3-23-2016

Experimental Studies of Simultaneous Nitrification Denitrification and Phosphorus Removal at Falkenburg Advanced Wastewater Treatment Plant

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Experimental Studies of Simultaneous Nitrification Denitrification and Phosphorus Removal at Falkenburg Advanced Wastewater Treatment Plant

by

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A thesis submitted in partial fulfillment
of the requirements for the degree of
Master of Science in Environmental Engineering
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College of Engineering
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Date of Approval:
March 16, 2016

Keywords: Activated Sludge, Centralized Wastewater Treatment, Nitrogen Removal, Oxidation Ditch, Wastewater Optimization

DEDICATION

I dedicate this thesis to all of the people who would laugh and joke with me about the subject matter I was studying and to the 2009 University of New Hampshire Sailing Team who convinced me I should be an engineer.

ACKNOWLEDGMENTS

This material is based upon work supported by the National Science Foundation under Grant No. DUE 0965743. Any opinions, findings, and conclusions or recommendations expressed in this material are those of the author and do not necessarily reflect the views of the National Science Foundation.

I am thankful for the American Water Works Association- Florida Section for their support through the Roy W. Likins Memorial Scholarship.

This thesis was possible because of Hillsborough County Public Utilities Department (HCPUD). I greatly appreciate the support, encouragement and friendship of Marcus Moore and Dan Orloksy. My visits to Falkenburg Advanced Wastewater Treatment Plant were the highlights of this work. Additionally, the assistance from Gita Iranipour, Luke Mulford and everyone at HCPUD was critical to the success of this project - I am especially thankful for their advice and time.

Many thanks to my advisor and mentor, Dr. Sarina Ergas for her patience and knowledge.

I wish to express my deepest gratitude to Laura Rodriquez Gonzalez for the many miles carpooling brainstorming, to the many hours of being interrupted by me and for her support and friendship. I gratefully acknowledge Kyle Cogswall and Dr. Aydin Sunol for their teamwork and modeling assistance; Kathryn Harring for her assistance in the lab and to Sarah Sanford for her assistance in the lab, motivation and friendship; Jose Jimenez from Brown and Caldwell for his assistance with my lab protocol; the Tampa office of Hazen and Sawyer for their patience and understanding as I completed my thesis and for their assistance in analyzing my results and

conclusions; Leslie Knapp, my co-author on publications, who gave me some pretty big shoes to fill. I appreciate her words of inspiration, motivation and cooperation, it was a pleasure to work along side her.

Finally, to my friends who supported me and forgave me when I disappeared for days at a time in the lab, who kept me awake during late nights and early mornings and those who gave me a couch to sleep on and to Colleen Naughton, the MI's and MI Groupies for guiding me through my time as a graduate student and for your friendship. I would like to extend my deepest appreciation to Nancy and Jim for being my Florida parents, for keeping up on exactly what I was working on and giving me motivation to be my best and lastly to my parents, sister and brother, aunts, uncles and cousins- I thank them for helping me along by supporting and encouraging me - I could not have done this without them.

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ABSTRACT

The discharge of point- and non-point source pollutants into surface waters resulting from industrial and/or municipal activities is a major focus of environmental regulation in the United States. As a result, the National Pollutant Discharge Elimination System (NPDES) permit program was established in 1972 in an effort to regulate discharges from industrial or municipal sources, including wastewater treatment plants (WWTP). To further protect Florida water quality, in 1978, State legislation enacted the Grizzle-Figg Act for Tampa Bay, which requires advanced wastewater treatment for any discharge into sensitive water bodies. A common use of wastewater effluent in the Tampa Bay area is for reclaimed water for irrigation. This leads to an estimated 90% reduction of total nitrogen (TN) load to the bay in comparison to direct discharge (TBEP, 2016).

One type of wastewater treatment process that has been shown to have low aeration and chemical requirements is simultaneous nitrification denitrification (SND), which can be carried out in an oxidation ditch. SND is a biological process for nitrogen removal where nitrification and denitrification occur at the same time within the same reactor. An oxidation ditch is a race-track type reactor that promotes the occurrence biological conversion of reactive nitrogen to nitrogen gas (N_2) and additionally can provide enhanced biological phosphorus removal (EBPR). Many theories exist as to the mechanisms that allow SND to occur, but the literature is inconclusive as to whether the presence of different zones within the floc, within the reactor itself, a combination of the two or unique microorganisms are responsible for SND. Advantages of SND include efficient (80-96%) nitrogen removal, with significant reductions in energy,

chemical, equipment and spatial requirements. Specifically, oxygen requirements are reduced and dedicated aerobic/anoxic zones, internal recirculation and supplemental carbon and alkalinity are not required. Despite these advantages, widespread use of SND is limited because of a lack of understanding of SND kinetics as well as interactions between factors affecting SND performance.

This research was carried out at the Falkenburg Advanced Wastewater Treatment Plant (AWWTP) in Hillsborough County Florida, which carries out SND, biological and chemical phosphorous removal in an oxidation ditch system. Although this facility continually meets and exceeds its permit requirements, improvements in process control strategies have the potential to improve energy efficiency, as well as decrease chemical use, sludge production, greenhouse gasses (GHG) emissions and costs. Therefore, the overall goal of this research was to investigate mechanisms of nitrogen and phosphorus removal at the Falkenburg AWWTP. These goals were achieved through bench scale SND studies carried out at varying temperatures. Kinetic parameters were determined using a simple kinetic model of nitrification/denitrification. Additionally, carrying out sampling campaigns completed the investigation of the fate of phosphorus in the Falkenburg AWWTP. The results were combined with information on alum dosing and sludge wasting to determine the overall fate of phosphorus in the system and make additional recommendations regarding the addition of alum.

