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Partial nitritation-anammox using pH-controlled aeration in submerged attached growth bioreactors

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PARTIAL NITRITATION-ANAMMOX USING pH-CONTROLLED AERATION IN SUBMERGED ATTACHED GROWTH BIOREACTORS

by

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CERTIFICATE OF APPROVAL

MASTER'S THESIS

This is to certify that the Master's thesis of

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has been approved by the Examining Committee
for the thesis requirement for the Master of Science
degree in Civil and Environmental Engineering at the May 2014 graduation.

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Gene F. Parkin, Thesis Supervisor

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Craig L. Just, Thesis Supervisor

_____
Timothy E. Mattes

To my loving parents, who raised me to cherish and protect the environment.

We shall never achieve harmony with land, any more than we shall achieve absolute justice or liberty for people. In these higher aspirations, the important thing is not to achieve but to strive.

-Aldo Leopold

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ABSTRACT

Total nitrogen and ammonia removal is a continuing challenge for wastewater treatment plants, especially those in the rural Upper Midwest United States. These challenges primarily consist of aeration costs and low removal efficiency in the winter. Submerged attached growth bioreactors (SAGBs) have been previously shown to remove total nitrogen and ammonium with timer-controlled aeration in cold climates. SAGB1 and SAGB2 were designed to remove total nitrogen via nitrification-denitrification with timer-controlled aeration. SAGB3 and SAGB4 with pH-controlled aeration were designed to remove total nitrogen and ammonium via partial nitritation-anammox from a low organic carbon synthetic wastewater at 20°C. Each SAGB was seeded with primary effluent from a local wastewater treatment plant and anammox seed from a wastewater treatment plant in Virginia that performs partial nitritration-anammox. Data, including nitrogen speciation, dissolved oxygen, pH, total organic carbon, and chemical oxygen demand, were routinely collected from each SAGB during a 48-hr study that occurred 3 months after anammox seeding. Effluent samples were taken every 30 minutes for the first 10 hours of the 48-hr study. Effluent data show that SAGB1 and SAGB2 removed 45% total nitrogen and 100% ammonium, while SAGB3 and SAGB4 removed 48-53% total nitrogen and 36-67% ammonium. It is estimated that 9.6-15.6 mg N/L and 7.3-15.8 mg N/L was removed via anammox in SAGB3 and SAGB4, respectively. Sample port data, collected every 2 hours, show an average accumulation of 1.7 mg NO_2-N/L in SAGB3 and up to 0.4 mg NO_2-N/L in SAGB4, which would facilitate anammox. The data also show 6-8 mg total inorganic nitrogen (TIN)/L less than the influent TIN in SAGB3 and SAGB4. Results from a DNA study suggest that anammox DNA was present in each SAGB and the anammox seed used in inoculation, 14 weeks after the 48-hr study. Over a 71-hr period, ammonium was removed (k=0.54/d) in the anaerobic anammox seed, suggesting that anammox activity occurred. Multiple lines of converging evidence,

including anammox inoculation, nitrite accumulation, and TIN removal, suggest that SAGB3 and SAGB4 operated in partial nitritation-anammox mode during the intensive study.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1

INTRODUCTION

1.1 Background

Excess nitrogen loading from agricultural runoff and wastewater discharges in the Mississippi River Basin has created a hypoxic dead zone the size of New Jersey in the Gulf of Mexico each year. As a result, it is expected that total nitrogen will be regulated in wastewater discharges in the near future in many states. In fact, the Iowa Department of Natural Resources (IDNR) has already begun regulating total nitrogen in wastewater effluent on new National Pollutant Discharge Elimination System (NPDES) permits. The total nitrogen discharge limit will be "no more stringent than 10 mg/L" (Iowa DNR, 2013). These more stringent regulations will pose challenges to rural communities located in the Upper Midwest. Even now, rural communities and other low-population density areas, often experience wastewater treatment challenges.

Decentralized wastewater treatment can be economically challenging for small communities trying to meet regulations in a cost-efficient manner. Those systems that discharge into surface water bodies are required to meet the ammonia standards of an NPDES permit. To meet these regulations, mechanical aeration is often required to remove ammonia and biochemical oxygen demand (BOD). These aeration requirements are often a primary operating cost for most wastewater treatment plants; especially those that are decentralized. Many of these decentralized wastewater treatment plants are located in small communities that have a small tax base, which makes it difficult to generate the revenue for operation and maintenance.

A further challenge for many communities with low population densities, primarily in the Upper Midwest, is biological wastewater treatment in winter months. Biological activity slows down in cold environments, causing constituent removal efficiency to decrease as temperature decreases. The regulations for constituents are not

temperature dependent, with the exception of ammonia, and require all communities, regardless of population and capital, to meet increasingly stringent standards throughout the year. The need for a wastewater treatment system that can remove ammonia, total nitrogen, BOD, and other regulated constituents with minimal aeration in cold climates has developed. Fortunately, a type of bacterium was discovered approximately twenty years ago that removes total nitrogen, nitrite, and ammonium in an anaerobic environment. These bacteria are called anammox bacteria, which is short for anaerobic ammonia oxidation. It is the engineering application of anammox bacteria that serves as the foundation of the following research.

1.2 Research Objectives and Hypotheses

The objectives of this research were to:

1. remove significant amounts of ammonium, total nitrogen, and chemical oxygen demand using a submerged attached growth bioreactor operated in partial nitritation-anammox mode; and
2. to do so at 20°C, which is below the typical range of 25-40°C for anammox bacteria.

It was hypothesized that in SAGBs with pH-controlled aeration, as compared to SAGBs with timer-controlled aeration, effluent concentrations of:

1. ammonium, nitrite, and COD would be statistically greater; and
2. total inorganic nitrogen and nitrate concentrations would be statistically less.

1.3 Thesis Organization

This thesis contains six chapters to address the research objectives and the requirements put forth by the University of Iowa Graduate College. Chapter 2 consists of a literature review of nitrogen transformations, anammox processes in wastewater treatment, and SAGB systems with respect to nitrogen removal. Chapter 3 is a

description of the methods and materials section. This chapter consists of the design and operation of the SAGBs, and the synthetic wastewater and analytical methods used throughout the experiments. Chapter 4 presents the results and discussion and consists of the results and discussion of the hydraulic residence time, an intensive two-day study, a long-term study, a genetics study, and an anammox activity study. Chapter 5 presents the conclusions of the research conducted over several months and describes the engineering significance. This chapter also contains recommendations for future research associated with submerged attached growth bioreactors, the process of partial nitritation-anammox, and cold temperature nitrogen removal. The appendix contains multiple images of the SAGB system, including construction and operation phases.

CHAPTER 2

LITERATURE REVIEW

2.1 Nitrogen Speciation in Wastewater

It is important to remove all forms of nitrogen during wastewater treatment for multiple reasons. Free ammonia (NH_3) is toxic to aquatic life and has an oxygen demand. Organic nitrogen also has an oxygen demand, and is regulated by the State of Iowa. These two nitrogen species, along with ammonium, are combined into Total Kjeldahl Nitrogen (TKN), which is the actual constituent that most permits limit. Furthermore, all forms of aqueous nitrogen species can contribute to eutrophication, especially in saltwater, due to their status as a limiting nutrient and their oxygen demand. Typical municipal influent primarily contains 40-70 mg TKN /L and during conventional wastewater treatment is oxidized to nitrite and then nitrate via nitritation and nitrification, respectively (Metcalf & Eddy, 2003). Both of these processes, commonly referred to as "nitrification," is an aerobic process and therefore requires aeration during treatment. Some treatment processes require the wastewater to become anaerobic, converting nitrate to nitrogen gas (N_2) via denitrification. It is the conversion of aqueous nitrogen to nitrogen gas when total nitrogen removal is achieved (Rittmann & McCarty, 2001). Several of the biological processes utilized during treatment are presented in Table 1.

Ammonium and organic nitrogen can be assimilated biologically whereby microorganisms use the nitrogen for protein, DNA, and RNA synthesis and other functions that require a nitrogen source. In treatment processes, such as submerged attached growth bioreactors, nitrogen may be adsorbed onto the media, thereby removing the total nitrogen from the wastewater. Organic nitrogen is readily converted ammonium via ammonification.

The concentration of nitrite in typical municipal wastewater influent and effluent is usually very low. Nitrite is easily oxidized to nitrate in the presence of *Nitrobacter*

species and oxygen. Excess oxygen is usually present in most aeration systems in order to ensure that permit discharge limits are met and therefore an appreciable nitrite concentration is seldom sustained. Nitrite-oxidizing bacteria can be inhibited, thus allowing higher levels of nitrite, in the presence of minimal oxygen or if the ammonia levels are too high (Anthonisen et al., 1976).

2.2 Anammox Bacteria

First discovered approximately 20 years ago, several species of anammox bacteria have been identified. Most of these species fall within the bacterial phylum, Planctomycetes. Regardless, anammox bacteria are slow-growing with a doubling time of 11-20 days (Jetten et al., 2009). They are autotrophic anaerobic bacteria that use bicarbonate as a carbon source and ammonium as the nitrogen source. They also all use ammonium and nitrite as the primary electron donors and acceptors, respectively (Strous et al., 1998). The anammox process likely occurs within a membrane-bound compartment called an anammoxosome, and comprises 50-70% of the total cell volume. It is estimated that up to 50% of the N_2 gas in the atmosphere is attributed to anammox activity (Jetten et al., 2009).

2.3 Partial Nitritation-Anammox

Various combinations of these processes readily occur in natural environments and engineered systems, such as wastewater treatment plants. One of these combinations is a lesser-known two-phase process and requires both aerobic and anaerobic conditions. The first reaction involves the aerobic process of partial nitritation, whereby some of the ammonium is converted to nitrite. The second reaction is the anaerobic oxidation of the remaining ammonia (anammox). In this reaction, the nitrite serves as an electron acceptor to oxidize the ammonia to nitrogen gas. This two-phase process, herein out known as partial nitritation-anammox, has been achieved in two-stage and single-stage systems (Strous et al., 1998). Two-stage systems consist of aerated and unaerated reactors where

partial nitritration and anammox reactions occur, respectively. Single-stage systems use cyclic aeration to achieve the appropriate conditions. It has been shown that by keeping the dissolved oxygen low, both types of systems select for ammonia oxidizing bacteria (AOB) over nitrite oxidizing bacteria (NOB). In doing so, the production of nitrate is limited and the extent of deammonification is maximized (O'Shaughnessy et al., 2008). This process is relatively new to the knowledge basis of environmental microbiology, requires various microbial species, and a low (non-zero) oxygen concentration.

Partial nitritation-anammox is a relatively new wastewater treatment approach that can remove total nitrogen while reducing aeration requirements (Strous et al., 1998; Wuertz et al., 2011). Partial nitritation requires dissolved oxygen to be kept at less than 1 mg O_2/L in order to select for AOBs while inhibiting NOB activity. Under these conditions, ammonium is oxidized to nitrite as alkalinity is consumed, thus driving the pH lower (Reaction 1) (Wuertz et al., 2011).

$$55\ NH_4^+ + 76\ O_2 + 109\ HCO_3^- \rightarrow C_5H_7O_2N + 54\ NO_2^- + 57\ H_2O + 104\ H_2CO_3 \qquad \text{(Rxn 1)}$$

With a desired level of NH_4^+ oxidation reached, aeration is turned off and anammox activity commences as dissolved oxygen tends toward zero. During this process, the anammox bacteria recover alkalinity as ammonium and nitrite are converted to nitrogen gas (N_2) and nitrate (Reaction 2) (Strous et al., 1998).

$$NH_4^+ + 1.32\ NO_2^- + 0.066\ HCO_3^- + 0.13\ H^+ \rightarrow 1.02\ N_2 + 0.26\ NO_3^- + 0.066\ CH_2O_{0.5}N_{0.15} + 2.03\ H_2O \qquad \text{(Rxn 2)}$$

The consumption and subsequent recovery of alkalinity during partial nitritation-anammox results in a characteristic "saw tooth" pH pattern over time when a single reactor is used. This pH signal can serve as an aeration control parameter toward the

creation of "smartly aerated" systems. The most common application of pH-controlled aeration to facilitate partial nitritation-anammox is at large side-stream treatment systems without attached growth media that are operated at 25-40°C (O'Shaughnessy et al., 2008; Third et al., 2001; Wett, 2005; Zeng et al., 2010; Zhao et al., 2013). A submerged attached growth bioreactor (SAGB) using hydrophilic acryl fiber netting as the growth medium treated high ammonium strength wastewater via partial nitritation-anammox at 35°C and dissolved oxygen maintained at 2-3 mg O_2/L achieved 60-80% ammonium removal (Furukawa et al., 2006).

2.4 Submerged Attached Growth Bioreactors

A SAGB is a reactor that uses fixed media to support biological growth while completely submerged with water. SAGBs are typically placed underground where they are less prone to freezing and thus offer an opportunity to be used in cold climates and offer a more aesthetic treatment method. In addition, SAGBs have a smaller land footprint than conventional treatment processes because of the greater available surface area from the media (Schlegel et al., 2007). Like all attached growth bioreactors, SAGBs support microbially diverse communities in the form of biofilms. Typically, the heterotrophs of the biofilm are on the outer edge, while the AOBs and NOBs reside within the biofilm and closer to the medium. This configuration gives a slight preference to the heterotrophs when it comes to oxygen use.

2.5 Current SAGB Systems

A variety of SAGB systems are currently being used in practice and in research to meet current regulations by removing ammonium and BOD, and in many instances, remove nitrogen. The major differences between these systems are the number of units required, aeration requirements, pumping requirements, and influent type, and specific process. SAGBs have been successfully utilized for decentralized wastewater treatment

due to relative ease of operation, favorable land area requirements, and underground placement which facilitates cold-climate treatment and minimizes aesthetic impacts.

SAGBs have been proven effective when continuously aerated to maximize the removal of carbonaceous biological oxygen demand (cBOD) and Total Kjeldahl Nitrogen (TKN) with greater than 95% and 90% cBOD and TKN removal, respectively (ATV, 1997). One planted SAGB, operated at a dissolved oxygen concentration of 0.2-0.6 mg O_2/L, achieved 87.2% cBOD removal and 68.7-85.1% total nitrogen removal (Zhang et al., 2010). A planted, vertical-flow SAGB with continuous aeration achieved a loading-rate dependent ammonium (NH_4^+) removal of 65-87% (Dong et al., 2012). A similar system with continuous aeration removed 97%, 99%, and 29% COD, NH_4^+, and total nitrogen (TN), respectively (Fan et al., 2013a). Additionally, a planted horizontal-flow SAGB removed 1.8-22.9% TN (Maltais-Landry et al., 2009). A planted, horizontal-flow SAGB removed 13-29% TKN in a winter climate and 4-42% TKN in a summer climate (Ouellet-Plamondon et al., 2006).

