄⁺-N that was less than or equal to and highlights the importance of NOB out-selection. During Phase V, the influent COD/NH₄⁺-N (12.3 ± 0.95), NAR (0.6 ± 0.22) and TIN removal rate (210 ± 43 mgN/L/d) and efficiency ($89\pm11\%$) were highest among all phases. However, the ratio of the TIN removal rate: COD removal rate was similar to Phase III ($p=0.23$). In intermittently aerated systems, COD that is not used for NO_x reduction is oxidized aerobically; therefore maintaining influent COD/NH₄⁺-N at an optimum level is important. The ratio of NH₄⁺-N and NO_x-N was maintained around 1 mg N/L as intended by the AvN controller during Phases II, III, IV, and V of the study (Figure 39a).

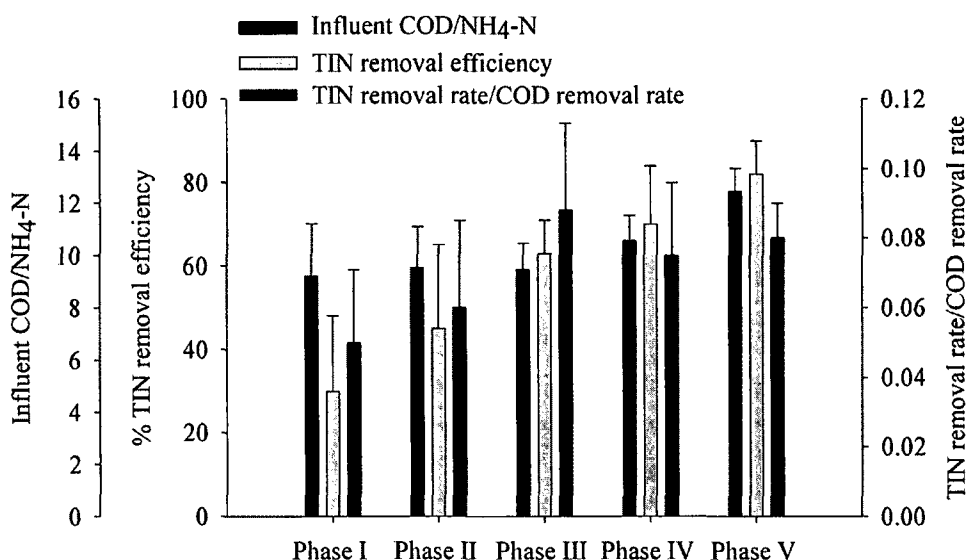


Figure 41. Different phases of the study showing variability and relationship between A) Influent COD/NH₄⁺-N, TIN removal efficiency and TIN removal rate/COD removal rate. Error bars represent standard deviation.

NOB out-selection in AvN CSTR

In this study, NOB out-selection was inferred through *ex-situ* AOB and NOB activity measurements, NAR, and targeted molecular analysis for bacterial populations. The AOB activity was greater than NOB activity (AOB: 391 ± 124 mgN/L/d, NOB: 233 ± 151 mgN/L/d, $p < 0.001$) during the entire study. Further, the results of targeted molecular analysis for AOB, NOB (*Nitrobacter sp.* and *Nitrospira sp.*) and total bacterial population clearly showed that the NOB population declined during the period of low NOB activity (Figure 42), which supports the NOB out-selection observed.

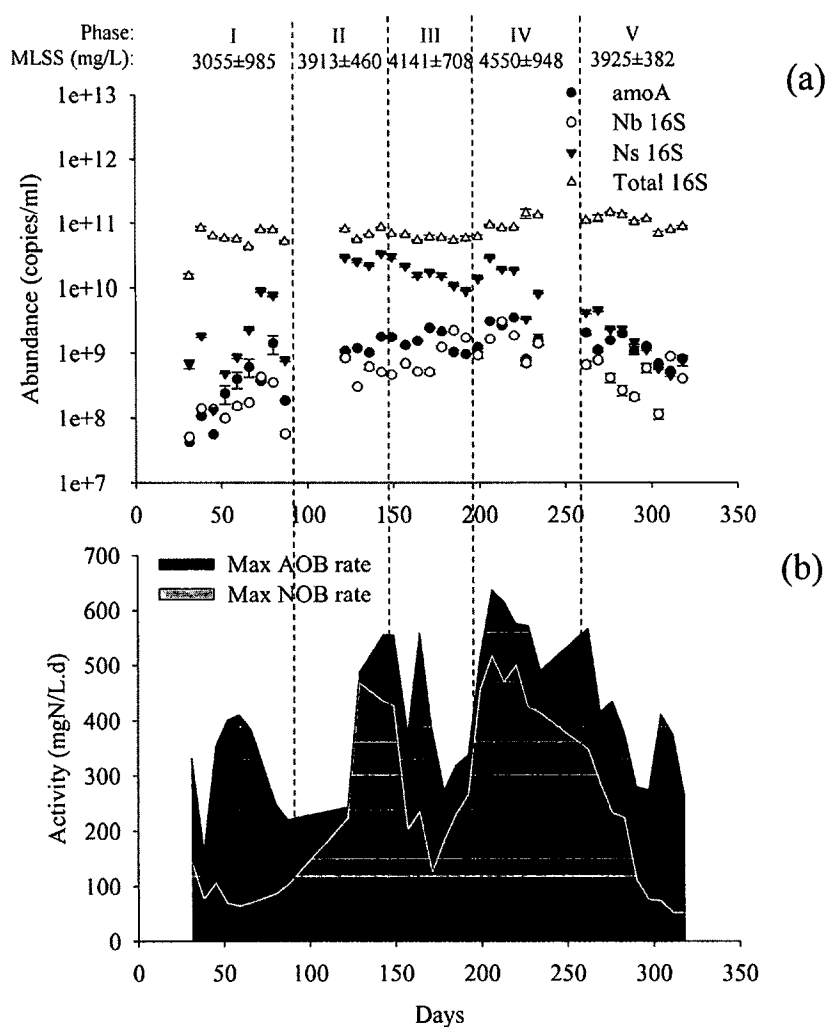


Figure 42. Trends of microbial populations (AOB, NOB and total bacteria) presented as copies of DNA per mL of sample from targeted qPCR (a) and weekly maximum AOB and NOB activities (b).

The dominant NOB were *Nitrospira sp.* which were 20 times more prevalent than *Nitrobacter sp.* The correlation of amoA abundance with AOB activity and *Nitrospira sp.* abundance with NOB activity can be seen in Figure 43a and Figure 43b.

NOB out-selection inferred from NAR and AOB/NOB activities was variable during the study (Figure 42, Figure 43). Therefore, variability in NOB out-selection warranted further investigation during the study. It was surmised that aggressive operation towards limiting the SRT for AOB is a key factor for washing out NOB. Under the AvN strategy, if the AOB are pushed towards washout, the aerobic fraction increases for the same influent COD/N. The trends of aerobic fraction and NAR in Figure 39c demonstrate that aggressive operation (combination of SRT and nitrogen loading rate) resulted in a higher aerobic fraction and NAR. Further, the ratio of nitrogen loading rate and maximum AOB rate (NLR/Max AOB rate) were analyzed for different phases of the study to assess variable NOB out-selection. This ratio captures how aggressively the system is operated towards AOB washout and is the result of variation in the systems SRT and NLR. In Phase I, it was observed that the NLR/Max AOB rate was greater than 1 and the NOB out-selection characterized by NAR was greater than 0.5 (Figure 43c). In Phase II and Phase IV NLR/Max AOB rate was around 0.7 and the NAR remained below 0.12 (Figure 43c). In Phase III and Phase V, the NLR/Max AOB rate was close to 1 which coincided with higher NAR (>0.3) and better NOB out-selection (Figure 42, Figure 43c).

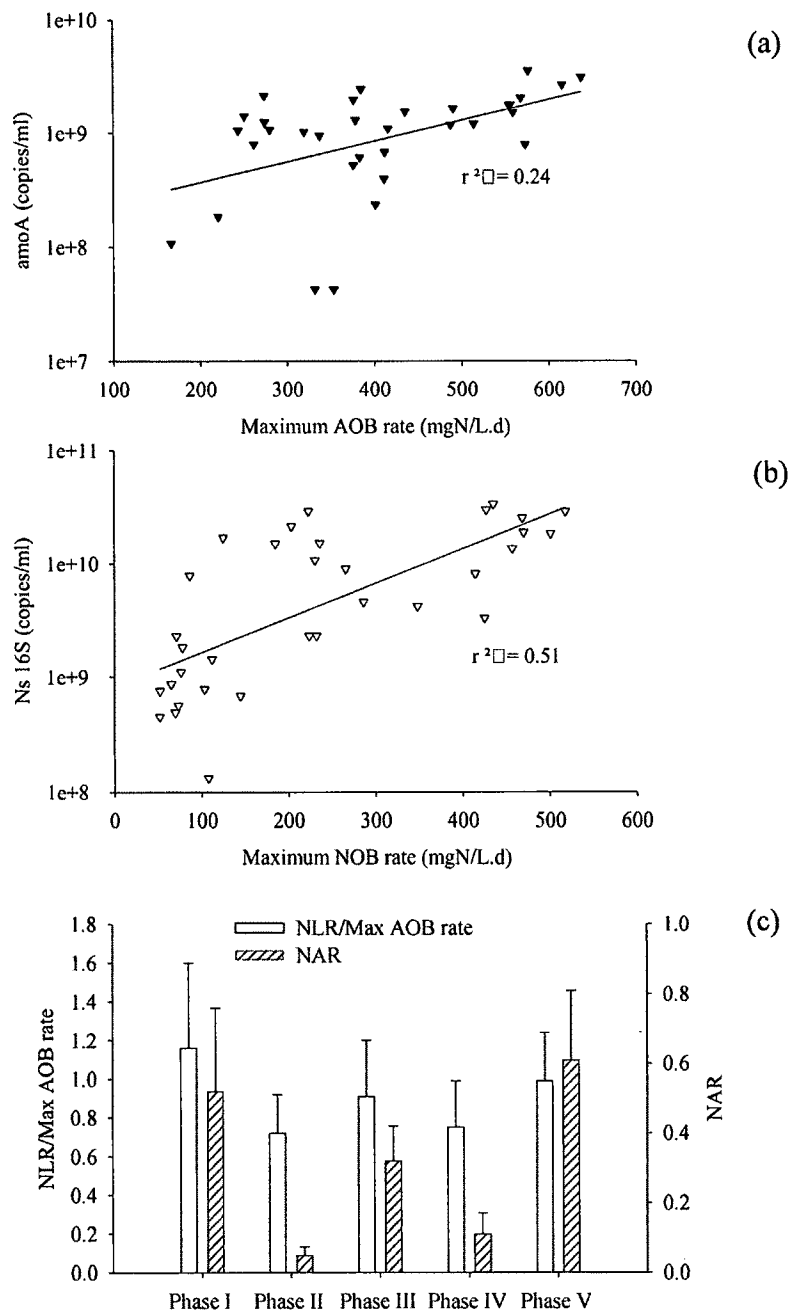


Figure 43. Correlation between a) *amoA* abundance and maximum AOB rates (weekly averages), b) *Nitrospira* sp. abundance and maximum NOB rates (weekly averages). c) Different phases of the study showing variability and relationship between NLR/Max AOB rate ratio and NAR. Error bars represent standard deviation.

Settling Performance

The settling characteristics of the AvN CSTR showed a sludge volume index (SVI) of 150 ± 38 mL/g. Moreover, there was seasonal variation in settling characteristics; during summer months settling was poor compared to other seasons (Figure 44). The weekly analysis of filaments showed *Thiothrix* was the dominant filaments during periods of poor settling (data not shown). *Thiothrix* related bulking was observed in the A-stage (Miller et al., 2012), which was believed to stem from high sulfide and organic acids in the influent raw wastewater during high temperature periods. The potential carryover of sulfide and *Thiothrix* as well as production of sulfide and organic acids in the over-sized A-stage clarifier might explain poor settling due to *Thiothrix*. High NO_2^- levels did not adversely affect the settling performance (Figure 44).

Nitrite accumulation has been linked with poor sludge settleability (Blackburne et al., 2008, Ma et al., 2009) although the cause of filamentous bulking due to nitrite is not understood clearly (Guo et al., 2013). Nitric Oxide (NO) which is an intermediate of denitrification and NO_2^- a precursor was hypothesized to inhibit the floc-formers over filamentous organisms by Casey et al. (1994). The validity of this hypothesis was questioned by Martins et al. (2004). Despite poor overall settling, NO_2^- accumulation did not negatively impact settling in this study. In fact, periods of good settling were marked by higher amounts of effluent NO_2^- (Figure 44). Further, bulking was not persistent throughout the study which suggests that operational parameters were not responsible for poor settling.

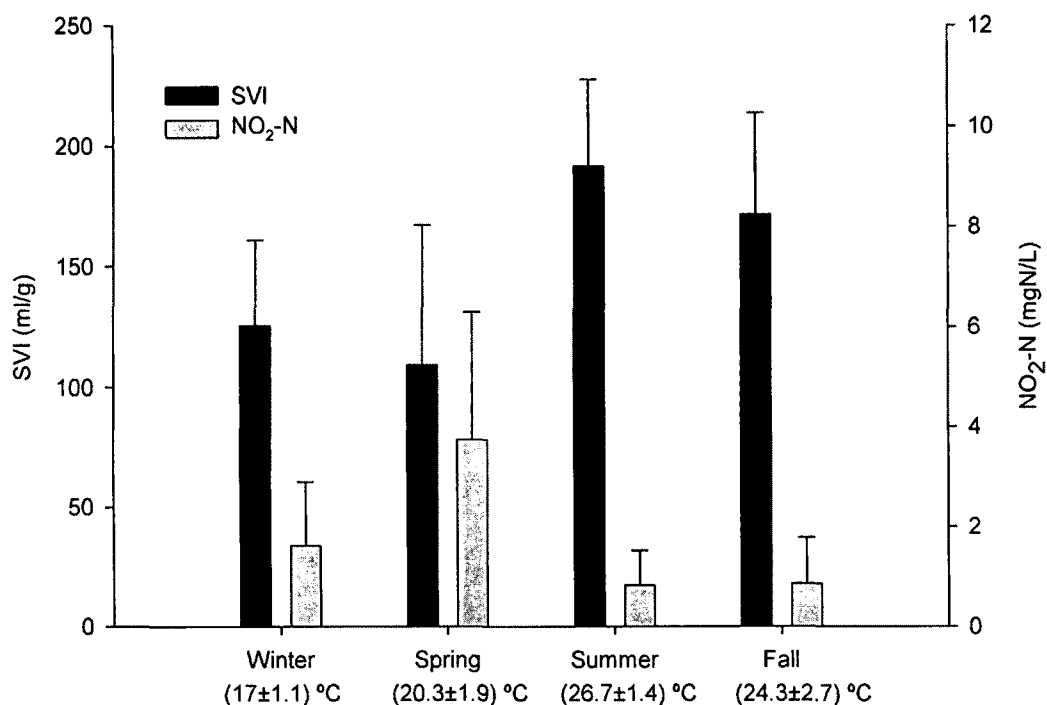


Figure 44. Seasonal variation in SVI, nitrite levels and temperature during the entire study.

6.4 Discussion

AvN nitrogen removal performance

The TIN removal rate 0.15 kg/m³/d observed in our study (influent at COD/N ~6.7 at 25°C) was comparable to short-cut nitrogen removal rates that were reported in the full-scale plants at the Strass wastewater treatment plant (WWTP) (Total N removal rate ~0.5-1.1 kg/m³/d, influent COD/N~ 15 at 9-19°C) and in the Changi water reclamation plant (WRP) (Total N removal rate ~0.13 kg/m³/d, influent COD/N ~7.5 at 28-32°C). In the Strass WWTP, AOB and AMX were bioaugmented from a sidestream deammonification reactor (Wett et al., 2012), while in the Changi WRP, higher N removal rates have been suspected to result from a very short aerobic SRT of 2.5 days in

combination with anaerobic ammonia oxidation by free cell AMX (Cao et al., 2013). A summary of these comparisons is provided in Table 26. During anaerobic batch testing (data not shown) of AvN CSTR samples, AMX activity was not detected. Therefore, AMX were not expected to contribute significantly to N-turnover.

Table 26. Comparison of performance and strategies used by recent studies to achieve NOB out-selection in mainstream conditions.

	CSTR	Strass WWTP	Changi WRP	SBR	RBC
Reference	This Study	Wett et al., 2012	Cao et al., 2013	Gao et al., 2013	De Clippeleir et al., 2013
COD/N	6.7	15	7.5	2.5	2
Temperature (° C)	25	9-19	28-32	12-27	15
N loading rate (kg/m ³ ·d)	0.25	0.55-1.6	0.17	N.R.	1.4
Total N removal rate (kg/m ³ ·d)	0.15	0.5-1.1	0.13	N.R.	0.5
Nitrite accumulation (mgN/L)	1.85	1-3	1.1	5-25	7
Ammonia residual (mgN/L)	7.3	2-3	1.7	5-25	1-4
Aeration pattern	cyclical (in time)	(in cyclical space)	(in cyclical (in space)	cyclical (in time)	cyclical (in space)
Frequency of aeration (min)	6-12	5	-	50-200	1
DO set-point during aeration	1.6	1.7	1.4-1.8	2-7	8
Total SRT (days)	6.5	10	5	30-40	N.C.
Aerobic SRT (days)	3.2	7	2.5	-	N.C.

N.C.: Not controlled. N/A: Not applicable. SBR: Sequencing batch reactor. N.R.: Not reported. CSTR: Continuously stirred-tank reactor.

RBC: Rotating biological contactor. SBR: Sequencing batch reactor.

Kinetic out-selection of NOB over AOB

Oxygen half saturation coefficients for AOB and NOB were evaluated, as no real consensus in the literature exists for mainstream conditions. The Monod curves for both groups are given in Figure 45, showing a half saturation coefficient of 0.16 and 1.14 mg O₂/L for NOB and AOB, respectively. It was therefore confirmed that the strategy of operating at a DO >1.5 mg O₂/L would help to increase the AOB/NOB activity differential under aggressive SRT operation.

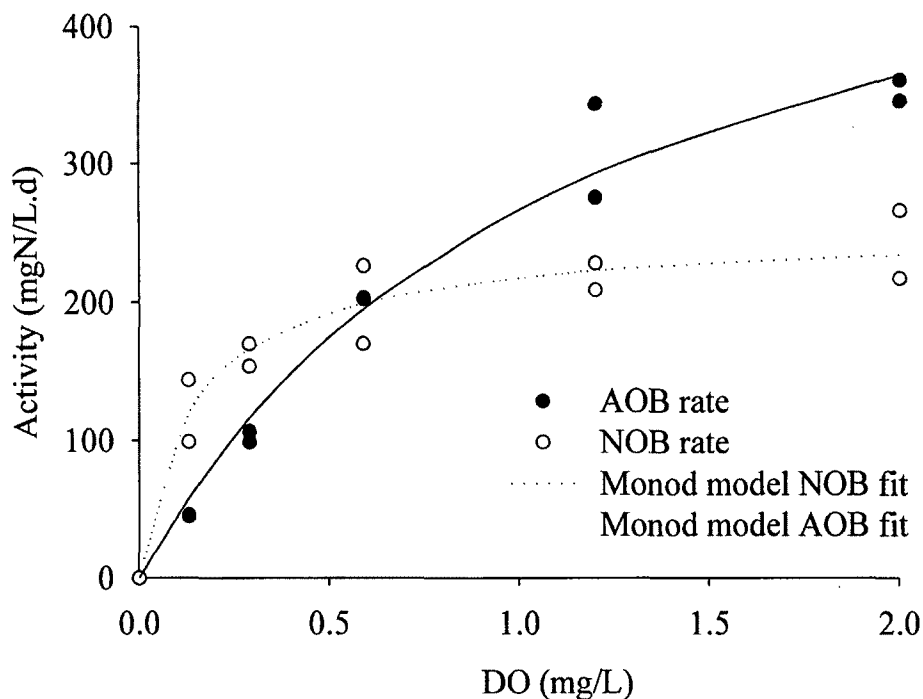


Figure 45. Dissolved oxygen Monod curves for AOB (model: $K_o = 1.16$ mg O₂/L, $r_{max} = 576.3$ mgN/L/d) and NOB (model: $K_o = 0.16$ mg O₂/L, $r_{max} = 254.6$ mgN/L/d) showing that NOB are well adapted at low DO compared to AOB.