To mimic an oxidation ditch at Falkenburg AWWTP, bench scale bioreactor experiments were set up in glass beakers at 22°C and 29.5 °C. Influent wastewater and return activated sludge (RAS) for these experiments were collected from the Falkenburg AWWTP. Bioreactors were constantly mixed and aeration was controlled to maintain a target dissolved oxygen (DO) concentration based on measurements of DO at the facility. Three phosphorous sampling

campaigns (October, November and December) were also carried out to understand the fate of phosphorous, nitrogen and organic carbon at the facility. In these campaigns, samples were taken at six locations at Falkenburg AWWTP and samples were analyzed for filtered and unfiltered total phosphorus, orthophosphate and polyphosphates, filtered and unfiltered total nitrogen, soluble, total and readily biodegradable COD (rbCOD), volatile acids, cations, anions, alkalinity, total suspended solids (TSS) and volatile suspended solids (VSS). pH and DO were also measured on site.

In the nitrification batch reactors, in four hours, 50% of ammonia was successfully removed at a rate of 6.31 mg-N/L/hr indicating that four hours is not sufficient time to achieve complete removal. In the denitrification batch reactors, in six hours, there was successful removal of nitrate and nitrite at a rate of 23.70 mg-NO_3^-/L/hr and 3.6 mg-NO_2^-/L/hr. In an SND batch reactor experiments at 22° C, ammonia oxidation successfully occurred in 12 hours but denitrification was inhibited due to insufficient rbCOD in the reactor. In an SND batch reactor at 29.5° C, no accumulation of nitrate or nitrite was observed, indicating successful SND. At a higher temperature, sludge bulking occurred in the reactor resulting in variations in TSS and VSS concentrations.

Results from the sampling campaigns at the treatment plant indicate that successful phosphorus removal was achieved. Alum addition varied before each sampling and a relationship between alum addition and sulfate can be made. rbCOD was consumed throughout the treatment process as expected and noticeable results can be noted when rbCOD was low in terms of phosphorus removal.

The results of the bench-scale experiments showed that the SND was successfully achieved at the Falkenburg facility and that temperature, DO and rbCOD are all important

factors controlling biological nutrient removal at SND facilities. DO is much more difficult to maintain and control at a higher temperature further supporting the idea that stricter operator control is needed in warmer months. Additionally, because SND removal still occurred with poor DO control at 29.5°C, it further supports the idea that SND occurs because of zones within the floc, the reactor or that novel microorganisms exist that allow denitrification to occur above ideal DO concentration and nitrification to occur below ideal concentrations of DO. A variation in rbCOD in the influent wastewater at the treatment plant caused nitrification and denitrification to be inhibited in different trials. With too much rbCOD, nitrification was inhibited and with too little rbCOD, denitrification was inhibited. Additionally, alkalinity consumption was minimal which supports the idea that supplemental alkalinity is not needed in SND processes.

The results from the phosphorous sampling campaign show how important influent COD is for successful phosphorus removal in the system.

The objectives were achieved and overall, the plant is achieving SND and EBPR and the plant is performing as designed. The addition of alum should continue to be studied to determine a better dose and save the county ratepayers money while still meeting permit regulations. Jar tests should be used to determine the proper dosing that will not hinder the settling properties further in the treatment train. Additionally, alum feed pipe sizes should be investigated at the plant to ensure no clogging occurs with a decrease in alum flow and automated aeration based on ammonia concentrations should be considered to remove the manual operation of aerators.

CHAPTER 1: INTRODUCTION

1.1 Background

The discharge of point and non-point source pollutants into surface waters resulting from industrial and/or municipal activities is a major focus of environmental regulation in the United States, specifically the Clean Water Act of 1972 (EPA, 2005). The Clean Water Act's primary objective is to restore and maintain the quality of water bodies throughout the United States. As a result, the National Pollutant Discharge Elimination System (NPDES) permit program was established in 1972 in an effort to regulate discharges from industrial or municipal sources, including wastewater treatment plants (WWTP). Minimum regulated criteria for discharge by WWTPs include, but are not limited to, biological oxygen demand (BOD) and total suspended solids (TSS). Total maximum daily load (TMDL) calculations for specified criteria are used to determine the maximum amount of a pollutant a water body can receive while still maintaining established water quality standards (WQS). Recently, increased sensitivity in TMDL's have driven down the NPDES effluent limits for nitrogen and phosphorus (Ergas and Aponte-Morales, 2013), as many water bodies, including Florida's fresh water springs and marine waters in the Florida Keys, are extremely sensitive to these pollutants. As a result, WQS call for more stringent control on wastewater treatment methodologies, and/or the design and operation of advanced wastewater treatment plants (AWWTP). To further protect Florida water quality, in 1978, State legislation enacted the Grizzle-Figg Act for Tampa Bay, which requires advanced wastewater treatment for any discharge into sensitive water bodies. A common use of wastewater effluent in the Tampa Bay area is for reclaimed water for irrigation. This leads to an estimated

90% reduction of total nitrogen (TN) load to the bay in comparison to direct discharge (TBEP, 2016).