Other SAGBs have been operated with intermittent aeration to facilitate nitrification and denitrification, resulting in reduced aeration costs and increased TN removal, relative to continuously aerated systems. Previous studies demonstrated that intermittently aerated, horizontal-flow SAGBs dosed with municipal primary effluent removed 84- 93% cBOD and 65-95% TN in planted and unplanted cells (Redmond et al., 2014). A planted, intermittently aerated vertical flow SAGB removed 54-78% of NH_4^+ and 29-57% of TN as a function of hydraulic loading (Dong et al., 2012). A similar system achieved 96% COD, 99% NH_4^+, and 90% TN removal (Fan and Wang et al., 2013) and another achieved 96% COD, 97% NH_4^+, and 74% TN removal (Fan et al., 2013).

The Amphidrome® SAGB claims to treat domestic wastewater flows of 440-150,000 gallons per day to less than 30 mg/L cBOD and less than 10 mg/L TN (Pedros et al., 2006). With methanol addition, a similar system removed over 94% cBOD and 52-

72% TN from municipal wastewater (Pedros et al., 2007a). Another study, performing side-stream treatment of dewatering centrate, achieved an average TN removal of 85% when methanol was added (Pedros et al., 2008). A similar SAGB with limited aeration favored nitrite formation from ammonium while minimizing nitrate production and with the addition of methanol and sodium bicarbonate, 25% of the TN was removed (Pedros et al., 2007b).

Table 1. A modified table of biogeochemical transformations of nitrogen in wetlands

Process	**Transformation Reaction**
Ammonification	$Organic\text{-}N_{(aq)} \rightarrow NH_{3(aq)}$
Nitritation	$2NH_4^+{}_{(aq)} + 3O_2 \rightarrow 2NO_2^-{}_{(aq)} + 2H_2O_{(l)} + 4H^+{}_{(aq)}$
Nitrification	$2NO_2^-{}_{(aq)} + O_2 \rightarrow 2NO_3^-{}_{(aq)}$
Denitrification	$2NO_3^-{}_{(aq)} \rightarrow 2NO_2^-{}_{(aq)} \rightarrow 2NO_{(g)} \rightarrow N_2O_{(g)} \rightarrow N_{2(g)}$
Biological Assimilation	$NH_{3(aq)}, NO_2^-{}_{(aq)}, NO_3^-{}_{(aq)} \rightarrow Organic\text{-}N_{(s)}$
ANAMMOX	$NH_{3(aq)} + NO_2^-{}_{(aq)} \rightarrow N_{2(g)}$

Source: “Biogeochemical transformations of nitrogen in wetlands” (Vymazal, 2007)

Based on this literature review, gaps in the application of partial nitritation in SAGB systems have been found. To our knowledge, there are no reports on partial nitritation-anammox SAGBs treating mainstream domestic wastewater in cool climates using pH-controlled aeration.

CHAPTER 3

METHODS AND MATERIALS

3.1 Experimental Apparatus

Four pilot-scale SAGBs (Figure 1) were constructed within a temperature controlled chamber operated at 20°C. The SAGBs were dosed by a 950 L polypropylene head tank positioned approximately 4 m above the ground. The head tank connected via 3.8 cm diameter PVC pipe to four 45 L polypropylene dosing tanks positioned above the inlet of each 61 cm x 61 cm x 46 cm SAGB (Figure 2). The dosing tank outlets were connected to an electronic valve and that to an inlet manifold. The inlet manifold was a 1.3 cm diameter PVC down pipe connected to a 1.3 cm diameter, 55 cm long, horizontal pipe with eight 2.4 mm dosing holes. Treated wastewater exited each SAGB via a horizontal, 3.8 cm diameter PVC pipe, 55 cm long with sixteen 3.6 mm diameter holes. The effluent manifold piping penetrated the SAGB wall before connecting to a water level control apparatus. The level control apparatus was a vertical 3.8 cm diameter, 30.5 cm tall PVC pipe open to atmosphere. The level control apparatus was contained in a 19 L bucket that drained to the sanitary sewer via 3.8 cm diameter pipe. Additional images and descriptions of the SAGB system are located in the appendix.

Two SAGBs (SAGB1 and SAGB2) contained aeration manifolds consisting of four 50 cm long PVC pipes with a diameter of 1.3 cm and 3.2 mm diameter outlet holes, connected via 61 cm long distribution piping including a 76 cm tall inlet pipe. Compressed air was provided by a Pondmaster AP 100 pump. The compressed air was controlled by a distribution manifold with needle valve regulators capable of distributing 0-2.25 liters per minute (LPM) of air and controlled by a timer.

The other SAGBs (SAGB3 and SAGB4) were aerated via a 2.5 cm diameter diffuser stone placed 13 cm from the inlet 13 cm above the bottom of the SAGB. Compressed air was provided by a Pondmaster AP 100 pump. The compressed air was

controlled by a mass flow controllers (FMA 5400/5500, Omega, Stamford, CT), each capable of distributing 0-5 LPM of air, and regulated by a pH probe (pHDsc Digital Differential pH Sensor, Hach, Loveland, CO) coupled with an SC200 (Hach, Loveland, CO).

Approximately 0.1 m^3 of washed pea gravel was added to each SAGB before inoculation with 38 L of municipal primary effluent from Iowa City's North Wastewater Treatment Plant. Approximately 45 L of synthetic wastewater was slowly added over 7 days to avoid washout and to promote bacterial attachment. At system startup, the dosing valves were microcontroller programmed, using an Arduino UNO R3, to deliver 11.4 L of synthetic wastewater every 24 hours. Specifically, the dosing schedule consisted of a 40-sec does every 6 hours. After 19 weeks, 0.25 L of an anammox bacteria seed culture, in the form of underflow, was added to each SAGB.

3.2 Anammox Seed

The Anammox seed was obtained from the Hampton Roads Sanitation District located in southeastern Virginia that uses partial nitritation-anammox to treat side-stream digester centrate. Approximately 1.6 L of anammox seed culture was collected from the treatment system's underflow and shipped overnight on ice. Upon arrival, it was placed in a 2 L glass container, which was subsequently placed in an incubated shaker table with 100 RPM and at 38.5°C. A nitrogen gas (N_2) purge line with diffuser stone was placed into the seed culture in order to maintain anaerobic conditions. The seed culture was periodically fed ammonium chloride and sodium nitrite and anammox activity was monitored.

3.3 Synthetic Wastewater

The synthetic wastewater was designed to be similar in constituents and concentrations to that of a typical municipal primary effluent. Specifically, the low total organic carbon synthetic wastewater was developed and modified after a mixture (Table 2) used by Klatt

& LaPara (2003). The synthetic wastewater, (theoretical COD of 400 mg/L, theoretical TOC of 103 mg C/L, and theoretical TKN of 54 mg/L) was made in the head tank (908-L batches) with continuously bubbled nitrogen from a nitrogen generator (Domnick Hunter n, Parker) to minimize microbial degradation prior to dosing.

In addition to the above constituents, 0.1 mL of SL7 Mineral Solution (Table 3) was added for each liter of synthetic wastewater. This mineral solution provides the trace minerals commonly found in municipal wastewater and required as micronutrients to many microorganisms.

3.4 Analytical Methods

Ammonium was analyzed with a Dionex CS2000 ion chromatograph with a CS15 column and equipped with computer control and data processing (Chromeleon, version 7). Nitrite and nitrate were analyzed with a Dionex AS900 ion chromatograph with an AS22 column and equipped with Chromeleon software, as well. Prior to analysis, samples of anammox seed culture were centrifuged at 1000 RPM for 5 minutes and then filtered with a vacuum pump setup and a 0.45 micron filter. SAGB samples were filtered with 0.45 micron syringe filters prior to analysis.

Total nitrogen was measured by persulfate digestion method 4500-N C (APHA 2012), while COD was determined by USEPA Approved method (5220 D). Total organic carbon was measured by direct method 415.3 (40 CFR 2012) using Hach TOC low-range (0.3-20.0 mg C/L) reagent sets. Dissolved oxygen was measured by luminescence with an electronic probe (IntelliCal^{TM} LDO10101, Hach, Loveland, CO.) and meter (HQ40d, Hach, Loveland, CO). The pH was measured either by the continuously operating probes described earlier or by a glass electrode (Combination pH, Orion Thermo, Fisher Scientific, Waltham, MA) and meter (AB15 pH meter, Fisher Scientific, Waltham, MA).

The Mann-Whitney Rank Sum test was used to estimate the significance of removal efficiencies between the various SAGB types. Statistical analyses were performed using SigmaPlot Software Version 12.5.

3.5 Hydraulic Residence Time Study

The hydraulic residence time (HRT) was calculated with two methods in order to better understand the flow regime. The theoretical HRT was calculated as a function of flow, the dimensions of the SAGB media, and typical porosity of pea gravel (Equation 1). The porosity of the pea gravel was then confirmed with a sample of media in a graduated cylinder, noting the volumetric displacement as a known amount of water was added.

$$Theoretical\ HRT = \frac{Volume\ of\ SAGB}{Flow\ of\ Water} \times porosity \quad \text{(Eq. 1)}$$

The mean HRT of SAGB1 was determined using a bromide tracer study. During this study, potassium bromide was placed at the influent manifold and the dose valve opened manually. The water level in the dose tank was maintained at its normal level using a hose as needed. Conductivity was measured using a probe (IntelliCAL™ CDC40101 Standard Conductivity Probe, Hach, Loveland, CO) coupled with a meter (HQ40d, Hach, Loveland, CO) during the duration of the study and automatically recorded. The mean HRT was calculated as a function of the continuous flow HRT and the daily dose time (Equation 2).

$$Mean\ HRT = \frac{Continuous\ flow\ HRT\ (minutes)}{Dose\ time\ (\frac{min}{d})} \quad \text{(Eq. 2)}$$

3.6 Intensive Study

An intensive study was conducted over a 2-day period for each SAGB during which port samples were collected every two hours and effluent samples every half hour for ten hours, from the water-level control apparatus. The study was conducted in order to

characterize the nitrogen dynamics and performance of the SAGBs. Influent samples were collected from the dose tans at the beginning of the study. The sample port was a 3.8 cm diameter tube installed 13 cm from the inlet. The tube spanned the buried depth of the pea gravel and had several 2.5 cm diameter holes along its length and a porous fabric wrapping prevented pea gravel intrusion. Data obtained from each port included pH, dissolved oxygen, and nitrogen species. The pH of SAGB1 and SAGB2 was measured using the pH probe as previously described and measured every two hours during sample collection. The pH in SAGB3 and SAGB4 was measured and stored every 5 minutes by reading the pH using the SC200 controller. Additionally, effluent samples were collected from each SAGB every 30 minutes for 10 hours from the water level control apparatus that is open to the atmosphere. These effluent samples were subsequently analyzed for nitrogen species, TOC, and COD.

SAGB1 and SAGB2 were aerated on a 6-hr on/off schedule controlled by a timer at 1.25 LPM, while SAGB3 and SAGB4 were aerated at 5.0 LPM as a function of pH. SAGB3 was programmed to begin aeration at pH 7.25 and to cease aeration at pH 7. Initially, SAGB4 had set points of pH 7.50 and 7.30. The set points of SAGB4 were changed to 7.45 and 7.25, after 27 hours. The effluent in SAGB4 was continuously recirculated at 0.1 LPM (Recycle ratio = 12.6) to the sample port near the influent manifold using a peristaltic pump and flex tubing to induce mixing of the wastewater within the SAGB. Recirculation of SAGB4 was conducted in order to compare the effects of mixed and non-mixed SAGBs.

3.7 Long-term Study

Following the intensive study, influent and effluent samples were collected from SAGB4 approximately every four days (~1 HRT). These samples were analyzed for total nitrogen, ammonium, nitrite, and nitrate as previously described.

The aeration capacity of SAGB4 was doubled with the addition of a second diffuser stone approximately 13 cm away from the original diffuser stone. This diffuser stone was connected to an additional mass flow controller that was connected to the pH probe and SC200 controller combination. SAGB4 was also re-inoculated with approximately 38 L of primary effluent from the Iowa City South Wastewater Treatment Plant on February 25th, 2014.

3.8 DNA Analysis

A DNA analysis was conducted by Lee Hauser and Xikun Liu on the anammox seed culture to confirm the presence of anammox bacteria. A 5 mL sample was taken from the MLSS and diluted to 100 mL. Approximately 14 weeks after the intensive study, 500 mL of pea gravel and wastewater were collected from various regions in each SAGB. The SAGB and anammox seed culture samples were then analyzed. A DNA isolation kit (14600-50-NF, PowerWater® Sterivex™ DNA Isolation Kit, MO Bio Laboratories, Carlsbad, CA) was used to harvest DNA from filter-extracted (Sterivex™ filter units, SVGPL10RC, Millipore) samples. After the DNA was isolated, it was eluted from the binding column membrane in order to conduct polymerase chain reaction (PCR) amplification. PCR was performed using a published forward/reverse primer set S-P-Planc-0046-a-a-18/Amx-0368-a-A-18 (Schmid et al., 2005). The bacteria were cloned using *E. coli* and then PCR was repeated using M13 Forward and M13 Reverse. These PCR products were then sequenced by DNA Core.

3.9 Anammox Activity Study

Five samples were collected from the anaerobic anammox seed culture over the course of 71 hours and filtered as previously described. The first three samples were diluted 1:10 prior analysis in order to ensure that the values would be less than the maximum concentration that the ion chromatograph can accurately measure (50 mg N/L). The last two samples were not diluted. The volume of the anammox seed culture would

decrease over time as a result of evaporation and this was counteracted by adding synthetic wastewater to keep the volume at 1.2 L.