Under the AvN strategy, the AvN CSTR was operated transiently at a DO equal to or greater than 1.5 mg O₂/L. Although the hypothesis that low DO operation favors AOB over NOB is very widespread (Sin et al., 2008), this study confirmed other research results pointing in the opposite direction (Daebel et al., 2007, Manser et al., 2005) for systems like this study, which were selectively enriched with *Nitrospira* sp. rather than *Nitrobacter* sp. (Figure 6). *Nitrospira* are known for successful adaptation in most nitrifying ecosystems and hypoxic environmental niches (Lücker et al., 2010). Additionally, *Nitrospira* sp. has been reported to lack common protection mechanisms against oxidative stress which might be attributed to the hypothesis from Lücker that *Nitrospira* sp. evolved from an anaerobic or microaerophilic origin. The earlier reports of higher oxygen affinity of AOB compared to NOB might have considered *Nitrobacter* sp., which function as *r*-strategists (higher specific growth rates and low substrate affinity), as opposed to *Nitrospira* sp., which function as *K*-strategists (lower specific growth rates and higher substrate affinity). Therefore, our strategy of intentionally operating at a high DO concentration (≥ 1.5 mg O₂/L) to provide competitive advantage for AOB over NOB (especially *Nitrospira* sp.) would be justified against other reports in the literature that might have overlooked *Nitrospira* sp. completely. Table 26 shows other studies where high DO was used to facilitate NOB out-selection in mainstream treatment processes.

The use of transient anoxia has been a common approach to achieve NOB out-selection (Li et al., 2012, Ling, 2009, Pollice et al., 2002, Rosenwinkel et al., 2005, Zekker et al., 2012). Transient anoxia was a common approach to induce NOB out-selection in mainstream conditions in the studies presented in Table 26. Transient anoxia allows for a measured approach to control the aerobic SRT, as well as to introduce a lag-time for NOB to transition from the anoxic to aerobic environment, either due to nitrite limitation (Knowles et al., 1966, Chandran et al., 2000) or by an enzymatic lag (Kornaros et al., 2010). Kornaros showed a delay in NOB recovery and NOB lag adaptation in aerobic conditions following transient anoxia lasting 1.5 hr to 12 hr (the delay in recovery was shown to be a function of the length of anoxic disturbance), thus confirming the observations of the usefulness of transient anoxia by many others (Alleman et al., 1980, Katsogiannis et al., 2003, Sedlak, 1991, Sliverstein et al., 1983, Yang et al., 2011, Yoo et al., 1999). However, the low nitrite in the beginning of the aerobic phase was not

discussed as a factor for the lag in NOB activity. Although transient anoxia has been used successfully in high strength wastes (Wett, 2007) and the ability to use it in low strength wastes has been suggested (Peng et al., 2004), the control features associated with transient anoxia remains a challenge for NOB out-selection.

The influent COD in the AvN CSTR provided conditions for NO_2^- to be consumed by heterotrophs, while no NH_4^+ oxidation takes place during the anoxic phase (data not shown). By consuming NO_2^- in anoxic conditions, heterotrophs restrict NO_2^- availability for NOB in the aerobic phase. Further, over many cycles this can potentially limit NOB population as a result of lower substrate utilization by NOB compared to substrate utilization by AOB. Lemaire et al. (2008) attributed this positive feedback as one of the primary mechanisms for NOB out-selection in aeration duration controlled SBR treating abattoir wastewater. The AvN aeration controller used in the AvN CSTR successfully allowed maintenance of residual NH_4^+ ($7.3 \pm 4.4 \text{ mgNH}_3\text{-N/L}$) throughout the study, allowing the AOB growth rate to be close to the maximum. Free ammonia (FA) concentration levels in the AvN CSTR were too low to cause NOB inhibition since FA was $0.0314 \pm 0.0189 \text{ mgNH}_3\text{-N/L}$ compared to $0.1 - 0.8 \text{ mgNH}_3\text{-N/L}$ that is considered inhibitory (Anthonisen et al., 1976). Similar trends have also been observed in the mainstream deammonification testing at the Strass WWTP, which showed higher NOB out-selection (indicated by less NO_3^- production) during late December where effluent NH_4^+ levels were high (NH_4^+ set-point = 2.5 mgN/L compared to normal NH_4^+ set-point = 1.5 mgN/L) at significantly higher loadings and low temperatures, therefore lowering the SRT to its minimum (Wett et al., 2012). Alternating aerobic and anoxic conditions and maintaining residual NH_4^+ has proven effective for NOB out-selection in recent studies in mainstream conditions (Table 26).

Metabolic out-selection of NOB over AOB

The AvN CSTR was operated at a relatively low total SRT (6.5 ± 4.3 days) during the study period. The intent of limiting the SRT of the system was to operate very close to the AOB washout SRT such that NOB were out-selected. It is very important to recognize that heterotrophic denitrification pressure, high DO, and intermittent aeration

provides unfavorable conditions for NOB, without adversely affecting the AOB population. However, it was surmised that it was the ability of the system to be operated at aggressive SRTs would out-select NOB over AOB.

The use of AvN strategy allowed control of the aerobic SRT of the system such that NH_4^+ oxidation was always maintained at the optimum level for a given influent COD/TKN and SRT, thus allowing the system to be run at the minimum SRT which eliminates NOB from the system. We clearly showed that when the system was operated aggressively at low SRTs that corresponded to NLR/Max AOB rate ~ 1 , NOB out-selection was more rampant. The use of online aeration controllers and intentional SRT control towards critical AOB washout demonstrated in this study was a novel approach to out-select NOB in mainstream conditions.

6.5 Conclusions

In this study, it was demonstrated that mainstream NOB out-selection in a continuous process is possible without using known factors that aid NOB out-selection in sidestreams with high strength ammonia. A novel aeration control strategy based on direct *in-situ* measurement of NH_4^+ , NO_3^- , and NO_2^- was demonstrated to be capable of facilitating the proposed strategies and exploiting NOB out-selection mechanisms. In this study, higher DO affinity of NOB versus AOB was demonstrated and a high DO (>1.5 mg/L) was used to out-select NOB along with intermittent aeration and low SRT. Therefore, this study presents a new paradigm in biological nitrogen removal that utilizes advanced process control strategies. As we move closer to mainstream nitrite-shunt based processes, the findings of this study are expected to help existing biological nitrogen removal (BNR) plants be optimized for cost-effective and efficient nitrogen removal.

CHAPTER 7

NITROGEN POLISHING IN A FULLY ANOXIC ANAMMOX MBBR TREATING MAINSTREAM NITRITATION-DENITRITATION EFFLUENT

Note: The contents of this chapter have been submitted to the Journal of Applied and Environmental Microbiology. Regmi, P., Holgate, B., Miller, M.W., Park, H., Chandran, K., Wett, B., Murthy, S., Bott, C.B., Nitrogen polishing in a fully anoxic tertiary anammox MBBR treating mainstream nitrification-denitrification effluent.

7.1 Introduction

Wastewater treatment plants (WWTPs) are often required to meet stringent nitrogen limits to prevent eutrophication of receiving water bodies. Most commonly, nitrogen removal is achieved by biological nitrification-denitrification (Grady et al., 1999). Due to the high cost of biological nitrification-denitrification, alternatives are being explored. Anaerobic ammonia oxidizing (anammox) bacteria, capable of NH_4^+ oxidation with NO_2^- as the electron acceptor (Strous et al., 1998), has been successfully implemented as a cost effective alternative to treat ammonia rich wastewater at mesophilic temperatures (Abma et al., 2010, Sliekers et al., 2003, van der Star et al., 2007, Wett, 2007). Deammonification, which relies on NH_4^+ oxidizing bacteria (AOB) to partially convert NH_4^+ to NO_2^- and anammox bacteria (AMX) to convert the remaining NH_4^+ and NO_2^- to N_2 , has emerged as an innovative and efficient alternative to treat high strength NH_4^+ wastewater streams such as recycle streams from the dewatering of anaerobically digested sludge. Following the success of sidestream deammonification there is a great interest in leveraging the know-how to the mainstream application.

Since the dilute and cold conditions of mainstream are not well-suited for the suppression of nitrite oxidizing bacteria (NOB), short-cut nitrogen removal, in particular deammonification, is still a challenge at full-scale implementation. Due to lower growth rates and activities associated with AMX, nitrogen removal rates decrease substantially at lower temperatures (Isaka et al., 2008; Vazquez-Padin et al., 2011). In lab-scale reactors, anammox processes have been successfully operated at temperatures $\leq 20^\circ\text{C}$ for low

chemical oxidation demand: nitrogen ratio (COD/N) waste streams (Cema et al., 2007, Dosta et al., 2008, Isaka et al., 2008, Vazquez-Padin et al., 2011). AMX processes have been demonstrated at 20°C (Hendrickx et al., 2012) and a stable long-term operation of a nitrification-anammox was shown with low influent NH_4^+ concentrations in lab- scale sequencing batch reactors (SBR) (Hu et al., 2013) and rotating biological contactors (RBC) (De Clippeleir et al., 2013) at 12 °C and 15 °C, respectively. At full-scale, long-term stability of anammox processes treating high COD/N waste streams at lower temperatures has yet to be demonstrated.

To meet stringent nitrogen removal requirements, tertiary nitrogen polishing after the secondary biological nitrogen removal (BNR) step may be required. Nitrogen polishing is achieved by employing treatment processes involving denitrification of residual nitrate from the secondary BNR process, and external electron donors are supplied. Tertiary denitrification technologies such as biologically active filters (BAF), moving bed biofilm reactor (MBBR), and fluidized bed biofilm reactors (FBBR) have been used successfully to achieve nitrogen polishing with added organic carbon usually in the form of methanol (WEF, 2009). Recent studies show that maintaining NH_4^+ residual is important for achieving nitrification-denitrification in mainstream conditions (Regmi et al., 2014). Therefore, removing residual NH_4^+ to meet permits becomes important in these processes. Also, residual NO_2^- removal becomes necessary to avoid high chlorine demand during secondary effluent disinfection and to avoid effluent toxicity. To treat the effluent of nitrification-denitrification systems that typically contain residual NH_4^+ and NO_2^- , AMX nitrogen polishing can be considered.

In this study, it is shown for the first time the feasibility of using anammox nitrogen polishing in an MBBR receiving effluent from a pilot-scale mainstream nitrification-denitrification system.

7.2 Material and Methods

The pilot

Setup

The pilot process described in this study was part of a larger configuration including an adsorption-style high rate activated sludge A-stage (HRT= 30 minutes, SRT = 0.25 days) for COD removal providing the influent for the AOB versus NOB (AvN) CSTR (Miller et al., 2012, Regmi et al., 2014) and a post anoxic anammox moving bed bioreactor (MBBR) after the AvN reactor (Figure 46) allowing for final polishing of the treated sewage. The AvN CSTR was operated at a 3 h HRT at a flow rate of 1.9 L/min. The total SRT was targeted to be around 6 days and temperature was maintained at 25 °C during the entire study. The aerobic SRT was controlled by an online aeration controller to achieve the desired $\text{NH}_4^+\text{-N}$: $\text{NO}_2^-\text{-N}$ ratio (Regmi et al., 2014). In this study, on the performance and operation of the post N-polishing MBBR is being focused on. The entire pilot process flow layout can be seen in Figure 37.

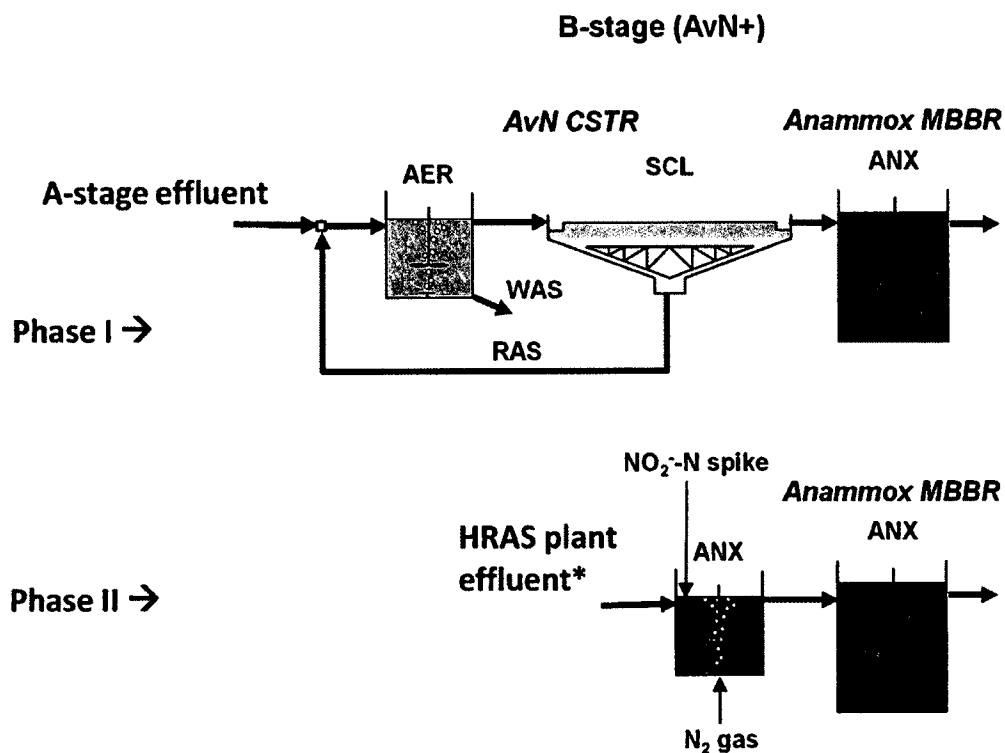


Figure 46. Pilot study process flow diagram during Phase I and II. *High-rate non-nitrifying activated sludge plant effluent.

The anammox MBBR had a volume of 0.45 m^3 where 50% of the volume was filled with K3 biofilm carriers (AnoxKaldnes, Sweden). The effective surface area of the carriers was $500 \text{ m}^2/\text{m}^3$. Mechanical mixing of carriers was achieved by a variable speed mixer ($G = 14 \text{ s}^{-1}$). The pH was recorded continuously by an online pH probe and the reactor was covered with Styrofoam to avoid oxygen transfer from the atmosphere. During startup, the anammox MBBR was operated with a temporary clarifier to recycle sludge back to the MBBR (Startup: 0-78 d). The anammox MBBR received the effluent of the AVN CSTR (Startup: 0-78 d, Phase I: 79-253 d) and non-nitrifying high rate activated sludge (HRAS) plant final effluent (FNE) spiked with NO_2^- -N (Phase II: 286-385 d). The two phases of operation can be seen in Figure 46.

Startup

The anammox MBBR was seeded with 10 L sidestream deammonification granular sludge (cyclone underflow) from the Strass WWTP, Austria. The influent feed was AvN CSTR effluent characterized by $\text{pH} = 6.8 \pm 0.1$, $\text{COD} = 45.0 \pm 11.8 \text{ mg/L}$, $\text{NH}_4^+ = 5.5 \pm 2.2 \text{ mgN/L}$, $\text{NO}_2^- = 0.7 \pm 0.6 \text{ mgN/L}$, $\text{NO}_3^- = 2.9 \pm 1.1 \text{ mgN/L}$, $\text{Ortho-P} = 3.0 \pm 1.2 \text{ mgP/L}$, and $\text{Alkalinity} = 69.3 \pm 9.1 \text{ mgCaCO}_3/\text{L}$. The anammox MBBR was operated at a 4 h HRT with the flow rate of 1.9 L/min.

Influent/Effluent monitoring

Performance of the AvN pilot was monitored by collecting 24-hr flow-weighted composite samples from the influent and effluent. Samples were analyzed for TSS, VSS, total and soluble COD, TKN, TP, OP, NO_3^- -N, NO_2^- -N, NH_4^+ -N and alkalinity. All relevant analytical methods for solids and liquids are presented in Table A1 and Table A2.

AMX activity tests

To measure maximum AMX activity, the anammox MBBR was isolated from the system. A sample was taken for sCOD, NH_4^+ -N, NO_2^- -N, and NO_3^- -N analysis. The MBBR was spiked with 8 mg/L NH_4^+ -N (ammonium chloride) and 10 mg/L NO_2^- -N (sodium nitrite) and sampled continuously at 20-minute intervals until the NO_2^- -N was less than 1.5 mg/L NO_2^- -N. On the last sample of the activity measurement, a sCOD sample was taken along with a sample for NH_4^+ -N, NO_2^- -N, and NO_3^- -N analysis. The dissolved oxygen (DO) was maintained close to 0 mg O_2/L and was recorded at every 20 minute intervals along with the pH. NH_4^+ -N and NO_2^- -N uptake rates were calculated as the slope of the NH_4^+ -N and NO_2^- -N values taken during the activity test. NO_3^- -N production rates were calculated as the slope of the NO_3^- -N values. The results of the tests are presented in terms of the unit of $\text{gN}/\text{m}^2/\text{d}$. AMX activity tests were performed weekly from day 136 to 385.

Biomass density

Biomass density measurements were performed according to the method described by Regmi et al. (2011). Measurements were performed bi-weekly during Phase II (day 337 to 385).

Molecular methods for microbial quantification

DNA extraction was conducted using the DNeasy mini kit (Qiagen, CA). Resulting DNA concentrations and quality were measured by Nanodrop Lite UV spectrophotometry (Thermofisher, MA). The abundance of AMX was quantified via SYBR® Green chemistry quantitative PCR (qPCR) assays targeting AMX 16S rRNA gene (van der Star et al., 2007). *C. “Brocadia fulgida”* specific qPCR assay was applied based on the highly variable region of the *hzsA* gene (Park et al., in submission). qPCR primers were used with TaqMan chemistry (forward, 5'-AGT TAG TGA GTG TGG ATG GCG TGT-3'; reverse, 5'-TCA TCC TGC GTG AGG AAC TTG TCA-3'; probe, 5'-/56-FAM/AT TCA GCC G/Zen/T GCG TAC ACC AGC TTG CTT /3IABkFQ/-3') (IDTDNA, IA).

qPCR assays were conducted on a iQ5 real-time PCR thermal cycler (BioRad Laboratories, CA). Standard curves for qPCR were generated via serial decimal dilutions of plasmid DNA containing specific target gene inserts. qPCR for standard plasmid DNA and sample DNA were conducted with duplication and triplication, respectively. DNA grade ddH₂O (Fisher Scientific, MA) was used for non-template control. Primer specificity and the absence of primer-dimers were confirmed via melt curve analysis.