If WQS are ignored, impacts on water bodies include increased potential for eutrophication. Eutrophication occurs when excess nutrients, including nitrogen and phosphorus, result in excess algal growth. Typically, the availability of these nutrients act as a limiting factor with respect to algal growth. Unchecked, algal growth can block filters and intake pipes for water treatment plants, or pass through them causing bad odors, taste, and potential health impacts in the treated waters (Goel and Motlagh, 2013). Subsequent die off and decomposition of biomass results in degradation of water quality with respect to decreased dissolved oxygen (DO) levels, and increased turbidity (Fuerhacker, 1999). These factors are significant contributors to the decline of aquatic and marine habitats, including coastal nurseries and sea grass beds, upon which numerous species depend for both food and habitat. In addition to the dangers to marine and aquatic life, human health can be affected if nitrate-rich water is ingested. Methemoglobinemia, or blue baby syndrome, occurs in infants when nitrate oxidizes iron in hemoglobin in red blood cells to methemoglobin back to hemoglobin too slowly, hindering the infants' ability to carry oxygen in blood (Ergas and Aponte-Morales, 2013).

The advancement and evolution of wastewater treatment methodologies continues pursuant to four basic principles: (1) human health (2) environmental concerns, (3) cost effectiveness and 4) sustainability. Combined, these principles have led to the design and operation of facilities that promote the biological and chemical removal of solids, organics, nutrients, metals, toxic compounds and pathogens from wastewater prior to the discharge of effluent waters into the environment. The need to conserve resource use has incentivized improving process sustainability through the optimization of treatment processes. Sustainability

issues, including energy consumption, process related greenhouse gas (GHG) emissions, chemical usage and carbon footprint, are now of critical importance to the design, construction and operation of any WWTP (Metcalf & Eddy, 2014). Aeration is the single largest energy consuming operation at a WWTP, accounting for 45-75% of treatment energy costs. As a result, investigations of wastewater treatment processes with lower oxygen requirements are increasing (Arnaldos et al., 2014). Another large cost of wastewater operations are for chemicals, such as those used for phosphorus removal and alkalinity consumption, resulting in the need for research on optimization of chemical use in treatment plants.

One type of wastewater treatment process that has been shown to have low aeration and chemical requirements is simultaneous nitrification denitrification (SND), which can be carried out in an oxidation ditch. An oxidation ditch is a race track type reactor that promotes the occurrence biological conversion of reactive nitrogen to nitrogen gas (N_2) and additionally can be configured to provide enhanced biological phosphorus removal (EBPR). SND is a biological process for nitrogen removal where nitrification and denitrification occur at the same time within the same reactor. Many theories exist as to the mechanisms that allow SND to occur, but the literature is inconclusive as to whether the presence of different zones within the floc or within the reactor itself or a combination of the two are responsible for SND. Advantages of SND include efficient (80-96%) nitrogen removal, with significant reductions in energy, chemical, equipment and spatial requirements. Specifically, oxygen requirements are reduced and dedicated aerobic/anoxic zones, internal recirculation and supplemental carbon and alkalinity are not required. Despite these advantages, widespread use of SND is limited because of a lack of understanding of SND kinetics as well as interactions between factors affecting SND performance (Jimenez et al, 2010).

The Falkenburg AWWTP in Hillsborough County, Florida uses oxidation ditches preceded by anaerobic selectors for treatment of domestic and a small fraction of industrial wastewater. Total phosphorous (TP) is removed using a combination of EBPR and aluminum sulfate (alum) coagulation. The average influent flow rate is 9.27 million gallons per day (MGD), with a permitted annual average daily flow rate of 12.0 MGD. The plant's NPDES permit requires the removal of carbonaceous Biochemical Oxygen Demand (cBOD5), TSS, total nitrogen (TN) and TP to levels of 5, 5, 3, and 1 mg/L (annual averages), respectively.

1.2 Research Objectives

Although the Falkenburg AWWTP continually meets and exceeds its permit requirements, improvements in process control strategies have the potential to improve energy efficiency, as well as decrease chemical use, sludge production, GHG emissions and costs. In addition, there is a limited understanding of the mechanisms behind SND and EBPR in SND systems. Therefore, the overall goal of this research was to investigate mechanisms of nitrogen and phosphorus removal at the Falkenburg AWWTP in Hillsborough County, Florida. This was achieved through the following:

- Bench scale SND studies carried out at varying temperatures. The experimental results at each temperature were compared and then compared to results from full plant nitrogen results.
- Investigation of fate of phosphorus in the Falkenburg AWWTP by carrying out sampling campaigns. The results were combined with information on alum dosing and sludge wasting to determine the overall fate of phosphorus in the system and make additional recommendations regarding alum addition.

CHAPTER 2: LITERATURE REVIEW

This chapter focuses on biological nitrogen removal in SND systems and combined EBPR and chemical phosphorous removal in wastewater treatment.

2.1 Nitrogen Removal

2.1.1 Nitrification

Nitrification is the biological oxidation of ammonia (NH_4^+) to nitrite (NO_2^-) and then to nitrate (NO_3^-) by autotrophic nitrifying prokaryotes that use oxygen as a terminal electron acceptor through a two- step process (Figure 2.1). The first step is nitritation, which is carried out by ammonia oxidizing bacteria (AOB) and ammonia-oxidizing archaea (Eq. 1):

$$2NH_4^+ + 3O_2 \rightarrow 2NO_2^- + 4H^+ + 2H_2O \quad [1]$$

The second step is the oxidation of nitrite to nitrate, which is carried out by nitrite oxidizing bacteria (NOB) (Eq. 2):

$$2NO_2^- + O_2 \rightarrow 2NO_3^- \quad [2]$$

The overall reaction, if biosynthesis is included, can be shown as (Ergas and Aponte-Morales, 2013):

$$NH_4^+ + 1.86\ O_2 + 0.098\ CO_2 \rightarrow 0.0196C_5H_7O_2N + 0.094H_2O + 1.92\ H_2CO_3 + 0.98NO_3^-\ 1.98H^+ \quad [3]$$