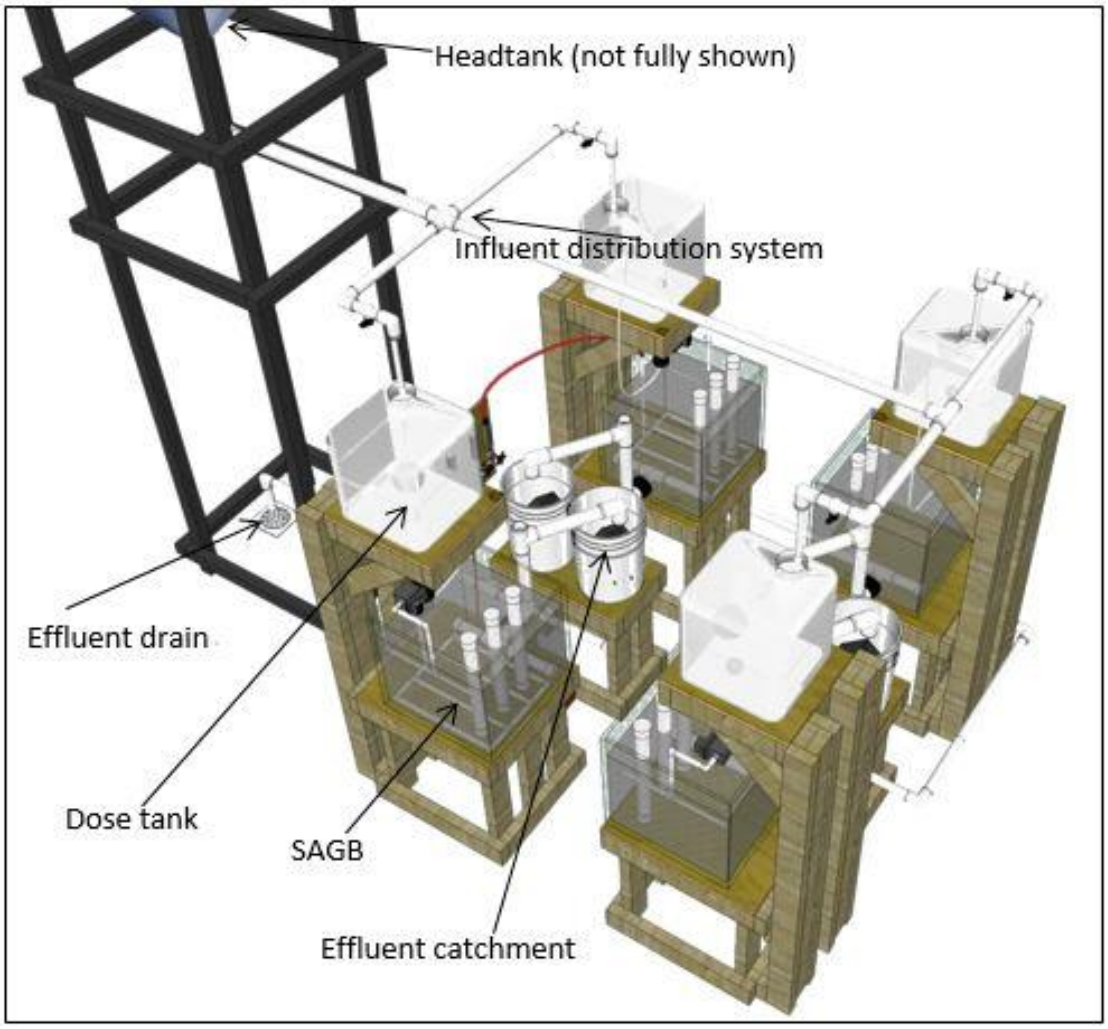

Figure 1. A conceptual drawing of the SAGB system.

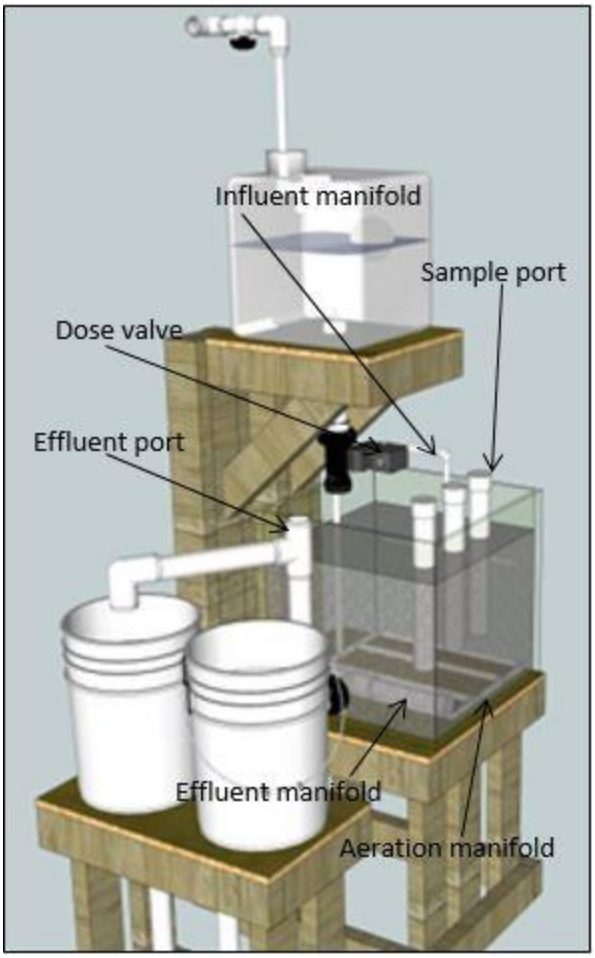

Figure 2. A conceptual drawing of an individual SAGB.

Table 2. Constituents of the synthetic wastewater

Constituent	**Concentration (mg/L)**
Yeast Extract	10
Casamino Acids	10
Ammonium Chloride	150
Sodium Bicarbonate	100
Sodium Phosphate monobasic	25
Potassium Phosphate dibasic	30
Magnesium Chloride	40
Calcium Chloride	60
Sodium Acetate (as NaAc)	110
Glucose	100
Glycine	67

Table 3. Constituents in SL7 Solution

Constituent	**Concentration (mg/L)**
Zinc Chloride	70
Manganese Chloride·tetrahydrate	100
Boric Acid	60
Cobalt Chloride·hexahydrate	200
Cupric Chloride·dihydrate	20
Nickel Chloride·hexahydrate	20
Sodium Molybdate·dihydrate	40
Hydrochloric Acid (25% v/v)	1%

CHAPTER 4

RESULTS AND DISCUSSION

This chapter is broken into five distinct studies. A study was conducted in order to determine the theoretical and actual hydraulic residence time. A 2-day intensive study was conducted in order to determine the dynamics of pH, dissolved oxygen, and nitrogen species within each SAGB. A long-term study was conducted to determine the removal efficiency of SAGB4 over a period of several weeks. A DNA analysis was conducted on an anammox seed culture and each SAGB to determine if anammox bacteria DNA was present. The anammox seed culture was also analyzed to show that anammox activity was occurring.

4.1 Hydraulic Residence Time Study

The theoretical hydraulic residence time (HRT) of SAGB1 was calculated to be 3.7 days using Equation 2. The volume of the SAGB used in the calculation was 113 L. The porosity of the pea gravel in the SAGB was determined to be 37%. The influent flow used in the calculation was 11.4 L/d.

The mean HRT of SAGB1 was determined to be 4.5 days using tracer study data (Figure 3). Using the area under the tracer curve, the mean continuous flow HRT of the bromide tracer was11.2 minutes. Dividing this value by the dose time of 2.5 minutes/day, a mean HRT of 4.5 days was obtained.

The porosity of SAGB1 was within the range of values (32-40%) found in literature. The tailing off of conductivity was expected of a system like this and indicates typical advection and dispersion. Because the tracer study was conducted over 20 minutes (i.e. the dose valve was open for 20 minutes), the true HRT is expected to be greater than the one determined in the study and theoretically. This is because the intermittent dosing would increase the dispersion of the bromide tracer, causing a greater tailing effect.

4.2 Intensive Study

4.2.1 Influent Characterization

The influent synthetic wastewater varied slightly between SAGB types (Table 4 Table 4). SAGB1 and SAGB2 influent had average ammonium (total inorganic nitrogen) and total nitrogen concentration of 30 mg N/L and 58 mg N/L, respectively. The average influent COD concentration was 77 mg/L. The average influent of SAGB3 had total inorganic nitrogen (TIN) and total nitrogen concentrations were 34 mg N/L and 55 mg N/L, respectively. Average influent COD and TOC concentrations of SAGB3 were 67 mg/L and 15 mg C/L, respectively. The average influent total inorganic nitrogen and total nitrogen concentrations of SAGB4 were 33 mg N/L and 61 mg N/L, respectively. Average COD and TOC concentrations of SAGB4 were 86 mg/L and 17 mg C/L, respectively. Influent concentrations of COD and TOC were much lower (19% and 16% of theoretical COD and TOC, respectively) than estimated from the chemical makeup of the synthetic wastewater due to biological degradation in the head tank and dose tanks. Influent TIN concentrations were typical for municipal primary effluent. The influent of SAGB4 had a pH of 7.5 to 7.8 a week prior to the intensive study.

4.2.2 Sample Port Results

Wastewater located in the sample port of each SAGB was measured for pH, nitrogen species, and dissolved oxygen every two hours during the intensive study.

pH

The pH of SAGB1 and SAGB2 fluctuated between 7.25 and 7.95 over the course of the intensive study (Figure 4 and Figure 5). The pH decrease during aeration was expected as ammonium was oxidized by AOBs; producing H^+ ions and consuming alkalinity. The subsequent rise in pH during periods of no aeration was expected, as heterotrophic denitrification occurred, which recovered alkalinity. In some instances, pH

decreased during periods of no aeration and increased during aeration. In essence, the port pH data for timer-controlled aeration showed no distinct pattern.

The pH of SAGB3 fluctuated between 6.94 and 7.38 during the intensive study, which was outside of the set-control pH values (Figure 6). The pH decreased during aeration as a result of AOB and NOB activity and continued to decrease after aeration ceased. Following each dose, the pH increased to the maximum pH set point over a matter of minutes.

The sharp pH spike in SAGB3 after each dose suggests that a dose-effect occurred. Prior to the intensive study, the pH of the synthetic wastewater ranged from 7.5 to 7.8; which is much greater than the maximum operating pH of 7.25 for SAGB3, allowing the synthetic wastewater to have a greater influence on the pH during doses. This dose-effect makes it difficult to discern as to whether or not anammox occurred during periods of non-aeration.

The pH of SAGB4 fluctuated between 7.21 and 7.54 during the intensive study. During the first 17 hours of the study, the pH rose and then remained roughly constant for 9 more hours (Figure 7). Shortly after a dose around hour-27, the pH rose and aeration turned on. During this period of aeration, the pH initially increased for approximately 1.5 hours and then rapidly decreased. After aeration ceased, the pH continued to decrease for a couple hours, after which, the pH increased for the remained of the study.

The steady pH increase in SAGB4 during the first 17 hours of the experiment may be the result of a dose-effect. SAGB4 was not aerated prior to the intensive study and during these 17 hours. Unlike the sharp pH spikes in SAGB3, SAGB4's pH increase was less dramatic, which was expected due to recirculation. This is further evidenced by the pH becoming fairly constant after a period of time. It is possible that anammox and/or denitrification occurred during the time prior to aeration; however denitrification would be unlikely because of the low levels of TOC in the synthetic wastewater. Anammox

activity could also explain the pH increase, but like SAGB3, it is difficult to discern from these data

The rise in pH during aeration was most likely due to the dose-effect, much like SAGB3. The pH rise was slower than that in SAGB3 for a few possible reasons. Because the operating pH was higher, the dose effect would not be expected to have as great an impact as it would in SAGB3. Furthermore, the recirculation of SAGB4 certainly would make the dose-effect less dramatic, just as well-mixed reactors are less vulnerable to shock loads than unmixed reactors.

The pH decrease for the remainder of aeration was expected as ammonium was biologically oxidized, generating H^+ ions and consuming alkalinity. As expected, pH increased while aeration was turned off. This pH recovery suggests that denitrification and/or anammox activity may have taken place during this time. Like SAGB3, denitrification is possible, but less likely due to the low TOC in the synthetic wastewater. Therefore, the data suggest that anammox activity may have occurred during this final anaerobic period.

Nitrogen and Dissolved Oxygen

SAGB1 and SAGB2 underwent four 6-hr aeration periods, interspersed by four 6-hr non-aeration periods, causing nitrogen species and dissolved oxygen concentrations to vary (Figure 8 and Figure 9). The data presented in these plots are from the sample ports located 13 cm in front of each influent manifold. Ammonium concentrations ranged from 0 to 6.3 mg N/L. Nitrate concentrations fluctuated between 26 and 37 mg N/L. Nitrite concentrations fluctuated between 0 and 0.64 mg N/L. The dissolved oxygen in SAGB1 fluctuated between 0 and 8.2 mg O_2/L.

Ammonium concentrations decreased while nitrate concentrations increased during aeration in SAGB1 and SAGB2, except in two instances. During these two periods, ammonium peaked midway during aeration as a dose of synthetic wastewater

occurred, causing the ammonium and oxygen demand levels to rise. This dose-effect also explains the decreasing dissolved oxygen concentration even though aeration is occurring. The removal of ammonium and production of nitrate during aeration were expected because of AOB/NOB activity that was facilitated by aerobic conditions. TIN was higher than the influent TIN within the port, most likely due to the low TOC influent levels.

SAGB3 underwent five periods of aeration, ranging from 2 to 5-hrs, causing nitrogen species to vary (Figure 10). Ammonium concentrations ranged from 8.2 to 24.6 mg N/L. Nitrate concentrations ranged from 2.5 to 13.5 mg N/L. Nitrite concentrations ranged 0.62 to 2.1 mg N/L, with an average of 1.7 mg N/L. The average concentration of total inorganic nitrogen in the port of SAGB3 was 6 mg N/L less than the average influent, while dissolved oxygen ranged from 0 to 0.03 O_2/L.

AOBs and NOBs in SAGB3 oxidized ammonium to nitrate as evidenced in the removal of ammonium and production of nitrate. As with SAGB1 and SAGB2, a spike in ammonium concentration occurred during doses in SAGB3. Nitrite concentrations also decreased during aeration as it was being oxidized to nitrate via NOB activity. Despite some nitrite oxidation occurring, nitrite concentrations remained high throughout the experiment, allowing anammox activity to occur. With an average total inorganic nitrogen removal of 6 mg N/L in the presence of anaerobic conditions with low influent TOC and high port nitrite concentrations, removal via partial nitritation-anammox is suggested.

SAGB4 underwent one period of aeration lasting about 4 hours, causing nitrogen species to vary (Figure 11). Ammonium concentrations ranged from 20 to 25.8 mg N/L. Nitrate concentrations ranged from 0 to 3.7 mg N/L. Nitrite concentrations ranged from 0 to 0.44 mg N/L, with an average of 0.12 mg N/L. The average concentration of total inorganic nitrogen in the port of SAGB4 was 8 mg N/L less than the average influent, while dissolved oxygen concentrations ranged from 0 to 0.67 mg O_2/L.