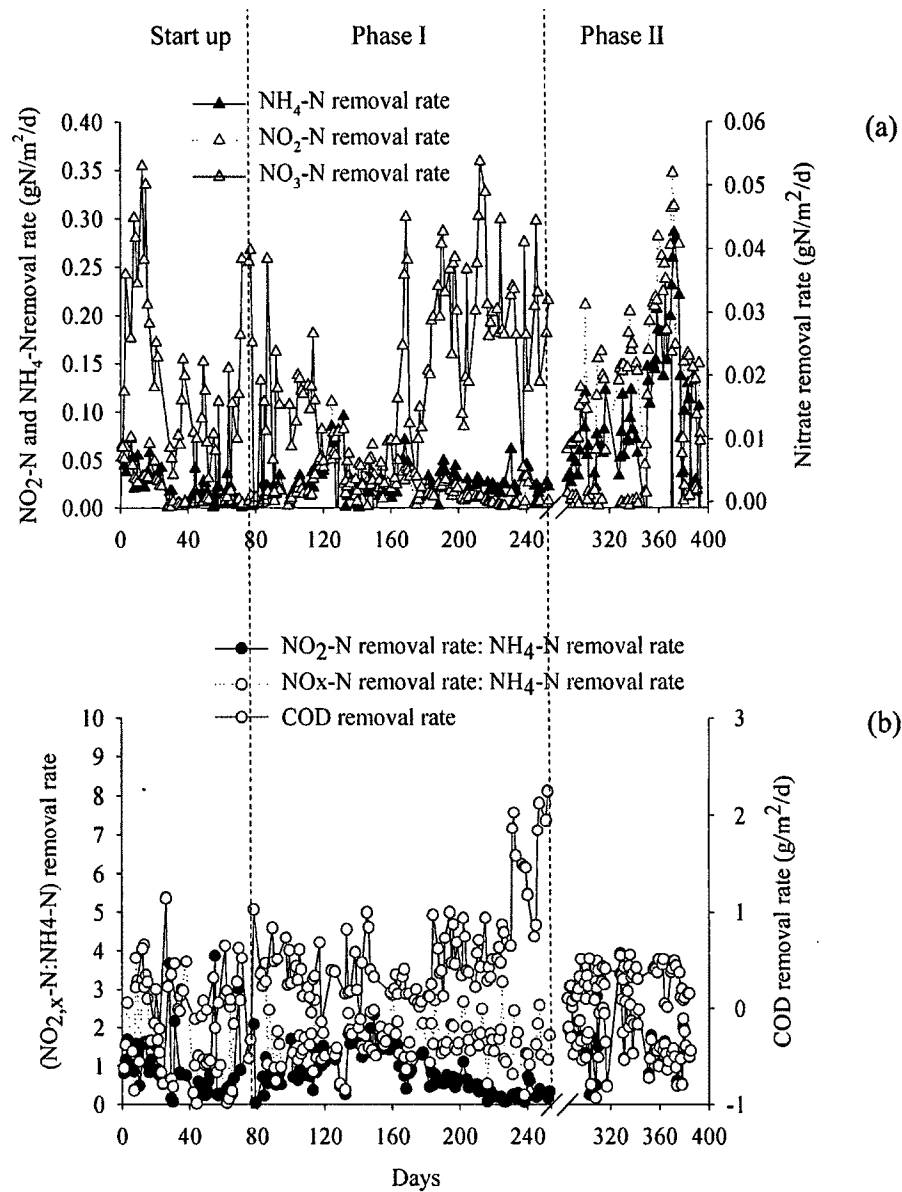
Statistical analysis

Statistical comparison between variables was performed using the t-test (for a normally distributed data set) and Mann-Whitney rank sum test (for not a normally distributed data set) on Sigma Plot (Systat Software, San Jose, CA). A Shapiro-Wilk test was used to determine the normality of the data set. A *p*-value of 0.05 or lower indicates that variables being compared are statistically different at the 95% confidence level.

7.3 Results

Startup and long-term operation

The anammox MBBR was operated in three phases over a period of 385 days. During the startup phase the reactor was operated with a clarifier and sludge recycle to retain seed sludge without wasting. This strategy was not entirely successful as most of the seed sludge floated and washed out from the clarifier in the first week of operation. Despite unintentional wasting of seed sludge, AMX activity was maintained, as seen from the NH_4^+ removal rate ($0.025 \pm 0.021 \text{ gN/m}^2/\text{d}$), throughout the startup period (Figure 47a). The trends of NH_4^+ , NO_2^- , NO_3^- and COD removal rates during the entire study can be seen in Figure 47. The NH_4^+ and NO_2^- removal rates were highest during Phase II. In Phase II, the anammox MBBR was fed plant FNE augmented with NO_2^- resulting in highest N loading (Figure 48). The ratio of NO_2^- removal rate and NH_4^+ removal rate as well NO_x removal rate and NH_4^+ removal rate can be seen in Figure 47b. During the Startup and Phase I, there was a net NO_3^- removal and there was a slight NO_3^- production in Phase II (Figure 47).



	Start up	Phase I	Phase II
NH ₄ -N removal rate (gN/m ² /d)	0.023±0.016	0.026±0.016	0.10±0.069
NO ₂ -N removal rate (gN/m ² /d)	0.017±0.018	0.021±0.018	0.14±0.08
NO ₃ -N removal rate (gN/m ² /d)	0.018±0.015	0.019±0.013	-0.003±0.010
NO ₂ -N removal rate: NH ₄ -N removal rate	1.05±1.14	0.83±0.62	1.66±0.73
NO _x -N removal rate: NH ₄ -N removal rate	1.78±1.42	1.71±0.75	1.64±0.71
COD removal rate (g/m ² /d)	0.195±0.374	0.58±0.51	0.31±0.144

Figure 47. Temporal trends during the study a) NH₄⁺-N, NO₂⁻-N, and NO₃⁻-N removal rate, b) COD removal rate, ratio of NO₂⁻-N removal rate: NH₄⁺-N removal rate, and NO_x-N removal rate: NH₄⁺-N removal rate.

In this study, $\text{NH}_4^+\text{-N}$ removal in the anoxic conditions of the anammox reactor was attributed to the AMX activity (release of $\text{NH}_4^+\text{-N}$ due to biomass decay and heterotrophic uptake of $\text{NH}_4^+\text{-N}$ during denitrification were assumed not to impact overall $\text{NH}_4^+\text{-N}$ removal). $\text{NO}_2^-\text{-N}$ in AvN CSTR effluent feeding anammox reactor was 0.73 ± 0.62 mg N/L during startup (Figure 48a). In fact, $\text{NO}_2^-\text{-N}$ accumulation in AvN CSTR was limiting AMX activity during startup as near complete $\text{NO}_2^-\text{-N}$ removal was observed (effluent $\text{NO}_2^-\text{-N} = 0.13 \pm 0.11$ mgN/L, Figure 48b). Since, the influent $\text{NH}_4^+\text{-N}$ (6.13 ± 2.86 mgN/L) was much greater than the $\text{NO}_2^-\text{-N}$, the overall TIN removal through the AMX pathway was limited (Figure 48a).

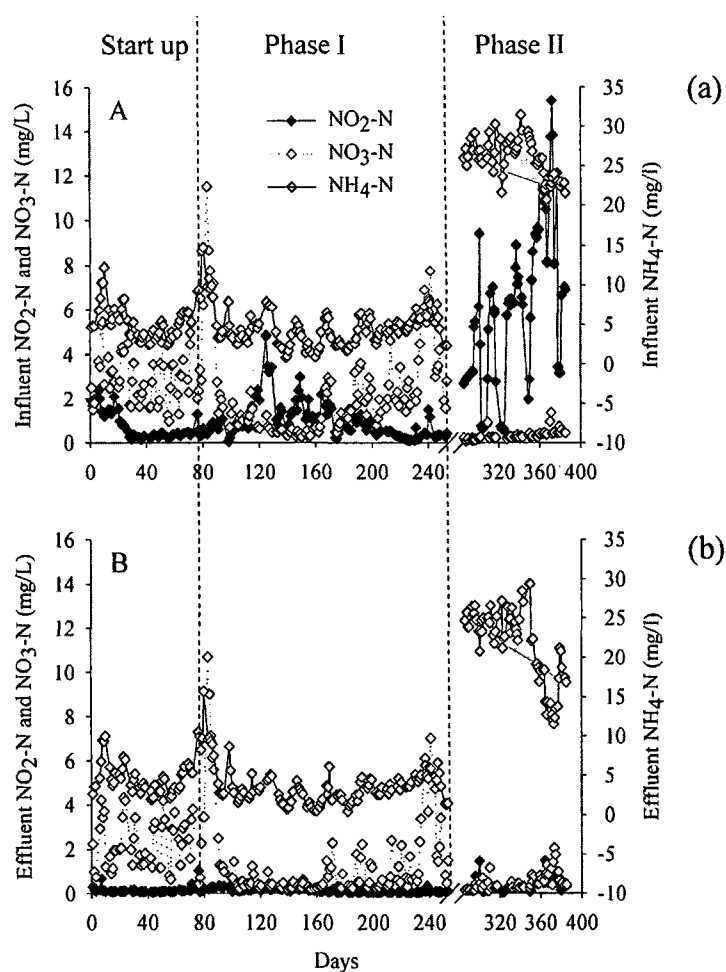


Figure 48. Temporal trends during the study a) Influent $\text{NH}_4^+\text{-N}$, $\text{NO}_2^-\text{-N}$, and $\text{NO}_3^-\text{-N}$, b) Effluent $\text{NH}_4^+\text{-N}$, $\text{NO}_2^-\text{-N}$, and $\text{NO}_3^-\text{-N}$.

The anammox MBBR was not fed between days 254-285, however, there was instant AMX activity during initiation of Phase II (Figure 47 and Figure 48). In Phase II, plant FE, which contained $\text{NH}_4^+\text{-N}$ (26 ± 2.45 mg N/L), was spiked with varying concentration of $\text{NO}_2^- \text{-N}$ and fed to the anammox MBBR. During Phase II, influent $\text{NO}_2^- \text{-N}$ concentrations were increased over time, however, $\text{NO}_2^- \text{-N}$ breakthrough was not observed (Figure 48b). As the $\text{NO}_2^- \text{-N}$ input was increased, AMX responded with greater $\text{NH}_4^+\text{-N}$ removal and subsequent TIN removal rates (Figure 49). There was slight reduction in the $\text{NO}_2^- \text{-N}$ removal rate when the $\text{NO}_2^- \text{-N}$ loading rate was increased up to 0.34 gN/m²/d during Phase II (Figure 49b).

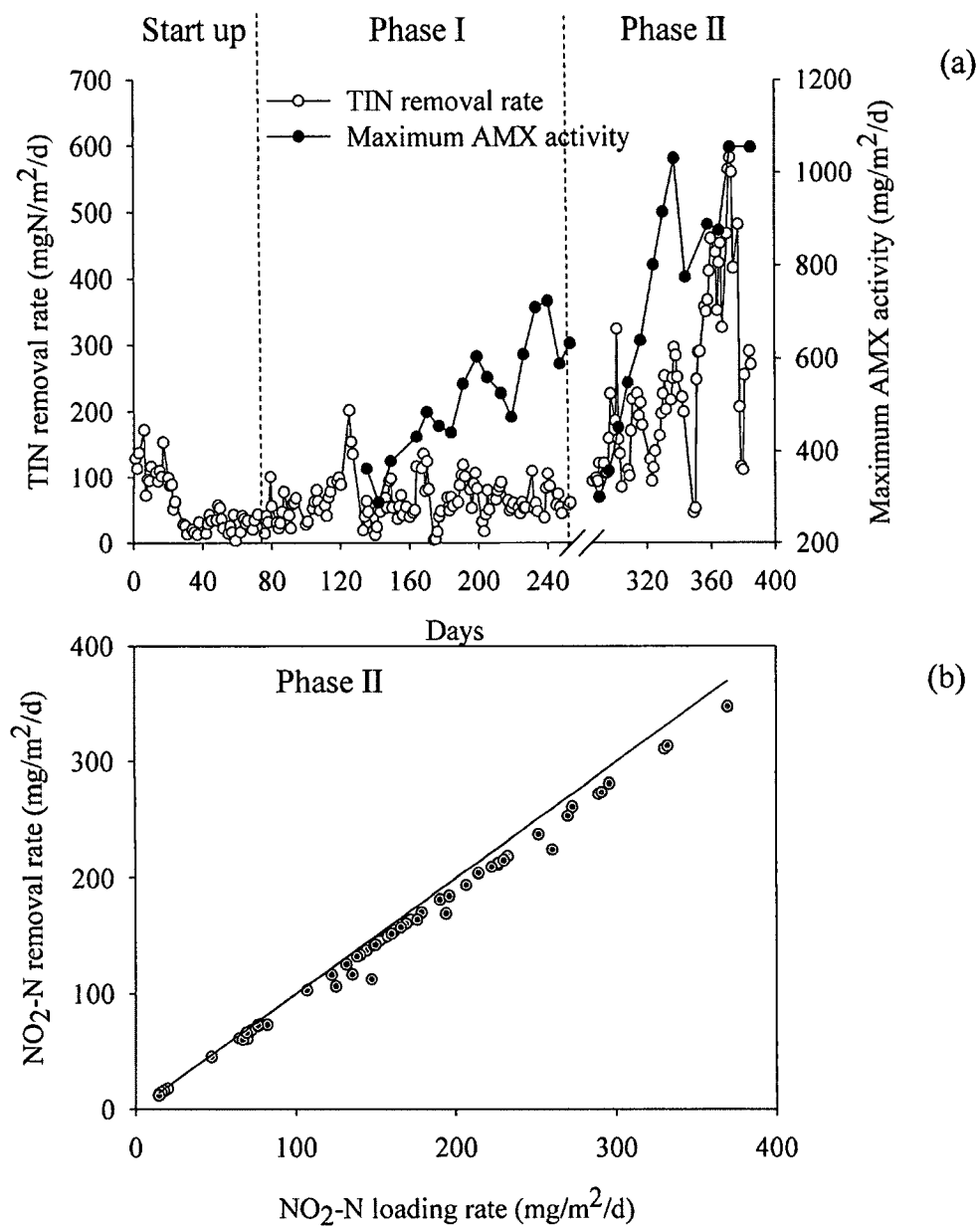


Figure 49. a) Trends of the TIN removal rate and the maximum AMX activity (Phase I and Phase II) b) NO₂⁻-N loading rate compared to the NO₂⁻-N removal rate during Phase II at influent NH₄⁺-N concentration of 26±2.5 mgN/L.

AMX activity and N removal rates

To assess the N turnover potential of the anammox MBBR weekly maximum rate measurements were performed during this study. The results of the maximum AMX tests conducted on day 136 (Phase I) and day 366 (Phase II) are presented in Figure 50. The maximum activity test clearly demonstrate the NH_4^+ , NO_2^- removal and slight NO_3^- production in anoxic conditions, which is expected from anammox metabolism.

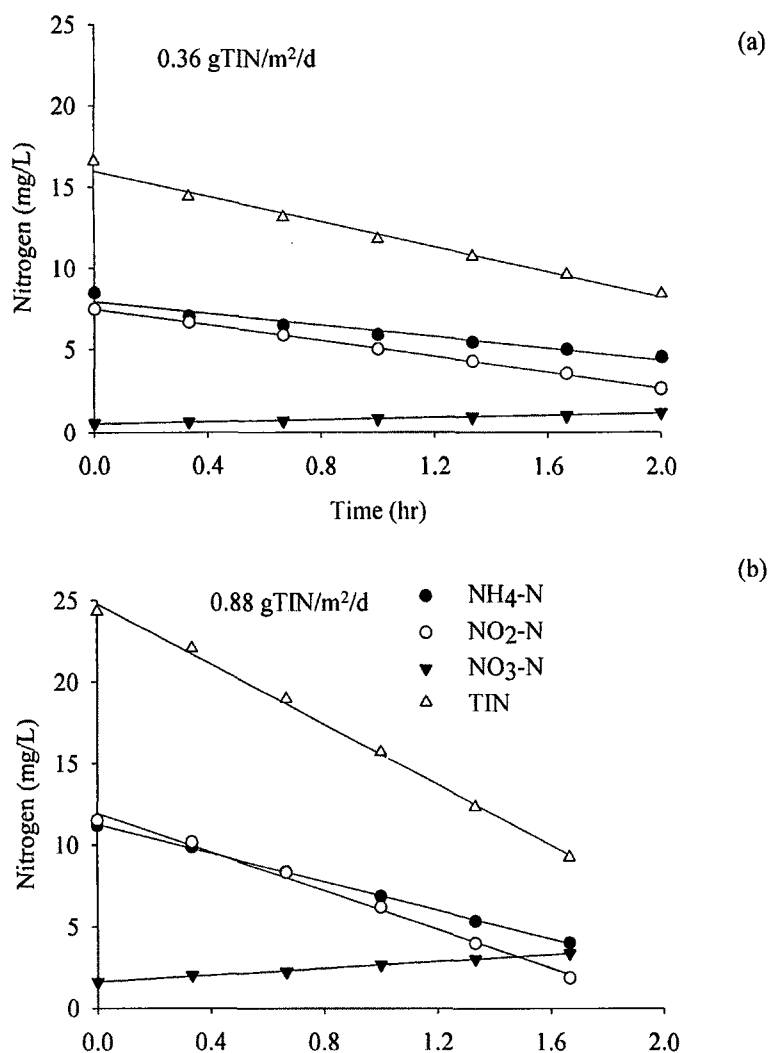


Figure 50. Maximum AMX activity test results a) During Phase I on day 136, b) During Phase II on day 366.