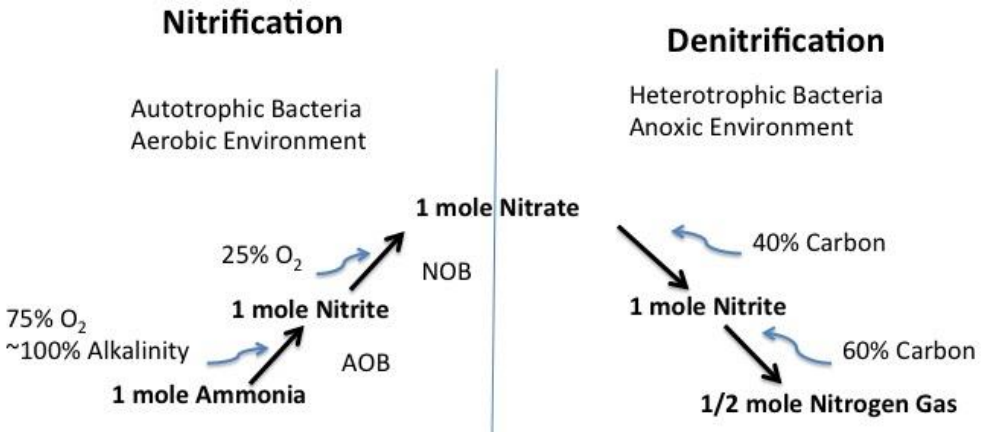

Figure 2.1 Nitrification and Denitrification

2.1.2 Nitrification Growth Kinetics

The rate of nitrification depends on the DO concentration, pH, temperature and the presence of metals and other toxic compounds. To achieve nitrification in WWTPs, it is common to operate the biological process at bulk DO levels above 2.0 mg/L. The system costs are increased and their energy requirements tend to increase with increasing aeration rates so the investigation of the ability of nitrifying communities to carry out nitrification at low DO concentrations is of great importance (Arnaldos et al., 2014). The optimum pH for nitrification is between 7.2 and 9.0 and below a pH of 6.8 the rates significantly decline (Ergas and Aponte-Morales, 2013; Metcalf & Eddy, 2014). Additionally, alkalinity is destroyed at a rate of 7.07 mg/L for every NH_4^+-N oxidized (Eq. 3). Reaction rates increase with increasing temperature until a maximum rate is reached (Rabionowitz, 2004). Therefore, a longer SRT will be necessary for nitrification at low temperatures (Ergas and Aponte- Morales, 2013). At temperatures greater than 25° C, the rate controlling factor isn't temperature but the conversion of nitrite to nitrate. Between 30 and 35° C, nitrification will not fail, but nitrite accumulation will become controlling (Rabinowitz, 2004).

The Hoff-Arrhenius equation (Metcalf and Eddy, 2014) describes the variations of rate with temperatures where:

$$k = Ae^{-\mu/RT} \quad [4]$$

k = reaction rate coefficient, d^{-1}
A= pre-exponential constant for the reaction, d^{-1}
μ = temperature coefficient, J/mol
R= ideal gas constant, 8.314 J/mol*K
T= absolute temperature, K

The presence of toxic compounds, such as amines, proteins, tannins, phenolic compounds, alcohols, cyanates, ether, carbamates and solvents can inhibit nitrification (Ergas and Aponte-Morales, 2013). Complete inhibition of nitrification occurs at 0.25 mg/L nickel, 0.25 mg/L chromium and 0.10 mg/L copper. In addition, nitrification is inhibited by un-ionized ammonia (Metcalf & Eddy, 2014).

2.1.3 Denitrification

Denitrification is the biological reduction of nitrate to nitrogen gas under anoxic conditions (Metcalf & Eddy, 2014; Ergas and Aponte-Morales, 2013; Critteneden and Trussel, 2005) (Figure 2.1). When DO concentrations are below 0.5 mg/L and NO_3^- is present, denitrifying bacteria will couple oxidation of organic carbon compounds to CO_2 with the reduction of NO_3^- to N_2 gas. At higher DO concentrations, denitrifiers utilize more thermodynamically favorable O_2 as an electron acceptor, which inhibits denitrification (Ergas and Aponte-Morales, 2013).

The overall denitrification reaction can be shown as:

$$NO_3^- \rightarrow NO_2^- \rightarrow NO \rightarrow N_2O \rightarrow N_2 \quad [5]$$

The stoichiometry depends on the electron donor, but with organic carbon from wastewater, the overall reaction can be shown as (Ergas and Aponte-Morales, 2013):

$0.02C_{10}H_{19}O_3N + 0.13429NO_3^- + 0.13429\ H^+ \rightarrow 0.01429C5H_7O_2N + 0.06N_2 + 0.10857CO_2 + 0.15714H_2O + 0.02HCO_3^-$ [6]

Denitrifying bacteria are facultative bacteria capable of using nitrite or nitrate as terminal electron acceptors for respiration under anoxic conditions (Ergas and Aponte-Morales, 2013). Denitrifying bacteria will use either the biodegradable soluble chemical oxygen demand (COD) in the influent wastewater, the COD produced during endogenous decay or an exogenous source, such as methanol, acetate, ethanol, elemental sulfur or hydrogen as the electron donor (Metcalf & Eddy, 2014). Low concentrations of electron donors, high DO, pH outside the range of 7-8 lead to an accumulation of NO_2^- and N_2O (Ergas and Aponte-Morales, 2013).