AOB and NOB activity caused ammonium removal and nitrate production during aeration, as expected. Nitrate was produced at the beginning and end of this time, along with a small amount of nitrite formation. SAGB4 was anaerobic several hours prior to the experiment and during the first 27 hours. Samples collected in days prior to the intensive study yielded a port nitrite concentration of 0.3 mg N/L, which suggests that the conditions were favorable for anammox activity to occur. The removal in total inorganic nitrogen from a low TOC influent, high nitrite concentration, and decreasing ammonium levels several hours after aeration in anaerobic conditions, suggests that partial nitritation-anammox activity occurred.

4.2.3 Effluent Results

SAGB1 and SAGB2 removed 45% of the total nitrogen, achieving a total nitrogen effluent concentration of 32 mg N/L (Table 5). Because dosing occurred during periods of no aeration, TOC was introduced under anaerobic conditions in the SAGBs. These doses of TOC during anaerobic conditions would likely support denitrification and denitritation, which would explain the high total nitrogen removal. These two SAGBs removed 100% of the ammonium and had an average effluent of concentration of 36 mg NO_3^--N/L and nitrite was not detected. Approximately 97% of the COD was removed, achieving an effluent COD concentration of 2.3 mg/L. The effluent TOC concentration was 1.4 mg C/L. Throughout the experiment, the average dissolved oxygen concentration in the two SAGBs was 4.6 mg O_2/L.

Effluent samples show that SAGB1 and SAGB2 removed more total ammonium than the SAGBs with pH-controlled aeration. The high COD and ammonium removals suggest that there was sufficient oxygen. As expected, SAGB1 and SAGB2 produced no nitrite as effluent, which most likely means that the nitrite was further oxidized to nitrate due to an excess of dissolved oxygen. The high levels of nitrate further support this and

suggest that total nitrogen removal was limited based on low TOC influent concentrations.

SAGB3 removed 48% of the total nitrogen, thus achieving a total nitrogen effluent concentration of 28 mg N/L. On average, all of the organic nitrogen was removed. This SAGB removed 67% of the ammonium and had an average effluent concentration of 11 mg NH_4^+-N/L. The average effluent of SAGB3 consisted of 17 mg NO_3^--N/L and 0.8 mg NO_2^--N/L, meaning that roughly half of the nitrite found in the sample port was removed by the time it reached the effluent port via aerobic oxidation by NOBs, anammox bacteria activity, or denitritation. SAGB3 removed 5.2 mg N/L (15%) of the average influent total inorganic nitrogen, of which it went to N_2 gas via denitrification and/or anammox activity. SAGB3 removed 76% of the COD and achieved an average effluent concentration of 16 mg/L. SAGB3 removed 87% of the TOC, achieving an average effluent concentration of 2 mg C/L. Throughout the intensive study, dissolved oxygen was not present in the sample port.

The removal of total nitrogen in SAGB3 suggests that denitrification, denitritation, biomass production, and/or anammox occurred, however with low TOC influent concentrations; it is likely that anammox activity occurred to some extent. Assuming that 100% of the COD removal was due to denitrification, the maximum potential nitrate removal was 17.8 mg NO_3-N/L. Likewise, the maximum nitrite removal via denitritation was 29.8 mg NO_2-N/L. In an effort to determine how much, if any, role anammox activity played in nitrogen removal, it was assumed that 50% of the COD removal was done aerobically and that the remaining 50% of the COD removal was from heterotrophic denitrification/denitritation. This assumption was made on the basis that aerobic heterotrophs utilize COD faster than anaerobic heterotrophs and that typical biofilm reactors have a larger heterotrophic population than anaerobic heterotrophs. It was assumed that 2.86 mg COD is required to remove 1 mg NO_3-N via denitrification and 1.71 mg COD is required to remove 1 mg NO_2-N via denitritation. With these

assumptions, 8.9 mg NO_3-N/L and 14.9 mg NO_2-N/L removal was estimated to be a result of denitrification and denitritation, respectively. Nitrogen removal from biomass production was also considered. It was assumed that the net yield (Y_{net}) was 0.4 and that biomass was 12.4% nitrogen by mass, resulting in an estimated 2.5 mg N/L remove. With these assumptions, it was estimated that 9.6-15.6 mg N/L was removed via anammox. It is difficult to discern the primary cause of ammonia removal; anammox or aerobic oxidation. Aeration occurred, but not for very long prior to and during the intensive study. However, the formation of nitrate implies that aerobic ammonia oxidation definitely occurred. Nitrate accumulation further suggests that denitrification did not occur, as most of it would have been removed. Furthermore, it suggests that SAGB3 was over-aerated in at least one region. The accumulation of nitrite in SAGB3 effluent shows that anammox could have taken place since nitrite is a key component of the anammox process. The COD results from SAGB3 show that an appreciable amount of COD can be removed while a SAGB performs partial nitritation-anammox, assuming that this treatment method occurred.

SAGB4 removed 53% of the total nitrogen, thus achieving a total nitrogen effluent concentration of 29 mg N/L. On average, SAGB4 removed 71% of the organic nitrogen, with an average effluent concentration of 8 mg Org-N/L. The SAGB removed 36% of the ammonium, achieving an average effluent concentration of 21 mg NH_4^+-N/L. Nitrate and nitrite were not detected in the effluent. On average, SAGB4 removed 12 mg N/L (36%) of the average influent total inorganic nitrogen, implying that N_2 gas was generated via denitrification and/or anammox activity. SAGB4 removed 83% of the COD and 92% of the TOC, achieving an average effluent concentration of 14 mg/L of COD and 1.4 mg C/L. Throughout the intensive study, the average dissolved oxygen concentration in the sample port of SAGB4 was 0.04 mg O_2/L. Assuming that 100% of the COD removal was due to denitrification, the maximum potential nitrate removal was 25.2 mg NO_3-N/L. Likewise, the maximum nitrite removal via denitritation was 42.1 mg

NO_2-N/L. The aforementioned assumptions made for SAGB3, regarding nitrogen removal, were also made for SAGB4, which resulted in an estimated 12.6 mg NO_3-N/L or 21.1 mg NO_2-N/L removal from denitrification or denitritation, respectively. Making the same biomass yield assumptions as done for SAGB3, an estimated 3.6 mg N/L were remove. With these assumptions, an estimated 7.3-15.8 mg TN/L remains unaccounted for, suggesting that anammox occurred.

SAGB4 achieved a total nitrogen removal comparable to the other three SAGBs. This nitrogen removal was most likely achieved via anammox because of the low TOC synthetic wastewater. Effluent samples taken prior to the intensive study had a nitrite concentration of 0.3 mg N/L from SAGB4, which further suggests that this nitrogen removal had occurred as a result of anammox. The lack of nitrate suggests that very little had been formed and that it had been denitrified from the minimal TOC amount. The absence of nitrite in the effluent does not support the conclusion that anammox activity occurred. However, it is very likely that the nitrite that was formed prior to the intensive study had been used up during anammox. The effluent results show that SAGB4 was able to remove a significant amount of COD with very minimal aeration.

In SAGBs with pH-controlled aeration, as compared to SAGBs with timer-controlled aeration, effluent concentrations of:

1. ammonium were statistically greater ($p < 0.001$);
2. COD concentrations were statistically greater ($p = 0.019$); and
3. total inorganic nitrogen concentrations were statistically less ($p < 0.001$).

SAGB3, as compared to SAGBs with timer-controlled aeration, effluent concentrations of:

1. nitrate were statistically less ($p < 0.001$); and
2. nitrite were statistically greater ($p < 0.001$).

4.3 Long-term Study

Between January 31st and March 5th, the pH ranged from 6.85 to 7.54 throughout operation (Figure 12). On February 5th, it was discovered that aeration had not entirely ceased for a two-day period. The flow rate was at 0.25 LPM, which drove the pH below the aeration "shutoff" level of pH 7.15. The pH below 6.85 and the sharp pH decrease on February 21st were from calibration procedures and not biochemical reactions or dose-effects. During the same timeframe, two periods of aeration occurred. These periods of aeration spanned for a day or more with a total flow rate of 10 LPM. The sharp pH increase that occurred around February 8th is due to a dose-effect in which approximately 380 liters of synthetic wastewater was dosed into SAGB4. As expected, the pH decreased during both periods of aeration as ammonium was oxidized. The sharp rise in pH on February 25th was due to a dose effect when SAGB4 was inoculated with 38 liters of primary effluent, which added TOC to the SAGB, allowing for denitrification and a subsequent rise in pH on February 28th.

The influent total nitrogen and ammonium remained fairly constant for approximately a month (Figure 13). The average total nitrogen influent concentration was 53.2 mg N/L and the average ammonium influent concentration was 39.1 mg N/L. Samples collected between February 5th and February 15th show effluent ammonium concentrations decreased. After February 15th, the effluent ammonium concentrations rose over time, but remained much lower than the influent ammonium concentrations.

Effluent nitrate and total nitrogen concentrations decreased between February 5th and February 15th. Due to this excess aeration that occurred February 3rd to February 5th, it is likely that a reservoir of slow-degrading organic nitrogen had undergone ammonification and was then oxidized to nitrite and then nitrate. This is supported by the spike in effluent nitrate to levels not seen in any of the SAGBs. The disappearance of nitrate after the spike on February 5th suggests that the nitrate was washed out and/or underwent denitrification. Because of the 380-L dose and no aeration during the dose, the

TOC levels were likely sufficient for denitrification, unlike normal operating T OC levels. Furthermore, the volume of the dose itself likely played a role in the removal of nitrate from the SAGB.

While the total inorganic nitrogen and effluent ammonium concentrations remained much lower than that of the influent after February 5th, it is apparent that anammox and/or denitrification occurred. The fact that the SAGB was dosed with low TOC synthetic wastewater suggests that denitrification was minimal and that anammox may have occurred between February 5th and February 25th. This is further supported by the high average effluent nitrite concentrations (0.6 mg N/L) measured beginning on February 5th, which would allow anammox to occur.

4.4 DNA Study

The presence of anammox DNA in the anammox seed culture and each SAGB was confirmed through DNA isolation, PCR amplification, gel electrophoresis, and sequencing (Figure 14). The DNA from the anammox seed culture was 246 base pairs (bp) long, which fell within the expected range of 200-250 bp. The presence of anammox in the seed culture was expected since partial nitritation-anammox is used at the wastewater treatment plant where the MLSS, in the form of underflow, was obtained. The overall significance of the presence of anammox in the MLSS is that it was used to inoculate the SAGBs, therefore directly adding large amounts of anammox bacteria. The presence of anammox bacteria DNA in SAGB3 and SAGB4 suggests that partial nitritation-anammox could have occurred.

4.5 Anammox Activity Study

The activity of anammox bacteria in the anammox seed culture was confirmed over a 71-hour study. Ammonium concentrations decreased from 25.5 mg N/L to 5 mg N/L over 71 hours in the anaerobic anammox seed culture, resulting in a k value of 0.54/d (Figure 15). Nitrate concentrations decreased from 0.55 mg N/L to 0.19 mg N/L via

denitrification. Over this same time frame, nitrite concentrations decreased from 23.9 mg N/L to 8.7 mg N/L, resulting in a k-value of 0.34/d.

The rates of utilization of ammonium and nitrite were not consistent with those suggested by the stoichiometry of anammox activity (Equation 2, Chapter 2). According to this stoichiometry, nitrite should be utilized at a rate approximately 1.32 times faster than ammonium. Instead, the results suggest that the rate utilization of ammonium is 1.59 times faster than nitrite, thus creating a stoichiometry of 1.59M NH_4^+ for every 1M NO_2^- during anammox. In addition, nitrate generation to a concentration greater than 1 mg N/L should have occurred. The presence of heterotrophic dentrifiers would explain the lack of nitrate accumulation, but would also cause the nitrite utilization rate to be faster than expected. Regardless, the removal of ammonium in anaerobic conditions suggests that anammox activity occurred, in the anammox seed culture, and not just the presence of anammox bacteria.

Table 4. Influent characterization during the intensive study

	Influent Concentration (mg/L) Average ± S.D.			
SAGB	**Total N** *n=4*	**NH_4^+-N** *n=1*	**COD** *n=4*	**TOC** *n=4*
1 & 2	58 ± 7	30	77 ± 25	N.A.
3	55 ± 3	34	67 ± 6	15 ± 4
4	61 ± 9	33	86 ± 34	17 ± 2