The trends of maximum $\text{NH}_4^+\text{-N}$ and $\text{NO}_2^- \text{-N}$ removal rates and $\text{NO}_3^- \text{-N}$ production rates can be seen in Figure 51a. The theoretical AMX stoichiometry ratios proposed by Strous et al. (1998) for $\text{NO}_2^- \text{-N}$ removed: $\text{NH}_4^+ \text{-N}$ removed and $\text{NO}_3^- \text{-N}$ produced: $\text{NH}_4^+ \text{-N}$ removed is 1.32 and 0.26, respectively. Maximum AMX activity measurements reveal that in Phase I, the ratio of $\text{NO}_2^- \text{-N}$ removed: $\text{NH}_4^+ \text{-N}$ removed was 1.47 ± 0.17 which is greater than 1.32 ($p < 0.001$), while the ratio of $\text{NO}_3^- \text{-N}$ produced: $\text{NH}_4^+ \text{-N}$ removed was 0.266 ± 0.033 , which is not statistically different from 0.26 ($p = 0.454$) (Figure 51b). In Phase II, the ratio of $\text{NO}_2^- \text{-N}$ removed: $\text{NH}_4^+ \text{-N}$ removed was 1.27 ± 0.10 , which is not statistically different from 1.32 ($p = 0.168$), while the ratio of $\text{NO}_3^- \text{-N}$ produced: $\text{NH}_4^+ \text{-N}$ removed was 0.243 ± 0.025 , which is less and statistically different than 0.26 ($p = 0.032$) (Figure 51b). The influent $\text{NH}_4^+ \text{-N}$ and $\text{NO}_2^- \text{-N}$ were much higher in Phase I compared to Phase II (Figure 48a). TIN removal rates were lower during startup (0.056 ± 0.042 $\text{gN/m}^2/\text{d}$) and Phase I (0.065 ± 0.032 $\text{gN/m}^2/\text{d}$) compared to Phase II (0.24 ± 0.013 $\text{gN/m}^2/\text{d}$) (Figure 47a). The maximum AMX activities measured during Phase I and Phase II were higher than the actual TIN removal rates during the same periods (Figure 47a). Maximum AMX activity over 1.0 $\text{gN/m}^2/\text{d}$ was observed towards the end of Phase II (Figure 47a). The bi-weekly measurements (day 337 to 385) show that biomass density remained stable around 3.2 ± 0.2 gTSS/m^2 . However, there was an increase in maximum specific AMX activity from 0.21 gN/gTSS/d to 0.33 gN/gTSS/d during the same time. The AMX reactor was not fed between Phase I and Phase II (day 254 to 285), which resulted in a drop in maximum AMX activity from 0.63 $\text{gN/m}^2/\text{d}$ to 0.30 $\text{gN/m}^2/\text{d}$. However, with the increase in $\text{NO}_2^- \text{-N}$ loading, the maximum activity increased from 0.30 $\text{gN/m}^2/\text{d}$ to 1.05 $\text{gN/m}^2/\text{d}$ during Phase II (day 286 to 385).

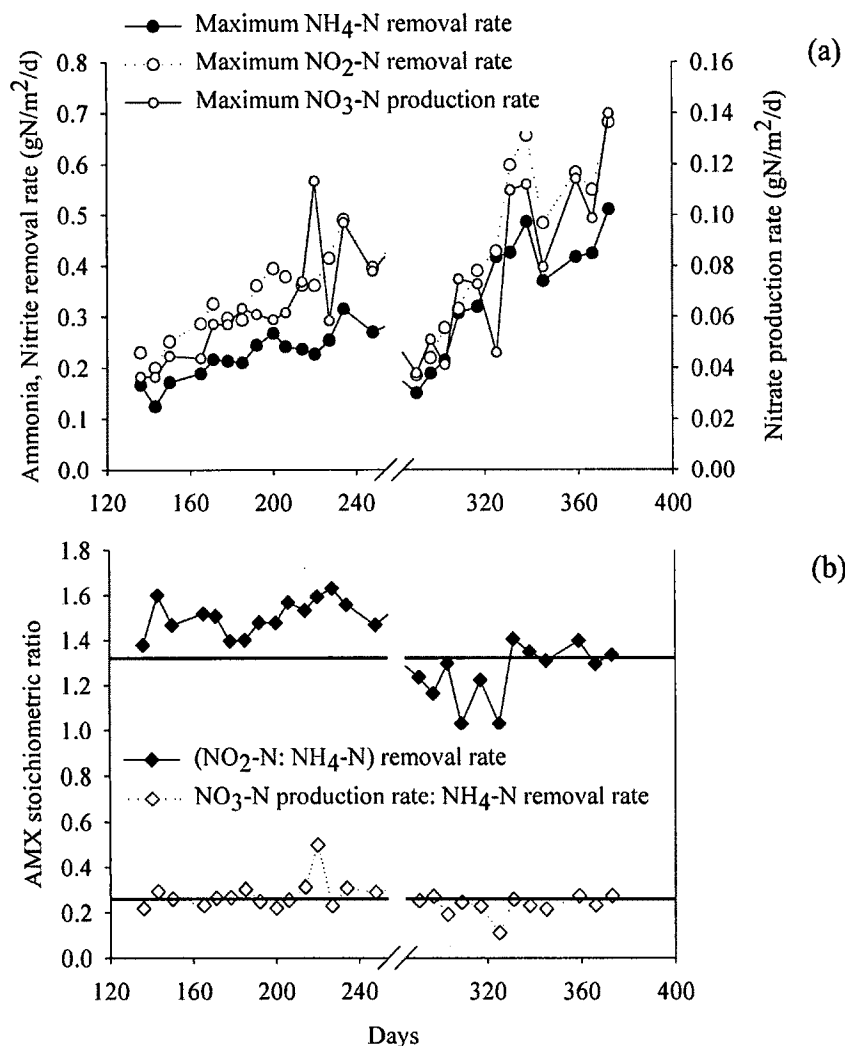


Figure 51. Temporal trends of a) Maximum $\text{NH}_4^+\text{-N}$, $\text{NO}_2^-\text{-N}$ removal rate and $\text{NO}_3^-\text{-N}$ production rate during weekly maximum AMX activity test b) AMX stoichiometric ratio of $\text{NO}_2^-\text{-N}$ removal rate: $\text{NH}_4^+\text{-N}$ removal rate and $\text{NO}_3^-\text{-N}$ production rate: $\text{NH}_4^+\text{-N}$ removal rate.

Nitrate removal and AMX contribution

There were two major observations: 1) $\text{NO}_3^-\text{-N}$ removal as opposed to production during Startup and Phase I of the study 2) The ratio of $\text{NO}_2^-\text{-N}$ removed: $\text{NH}_4^+\text{-N}$ removed less than the generally accepted ratio of 1.32 (Figure 47), which highlighted $\text{NO}_3^-\text{-N}$ removal in the anammox MBBR and AMX's involvement. $\text{NO}_3^-\text{-N}$ removal rates during Start up

and Phase I were 0.02 ± 0.021 gN/m²/d and 0.02 ± 0.014 gN/m²/d, respectively (Figure 47a). The influent NO₃⁻-N during Startup (3.41 ± 1.92 mgN/L) and Phase I (1.82 ± 1.51 mgN/L) were greater than during Phase II (0.34 ± 0.22 mgN/L) (Figure 48a). The ratio of NO_x-N removal rate: NH₄⁺-N removal rate was greater than the ratio of NO₂⁻-N removal rate: NH₄⁺-N removal rate during Start up and Phase I (Figure 47b). Significant COD removal by the anammox MBBR was observed during the study (Figure 47b). The COD removal rate during Start up and Phase I were 0.22 ± 0.37 g/m²/d and 0.58 ± 0.52 g/m²/d, respectively, compared to a COD removal rate of 0.29 ± 0.16 g/m²/d during Phase II. The higher NH₄⁺-N removal compared to NO₂⁻-N removal compared to expected AMX stoichiometry can result from either i) NO₃⁻-N being converted to NO₂⁻-N which was there available for AMX metabolism or ii) NH₄⁺-N uptake by heterotrophic denitrifiers assimilation during NO₃⁻-N and NO₂⁻-N reduction to N₂. Since NO₃⁻-N removal compared to NH₄⁺-N removals were much higher than would be expected from the heterotrophic uptake of NH₄⁺-N (calculations not shown), it is highly likely that NO₃⁻-N being converted to NO₂⁻-N was used by AMX, which resulted in higher TIN removal rates.

7.4 Discussion

Feasibility of anammox N polishing in an MBBR

In this study, the startup of anammox was immediate without any lag as a final polishing step. However, the seed sludge used and N loading could be different in a full-scale startup. One of the biggest drawbacks of the anammox based processes is the long startup times resulting from the slow growth rates of AMX (van der Star et al., 2007). Unlike one-stage deammonification systems, where AOB and AMX are required to be managed within the same system requiring complex controls, AMX polishing was possible without process control. The anammox step of the sidestream two-stage deammonification systems are prone to NO₂⁻-N inhibition (Wett, 2007), which often requires a very strict control over the NO₂⁻-N loading. The NO₂⁻-N levels that are expected in mainstream polishing applications can be considered below inhibitory levels.

As a final step, the anammox MBBR received low COD/N ratio influent, therefore, heterotrophs were not able to out-compete AMX for NO_2^- -N. The retention of slow growing AMX, which is often the main challenge (Fernández et al., 2008), was resolved by providing supporting material in the form of biofilm carriers.

The maximum AMX activities (from batch activity experiments) are much higher compared to in-tank N removal rates, which suggest enrichment of the biofilm carriers. In fact, the TIN removal rate was around $0.064 \pm 0.028 \text{ gN/m}^2/\text{d}$ during Phase I (day 136 to 253), while the maximum AMX activity doubled over the same time (Figure 49a). The trend of maximum AMX activity continued to increase in both Phase I and Phase II (Figure 49a). The lower decay rates ($0.002 - 0.004 \text{ d}^{-1}$) of AMX (Dapena-Mora et al., 2004, Udert et al., 2008, Ni et al., 2009) and the effective retention through biofilm carriers may have contributed to such enrichment. The process stability was demonstrated in Phase II of the study when the NO_2^- -N loading rate was increased rapidly without any loss of the NO_2^- -N removal rate. This can be attributed to the extra capacity that existed because of the AMX enrichment.

The N removal in anammox polishing was limited by the influent NO_2^- -N/ NH_4^+ -N ratio and not by the AMX retention and enrichment. It emphasizes the fact that to maximize benefits of anoxic autotrophic N removal through anammox, greater stability of NOB suppression and a tight control of the effluent NO_2^- -N/ NH_4^+ -N ratio in a nitrification-denitrification system is needed.

Nitrate removal in AMX MBBR

The influent COD to the anammox MBBR was mostly comprised of effluent TSS, refractory and particulate COD fractions that were not degraded by the AvN CSTR (Startup + Phase I) and HRAS plant (Phase II). However, COD removal was observed in the anammox MBBR, and as a consequence, there was a limited heterotrophic contribution to the N removal observed. It was reported that in the presence of a certain level of organic matter, AMX cannot compete with heterotrophic denitrifiers due to their slower growth rate (Udert et al., 2008). Tang et al. (2010) demonstrated that when the influent COD/ NO_2^- -N ratio was 2.9, heterotrophic denitrification dominated over AMX

in an upflow anaerobic sludge blanket (UASB) reactor. Many studies lately have showed that with a ratio of COD/N less than 0.5 in the influent, AMX can outcompete heterotrophic bacteria with mainly nitrite in the influent (Lan et al., 2011, Chen et al., 2009, Xu et al., 2010). In this study in spite of relatively high influent COD/NO₂⁻-N ratio of ~4, the anammox metabolism remained the primary N turnover pathway.

Recently it was shown that AMX bacteria have the ability to use short-chain fatty acids (SCFA) with NO₃⁻ as the electron acceptor (Guyen et al., 2005, Kartal et al., 2008, Winkler et al., 2012). AMX bacteria completely oxidize organic matter into CO₂ without assimilation, which results in a low biomass yield (Winkler et al., 2012). *Ca. "Brocadia fulgida"*, known for its capability to use acetate with NO₃⁻ as the electron acceptor (Kartal et al., 2008) was not significant in the anammox MBBR in this study. In an anoxic reactor, Guven et al. (2005) showed that heterotrophs outcompete AMX for nitrate if the COD/N ratio exceeds 1. Since the anammox MBBR was not fed external acetate (VFAs in upstream nitrification-denitrification effluent was below detection) nor was *Ca. "Brocadia fulgida"* dominant (Figure 52), AMX using NO₃⁻-N was not completely justified. In fact, the species of AMX remained unknown and further investigation is ongoing. Therefore, the possibility of heterotrophs converting some fraction of NO₃⁻-N to NO₂⁻-N under limited COD availability and AMX using this produced NO₂⁻-N with NH₄⁺-N cannot be overruled. Regardless of the exact pathway, this study has shown that NH₄⁺-N, NO₂⁻-N and NO₃⁻-N removal was possible in a mainstream fully anoxic anammox MBBR with limited influent COD.

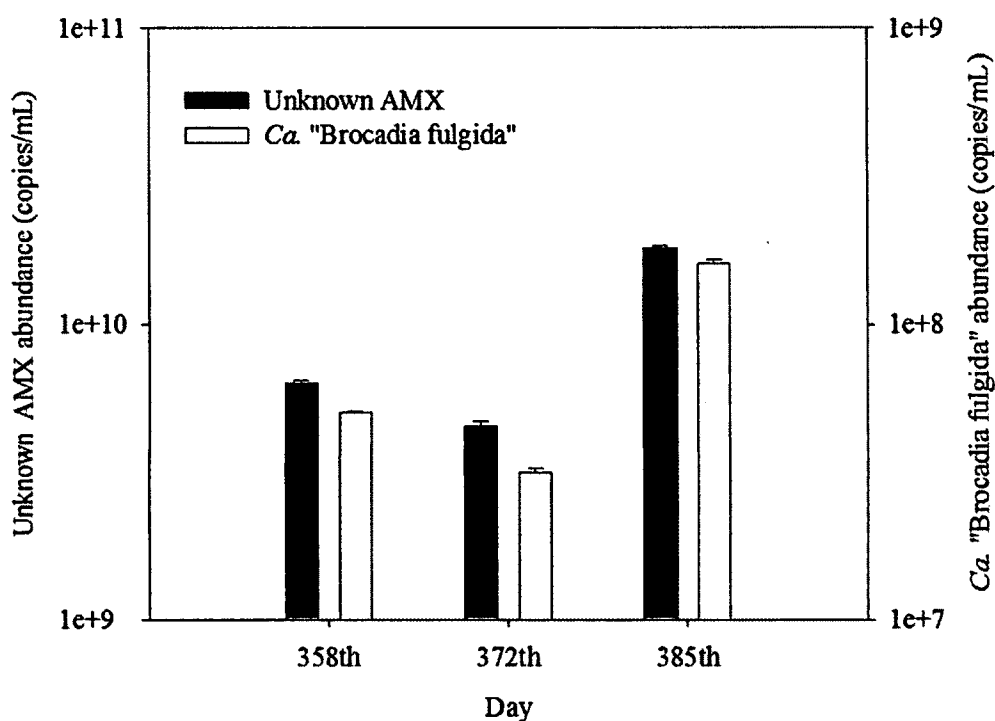


Figure 52. Abundances of AMX species identified during Phase II of the study (Day: 358, 372, and 385).

7.5 Conclusion

In this study, mainstream application of anammox for nitrogen polishing in an anoxic MBBR was demonstrated. The startup was fast despite a very low NO_2^- concentrations in the effluent of the nitrification-denitrification system feeding the anammox MBBR. A highly stable nitrogen removal performance was demonstrated within a wide range of influent nitrogen species concentrations. The anammox MBBR demonstrated maximum nitrogen removal rates of close to $1 \text{ gN/m}^2/\text{d}$. The production of NO_3^- -N limits the applicability of anammox to meet stringent nitrogen permits, however, in this study it was showed that NO_3^- -N removal is possible. Although, the exact pathway of NO_3^- -N removal remained unclear, it will be explored in future research. Therefore, for the first time this study shows that anammox nitrogen polishing in an MBBR is possible for nitrification-denitrification system with wide range of NO_2^- concentrations in the effluent.

CHAPTER 8

OPTIMIZATION OF A MAINSTREAM NITRITATION-DENITRITATION PROCESS AND ANAMMOX POLISHING

Note: The contents of this chapter will be submitted into two joint papers for publication in Water Research. Regmi, P., Holgate, B., Fredericks, D., Miller, M.W., Park, H., Chandran, K., Wett, B., Murthy, S., Bott, C.B., Optimization of a mainstream nitrification-denitrification process and anammox polishing.

8.1 Introduction

Wastewater treatment plants (WWTPs) around the world are facing technical and financial challenges to meet ever more stringent water quality standards. For the WWTPs required to remove nitrogen, the cost of energy and resources (e.g., external carbon and alkalinity) for nitrogen removal from wastewater is increasing while nitrogen limits are becoming lower. Recently, there has been an explosion of new innovative technologies to achieve high levels of total nitrogen removal for less energy, fewer resource demands, and over less space. However, most of these technologies are limited to nitrogen rich waste streams with low carbon to nitrogen ratio (COD/N hereafter). The very efficient partial nitrification-anaerobic ammonia oxidation (anammox) or deammonification based technologies have already been proven to treat high ammonia strength reject water with more than 100 full-scale installations (Lackner et al., 2014), while the mainstream implementation is currently under development by several research groups around the world. The latter holds the key to intensification of wastewater treatment for biological nitrogen removal (BNR), recovery of energy from carbon content of raw wastewater and minimization of energy and resources for nitrogen polishing to meet stringent permits.

Successful mainstream deammonification depends on stable anammox activity, however, effectively suppressing nitrite oxidation is the major precondition. The most common approaches to suppress nitrite oxidizing bacteria (NOB) are specific to unique conditions

of high temperature and high ammonia strength waste streams such as the reject stream generated from anaerobic digestion of municipal sludge. The implementation of partial nitrification + anammox is successfully applied in highly loaded sidestream processes that use one or more of the following conditions to out-select NOB: high temperature (Hellinga et al., 1998); low DO (Joss et al., 2009); low solids retention time (SRT) (van Dongen et al., 2001); and free ammonia (FA) inhibition (Anthonisen et al., 1976). However, since FA inhibition is not available at the lower total ammonia concentrations that occur in domestic wastewater and temperatures vary enough so that reliable high temperature selection is not viable, different strategies are needed for NOB out-selection in mainstream processes. In a preliminary study using a continuous flow single CSTR, Regmi et al. (2014) demonstrated unique mainstream strategies for achieving sustained NOB out-selection (Table 27).

Table 27. Strategies used by Regmi et al. (2014) to achieve NOB out-selection during mainstream treatment.

Strategy	Action	Impact	Control Basis
1	$\frac{\text{NH}_4^+}{\text{NO}_2^- + \text{NO}_3^-} = 1$	Optimum aerated and unaerated volume for nitrification and denitrification. Optimum alkalinity for AOB growth. Residual ammonium supports higher AOB growth rates	Control based on real-time effluent NH_4^+ , NO_2^- , NO_3^- signals
2	Intermittent aeration and bioavailable COD	Allow NO_2^- consumption by denitrifiers	Upstream organic carbon treatment system
3	DO = 1.6 mg/L	AOB grows faster than NOB	Control DO set-point
4	Aggressive low SRT	AOBs to grow rapidly, making them competitive relative to stressed NOBs due to strategies 1, 2 and 3.	Wasting

These strategies, however, have not yet been tested for reliable NOB out-selection in a wide range of loading conditions in more practical plug-flow configurations.

The slower doubling times and sensitivity of anammox bacteria (AMX) towards dissolved oxygen and high carbon concentrations were thought to be the obstacles for mainstream implementation. In a preliminary study, the mainstream anammox polishing moving bed biofilm reactor (MBBR) coupled to the AvN CSTR demonstrated a highly stable mainstream deammonification (Chapter 7). The limitation of NOB out-selection in the AvN reactor resulting in more NO_3^- -N than NO_2^- -N severely limited the nitrogen turnover through the anammox metabolism.

Recently, the capability of certain AMX species to oxidize volatile fatty acids with NO_3^- as the electron acceptor has been demonstrated (Güven et al., 2005, Kartal et al., 2007, Winkler et al., 2012). Since, AMX converts the fatty acids directly to CO_2 without incorporating into the biomass, the yield associated with such metabolism is low (Winkler et al., 2012) and can be considered advantageous. On the other hand, at a carbon to nitrogen ratio of greater than 1, heterotrophs are shown to out-compete AMX (Güven et al., 2005). The removal of NO_3^- produced from the AMX reaction or resulting from uninhibited NOB is of significant importance when anammox is used for nitrogen polishing to meet stringent permits.