2.1.4 Nitrite Shunt

Nitrite Shunt (or Shortcut Nitrogen Removal), is a nitrogen removal process that involves the autotrophic oxidation of ammonium to nitrite and the heterotrophic reduction of nitrite to nitrogen gas. If nitrification stops at nitrite, skipping the conversion of nitrite to nitrate, then denitritation can occur (Jimenez et al, 2013; Ju et al., 2007), as shown in Figure 2.2. Advantages of nitrite shunt include 25% reduction in oxygen demand (which saves energy), 40% reduction in carbon demand and 40% reduction in biomass production (which reduces sludge disposal costs) in comparison to conventional nitrification/denitrification (Jimenez et al, 2014 and AECOM, 2012). Disadvantages include a lack of complete understanding of the underlying mechanisms and proper design, control and operational guidelines. Low DO has been shown to suppress NOB while high DO tends to favor AOB over NOB in studies of mainstream nitrite shunt systems (Jimenez et al, 2013).

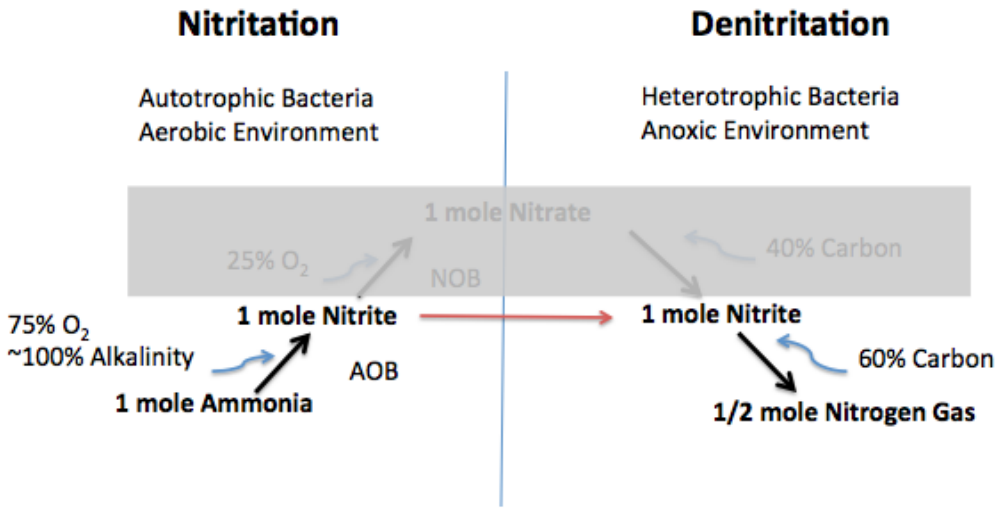

Figure 2.2 Nitritation and Denitritation

2.1.5 Simultaneous Nitrification Denitrification

SND is a biological process for nitrogen removal where nitrification and denitrification occur at the same time within the same reactor (Jimenez et al., 2010). Advantages and disadvantages of SND are listed in Tables 2.1 and 2.2. The literature is inconclusive as to the mechanisms of SND; however, several theories have been proposed:

- Anoxic micro-environments, or the presence of microscopic anoxic and aerobic zones within the sludge flocs caused by DO consumption on the outside of the floc (Figure 2.3; Kaempfer et al., 2000; Satoh et al., 2003; Stensel, 2001; Pochana and Keller, 1999);
- Aerobic denitrification (Zhao et al., 1999; Hippen et al., 1997);
- Shortcut nitrogen removal (Figure 2.2; Villaberde et al, 2000; Yoo et al, 1997);
- The macro-environment, or presence of aerobic and anoxic zones within the reactor;
- The presence of novel microorganisms (Daigger and Littleton, 2000).

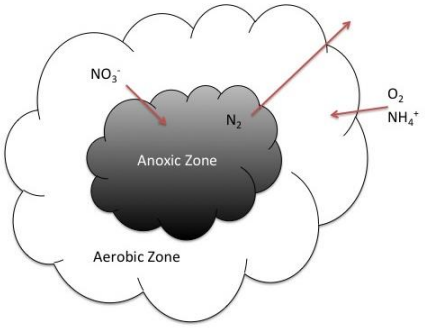

Figure 2.3 Conceptual Model of SND

A number of factors, including the concentration of DO, COD, TN, hydraulic residence time (HRT) and solids residence time (SRT), influence nitrifiers but DO is one of the most important (Puznava et al, 2000). Rittman et al. (1985) suggested that SND occurs in a micro-environment with the appropriate carbon supply, DO concentrations and floc size. Barnard et al. (2004) found that while SND is the result of many factors, the main factor is the DO gradient within the floc. Puznava et al. (2000) stated that the main physical explanation for SND is the occurrence of SND within microbial flocs as a result of oxygen diffusion. The authors found through investigation of aeration strategies at treatment plants that nitrifying organisms arrange themselves in the outer layer of the floc where oxygen is available. As long as the floc is not broken up by aeration, denitrification will occur in anoxic zones in the floc. If this theory were true, there would not be enough of a carbon source to promote denitrification in the anoxic environment in the inner part of the floc, leading the authors to believe that a combination of both zones within the floc and zones within the reactor cause SND to occur. With aeration, the floc is able to utilize some of the carbon from the outer environment and thus is able to promote denitrification within the floc (Barnard et al., 2004).

Pochana and Keller (1999) proposed that an increase in readily biodegradable COD (rbCOD) increased SND activity and that increases in DO caused a decrease in SND activity. Additionally, Pochana and Keller (1999) and Pochana et al (1999) found that with a decrease in

floc size came a decrease in nitrogen removal, as the flocs were unable to withstand the sheering, eliminating the different anoxic and aerobic zones.