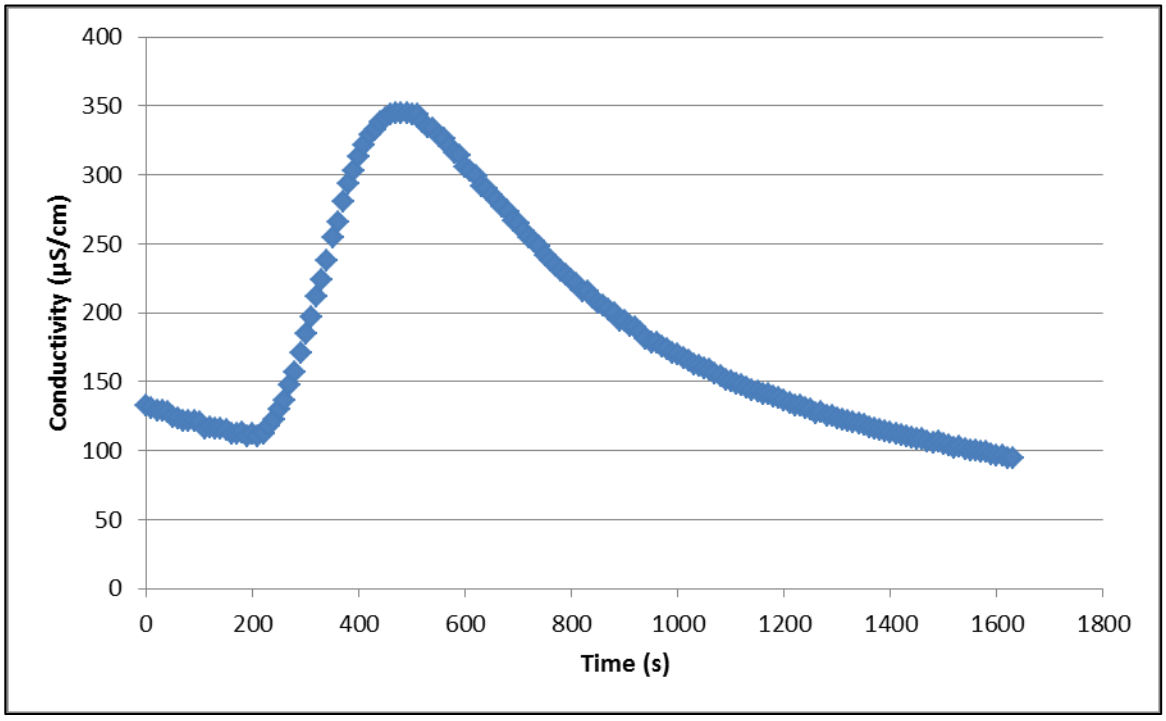

Figure 3. Tracer study for SAGB1

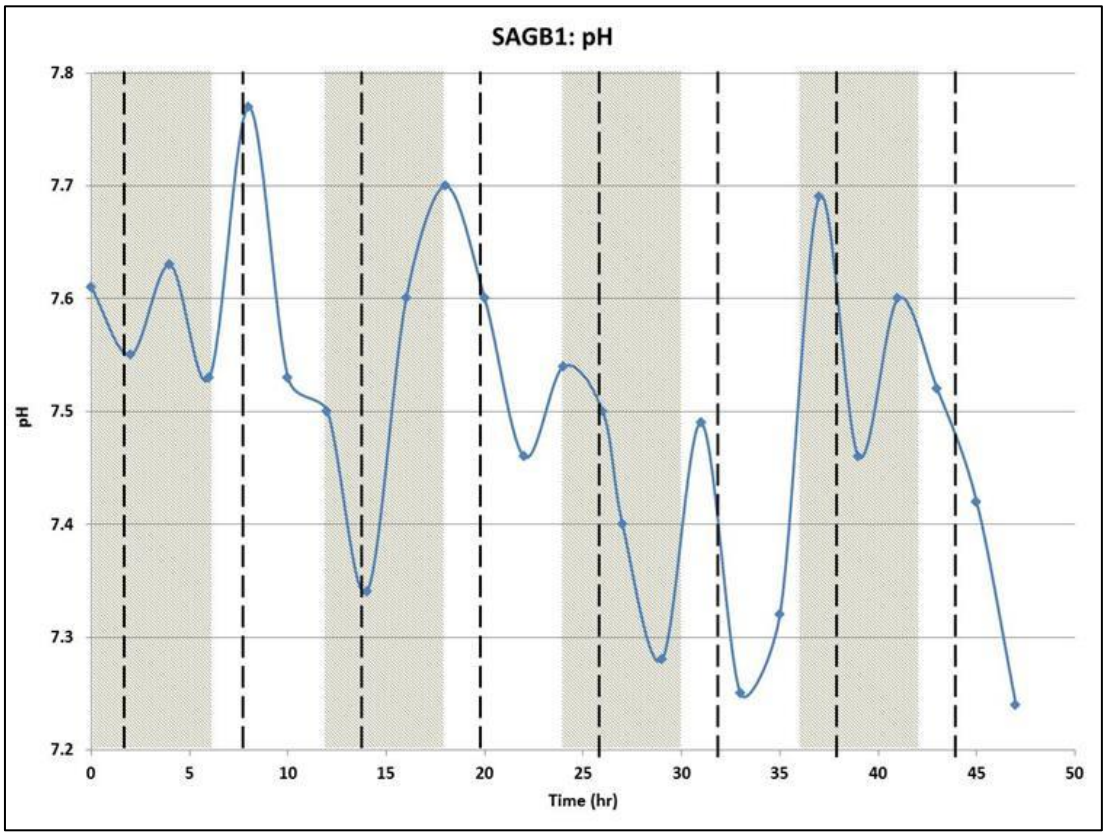

Figure 4. Intensive study pH data for SAGB1. Shading indicates a period of aeration. Vertical dashed lines indicate a dose of synthetic wastewater.

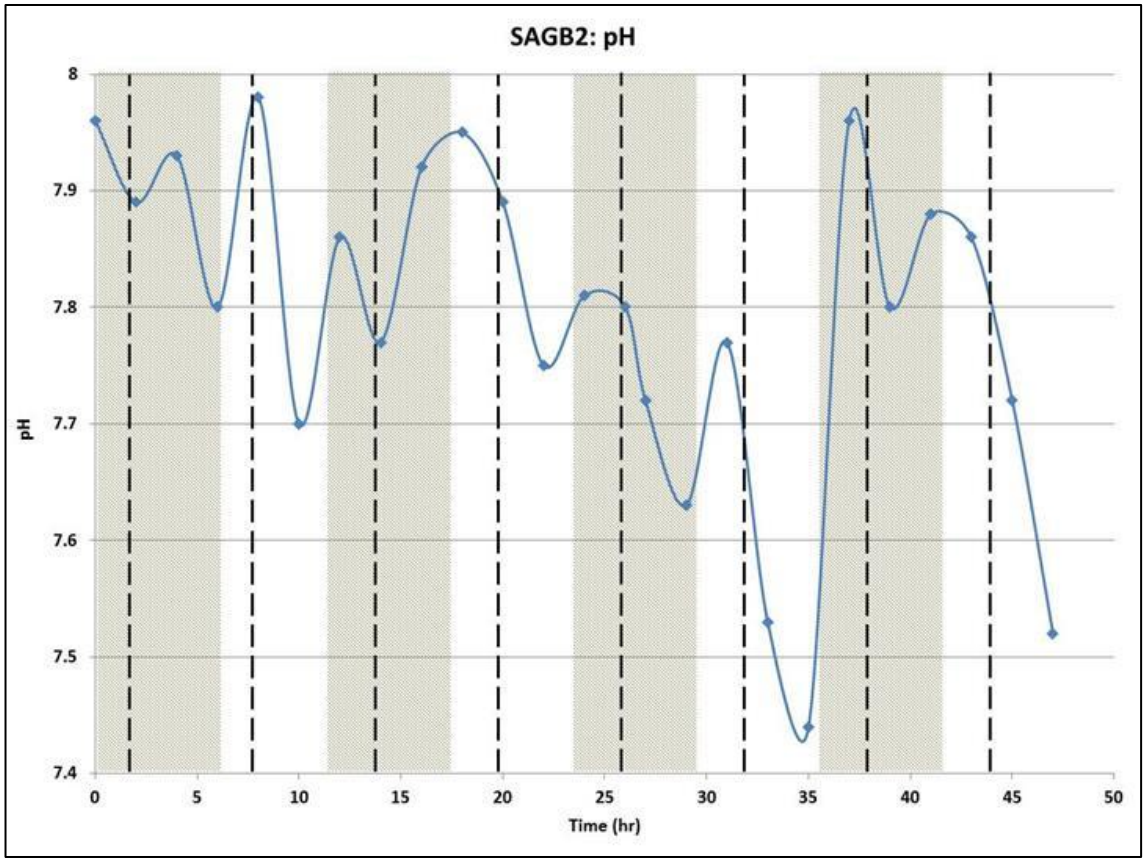

Figure 5. Intensive study pH data for SAGB2. Shading indicates a period of aeration. Vertical dashed lines indicate a dose of synthetic wastewater.

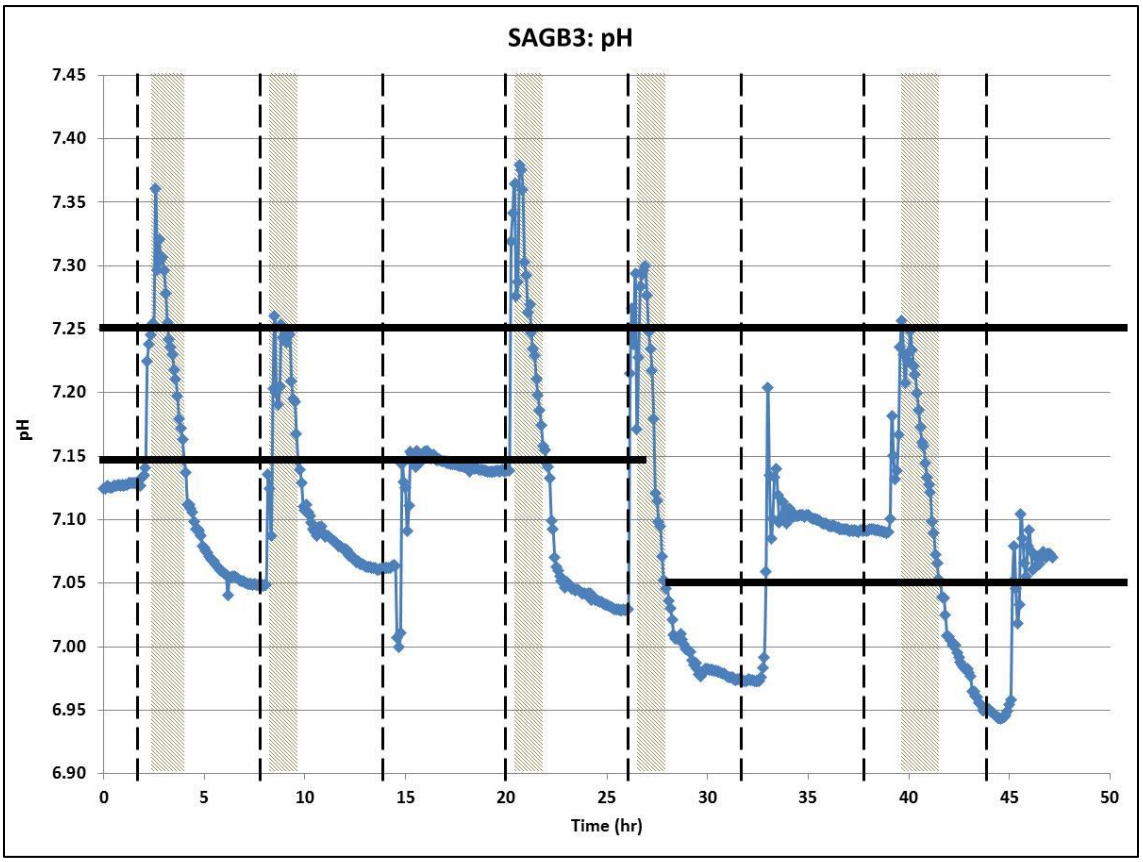

Figure 6. Intensive study pH data for SAGB3. Shading indicates a period of aeration. Vertical dashed lines indicate a dose of synthetic wastewater. Horizontal black lines indicate pH/aeration settings.

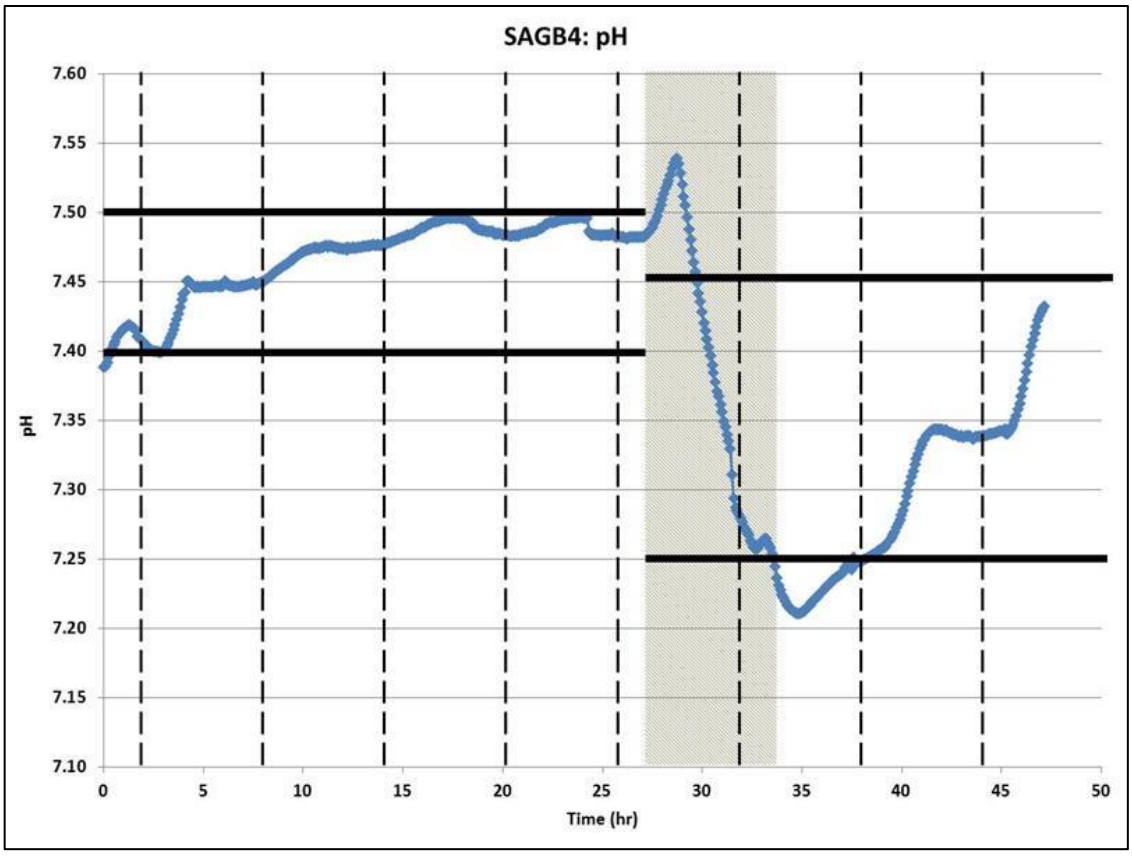

Figure 7. Intensive study pH data for SAGB4. Shading indicates a period of aeration. Vertical dashed lines indicate a dose of synthetic wastewater. Horizontal black lines indicate pH/aeration settings.