In this study, an A-B process was piloted with the principal objective of mainstream repression of nitrite oxidation and implementation of anammox. The A-stage (i.e., the high rate carbon removal process) was operated under strategies to control the effluent carbon to ammonia ratio ($8 < \text{COD}/\text{NH}_4^+\text{-N} < 11$) that would be optimum for the B-stage nitrogen removal. The B-stage, this paper's focus, consisted of an aggressively operated (i.e., short SRT and HRT) nitrification-denitrification process [named AOB versus NOB (AvN)] and was followed by an anammox moving bed biofilm reactor (MBBR) for nitrogen polishing. The combined AvN and the anammox MBBR was named AvN+. The B-stage AvN was operated under an intermittent aeration control strategy that targeted an effluent oxidized nitrogen to ammonia nitrogen $[(\text{NO}_2^-\text{-N} + \text{NO}_3^-\text{-N})/\text{NH}_4^+\text{-N}]$ ratio of 1. In this chapter, two aspects of the pilot study are presented: 1) Nitrite oxidizing bacteria (NOB) out-selection in AvN and nitrogen removal performance by the AvN+ process at

different loading and operating conditions 2) The enhancement of nitrate and ammonia removal in the anammox (MBBR) with acetate ($\text{COD}/\text{NO}_3^- - \text{N} < 1.5$) addition to meet stringent nitrogen permit limits.

8.2 Material and Methods

The AvN+ pilot

The pilot process described in this study was part of a larger configuration including a high rate activated sludge A-stage (HRT= 30 minutes, SRT = 0.25 days) for COD removal providing the influent for the AvN reactor (Figure 37). In this paper, the focus is only on the performance and operation of the AvN+ process.

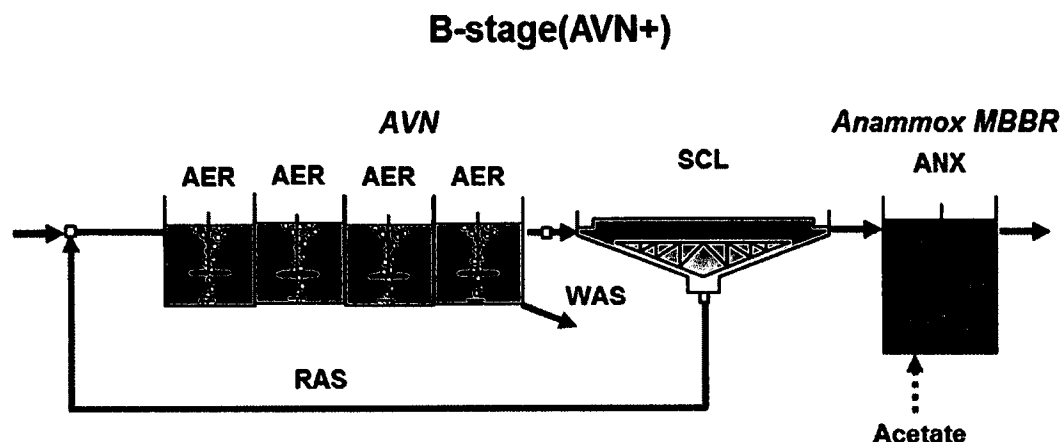


Figure 53. Process flow diagram of the AvN+ process.

The AvN process included four equal sized reactors with combined aeration tank volume of 0.6 m^3 followed by a clarifier. Each reactor was equipped with a variable speed mixer ($G = 106 \text{ s}^{-1}$) in order to maintain completely-mixed conditions. Return activated sludge (RAS) from the clarifier was returned to the first AvN reactor with a peristaltic pump at

100% of the influent flow rate. SRT was controlled by wasting solids from the last reactor with a programmable digital peristaltic pump. All AvN reactors were equipped with sensors to monitor DO (Hach LDO, CO, USA) while the last reactor was also monitored for NO_3^- -N, NO_2^- -N (s::can Spectro::lyser, Austria), and NH_4^+ -N (WTW VARiON, Germany). NH_4^+ -N, NO_3^- -N, NO_2^- -N signals were used to control aeration (Figure 38).

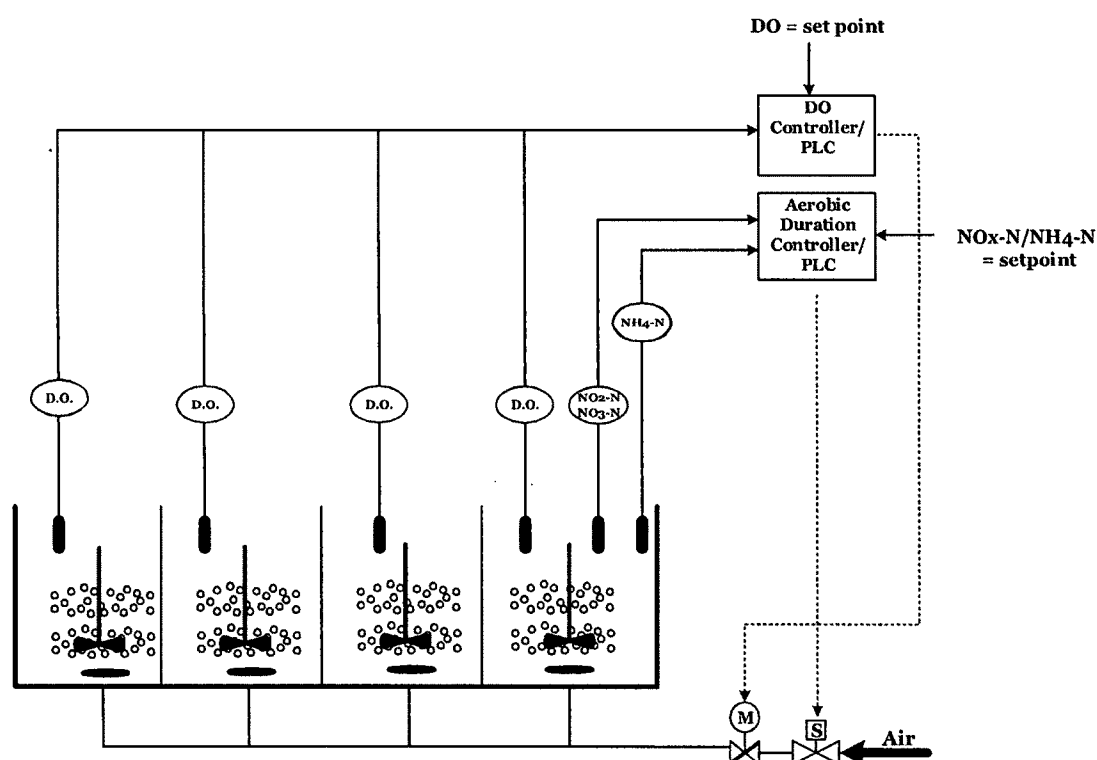


Figure 54. AvN controller depicting aerobic duration controller receiving NH_4^+ (WTW VARiON, Germany), NO_2^- and NO_3^- (s::can Spectro::lyser, Austria) signals and DO controller receiving dissolved oxygen (Hach LDO, USA) signal.

The anammox MBBR had a volume of 0.45 m^3 where 50% of the volume was filled with K3 biofilm carriers (AnoxKaldnes, Sweden). The volume was later changed to 0.34 m^3

on day 85. The effective surface area of the carriers was $500 \text{ m}^2/\text{m}^3$. Mechanical mixing of carriers was achieved by a variable speed mixer ($G = 14 \text{ s}^{-1}$). The pH was recorded continuously by an online pH probe and the reactor was covered with Styrofoam to avoid oxygen transfer from the atmosphere.

To assess the performance of the AvN+ process within different loading conditions the HRT of the entire system was changed during the study as seen in Table 28.

Table 28. Five phases of the study based on changes to the HRT.

	AvN HRT (h)	Anammox MBBR HRT (h)	AvN+ Total HRT (h)	Length (d)	Number of Days
Phase I	3	2.2	5.2	0-84	84
Phase II	4	2.2	6.2	85-118	34
Phase III	6	3.4	9.4	119-160	42
Phase IV	2	1.1	3.1	161-204	44
Phase V	3	1.7	4.7	205-220	16

To enhance the NO_3^- removal in anammox MBBR acetate (Sodium Acetate) was added from days 102- 161. The target COD/ NO_3^- -N ratio was around 1.

AvN (NH_4 - NO_x) aeration control

To impose conditions favorable for NOB out-selection and to provide effluent suitable for anaerobic ammonia oxidation (anammox) polishing, an aeration controller was developed which uses online *in-situ* DO, NH_4^+ , NO_2^- and NO_3^- sensors. The first component of AvN control was the aerobic duration controller with the goal of maintaining equal effluent NH_4^+ -N and NO_x -N ($\text{NO}_x\text{-N}/\text{NH}_4^+\text{-N} = 1$) in the AvN effluent at all times. The latter would guarantee a treatable effluent for the final polishing step with AMX. The other component of the AvN control was the DO controller, which maintains the DO at a desired set-point during the aerated period (Figure 54).

Under the AvN strategy, $\text{NH}_4^+\text{-N}$ was compared to the sum of $\text{NO}_2^-\text{-N}$ and $\text{NO}_3^-\text{-N}$ ($\text{NO}_x\text{-N}$). First, the cycle duration (aerobic duration + anoxic duration) had a defined minimum and maximum aerobic duration. The cycle duration was kept constant at 16 minutes during the entire experiment and minimum and maximum aeration times were set at 4 and 12 minutes, respectively. These set points were selected to avoid the $\text{NH}_4^+\text{-N}$ below 1.5 mg N/L. As the AvN controller aimed at ammonium concentrations equal to $\text{NO}_x\text{-N}$ concentrations, aerobic duration was increased up to a predetermined maximum aeration time set-point, while maintaining the cycle duration constant at $\text{NO}_x\text{-N}$ over $\text{NH}_4^+\text{-N}$ ratios less than 1. When $\text{NH}_4^+\text{-N}$ was less than $\text{NO}_x\text{-N}$, aerobic duration was decreased until it reached the minimum aeration time. When aerated, the proportional-integral-derivative (PID) controlled a mechanically operated valve (MOV) to maintain the target DO set-point of 1.6 mg $\text{O}_2\text{/L}$.

Influent/Effluent monitoring

Performance of the AvN pilot was monitored by collecting 24-hr flow-weighted composite samples from the influent and effluent. Samples were analyzed for TSS, VSS, total and soluble COD, TKN, TP, OP, $\text{NO}_3^-\text{-N}$, $\text{NO}_2^-\text{-N}$, $\text{NH}_4^+\text{-N}$ and alkalinity. All relevant analytical methods for solids and liquids are presented in Table A1 and Table A2.

Microbial activity measurements

To measure maximum AOB and NOB activity, 4 L samples were collected and dispensed into 4L vessels from the AvN CSTR and aerated for 30 minutes to oxidize excess COD, and spiked with 20-30 mg/L $\text{NH}_4^+\text{-N}$ (as ammonium chloride) and 2-4 mg/L $\text{NO}_2^-\text{-N}$ (as sodium nitrite), respectively, and sampled continuously for 1 hour at 20-minute intervals. All collected samples were analyzed for $\text{NH}_4^+\text{-N}$, $\text{NO}_2^-\text{-N}$, and $\text{NO}_3^-\text{-N}$. Mixing was provided by a magnetic stir bar. The DO concentration was maintained between 2.5 and 4 mg $\text{O}_2\text{/L}$. pH was maintained between 7-7.5 by adding sodium bicarbonate. The AOB rates were calculated as the slope of the $\text{NO}_x\text{-N}$ production and NOB rates were calculated as the slope of the $\text{NO}_3^-\text{-N}$ production.

AMX activity tests

To measure maximum AMX activity, the anammox MBBR was isolated from the system. A sample was taken for sCOD, $\text{NH}_4^+\text{-N}$, $\text{NO}_2^-\text{-N}$, and $\text{NO}_3^-\text{-N}$. The MBBR was spiked with 8 mg/L $\text{NH}_4^+\text{-N}$ (ammonium chloride) and 10 mg/L $\text{NO}_2^-\text{-N}$ (sodium nitrite) and sampled continuously at 20-minute intervals until the $\text{NO}_2^-\text{-N}$ was less than 1.5 mg/L $\text{NO}_2^-\text{-N}$. On the last sample of the activity measurement, a sCOD sample was taken along with $\text{NH}_4^+\text{-N}$, $\text{NO}_2^-\text{-N}$, and $\text{NO}_3^-\text{-N}$. The DO concentration was maintained close to 0 mg $\text{O}_2\text{/L}$ and was recorded at 20 minute intervals along with the pH. $\text{NH}_4^+\text{-N}$ and $\text{NO}_2^-\text{-N}$ uptake rates were calculated as the slope of the NH_4^+ and NO_2^- values measured during the activity test. NO_3^- production rates were calculated as the slope of the $\text{NO}_3^-\text{-N}$ values. The results of the tests are presented by the unit of gN/m²/d.

Biomass density

Biomass density measurements were performed according to the method described by Regmi et al. (2011). Measurements were performed bi-weekly during the study.

Molecular methods for microbial quantification

DNA and RNA extraction was conducted using the DNeasy and RNeasy mini kits (Qiagen, CA). Resulting DNA and RNA concentrations and quality were initially checked by UV spectrophotometry (Varian, CA). The abundance of AOB and NOB was quantified via SYBR® Green chemistry quantitative polymerase chain reaction (qPCR) assays, NH_4^+ monooxygenase subunit A (*amoA*) gene (Rotthauwe et al., 1997), *Nitrobacter* 16S rRNA gene (Graham et al., 2007) and *Nitrospira* 16S rRNA gene (Kindaichi et al., 2007), respectively. Total bacterial abundance was quantified using eubacterial 16S rRNA gene targeted primers (Ferris et al., 1996). qPCR assays were conducted on a iQ5 real-time PCR thermal cycler (BioRad Laboratories, Hercules, CA). Standard curves for qPCR were generated via serial decimal dilutions of plasmid DNA containing specific target gene inserts. qPCR for standard plasmid DNA and sample DNA were conducted with duplication and triplication, respectively. DNA grade

deionized distilled water (Fisher Scientific, MA) was used for non-template control. Primer specificity and the absence of primer-dimers were confirmed via melt curve analysis of each and every qPCR profile.

The abundance of AMX was quantified via SYBR® Green chemistry quantitative PCR (qPCR) assays targeting AMX 16S rRNA gene (van der Star et al., 2007). *C. “Brocadia fulgida”* specific qPCR assay was applied based on the highly variable region of the *hzsA* gene (Park et al., in submission). qPCR primers were used with TaqMan chemistry (forward, 5'-AGT TAG TGA GTG TGG ATG GCG TGT-3'; reverse, 5'-TCA TCC TGC GTG AGG AAC TTG TCA-3'; probe, 5'-/56-FAM/AT TCA GCC G/Zen/T GCG TAC ACC AGC TTG CTT /3IABkFQ/-3') (IDTDNA, IA).

qPCR assays were conducted on a iQ5 real-time PCR thermal cycler (BioRad Laboratories, CA). Standard curves for qPCR were generated via serial decimal dilutions of plasmid DNA containing specific target gene inserts. qPCR for standard plasmid DNA and sample DNA were conducted with duplication and triplication, respectively. DNA grade ddH₂O (Fisher Scientific, MA) was used for non-template control. Primer specificity and the absence of primer-dimers were confirmed via melt curve analysis. The samples are collected using methods described in A1 and A2.

8.3 Results and Discussion

AvN+ Nitrogen Removal Performance

The AvN process was started with non-nitrifying biomass from a high rate activated sludge plant, while the anammox MBBR was enriched with AMX on the biofilm carriers from a prior study (Chapter 7). AOB activity in the AvN process was established within two weeks into the study with the effluent NH₄⁺-N below 10 mgN/L (Figure 55a). NOB activity was lower than AOB activity during this time as high concentrations of NO₂⁻-N were observed in the effluent. The anammox MBBR removed NO₂⁻ and NH₄⁺-N without any indication of lag during the startup (Figure 57b). The influent NH₄⁺-N concentration to the AvN fluctuated between 25-40 mgN/L (Figure 55a).

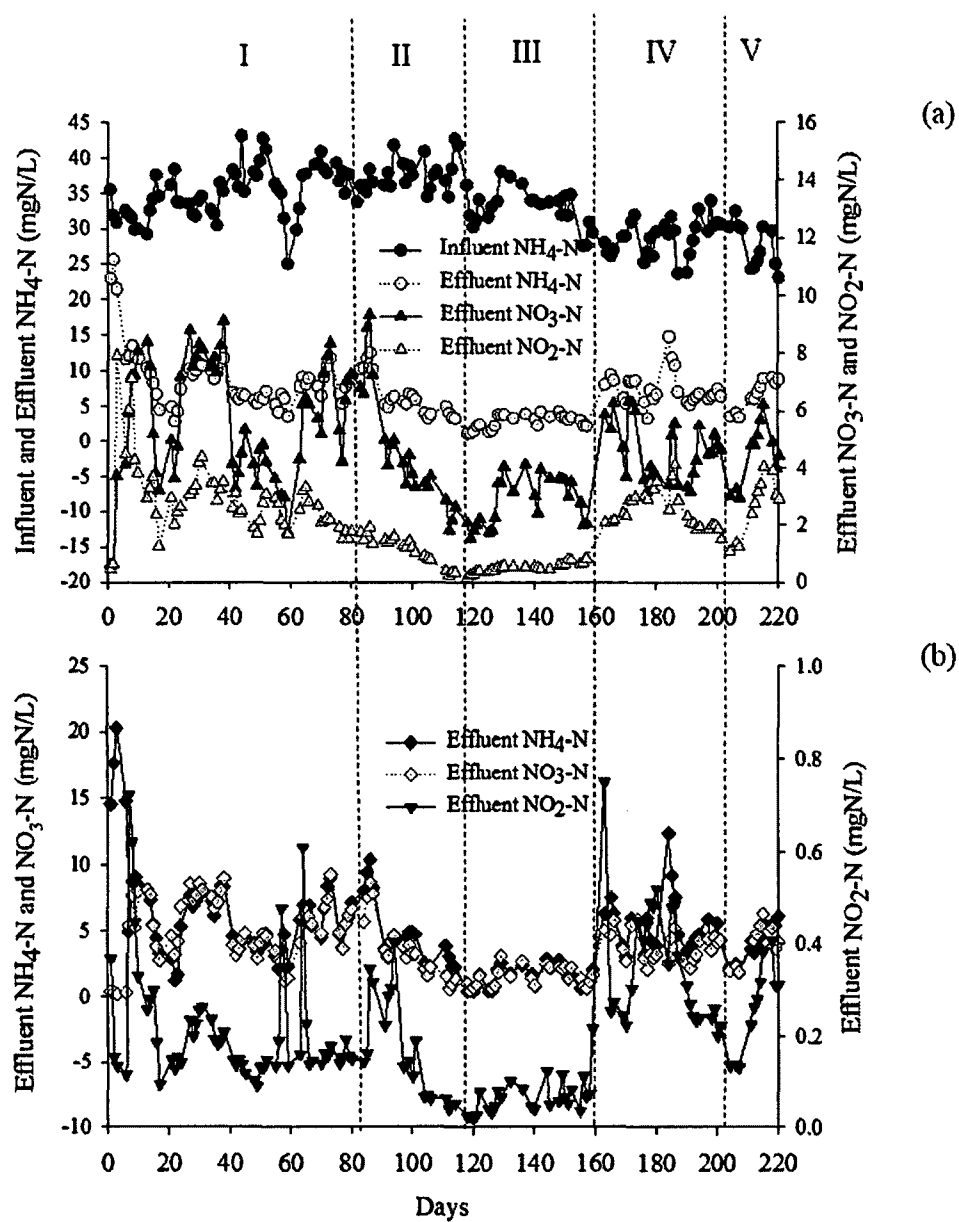


Figure 55. a) Trends of AvN influent $\text{NH}_4^+\text{-N}$ and effluent $\text{NH}_4^+\text{-N}$, $\text{NO}_3^-\text{-N}$, $\text{NO}_2^-\text{-N}$ during the entire study, b) Trends of anammox MBBR effluent $\text{NH}_4^+\text{-N}$, $\text{NO}_3^-\text{-N}$, $\text{NO}_2^-\text{-N}$ during the entire study.