In terms of novel microorganisms being responsible, two theories exist. The first is that the organisms responsible for denitrification within the anoxic zone are able to continue to reduce nitrogen after oxygen levels increase for an undetermined amount of time (Kugleman et al., 1991). The second is that microorganisms responsible for denitrification have a greater physiological variety than originally thought. Some of these denitrifying microorganisms could be autotrophic, which would reduce their rbCOD requirements (Tonkovic, 1998; Drysdale et al, 1999; Littleton and Daigger, 2002; Helmer and Kunst, 1998).

While many authors argue that different zones within the floc are responsible for SND, others argue that SND can be achieved within the same reactor with temporally separated aerobic and anoxic zones that are created by cyclic aeration (Alleman and Irvine, 1980; Randall et al., 1992; Sedlak, 1991; Silverstein and Schroder, 1983). In an oxidation ditch, the spatial separation of anoxic and aerobic zones within the ditch are created by adjusting aeration (Liu et al., 2010). The presence of a macroscopic anoxic and aerobic zones within the reactor are created by aerobic zones forming near the aerators and anoxic zones forming away from the aerators (Kaempfer et al., 2000; Satoh et al., 2003; Stensel, 2001). Ju et al. (2007) concluded that cyclic aeration (one hour at 0.8 mg/L, one hour at 0.2 mg/L) was better than constant aeration to avoid bulking along with shortening the required system SRT. Nitrogen removal during cyclic aeration resulted in more available nitrate and nitrite for denitrification. In these lab studies, nitrite shunt was observed in the low DO systems and results indicated that nitrite shunt likely took place because of the disrupted nitrification at low DOs. These lab studies were consistent with observations at full-scale wastewater treatment systems (Ju et al., 2007).

Holman and Wareham (2004) investigated the microbiological mechanisms involved in SND processes using bench scale sequencing batch reactor (SBR) systems. They found that the jumps in DO concentrations could be directly related to changes in COD and ammonia concentrations, which could cause an increase in DO. At low DO levels, the decrease in a DO concentration was believed to be due to microbial activity causing the DO to be utilized though COD and ammonia oxidation as quickly as it was supplied. The second increase in DO concentration was believed to have occurred when the COD was depleted. DO was also observed an increase when the ammonia concentrations were depleted. The authors stated that the lack of nitrate detected could indicate that the oxidation of nitrite to nitrate may not exist in SND or that nitrite could be reduced to nitrogen gas directly from nitrite, thus skipping the oxidation to nitrate and reduction to nitrite. The experiments concluded that at DO concentrations over 1.0 mg/L SND becomes inhibited. While the literature suggest that SND is inhibited at concentrations over 1.0 mg/L, the experimental results indicate that aerobic denitrifiers are likely able to continue to aerobically reduce nitrogen for a limited amount of time once the DO concentration is increased to about 1.0 mg/L. The authors concluded that SND is suppressed at high air flow rates further backing up the idea that SND is based on the mechanism of different zones within the floc.

Table 2.1 Advantages of SND

Advantages	**Source**
Achieves removal of 80-96% total nitrogen	Jimenez et al., 2010; Zeng, 2003; Fuerhacker, 1999
Eliminates the need to build separate tanks	Jimenez et al., 2010; Ergas and Aponte-Morales, 2013; Ju et al., 2007; Yoo, 1997
Produce less nitrous oxide emissions than conventional nitrogen removal processes	Jimenez et al., 2010
Simpler process design with a smaller total tank size	Kaempfer et al., 2000; Stensel, 2001; Ju et al., 2007

Table 2.1 (Continued)

Maintains a relatively neutral pH in the bioreactor without the addition of an external acid or base because alkalinity consumed by nitrification is partially recovered by alkalinity production in denitrification.	Ju et al., 2007; Grady et al., 1999; Ju et al., 2007

Table 2.2 Disadvantages of SND

Disadvantages	**Source**
Difficult to control	Jimenez et al., 2010
Requires an understanding of the kinetics and the interaction of the factors affecting its performance	Jimenez et al., 2010
Challenges in design, control and operation	Grady et al., 1999; Jenkins et al., 2003; Martins et al., 2004
More susceptible to sludge bulking, primarily because of the excessive growth of filamentous bacteria	Grady et al., 1999; Jenkins et al., 2003; Martins et al., 2004
Relies on achieving a balance between nitrification and denitrification	Grady et al., 1999; Jenkins et al., 2003; Martins et al., 2004
Long SRT	Ergas and Aponte-Morales, 2013
Strict control over DO concentration	Ergas and Aponte-Morales, 2013

2.1.6 Oxidation Ditch

An oxidation ditch is a term used to describe a loop shaped reactor with a continuous flow where all reactions occur at the same time in the same reactor (Rittmann and Langeland, 1985). It is a modified activated sludge system that utilizes a long SRT. As of January 2016, there were 58 oxidation ditches in use in Florida at domestic wastewater facilities (FDEP, 2016). An oxidation ditch is an economical and efficient technique for biological wastewater treatment (Yongzhen et al., 2007) that can achieve high removal of nutrients with low operational and energy requirements and operation and maintenance costs. Oxidation ditches have an added measure of reliability and performance due to the constant water level, continuous discharge, long HRT, and mixing which minimizes shock loading and surges, and long SRT's, which produce less sludge (EPA, 2002). Oxidation ditches are able to promote SND due to the establishment of alternating aerobic and anoxic zones, which are created by the distance and time

between aerators (Rittmann and Langeland, 1985). They have also been shown to have the ability to remove phosphorus without the high consumption of alkalinity (Yongzhen et al., 2007).