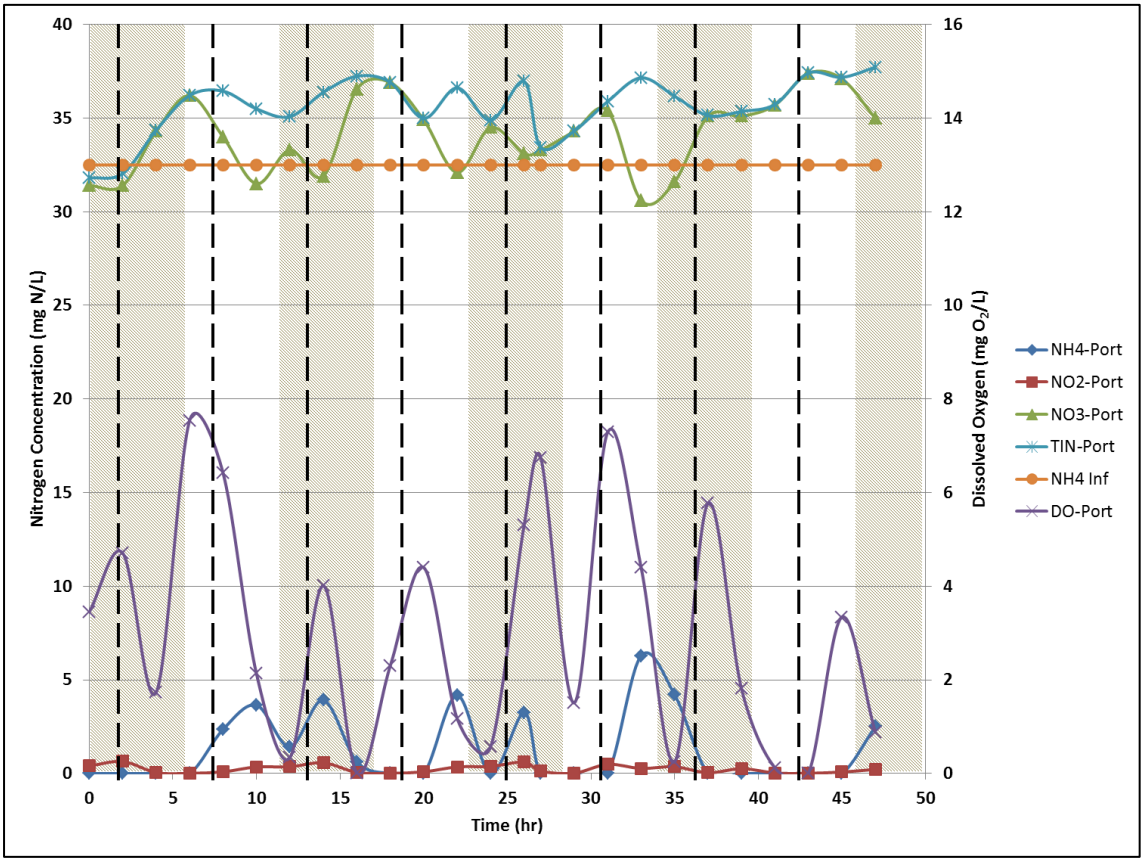

Figure 8. Intensive study nitrogen speciation data for SAGB1. Shading indicates periods of aeration. Vertical dashed lines indicate a dose of synthetic wastewater.

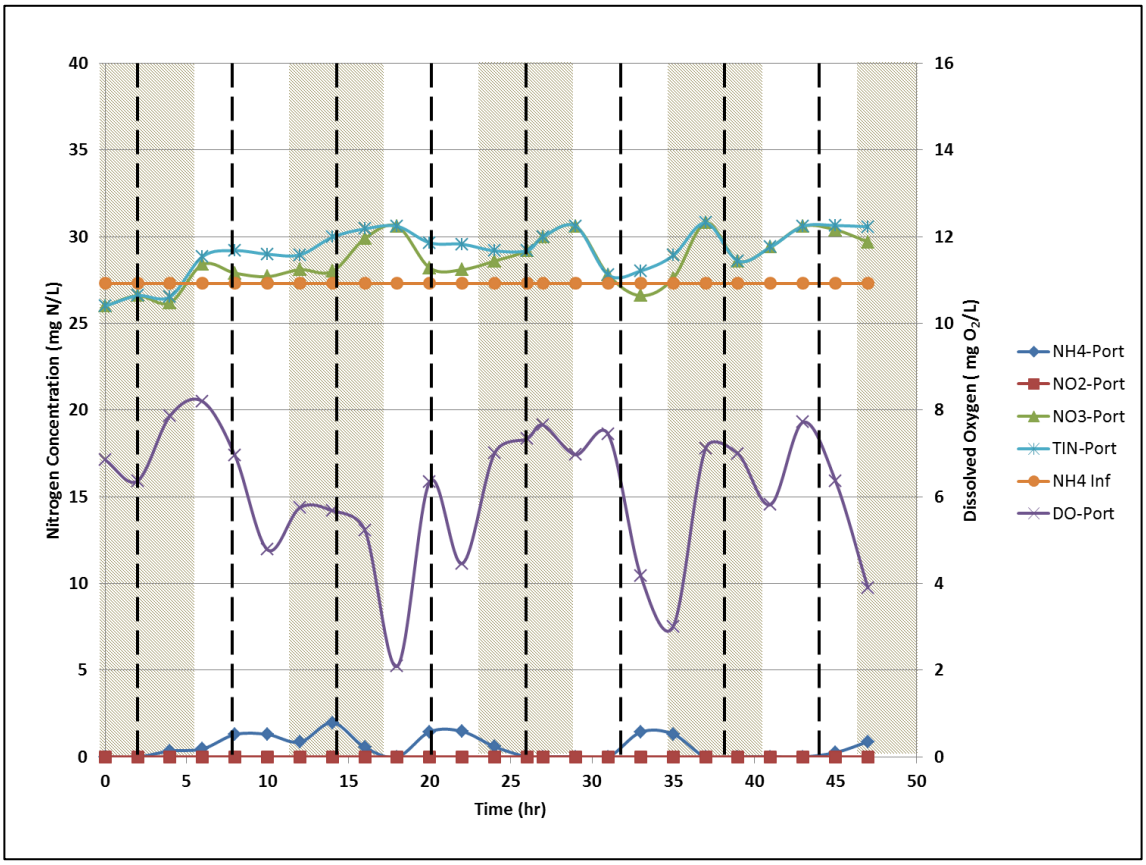

Figure 9. Intensive study nitrogen speciation data for SAGB2. Shading indicates periods of aeration. Vertical dashed lines indicate a dose of synthetic wastewater.

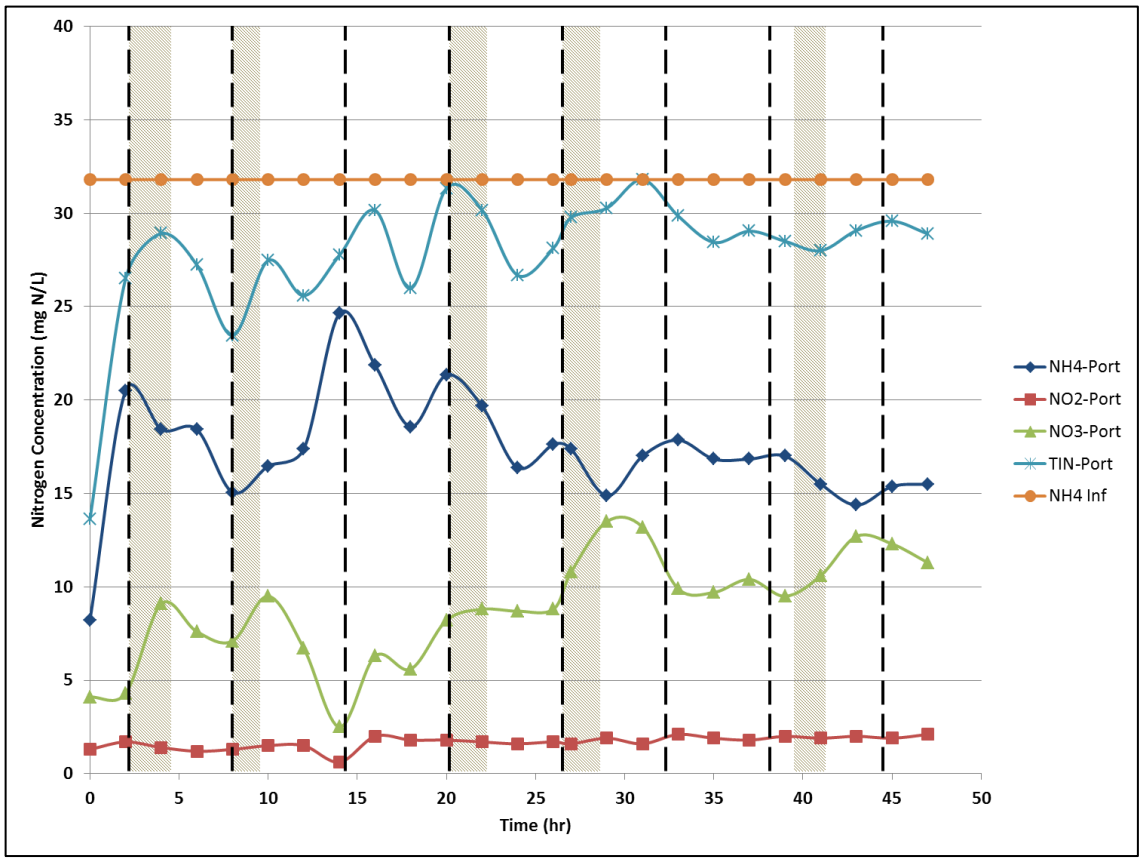

Figure 10. Intensive study nitrogen speciation data for SAGB3. Shading indicates periods of aeration. Vertical dashed lines indicate a dose of synthetic wastewater.

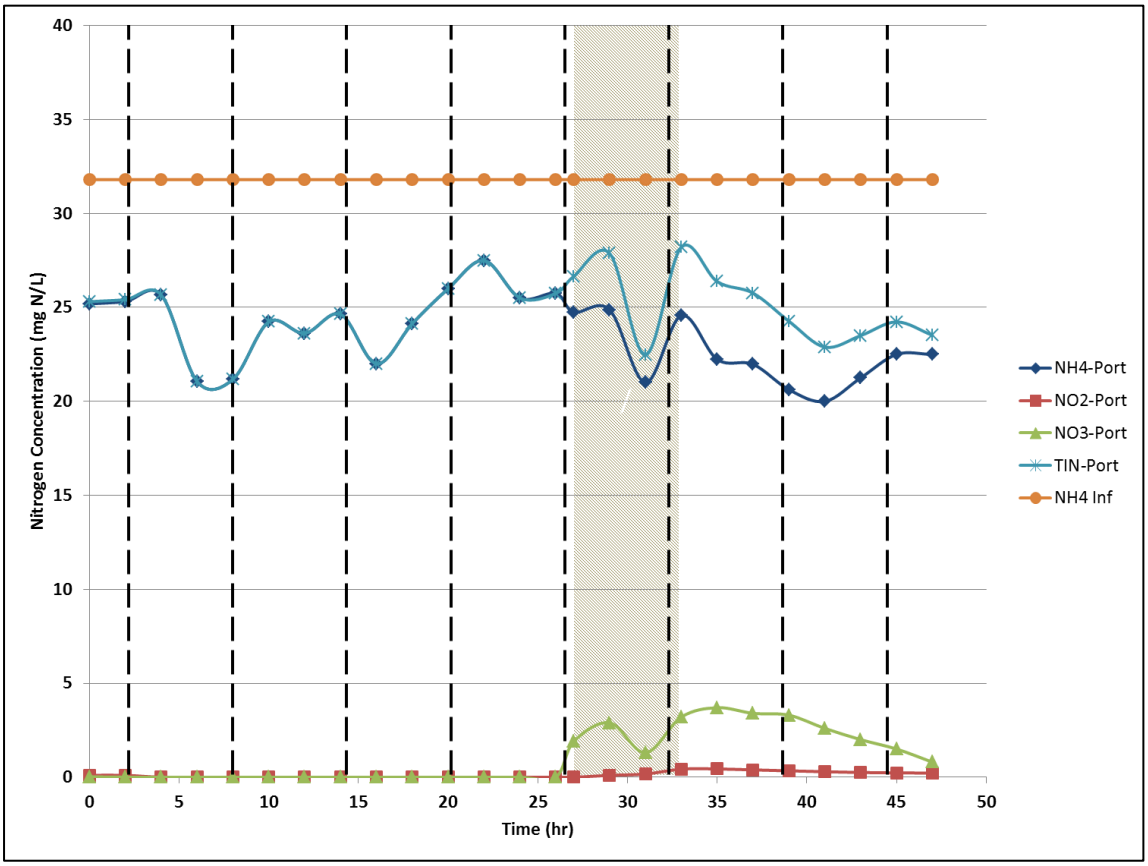

Figure 11. Intensive study nitrogen speciation data for SAGB4. Shading indicates periods of aeration. Vertical dashed lines indicate a dose of synthetic wastewater.

Table 5. Influent and effluent comparisons between SAGBs during the intensive study

	Influent Concentration (mg/L) Average ± S.D.				**Effluent Concentration (mg/L) Average ± S.D.**						**Sample Port (mg/L)**
SAGB	**Total N** ***n=4***	**NH_4-N** ***n=1***	**COD** ***n=4***	**TOC** ***n=4***	**Total N** ***n=4*** **(removal)**	**NH_4-N** ***n=21*** **(removal)**	**NO_3-N** ***n=21***	**NO_2-N** ***n=21***	**COD** ***n=4*** **(removal)**	**TOC** ***n=4*** **(removal)**	**DO** ***n=24***
1 & 2	58 ± 7	30	77 ± 25	N.A.	32 ± 3 (45%)	N.D. (100%)	36 ± 4	N.D.	2.3 ± 4.2 (97%)	1.4 ± 0.3	4.6 ± 2.6
3	55 ± 3	34	67 ± 6	15 ± 4	28 ± 0.7 (48%)	11 ± 1.5 (67%)	17 ± 1.5	0.8 ± 0.3	16 ± 10 (76%)	2.0 ± 2.4 (87%)	N.D.
4	61 ± 9	33	86 ± 34	17 ± 2	29 ± 2 (53%)	21 ± 2 (36%)	N.D.	N.D.	14 ± 5 (83%)	1.4 ± 0.9 (92%)	0.04 ± 0.14

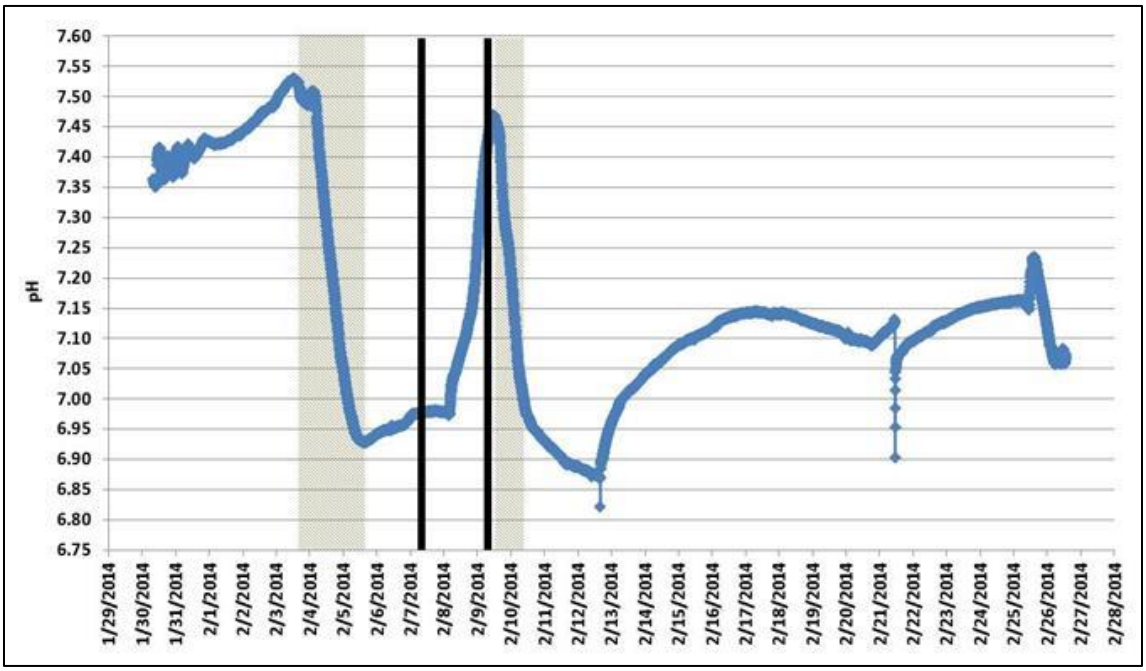

Figure 12. Time-series pH data of SAGB 4 following the intensive study. Shading indicates periods of aeration. Dark vertical lines indicate the period of time for the 380-L accidental dose occurred.