The key effluent parameters of the A-stage (influent to the AvN), AvN and anammox MBBR averaged over the 220 days of operation are presented in Table 29. The average influent COD/NH₄⁺-N ratio to the AvN was 8.9, which can be considered limiting to achieve a high degree of nitrogen removal in a combined carbon and nitrogen removal system through nitrification and denitrification. Almost half of the influent COD to the AvN was in the soluble form. The AvN effluent NH₄⁺-N averaged 6.6 and the average NO_x-N was 6.7 during the entire study, which demonstrates that the AvN aeration controller was able to achieve its goal.

Table 29. The key effluent parameters of the A-stage, AvN, and anammox MBBR during the study period of 220 days. (Average±Standard Deviation).

Parameter	A-stage effluent	AvN effluent	Anammox effluent
COD (mg/L)	296±71	52±14	41±11
sCOD (mg/L)	147±37	30±4	26±4
COD/NH ₄ ⁺ -N	8.9±1.8	-	-
NH ₄ ⁺ (mgN/L)	33.4±4.4	6.6±2.4	4.5±2.6
NO _x (mgN/L)	-	6.7±2.9	4.2±2.3
NO ₃ ⁻ (mgN/L)	-	4.6±1.9	4.0±2.2
NO ₂ ⁻ (mgN/L)	-	2.1±1.3	0.21±0.15
OP (mgP/L)	2.9±0.9	2.5±0.7	2.5±1.1
TSS (mg/L)	83±30	25±12	20±10
pH*	6.9±0.1	6.7±0.1	6.7±0.1
Temperature (°C)*	24.5±1.3	23.9±1.3	23.4±1.2

*Measured in the reactor with sensors

The TIN removal performance of AvN+ system was 75±15% during the study at a modest influent COD/NH₄⁺-N ratio of 8.9±1.8. The TIN removal efficiency of the AvN+ process within the HRT range of 3.1 to 9.4 h (Table 28) and at different A-stage COD removal efficiencies can be seen in Figure 56. In Phase III (AvN+ HRT = 9.1 h) the TIN

removal efficiency averaged 91%, which was the highest among all phases. In Phase III the A-stage COD removal efficiency averaged 41% which resulted in the influent COD/NH₄⁺-N ratio of 10.5. The relative TIN removal contribution of anammox MBBR was very stable within in the study period (Figure 56). Since, a significant amount of TIN removal was taking place in the AvN process during Phase III, the anammox contribution was limited to 8% which is lower than the average of 11% for the entire study. It is worthwhile to note that Phase III was also the period with the lowest NOB out-selection as indicated by nitrite accumulation ratio (NAR) of 0.16 and the ratio of maximum NOB activity and maximum AOB activity (NOB rate/AOB rate) of 1 (Table 30).

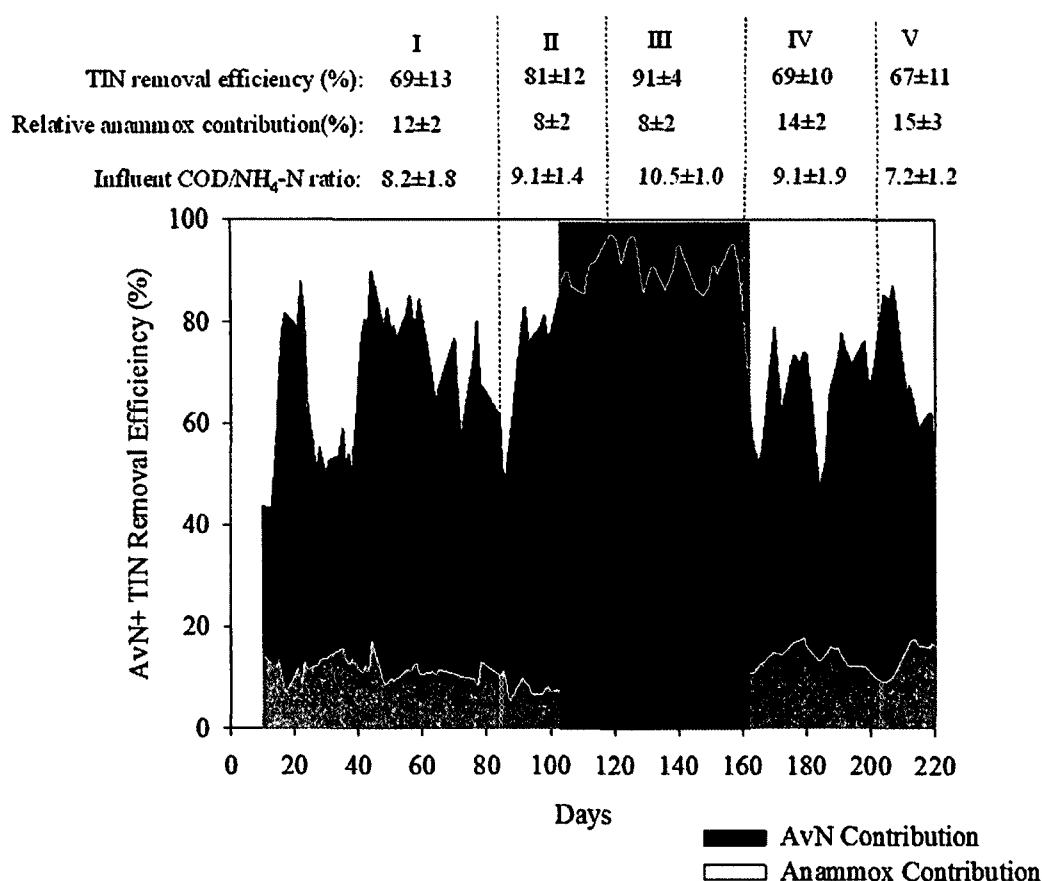


Figure 56. The TIN removal performance of the AvN+ process showing relative contribution from AvN and anammox MBBR during the study period at different influent COD/NH₄-N ratio. Acetate was added to anammox MBBR the days of 104 to 162.

In Phases I, IV and V, AvN+ was operated at a very low HRT (Table 28). The overall TIN removal efficiencies during these periods were similar, however, increased TIN removal contribution by anammox was observed (Figure 56). The high TIN removal contribution from anammox was due to relatively high NAR in the AvN effluent during those periods (Table 30). In fact, during Phase V TIN removal efficiency of 67% was achieved at an influent COD/NH₄⁺-N ratio of only 7.2. Consequently, it shows that AvN+ can be operated within a small volume to achieve relatively high nitrogen removal performance even with an aggressively operated upstream COD recovery system. Such performance was made possible by NO₂⁻ accumulation in the AvN process which allowed downstream nitrogen polishing *via* anammox metabolism.

AvN NOB out-selection and overall performance

The TIN removal performance of AvN process fluctuated during the study and was dependent on the influent COD/NH₄⁺-N ratio (Table 30). The NH₄⁺-N loading rate to the AvN process was varied by changing the HRT of the system during different phases of the study. During the Phase IV, the average NH₄⁺-N loading rate was 339 mgN/L/d with average TIN removal rate and TIN removal efficiency of 172 mgN/L/d and 51% respectively at an average influent COD/NH₄⁺-N ratio of 9.1 (Table 30).

The low average TIN removal efficiency during Phase IV was further inflicted by a sudden loss of mixed liquor suspended solids (MLSS) on day 178 due to clarifier malfunction (Figure 57). The consequence of rapid loss of AOB population during Phase IV was the increase in the aerobic fraction (which is controlled to maintain effluent NH₄⁺-N = effluent NO_x-N), which causes more aerobic oxidation COD, and less COD being available for NO_x-N reduction (Table 30). The result of this is reflected in a high ratio of COD removal rate and TIN removal rate (14.0±3.1) during Phase IV (Table 30). Similarly, in Phase I the ratio of COD removal rate and TIN removal rate was 14.0±4.1. The reason of this inefficient utilization of influent COD for nitrogen removal can be attributed to the low AOB rates during startup and sudden loss of mixed liquor that occurred on day 62 and overall high aerobic fraction (0.55±0.10) (Figure 57, Table 30).

In Phases II, III and V the ratio of COD removal rate to TIN removal rate were lower and the aerobic fraction also remained lower (Table 30).

Table 30. Performance and other relevant data of AvN during the study period of 220 days. (Average±Standard Deviation).

Phase	I	II	III	IV	V
HRT (hr)	3	4	6	2	3
NH ₄ ⁺ -N loading rate (mgN/L/d)	276±35	228±32	130±11	339±35	222±26
TIN removal rate (mgN/L/d)	142±56	157±59	105±9	172±38	104±46
TIN removal efficiency (%)	51±18	70±15	82±5	51±11	46±15
Influent COD/NH ₄ ⁺ -N	8.2±1.8	9.1±1.4	10.5±1	9.1±1.9	7.2±1.2
COD removal rate/TIN removal rate	14.0±4.1	11.2±2.1	11.1±1.3	14.0±3.1	11.7±1.3
Aerobic Fraction	0.55±0.10	0.43±0.06	0.38±0.04	0.60±0.10	0.36±0.07
NH ₄ ⁺ -N loading rate/AOB rate*	0.97±0.21	0.68±0.25	0.53±0.10	0.88±0.15	0.65±0.23
NAR	0.35±0.1	0.20±0.05	0.16±0.05	0.36±0.08	0.37±0.05
NOB rate/AOB rate * (%)	66±17	81±13	100±8	69±7	65±7
SRT (d)	7.3±2.6	6.2±1.2	4.6±0.8	5.5±1.6	3.3±0.5
MLSS (mg/L)	3336±808	3888±661	2675±451	3310±603	1790±512
SVI (mL/g)	138±47	133±29	111±27	120±29	133±19
Effluent NO _x -N/Effluent NH ₄ ⁺ -N	1.06±0.22	0.99±0.34	1.23±0.18	1.02±0.22	1.09±0.15

*From weekly *ex-situ* batch measurements for AOB and NOB maximum activity

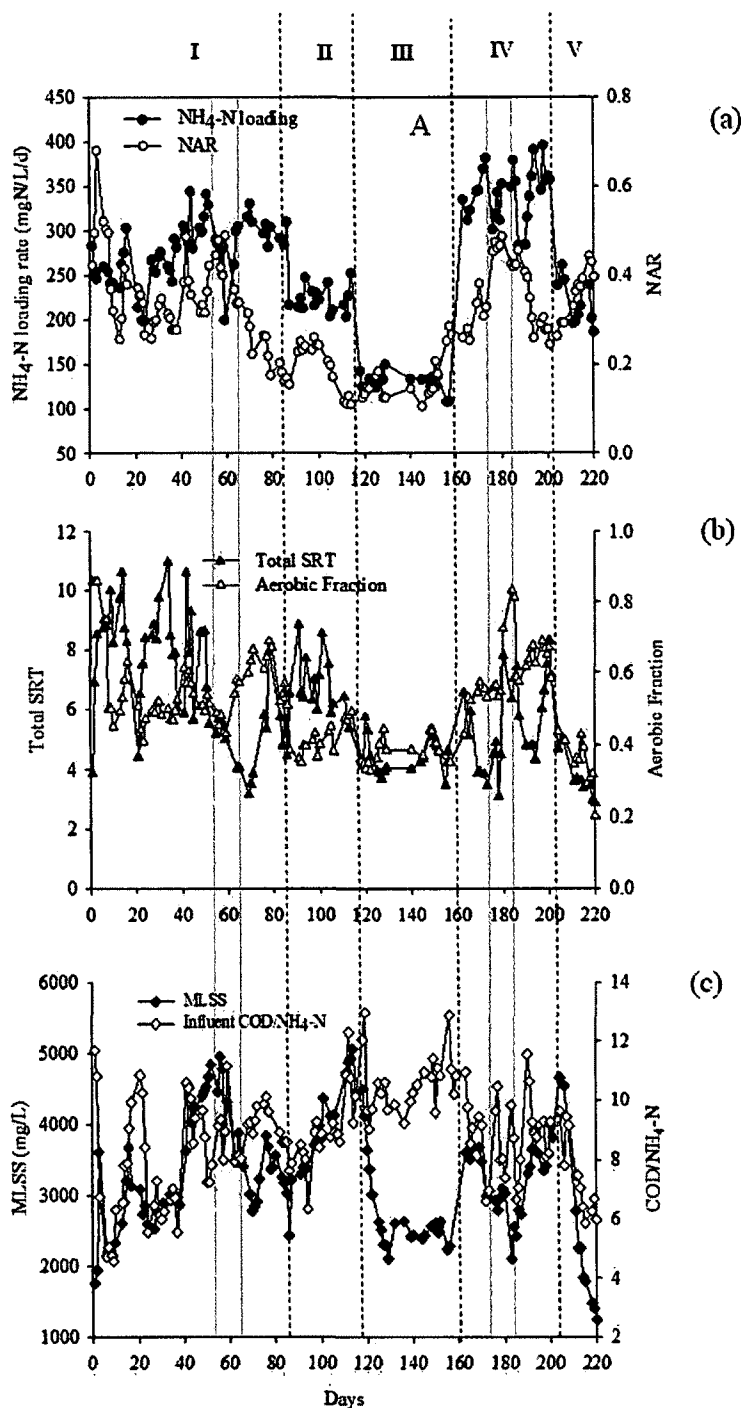


Figure 57. Trends of key parameters for the assessment of NOB out-selection a) $\text{NH}_4^+\text{-N}$ loading rate and nitrite accumulation ratio b) Total AvN SRT and aerobic fraction c) Mixed liquor suspended solids and influent COD/ $\text{NH}_4^+\text{-N}$ ratio. Note: There was a sudden drop in mixed liquor due to clarifier malfunction on day 62 and 178.

NOB out-selection was indicated through *ex-situ* maximum AOB and NOB activity measurements, NAR, and targeted molecular analysis for bacterial populations. The trends and averages of NAR during different phases of the study can be seen in Figure 57a and Table 30, respectively. The trends presented in Figure 58b show that maximum AOB activity remained greater than maximum NOB activity during most of the study duration. The results of the targeted molecular analysis for AOB, NOB (*Nitrobacter sp.* and *Nitrospira sp.*) and total bacterial population further substantiates that NOB population declined during the period of low NOB activity (Figure 58a).

The role of maintaining a residual effluent $\text{NH}_4^+\text{-N}$ (>1.5 mg N/L), operating at high DO (>1.2 mg $\text{O}_2\text{/L}$) and a short SRT in an intermittently aerated system (providing heterotrophic competition for $\text{NO}_2^-\text{-N}$) for NOB out-selection is documented by Regmi et al. (2014). The latter strategies were used in this study as well for NOB out-selection, however, the aggressive operation brought upon by changing the influent $\text{NH}_4^+\text{-N}$ loading was used as an additional feature to control NOB. The $\text{NH}_4^+\text{-N}$ loading rate had a positive relationship with NAR and therefore NOB out-selection except on two occasions when there was a sudden biomass loss (Figure 57a). When the system is operated aggressively towards wash out of AOB, the ratio of $\text{NH}_4^+\text{-N}$ loading rate/AOB rate is expected to be higher. This ratio remained high during Phases I and IV and NOB out-selection as indicated by NAR of 0.35 and 0.36, respectively (Table 30). However, the ratio of $\text{NH}_4^+\text{-N}$ loading rate/AOB rate was low (0.65 ± 0.23) during Phase V despite the very high NAR (0.37 ± 0.05). During Phase V, a strategy to tightly control the SRT was implemented which allowed the system to be operated at a very low SRT. The SRT during Phase V was lower than 4 days and the aerobic fraction was below 0.4 (Figure 57b). The low influent COD/ $\text{NH}_4^+\text{-N}$ during Phase V (Figure 57c) was another factor that was not expected to favor NOB out-selection due to reduced heterotrophic competition for $\text{NO}_2^-\text{-N}$ with NOB. The implications of sustaining NOB out-selection at low COD/ $\text{NH}_4^+\text{-N}$ would allow more COD capture upstream for energy production as well as increase the relative nitrogen removal contribution by anammox. Consequently, savings in terms of B-stage tank volume, aeration energy, and chemical additions can be maximized for more sustainable nitrogen removal.

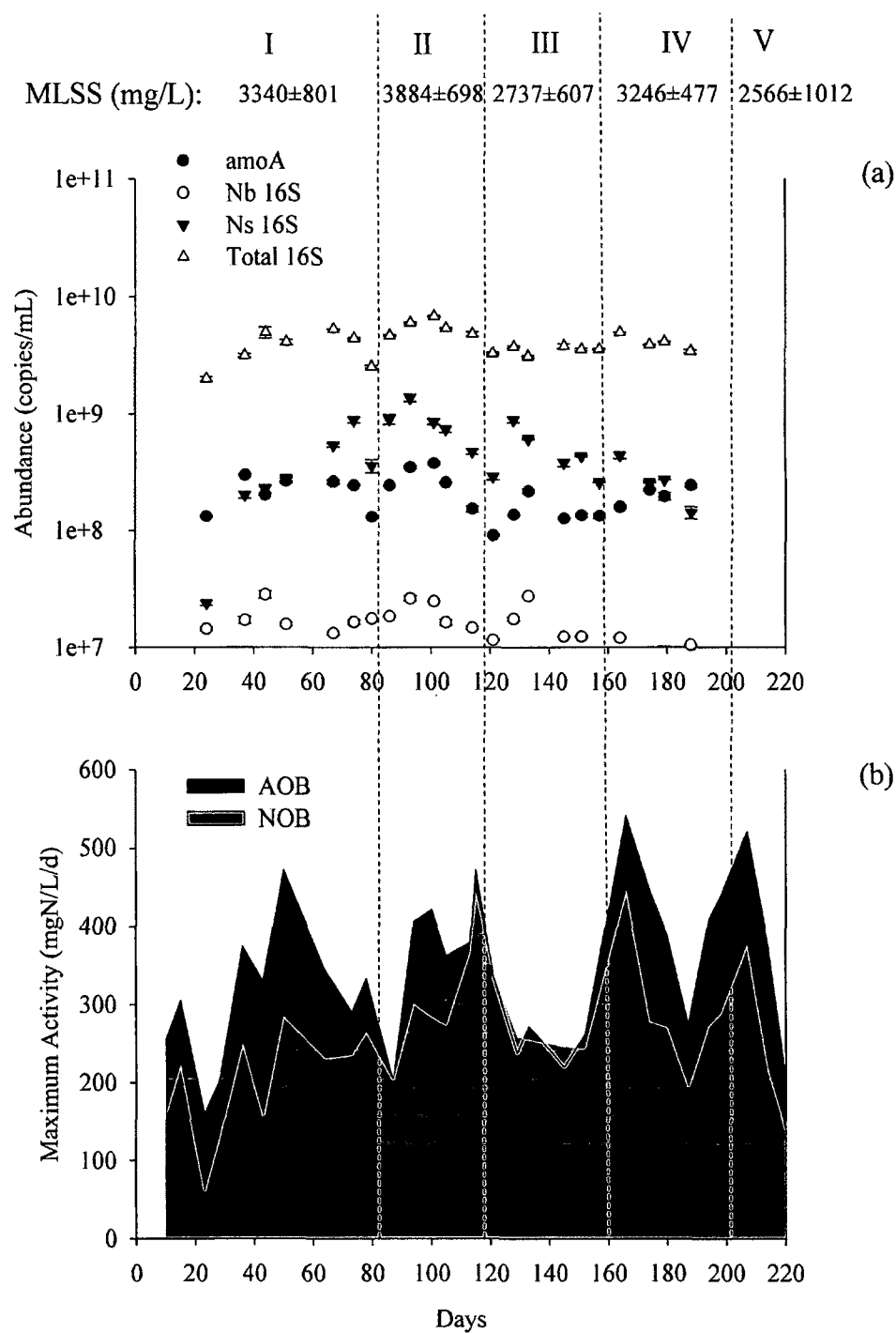


Figure 58. Trends of microbial populations (AOB, NOB and total bacteria) presented as copies of DNA per mL of sample from targeted qPCR (a) and weekly AOB and NOB activities (b).