Significant disadvantages of oxidation ditches include: high suspended solids concentrations, the large footprint required (EPA, 2000) and the absence of studies on how to create a feasible environment for SND to occur (Liu et al., 2010). A schematic of an oxidation ditch is shown in Figure 2.4.

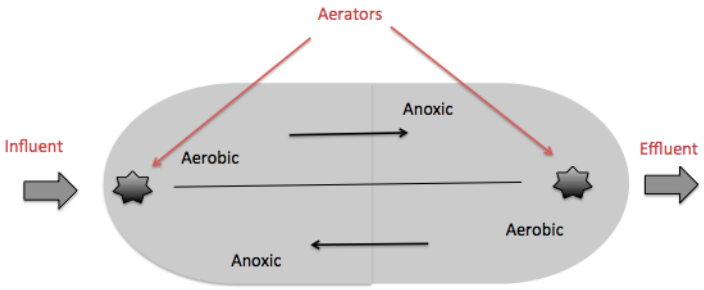

Figure 2.4 Schematic of an Oxidation Ditch

2.2 Phosphorus Removal

Phosphorus in municipal wastewater is often found in the form of orthophosphate, polyphosphate and organic phosphorus (Moore, 2009) (Figure 2.5). Orthophosphate can be soluble and can be precipitated using coagulants while polyphosphates cannot.

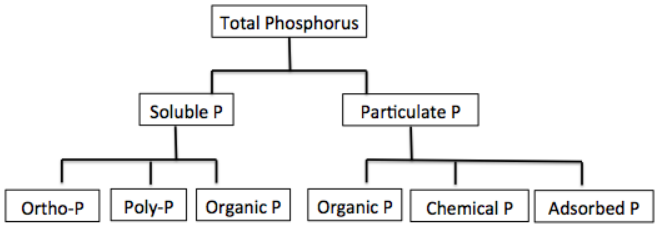

Figure 2.5 Phosphorus Species in Wastewater

Typical total phosphorus concentrations in municipal wastewater influent range from 6-8 mg-P/L, with concentrations of orthophosphate between 3-4 mg/L, polyphosphate of 2-3 mg/L

and organic phosphate of around 1 mg/L (WEF and ASCE, 2005). Phosphorus is commonly removed by a combination of both chemical and biological removal processes.

2.2.1 Enhanced Biological Phosphorus Removal

EBPR systems are constructed with an anaerobic zone followed be an aerobic and/or anoxic zone, as shown in Figure 2.7. These systems favor the growth of organisms that have the ability to accumulate polyphosphates in the aerobic zone, these are known as polyphosphate accumulating organisms (PAO) (Goel and Motlagh, 2013). In the anaerobic zone, PAOs assimilate fermentation products, such as rbCOD in the form of volatile fatty acids (VFA) (Metcalf and Eddy, 2014). PAOs have an advantage over other heterotrophic bacteria because other heterotrophs need an electron donor, such as oxygen, (Figure 2.6), which is not present in a reactor designed to put PAOs at an advantage over other organisms, like an anaerobic selector (Figure 2.7). In the aerobic zone, energy is produced in the oxidation stage, which allows for more growth and consumption of more phosphorous (Metcalf and Eddy, 2014; Jimenez et al., 2014).

To help with this process, primary clarifiers can act as a fermenter to produce many more VFAs on site (Metcalf and Eddy, 2014). Sewage contains a high proportion of VFAs, which are synthesized by fermentation under anaerobic conditions. These conditions may exist during sewage transport to the treatment plant (Arun et al., 1988). In flat topography and warmer climates, such as Florida, primary clarifiers are not frequently used, as the sewage has a long HRT in the transport process, allowing fermentation to occur before reaching the treatment plant. Phosphorous in the influent stream is incorporated into cell biomass, which is wasted during sludge wasting (Metcalf & Eddy, 2014). The phosphorus removed through treatment is

incorporated into sludge, which is then subject to a variety of different treatments, such as those which allow reuse.

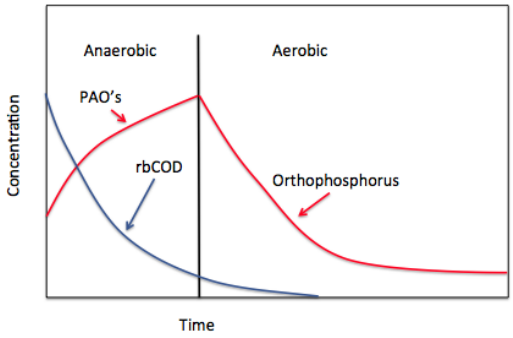

Figure 2.6 Fate of rbCOD and Phosphorus in EBPR

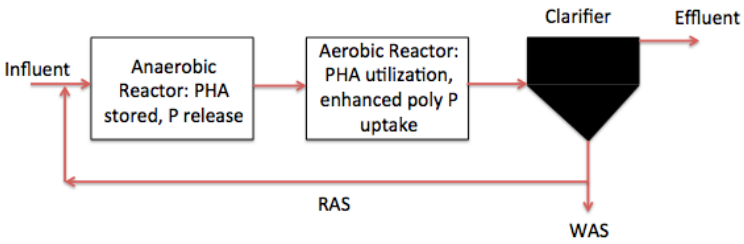

Figure 2.7 EBPR Typical Reactor Configuration

EBPR can be incorporated in a reactor that promotes the SND process, such as an oxidation ditch, by encouraging denitrification mediated by PAOs (Zeng et al., 2003; Meyer et al, 2005). EBPR occurs in an oxidation ditch by using the anaerobic zones (areas away from the aerators) as a fermentation zone for the production of VFAs and PAOs. In this area, there will also be a small release of phosphorus that accompanies fermentation. To improve EBPR in a configuration with an oxidation ditch, an anaerobic reactor may be added prior to the oxidation ditch for additional phosphorus removal (Figure 2.8). Yongzhen et al. (2008) achieved successful removal of nitrogen and phosphorus in a pilot scale oxidation ditch and concluded that an oxidation ditch is suitable to remove both. Ju et al. (2007) found through bench scale bioreactor experiments with cyclically aerated mixed liquor that phosphate concentrations increased during