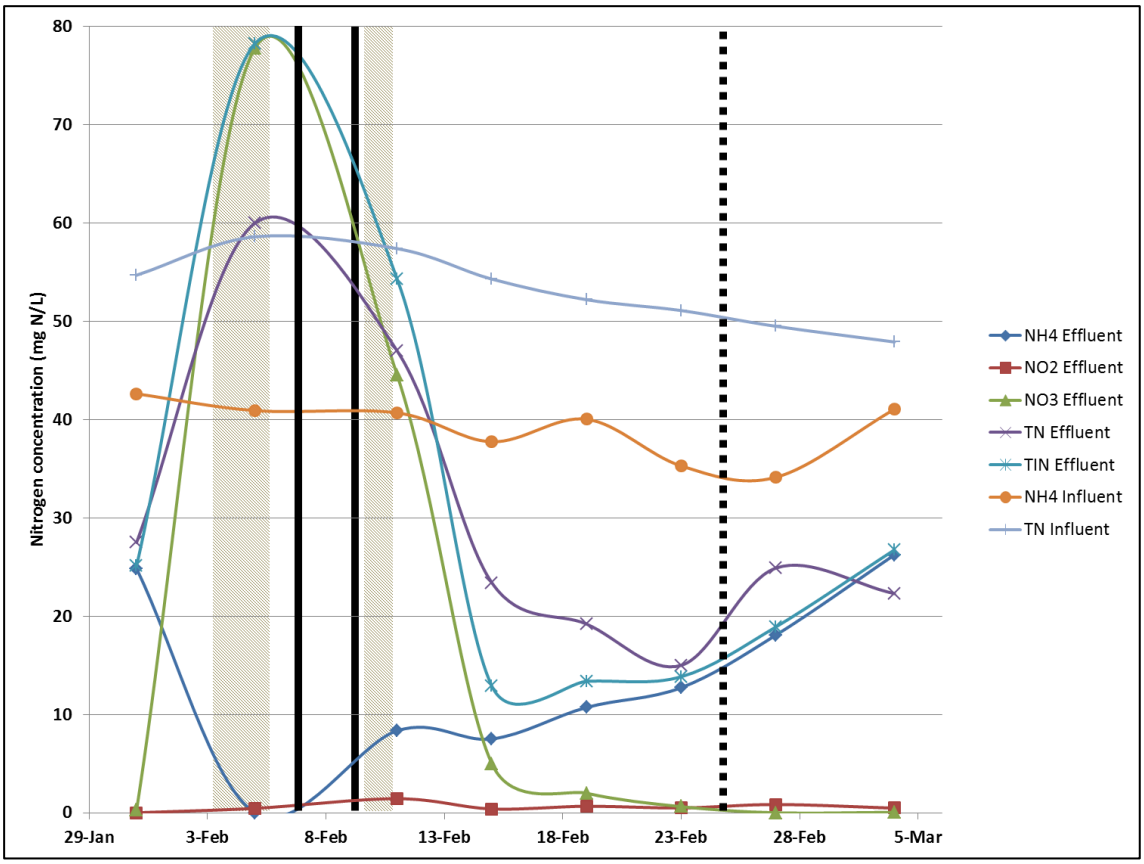

Figure 13. Time-series nitrogen speciation in SAGB4 following the intensive study. Shading indicates periods aeration. Dark vertical lines indicate the potential period of time for the 380-L accidental dose. The dashed line represents a 38-L inoculation of primary effluent.

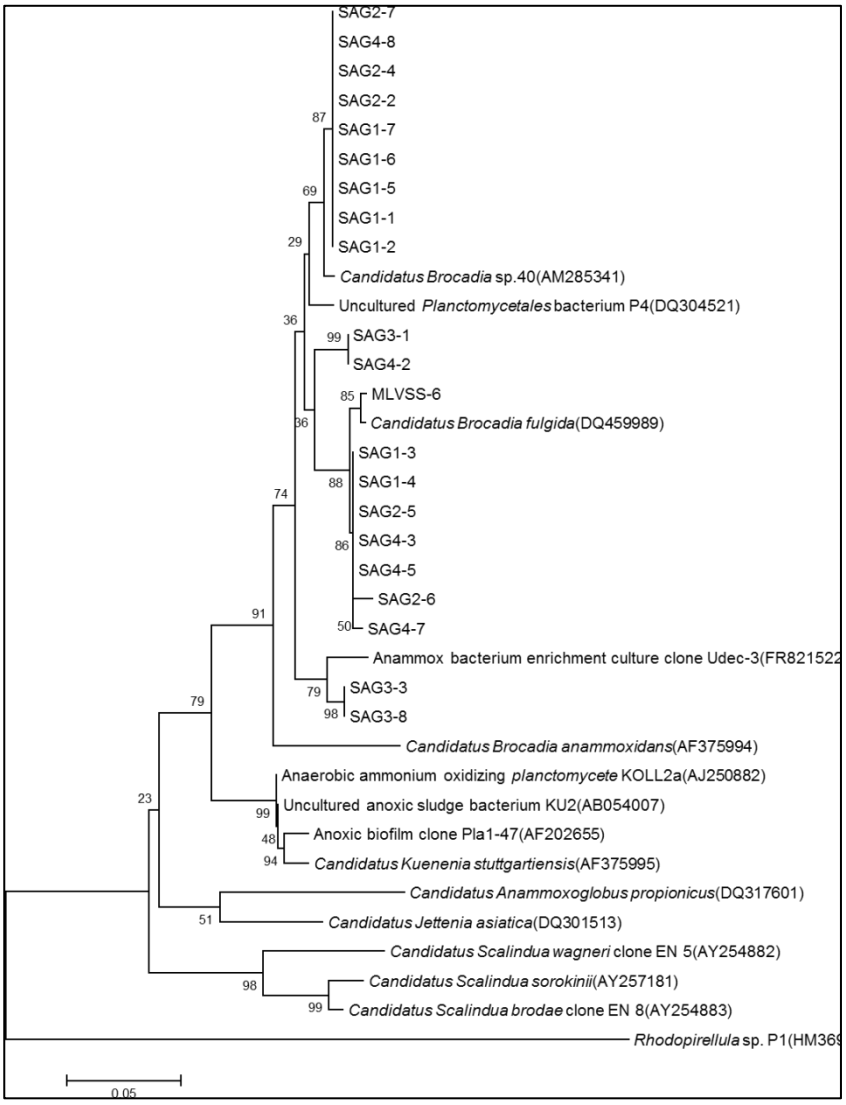

Figure 14. The phylogenetic tree of SAGB and MLSS samples that show Anammox bacteria present.

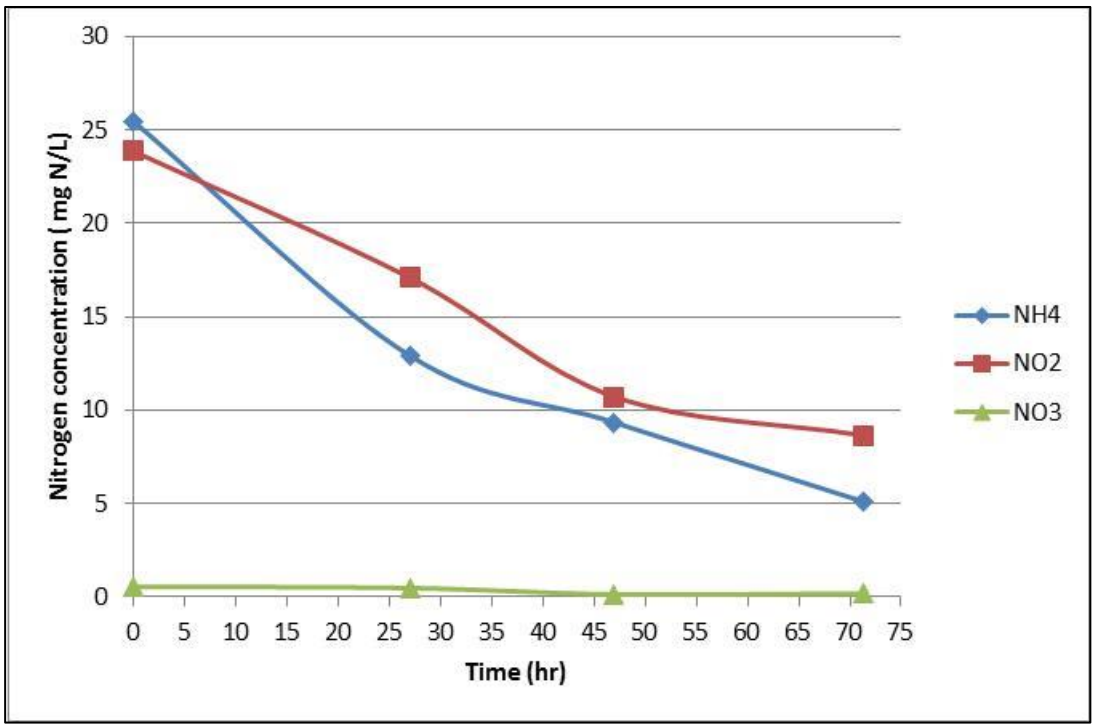

Figure 15. A plot of nitrogen species in an anammox seed culture over time.

CHAPTER 5
CONCLUSIONS, ENGINEERING SIGNIFICANCE, AND FUTURE RESEARCH

5.1 Conclusions

The hypotheses made were found to be correct in that SAGBs with pH-controlled aeration produced ammonium, nitrite, and COD effluent concentrations that were statistically greater than SAGBs with timer-controlled aeration. In addition, SAGBs with pH-controlled aeration produced TIN and nitrate effluent concentrations that were statistically less than SAGBs with timer-controlled aeration.

Multiple lines of converging evidence suggest that SAGB3 and SAGB4 operated in partial nitritation-anammox mode during the intensive study. The lines of evidence for this are:

1. Total nitrogen removal exceeded estimated removal from denitrification/denitritation and biological assimilation;
2. Elevated nitrite concentrations in port (SAGB3 & SAGB4) and effluent (SAGB3 only);
3. Significant ammonium removal during periods of anaerobic conditions;
4. Anammox bacteria DNA likely found in SAGB3 and the anammox seed culture; and
5. Anaerobic ammonium removal shown to have occurred in the anammox seed culture, which was used to inoculate the SAGBs.

While SAGB3 was most likely operated in partial nitritation-anammox mode, the aeration was likely not controlled as intended. The aeration, while a function of pH, seemed to be primarily affected by timed influent doses of a higher pH than of biological activity. This dose effect makes it difficult to discern anammox activity using pH fluctuations. However, it is clear that partial nitritation did occur in some regions of the

SAGB, and that total nitrification also occurred. The latter is due to a lack of aeration distribution. Regardless, the aerobic biological activity did cause the pH to move lower and thus turn off aeration.

The research conducted over two years presented in this thesis suggests that submerged attached growth bioreactors can be operated in partial nitritation-anammox mode. SAGB media can support a robust biofilm consisting of heterotrophic BOD-oxidizing bacteria, aerobic autotrophic ammonia-oxidizing bacteria, anammox bacteria, and heterotrophic denitrifiers. The data show that significant ammonia, total nitrogen, and COD can be removed with SAGB technology coupled with controlled aeration as a function of pH.

5.2 Engineering Significance

Of the three SAGB operating types, SAGB1 and SAGB2 produced an effluent that would meet typical NPDES permits for ammonia. The SAGBs with pH-controlled aeration were under-aerated in general, but as previously discussed, over-aerated near the pH port. It is difficult to conclude from these data that the SAGBs need more aeration. Rather, the lack of equal air distribution may have been a factor alone or in conjunction with a short aeration cycle. The difference between the SAGBs with and without recirculation may be attributed to greater anammox activity in the non-recirculated SAGB.

The average COD of the effluent of all SAGBs was less than 25 mg/L, which means that the average BOD of each effluent was less than 25 mg/L. This means that the BOD limit of a typical NPDES permit would be met with each SAGB. It further demonstrates that SAGBs with pH-controlled aeration are capable of producing allowable effluents in terms of BOD with the synthetic wastewater used.

The removal efficiencies obtained during the intensive study were compared to removal efficiencies in previous studies for three regulated constituents. SAGB1 and

SAGB2 had higher ammonia and COD removals than those obtained during a study by Redmond (Table 6). There are multiple possible reasons to explain this difference. The study conducted by Redmond used municipal primary effluent as influent, which had a greater oxygen demand. Because of this greater oxygen demand, more of the oxygen would have been used by faster-utilizing heterotrophs than the nitrifying autotrophs. In addition, the study by Redmond consisted of days throughout the year in Iowa, where temperatures range from less 0° C to greater than 32°C, while the intensive study consisted of a relatively constant temperature of 20°C. Biological activity would have certainly been affected by these temperature differences. Redmond achieved greater total nitrogen removal most likely because there was sufficient TOC to not limit denitrification. Since a low-TOC synthetic wastewater was used during the intensive study, less total nitrogen removal was expected. Effluent removal efficiencies at a SAGB in Walker, IA are also compared to those achieved during the intensive study. As expected, 100% of the ammonia was removed at the Walker SAGB, in order to meet NPDES permit requirements.