Anammox MBBR Overall Performance and Nitrate Removal

The TIN removal efficiency of the anammox MBBR was $38 \pm 12\%$. The TIN removal in the anammox MBBR was limited by the influent NO_2^- -N concentration as near complete NO_2^- -N removal was observed (Table 29). Since NO_2^- -N was the limiting substrate for the anammox reaction, the TIN removal rate responded to the changes in the NO_2^- -N loading rate (Table 31). During Phase IV, the TIN removal rate (0.36 ± 0.07 gN/m²/d) as well NO_2^- -N loading rate (0.182 ± 0.051 gN/m²/d) were highest for the entire study period (Table 31). The maximum TIN removal rates from batch testing were more than three times greater than observed TIN removal rates during high NO_2^- -N loading periods (Phase I, IV, and V) (Table 31). During the low NO_2^- -N loading periods (Phase II and III), the maximum TIN removal rates were more than eleven times greater than the observed TIN removal rates (Table 31). This relatively high enrichment of AMX in a biofilm provides the stability to the nitrogen polishing step providing safety during periods of peak loads. The stoichiometric ratio associated with anammox reaction during maximum activity measurements were close to theoretical values proposed by Strous et al., (1998). The biomass density increased steadily during the course of the study reaching 5.8 g/m² from the initial biomass density of 2.7 g/m² (Table 31). Although the NO_2^- -N loading was decreased during Phases II and III, the biomass density continued to increase (Table 31).

Table 31. Performance and other relevant data of AvN during the study period of 220 days. (Average±Standard Deviation).

Parameter	Phase I	Phase II	Phase III	Phase IV	Phase V
TIN removal rate (gN/m ² /d)	0.23±0.08	0.11±0.04	0.09±0.06	0.36±0.07	0.26±0.05
NO ₂ ⁻ -N Loading rate (gN/m ² /d)	0.118±0.05 8	0.038±0.02 1	0.012±0.00 4	0.182±0.05 1	0.129±0.04 3
TIN removal efficiency (%)	33±9	35±13	50±17	38±7	39±4
Max TIN removal rate (gN/m ² /d)*	0.71±0.06	1.35±0.16	1.03±0.09	1.17±0.20	1.06±0.11
NO ₂ ⁻ -N removed/NH ₄ ⁺ -N removed*	1.47±0.20	1.42±0.02	1.45±0.14	1.49±0.13	1.48±0.15
NO ₃ ⁻ -N produced/NH ₄ ⁺ -N removed*	0.29±0.06	0.26±0.02	0.23±0.04	0.22±0.06	0.33±0.10
Biomass density (g/m ²)**	2.7±0.33	3.3±0.21	3.7±1.1	5.1±2.0	5.8±1.0

*From weekly *in-situ* AMX maximum activity measurements. **From bi-weekly biomass density measurements of the MBBR media. The acetate was added to the anammox MBBR at the middle of phase II and entire period during phase III.

With the goal of improving TIN removal efficiency, a limited amount of acetate was added to the anammox MBBR from days 104-162, which included half of Phase II and the entire Phase III. During Phase III, the TIN removal efficiency averaged 50%, which was highest among all phases despite receiving the lowest amount of NO₂⁻-N (Table 31). This was possible due to the addition of acetate which was responsible for NO₃⁻-N removal (Figure 59). In fact, the NO₃⁻-N removal during the acetate addition was 2-3 times more than NO₃⁻-N removal that was observed without acetate addition (Figure 59).

Further, the $\text{NH}_4^+\text{-N}$ removal in the anammox MBBR during acetate addition was much higher than that could be accounted for by the anammox stoichiometric value ($\text{NO}_2^-\text{-N}$ removed: $\text{NH}_4^+\text{-N}$ removed = 1.32). The ratio of $\text{NO}_2^-\text{-N}$ removed: $\text{NH}_4^+\text{-N}$ removed was almost three times lower during the period of acetate addition (Figure 59). On the contrary the ratio of $\text{NO}_x\text{-N}$ removed: $\text{NH}_4^+\text{-N}$ removed was closer to the anammox stoichiometric value (Figure 59). This suggests that $\text{NO}_3^-\text{-N}$ reduction to $\text{NO}_2^-\text{-N}$ was supplying AMX their substrate for anaerobic $\text{NH}_4^+\text{-N}$ oxidation. In limited carbon addition (acetate) conditions certain species of AMX are known to reduce $\text{NO}_3^-\text{-N}$ to $\text{NO}_2^-\text{-N}$ to create their own substrate for $\text{NH}_4^+\text{-N}$ oxidation (Kartal et al., 2007). However, heterotrophic denitrification in carbon limited conditions could also provide $\text{NO}_2^-\text{-N}$ for anammox metabolism.

The molecular results reveal that both mechanisms might be responsible. The fully anoxic anammox MBBR was primarily colonized by AMX bacteria, which can be observed from the total 16S and AMX 16S qPCR measurements which were very close to one another (Figure 60). However, when the acetate addition was initially started, the gap between the AMX 16S and total 16S measurements widened indicating possible increase of heterotrophic population (Figure 60). Further, the difference in AMX 16S and total 16S measurements got smaller during the second half of the acetate addition period (Figure 60). This could be indicative of either AMX developed the capabilities to reduce $\text{NO}_3^-\text{-N}$ to $\text{NO}_2^-\text{-N}$ or the new species of AMX with such capability were selected within three weeks of acetate addition. However, the changes in AMX abundance over 2-3 weeks periods can be considered puzzling and needs further investigation in future considering slow growth and decay rates of AMX. Either of the two mechanisms are not likely to significantly affect advantages associated with $\text{NO}_3^-\text{-N}$ removal in an anammox reactor with limited acetate addition.

$\text{NO}_2\text{-N removed} / \text{NH}_4\text{-N removed}$:	1.02 ± 0.25	0.36 ± 0.11	0.92 ± 0.12
$\text{NO}_x\text{-N removed} / \text{NH}_4\text{-N removed}$:	1.19 ± 0.19	1.43 ± 0.26	1.22 ± 0.15
$\text{NO}_3\text{-N removed (mgN/L)}$:	0.31 ± 0.52	1.24 ± 0.33	0.60 ± 0.36

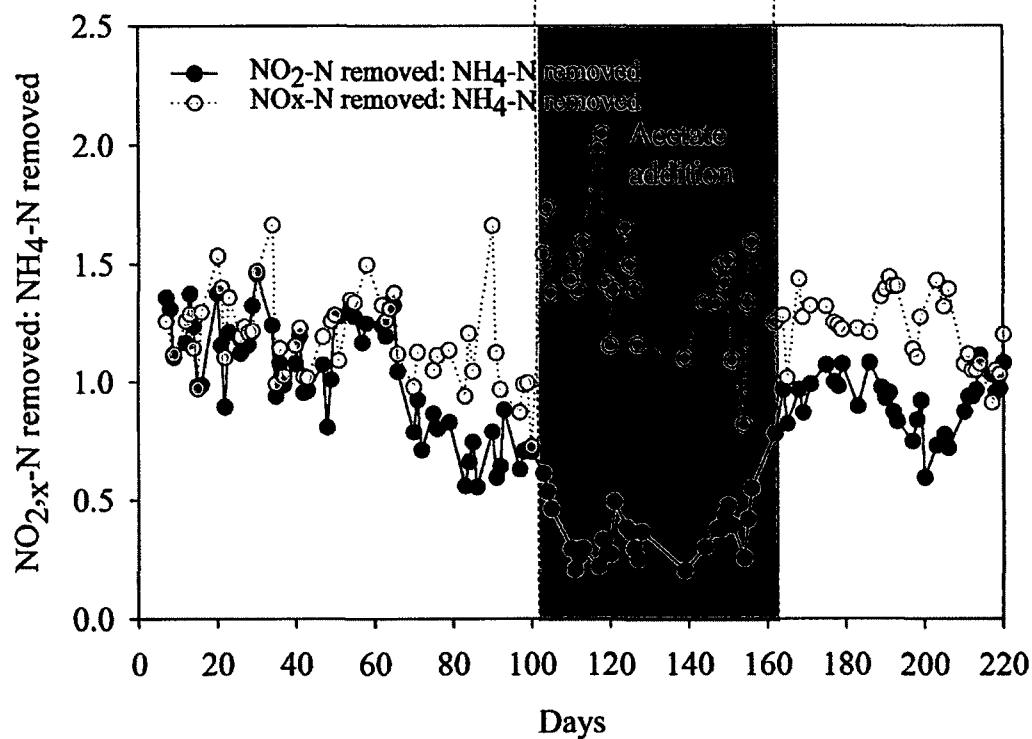


Figure 59. The ratio of $\text{NO}_2\text{-N removed} : \text{NH}_4^+\text{-N removed}$ and ratio of $\text{NO}_x\text{-N removed} : \text{NH}_4^+\text{-N removed}$. Acetate ($\text{COD}/\text{NO}_3\text{-N} = 0.9 \pm 0.6$) was added to the anammox MBBR between day 104 and day 162.

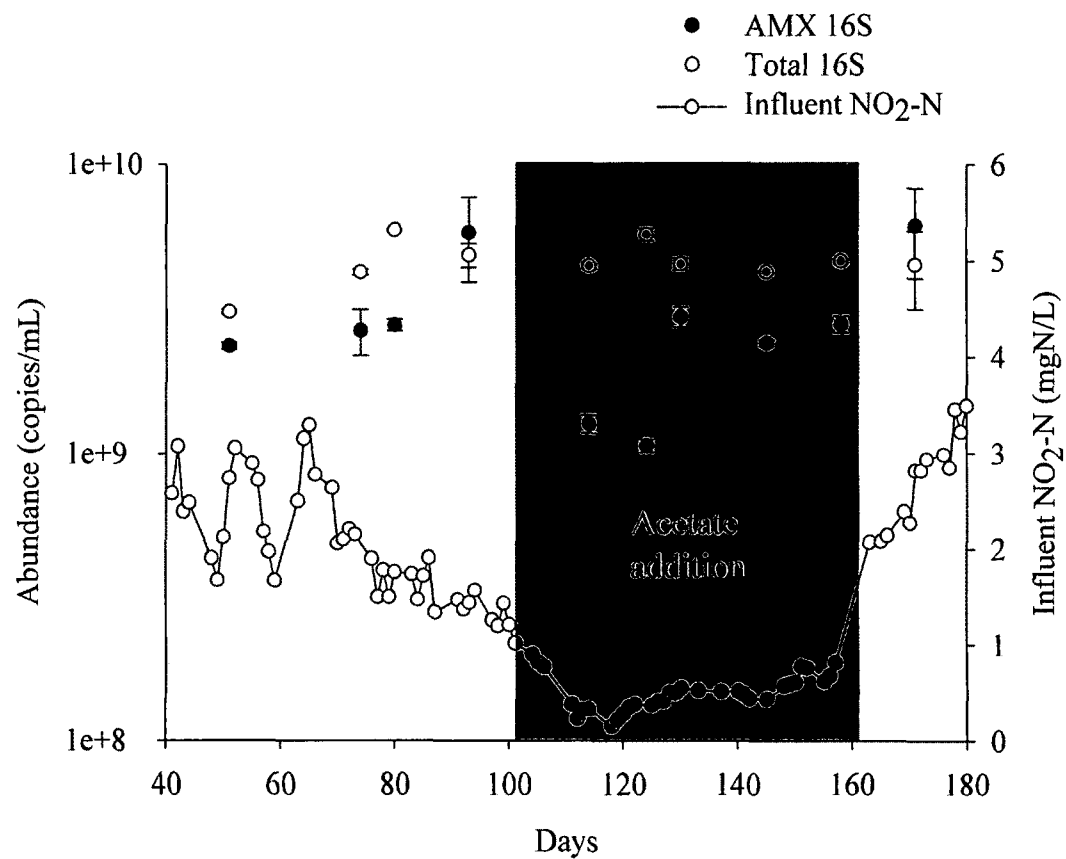


Figure 60. Trends of abundance of AMX bacteria and total bacteria and influent $\text{NO}_2\text{-N}$ during acetate addition (day 104-162) and before and after that period.

8.4 Conclusions

The AvN+ implemented downstream of a high rate activated sludge process (HRT = 30 min) operated at an average COD removal efficiency of 50% demonstrated average TIN removal efficiency of 75% at 24 °C. When the AvN was operated aggressively (i.e., low HRT and short SRT) NOB out-selection was more rampant than when it was operated with long HRT and SRT. The *ex-situ* maximum activity tests revealed that the ratio of NOB rate to AOB rate was 0.75 ± 0.18 during the study.

To meet stringent effluent nitrogen permit, energy and resource intensive tertiary treatments are used to eliminate remaining few percentages of nitrogen, which accounts for almost half of GHG emissions of the entire plant (Falk et al., 2013). In this study, nitrogen polishing in a small foot-print anammox MBBR without aeration and with little to no addition of external carbon sources (COD/NO₃⁻-N ratio of 0.9 ± 0.6) was demonstrated. Nitrate production during the anammox reaction often limits the nitrogen removal through anammox metabolism. The removal of nitrate and thus induced ammonia removal by a post-polishing MBBR with anammox was shown, which extends the applicability of anammox for mainstream nitrogen removal to meet lower effluent nitrogen permits at a limited external carbon dosage.

Therefore, the AvN+ system exploiting short-cut nitrogen removal showed that COD capture for energy production is possible without compromising nitrogen removal performance.

CHAPTER 9

CONCLUSIONS AND FUTURE PERSPECTIVES

In the context of rapid urbanization and population growth, the wastewater industry is faced with increasingly stringent regulations in order to protect receiving water quality. The very low nitrogen discharge limits intended to protect water quality often requires treatment technologies that are not sustainable. Technological advancements in wastewater treatment often follow changes to the regulatory requirements that demand increased efficiency and reduced capital or operating costs to remain sustainable. Innovative wastewater treatment technologies are expected to consider and minimize energy (e.g., electricity, natural gas, liquid fuels for transportation), chemicals (e.g., external carbon, alkalinity, polymers), and infrastructure (e.g., concrete, real estate/facility footprint) associated with advanced treatment requirements. In the U.S., the focus on restricting nutrient discharge has outweighed sustainability of corresponding treatment. Further, restrictions on nutrient discharge in nutrient-sensitive regions of the U.S. have resulted in incremental changes to conventional technologies that are inefficient and costly. Innovations in wastewater sustainability have primarily been centered in Europe over the last several decades. However, technical and financial challenges of meeting these regulations sustainably are forcing wastewater treatment in the U.S. to minimize and recover resources where possible. Recently, the rate of adoption of innovative technologies among wastewater utilities in the U.S. is increasing driven by a desire to reduce aeration energy demands, recover resources, reduce the footprint of treatment plants, and tighten design criteria. The very innovative and efficient sidestream deammonification is a prime example of such technology.

Traditional biological nitrogen removal systems are based on the conventional activated sludge systems designed predominantly for carbon removal. In these systems nitrogen and carbon removal occur within a single sludge as multiple microbial groups (e.g., ordinary heterotrophic organisms or OHO; ammonia oxidizing bacteria or AOB; nitrite oxidizing bacteria or NOB; polyphosphate accumulating organisms or PAO) co-exist

across a range of redox conditions. The shortcoming of combined carbon and nitrogen removal systems is that microbial groups spend a significant amount of time functioning outside the optimal growth range which impedes overall performance. Moreover, large safety factors are needed for sensitive microbial groups such as AOB (ammonia removal), resulting in more than adequate conditions for less sensitive heterotrophs (carbon removal). The A-B process is a two sludge system that consists of a very high-rate activated sludge (HRAS) A-stage for carbon removal followed by another activated sludge process, a B-stage for nitrogen removal. A highly compact A-stage concentrates carbon present in the raw wastewater, which can be digested to produce electricity. However, low carbon to nitrogen ratio in the B-stage limits the conventional nitrification-denitrification to meet nitrogen discharge limits sustainably. Short-cut nitrogen removal in the B-stage requiring less aeration and carbon could result in highly efficient wastewater treatment. NOB out-selection is key to the short-cut nitrogen removal, which has been easy to achieve in high ammonia and temperature wastewater. The mainstream NOB out-selection remains a topic of research and is critical for success of the energy efficient A-B process to achieve cost-effective nitrogen removal. The short-cut nitrogen pathway, exploiting AOB and AMX organisms (i.e., deammonification), is the most sustainable approach to remove nitrogen from wastewater. However, AMX are slow growing and require very long SRTs. The retention of AMX and enrichment to the significant extent to be dominant pathway for nitrogen removal in mainstream is a challenge.

This dissertation deals with challenges of implementation of mainstream deammonification in a highly efficient A-B process. The A-stage was a high rate activated sludge system for carbon removal and the B-stage consisted of activated sludge system that targeted NOB out-selection which was followed by a fully anoxic anammox MBBR. The major outcomes of this study can be summarized as follows:

An A-B process pilot study was conducted over a two-year period. The A-B pilot separated the carbon and nitrogen removal metabolic processes into distinct treatment steps. A high rate A-stage ($\text{HRT} = 30 \text{ min}$) was operated with carbon removal efficiency of $\sim 50\%$. B-stage activated sludge process was operated under controlled intermittent aeration targeting effluent ammonia and NO_x equal in the effluent, short SRT and HRT to achieve NOB out-selection and efficient nitrogen removal with low influent $\text{COD}/\text{NH}_4\text{-N}$ ratio (<10).

Anammox was implemented in a fully anoxic MBBR that received effluent from the activated sludge process. In a unique startup strategy, the anammox reactor filled with biofilm carriers was seeded with full-scale sidestream anammox granules with a temporary clarifier and a recycle line. The anammox activity was observed within 2 weeks of seeding. The nitrite concentration in the effluent of activated sludge process limited the overall nitrogen removal contribution by anammox.

The limited NOB out-selection in the activated sludge process resulted in nitrate in the effluent. With limited acetate addition ($\text{COD}/\text{NO}_3\text{-N} \sim 1$) the MBBR demonstrated nitrate removal and corresponding stoichiometric ammonia removal through anammox metabolism. A very high maximum anammox activity of $>1 \text{ gN}/\text{m}^2/\text{d}$ was achieved which provided high degree of stability since the nitrogen loading to the MBBR was below $0.25 \text{ gN}/\text{m}^2/\text{d}$ for the most part of the study.

Therefore, the application of mainstream deammonification as shown in this study has the potential to be a true game-changer and a new model for cost-efficient, space saving and potentially energy-neutral wastewater treatment. It not only reduces operational energy requirements and external carbon dosage, but also allows for more efficient use of the wastewater carbon for energy production. As this technology becomes available, it can be employed by utilities around the world to help preserve receiving waterways from the impacts of excessive nitrogen loading, and all at a reduced energy and land-use cost. In this context, integration of findings from this study into wastewater treatment plant

design criteria will provide opportunities for developing countries to leapfrog toward a more sustainable alternative.

The deployment of the technology developed in this study at a large scale would greatly benefit from strategic development of processes tailored around constraints of wastewater treatment throughout the world.

The prospect of converting wastewater treatment plants from consumers to the producers of energy is a fundamentally far-reaching idea that has the potential to change the entire industry and its impact on the environment and the people. The opportunities to mitigate short-comings and inefficiencies that have crippled the expansion and implementation of biological nitrogen removal technologies in terms of capital and operational cost, carbon and physical foot-print, energy and chemical usage can hardly be over-stated. This study involved a systematic and science-based characterization of the different factors and conditions, which are crucial for the successful full-scale implementation of mainstream deammonification or mainstream nitrite shunt. From a fundamental perspective, it has provided a significant knowledge into the microbial ecology, metabolic pathways and parameters of different microbial populations engaged in nitrogen-cycling in mainstream deammonification and nitrification-denitrification reactors. Development of practical design configurations and guidelines will be the next step towards the deployment of the knowledge acquired during this study. To this end computational simulation by developing process models will be a crucial and non-trivial effort due to the dynamic nature of the control systems employed in this work.

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APPENDIX

Hampton Roads Sanitation District
Chesapeake-Elizabeth Nutrient Removal Pilot Study
Process Flow Schematic

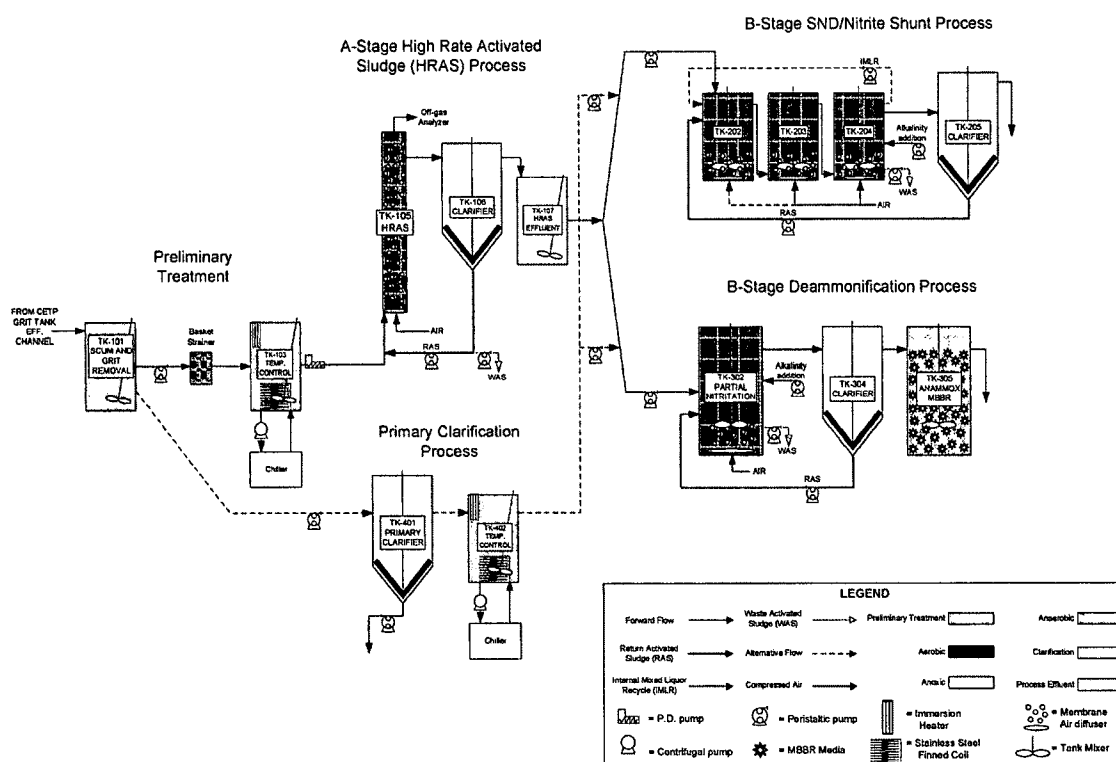


Figure A1. Process flow diagram of Pilot 1.0 with detailed preliminary treatment.

Hampton Roads Sanitation District
Chesapeake-Elizabeth Nutrient Removal Pilot Study
Process Flow Schematic

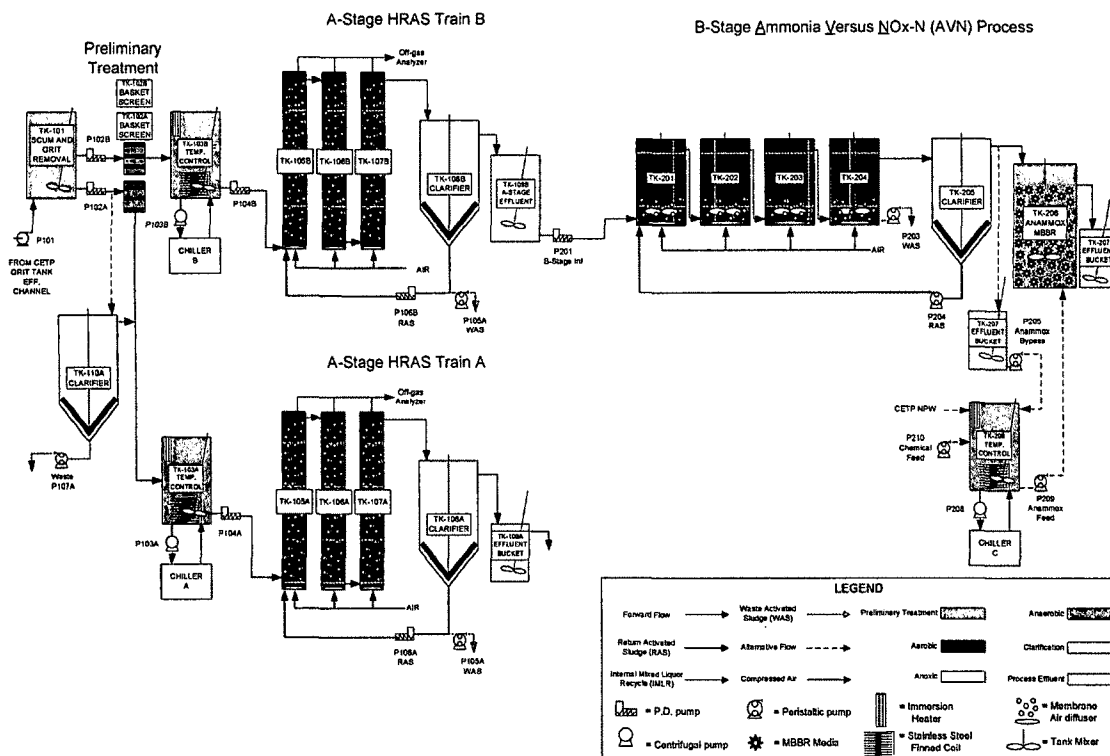


Figure A2. Process flow diagram of Pilot 2.0 with detailed preliminary treatment.

Table A1. Analyses performed by HRSD's Central Environmental Lab (CEL).

Parameter	Reference Method	Description
TSS	SM 20 th 2540D	TSS - Total Suspended Solids Dried at 103-105°C
TVSS	SM 18 th 2540E	TVSS – Fixed and Volatile Solids Ignited at 550°C
TKN	EPA 351.2 Lachat 10-107-06-2-I	Determination of Total Kjeldahl Nitrogen by Flow Injection Analysis Colorimetry (Block Digestion)
NH ₃ -N	EPA 350.1 Lachat 10-107-06-1-C	Determination of Ammonia by Flow Injection Analysis Colorimetry
NO ₂ , NO ₃ (NO ₃ by calc)	EPA 353.2 Lachat 10-107-04-1-C/A	Determination of Nitrate/Nitrite by Flow Injection Analysis Colorimetry
TP	EPA 365.1 Lachat 10-115-01-1-E	Determination of Total Phosphorous by Flow Injection Analysis Colorimetry (Acid Persulfate Digestion)
OP	Lachat 10-115-01-1-A	Orthophosphate in Waters
Alkalinity	EPA 310.2 Lachat 10-303-31-1-A	Determination of Alkalinity by Flow Injection Analysis Colorimetry
BOD	SM 18 th 5210B	Biochemical Oxygen Demand (BOD) 5 Day BOD Test
cBOD	SM 18 th 5210B	Carbonaceous Biochemical Oxygen Demand (cBOD) 5 Day BOD Test
COD	Hach 8000	Chemical Oxygen Demand, Reactor Digestion Method

Table A2. Analyses performed in the pilot lab.

Parameter	Reference Method	Description (Range)
TSS	SM 20 th 2540D	TSS - Total Suspended Solids Dried at 103-105°C
NH ₃ -N	Hach 10205	Ammonia TNTplus ULR (0.015 to 2.00 mg/L NH ₃ -N)
NH ₃ -N	Hach 10205	Ammonia TNTplus LR (1 to 12 mg/L NH ₃ -N)
NH ₃ -N	Hach 10205	Ammonia TNTplus HR (2 to 47 mg/L NH ₃ -N)
NO ₃ -N	Hach 10206	Nitrate TNTplus LR (0.23 to 13.5 mg/L NO ₃ -N)
NO ₃ -N	Hach 10206	Nitrate TNTplus HR (5 to 35 mg/L NO ₃ -N)
NO ₂ -N	Hach 10019	Nitrite NitriVer 3 TNT LR (0.002 to 0.500 mg/L NO ₂ -N)
NO ₂ -N	Hach 10237	Nitrite TNTplus HR (0.6 to 6.0 mg/L NO ₂ -N)
NO ₂ -N	Hach 10207	Nitrite TNTplus LR (0.015 to 0.600 mg/L NO ₂ -N)
OP	Hach 8048	Reactive Phosphorus TNT LR (0.6 to 5.00 mg/L PO ₄ ³⁻)
OP	Hach 10214	Reactive Phosphorus TNTplus (1.6 to 30.0 mg/L PO ₄ ³⁻ -P)
TP	Hach 10209	Total Phosphorus TNTplus (0.5 to 5 mg/L P)
COD	Hach 8000	COD TNTplus HR (20 to 1500 mg/L COD)
COD	Hach 8000	COD TNTplus LR (3 to 150 mg/L COD)
COD	Hach 8000	COD TNTplus ULR (1 to 60 mg/L COD)
Cl ⁻	Silver Titrant	Chloride (5 to 400 mg/L Cl ⁻)
K ⁺	Hach 8049	Potassium (0.1 to 7.0 mg/L K ⁺)
S ²⁻	LaMotte 4630	Pomeroy Methylene Blue Method (0 to 18 ppm S ²⁻)

A1. AOB and NOB Molecular Sampling

Molecular sampling was performed on a weekly basis. Grab samples were collected from AvN and AvN CSRT and 1.5 mL was transferred into a 1.7 mL micro centrifuge tube. The vial was placed into the centrifuge at 0°C and turned on for 3 minutes at 13,000 rpm. Supernatant was discarded. The vial containing the biomass was then filled with 1.5 mL of RNA Protect Solution and the biomass was re-suspended in this solution using a vortex mixer. Vials were incubated at room temperature for a period of 5 minutes and then placed back into the centrifuge at 0°C for 3 minutes at 13,000 rpm. Supernatant was discarded and samples were labeled with the date and immediately stored on dry ice and transferred to HRSD's Central Environmental Laboratory (CEL) for storage in freezer at -80°C. Vials were then shipped via Fed-Ex to Columbia University for qPCR analysis.

A2. AMX Molecular Sampling

Molecular sampling was performed on a bi-weekly basis, the same week as biomass density was performed. Kaldnes K3 media pieces were collected by a grab sample from the anammox MBBR. Three anammox media pieces were placed into approximately 50 mL of Tris-Acetate-EDTA 1x solution and swirled to remove any excess biomass not attached to the media. Using tweezers that were sterilized with isopropyl alcohol and an RNase AWAY Surface decontaminant, biomass was transferred from one media piece into a 1.7 mL micro centrifuge tube, with a minimum amount of 0.1 mL of biomass in the centrifuge tube (one piece of media per tube). The vial was placed into the centrifuge at 0°C and turned on for 3 minutes at 13,000 rpm. Supernatant was discarded. The vial containing the biomass was then filled with 1.5 mL of Tris-Acetate-EDTA 1x solution and the biomass was re-suspended in this solution using a vortex mixer. Vials were placed back into the centrifuge at 0°C for 3 minutes at 13,000 rpm. Supernatant was discarded and samples were labeled with the date and immediately stored on dry ice and transferred to HRSD's Central Environmental Laboratory (CEL) for storage in freezer at -80°C. Vials were then shipped via Fed-Ex to Columbia University for qPCR analysis.

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Winner, 5th Annual VA AWWA Student Water Challenge at Water JAM, 2008

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Golden Key International Honor Society, 2008

Chi Epsilon (*The Civil Engineering Honor Society*), 2011

PUBLICATIONS: PEER REVIEWED JOURNAL ARTICLES

- Regmi, P.**, Miller, M.W., Holgate, B., Bunce, R., Park, H., Chandran, K., Wett, B., Murthy, S., and Bott, C.B. 2014. Control of aeration, aerobic SRT and COD input for mainstream nitrification/denitrification. *Water Research*, 45(20), 6699-708.
- Regmi, P.**, Schafran, G., Thomas, W., Bott, C.B., Rutherford, B., and Waltrip, D. 2011. Nitrogen removal assessment through nitrification rates and media biofilm accumulation in an IFAS process demonstration study. *Water Research*, 45 (20), 6699-6708
- Regmi, P.**, Garcia Moscoso, J.L., Kumar, S., Cao, X., Mao, J., Schafran, G. 2012. Use of switchgrass derived HTC biochar for removing copper and cadmium from aqueous solution. *Journal of Environmental Management*, 109, 61-69.
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- Regmi, P.**, Bunce, R., Miller, M.W., Park, H., Chandran, K., Wett, B., Murthy, S., and Bott, C.B. 2014. Ammonia-based intermittent aeration control optimized for short-cut nitrogen removal (submitted).

PUBLICATIONS: SELECTED CONFERENCE PROCEEDINGS AND PRESENTATIONS

1. **Regmi, P.**, Holgate, B., Miller, M.W., Park, H., Chandran, K., Wett, B., Murthy, S., and Bott, C.B. 2013. NOB Repression for mainstream nitrite-shunt and deammonification: a pilot study. *Proceedings of the 86th Annual Water Environment Federation Technical Exposition and Conference*, Chicago, Illinois, October 5-9, 2013.
2. Bunce, R., Miller, M.W., **Regmi, P.**, Hingley, D., Kinnear, D., and Bott, C.B. 2013. Evaluation of a pilot scale A/B process utilizing SND and nitrite-shunt with a hydrocyclone for improved settleability. *Proceedings of the 86th Annual Water Environment Federation Technical Exposition and Conference*, Chicago, Illinois, October 5-9, 2013.
3. **Regmi, P.**, Miller, M.W., Holgate, B., Bunce, R., Park, H., Chandran, K., Wett, B., Murthy, S., and Bott, C.B. 2013 NOB out-selection in mainstream makes two-

- stage deammonification and nitrite-shunt possible. *Proceedings of WEF/IWA the Nutrient Removal and Recovery Conference*, July 28-31, 2013.
4. Bunce, R., Miller, M.W., **Regmi, P.**, Hingley, D., Kinnear, D., and Bott, C.B. 2013. Modification of a B-stage MLE to take advantage of SND and nitrite shunt in an A/B Process pilot study. *Proceedings of the WEF/IWA Nutrient Removal and Recovery Conference*, July 28-31, 2013.
 5. Jimenez, J., Bott, C.B., **Regmi, P.**, and Rieger, L. 2013. Process control strategies for simultaneous nitrogen removal systems. *Proceedings of the WEF/IWA Nutrient Removal and Recovery Conference*, July 28-31, 2013.
 6. Melcer, H., Bott, C.B., **Regmi, P.**, Rieger, L., Wan, J., Johnson, C., Chandran, K., and Ma, Y. 2013. Measuring nitrite -the key to controlling deammonification systems. *Proceedings of the WEF/IWA Nutrient Removal and Recovery Conference*, July 28-31, 2013.
 7. **Regmi, P.**, Miller, M.W., Bunce, R., Hingley, D., Kinnear, D., Wett, B., Murthy, S., and Bott, C.B. 2012. Nitrogen removal 3.0: a pilot study to evaluate the feasibility of mainstream deammonification. *Proceedings of the 85th Annual Water Environment Federation Technical Exposition and Conference*, New Orleans, Louisiana, September 29-October 3, 2012.
 8. Miller, M.W., Bunce, R., **Regmi, P.**, Hingley, D.M., Kinnear, D., Murthy, S., Wett, B., and Bott, C.B. 2012. A/B Process pilot optimized for nitrite shunt: high rate carbon removal followed by BNR with ammonia-based cyclic aeration control. *Proceedings of the 85th Annual Water Environment Federation Technical Exposition and Conference*, New Orleans, Louisiana, September 29-October 3, 2012.
 9. **Regmi, P.**, Schafran, G., Rutherford, B., Waltrip, D., Baumler, R., Thomas, W., and Bott, C.B. 2009. Relationships between operational conditions and nitrogen removal and media biofilm accumulation in an IFAS process demonstration study. *Proceedings of the 82nd Annual Water Environment Federation Technical Exposition and Conference*, Orlando, Florida, October 10-14, 2009.
 10. Thomas, W., Bott, C.B., **Regmi, P.**, Schafran, G., Pinto, A., Love, N.G., McQuarrie, J., Rutherford, B., Baumler, R., and Waltrip, D. 2009. Evaluation of

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PATENTS

U.S Non-provisional based on Provisional Application No. US20140091035A1
 U.S Non-provisional based on Provisional Application No. US20140069864A1
 U.S Non-provisional Application No. US20140091035A1
 International Publication No. WO2014/043547A1

REVIEWER ACTIVITY

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