5.3 Future Research

The research conducted for this thesis lays the groundwork for many future research opportunities related to optimization. Future research needs to be conducted in order to meet typical NPDES requirements. Recommendations for future research and modifications of the SAGB system include:

1. improving the aeration distribution system in order to create less pockets of concentrated dissolved oxygen;
2. determining the removal efficiencies of ammonium, total nitrogen, and COD using an influent that is not TOC-limited;

3. determining the removal efficiencies at lower temperatures in order to prove that this reactor type and operation process can be effective in cold climates;
4. performing in-situ DNA tests, such as Fluorescent In-Situ Hybridization (FISH), in order to establish the various microbial populations within the SAGBs;
5. conducting quantitative PCR (qPCR) analyses on concentrated effluent in order to determine the AOB/NOB ratio;
6. implementing nitrogen ion specific probes within the SAGBs in order to obtain a higher resolution of real-time nitrogen speciation; and
7. determining the removal of phosphorous with this technology.

Table 6. Comparison of removal efficiencies between SAGB types

SAGB	NH_4-N Removal (%)	Total Nitrogen Removal (%)	COD Removal (%)
1 & 2	100	45	97
3	67	48	76
4	36	53	83
Walker, IA	100	N/A	N/A
Redmond	82	80	89

APPENDIX

Figure A1. An image of the 908-L headtank

The pipe leads into the cooler. The synthetic wastewater constituents were added to this tank by climbing a ladder onto the supporting shelf. Water run through ion resin exchangers was used to fill the tank.

Figure A2. An image of the pipe connecting the headtank to the inside of the cooler.

The brass valve allows for small head tank samples to be collected, as well as draining the headtank. The blue-handled globe-valve allows for system isolation while the headtank is being filled.

Figure A3. An image of the influent piping system and dose tank.

The valve allows for an individual SAGB to be isolated. The vertical clear tubing allows for dose tank cleanout.

Figure A4. An image of an electronic dose valve placed directly under a dose tank.

Figure A5. An image of the influent manifold (PVC pipe) before the addition of pea gravel.

The dose valve has the electronic motor disconnected in this image to show that manual dosing using a wrench can be performed.

Figure A6. An image of the effluent manifold (perforated PVC penetrating the wall of the container).

The smaller-diameter PVC piping is a portion of the aeration distribution network before perforation.

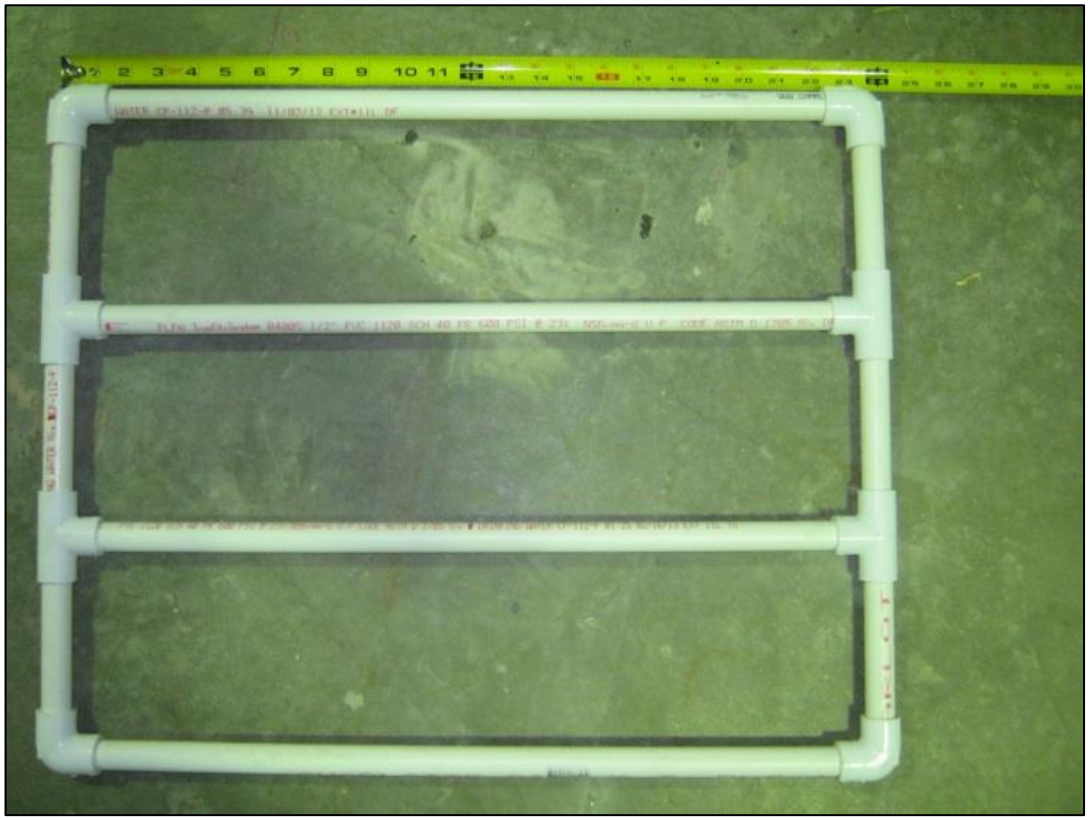

Figure A7. An image of the aeration distribution system (with dimensions) prior to perforations.

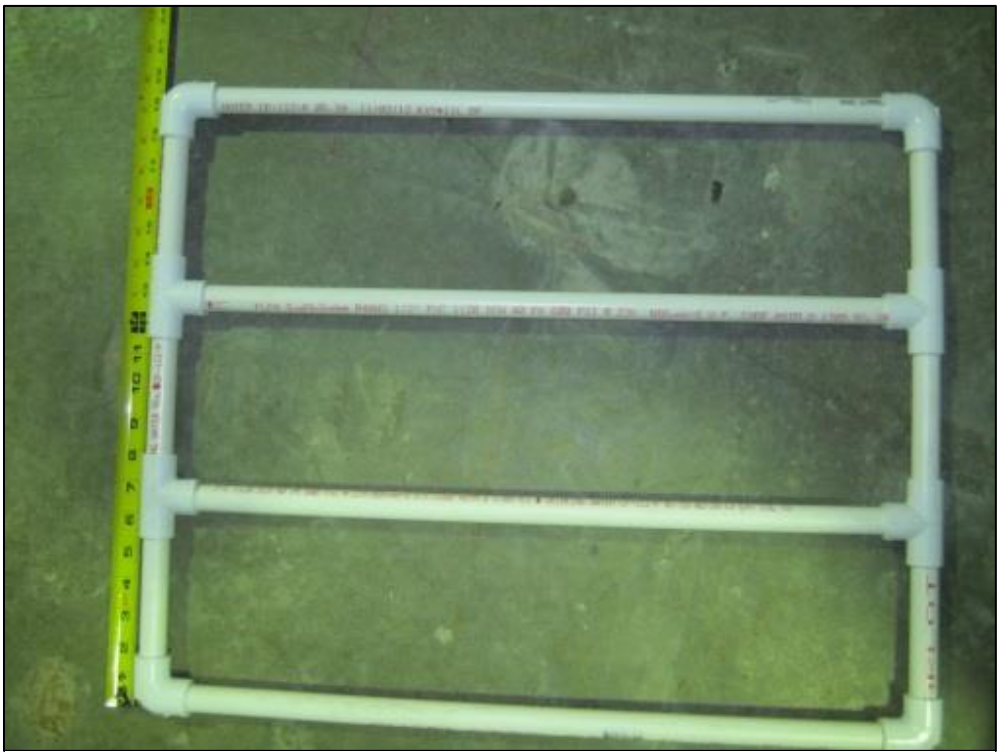

Figure A8. An image of the aeration distribution system (with dimensions) prior to perforations.

Figure A9. An image of an indivual SAGB and effluent catchment system.

Both stands were constructed with treated wood. This image was taken prior to placement in the cooler. The vertical piping that leads out of the container is the water level control apparatus that is open to atmosphere. This piping also served as an effluent sample port.

Figure A10. An image of the type of pea gravel used as the medium.

The pea gravel needed to be washed before being added to the SAGB container.

Figure A11. An image of the setup used to wash the pea gravel.

The perforated metal sheeting could support a bag of pea gravel at a time. A hose, along with spreading the pea gravel around with hands, was found to be the most efficient method of washing. With each bag, the first water to drain off was yellowish in color. The pea gravel was deemed to be washed once the water was clear for a few minutes.

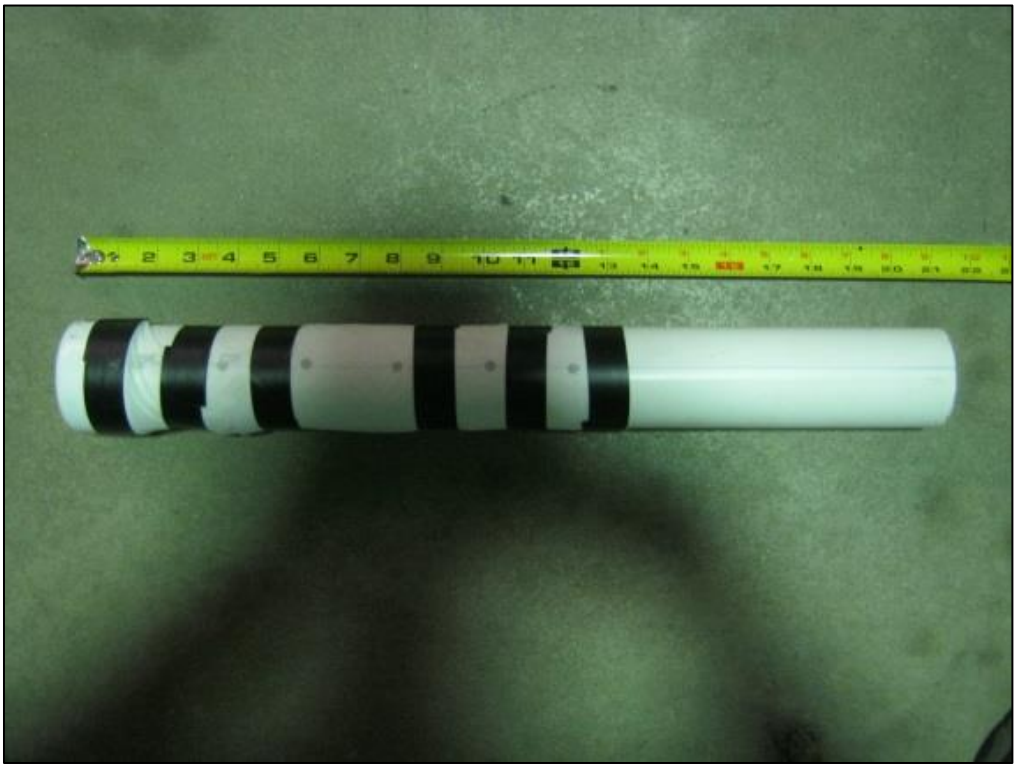

Figure A12. An image of a sample port prior to putting it into the SAGB.

The black electrical tape held a mesh fabric around the PVC. The holes were later widened to a 1" diameter in order to make the sample port more hydraulically-representative.

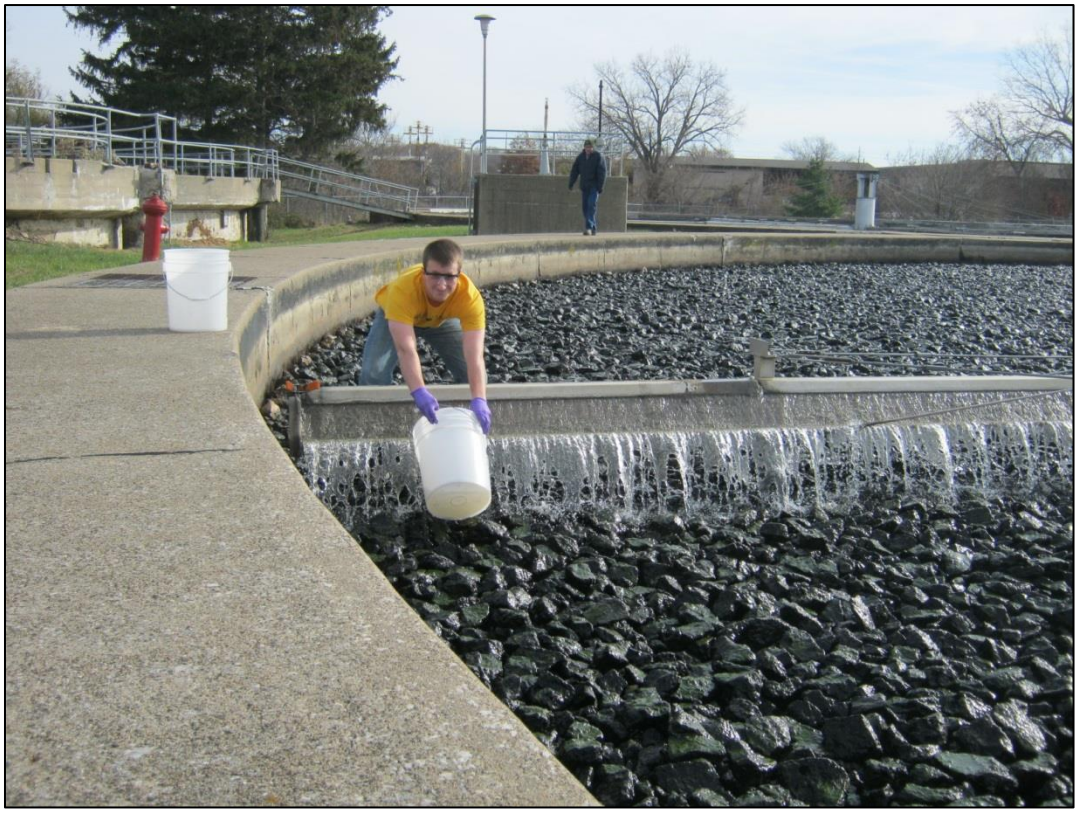

Figure A13. An image of James Shannon collecting primary effluent at the Iowa City North Wastewater Treatment Plant.

An operator stopped the distribution arms from moving and James walked out onto this trickling filter. The 5-gal buckets, collected from the UI Drinking Water Treatment Plant, were capped with lids and put into an IIHR Shop Truck. Note: this wastewater treatment plant is no longer in operation. (Image by: Katie Langenfeld)

Figure A14. An image of SAGB4, equipped with pH-controlled aeration and recirculation.

The gray wire is attached to an industrial-grade pH probe. The tubing going into this sample port is part of the recirculation system. The black apparatus in the upper right corner is the mass flow controller. The sample port with black electrical tape was used to measure dissolved oxygen using a Hach LDO probe.

Figure A15. An image of the aeration distribution manifold.

Figure A16. An image of the Hach SC200.

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