the low DO periods and decreased in high DO periods (similar to that of a separate basin tank). The authors concluded that cyclically aerated reactors had a higher phosphate removal than steady aeration, which supports the feasibility of enriching PAOs in a low DO SND system. The authors also completed plant case studies at a treatment plant with an oxidation ditch where they found that the plants showed similar phosphorus removal as the bench scale bioreactors. Typical influent TP concentrations were over 7.0 mg-P/L and effluent concentrations were 1.0 mg-P/L. Littleton et al. (2007) demonstrated through a theoretical model of an oxidation ditch that heterotrophs and PAOs were controlled by oxygen input, but that it was possible to achieve phosphorous removal in the same basin as biological nitrogen removal. Several studies have shown that a lower phosphorus removal rate is found at low DO levels compared to aerobic zones, but nitrate can be used as an electron acceptor for denitrifying PAOs, allowing phosphorus removal to occur in an SND reactor. Jimenez et al. (2013) investigated this theory at the Southwest WRF in St. Petersburg, FL (a simple A/O process configuration with no inter-reactor mixed liquor recycle, only return activated sludge (RAS) recycle) and found that the plant achieved effluent phosphate concentrations of approximately 0.1 mg-P/L, which contradicts the belief that a DO concentration of 1.5 mg/L is necessary for EPBR. Additionally, the authors found that phosphate uptake did not occur with nitrite as an electron acceptor.

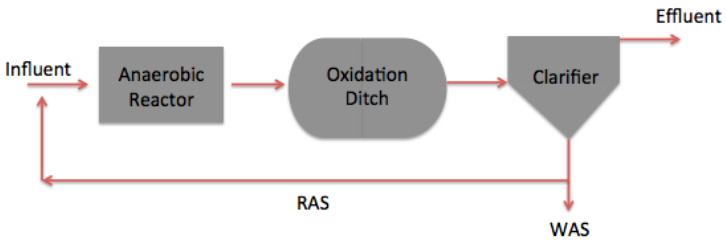

Figure 2.8 Common EBPR Reactor Configuration with an Oxidation Ditch

Activated sludge facilities can achieve greater than 90% phosphorus removal when the anaerobic tanks are configured before aerobic tanks. In this case, an effluent concentration of about 1.0 mg-P/L can be achieved. Improvement in phosphorus removal occurs because each reaction is optimized separately rather than all reactions occur in the oxidation ditch (Yeoman et al., 1988).

Operational considerations for EBPR include (Goel and Motlagh, 2013; Jeyanayagam, 2005):

- Maintain DO levels of 0.5-1.0 mg/L O_2 at the end of the aerobic zone
- Influent BOD: P ratio of at least 25:1
- Monitoring recycled phosphorous loading, as the sludge dewatering return flows can contain a high concentration of phosphorous, which increases the influent load to the WWTP and reduces the BOD:P ratio. With a decreased ratio, the biological process will be overwhelmed leading to insufficient VFA concentration in the anaerobic phase.
- If PAOs in the anaerobic tank release stored phosphates too soon and fail to uptake the available VFA's, secondary phosphorous release occurs in the clarifiers.

2.2.2 Chemical Phosphorous Removal

The most common way to achieve phosphorous effluent concentrations below 1.0 mg-P/L is by the chemical addition of metal salts (i.e. alum or ferric chloride) (Metcalf and Eddy, 2014). Chemical addition can be performed using four different strategies:

- Pre-precipitation, in which coagulants are added to the raw sewage. This process produces more sludge, which can be good for the production of biogas but adds to the amount of sludge handling needed;

- Co-precipitation, in which coagulants are added during or before/after activated sludge treatment. BOD, heavy metals and viruses are all removed in this process; however, the sludge volume increases and the aeration causes floc shearing and poor settlability;
- Post-precipitation, in which coagulants are added as a "polishing stage" after secondary sedimentation. Unlike the others, post-precipitation does not increase the amount of sludge produced, results in excellent effluent quality and has lower chemical requirements (Karlsson, 1985; Metsch et al., 1985);
- Two- Point Chemical Addition, which is applied at both the primary clarifier feed and before the secondary clarifier. This achieves the most efficient use of chemicals for phosphorous precipitation (Metcalf and Eddy, 2014).

Operations and maintenance costs are higher for chemical phosphorous removal than EBPR. The increase in chemical addition will result in the increase in sludge production. At treatment plants using alum, an increase in sludge production of up to ~26% has been reported (Boyko & Rupke, 1976).

2.2.3 Chemical Phosphorous Removal using Alum

Chemical processes for phosphate removal commonly rely on the formation of soluble phosphate that through precipitation by salts (such as alum) can be removed through solids separation processes (Sedlak, 1991) (Eq. 7). Yang et al. (2006) found through a serious of batch experiments designed to identify the characteristics of alum sludge for phosphorus adsorption, that alum has the ability for phosphorus removal, though there are many factors that affect the adsorption rate and capacity. Their results showed that alum has a higher phosphate adsorption capacity in an acid pH region than in an alkaline pH region.

$$Al_2(SO_4)_3 \cdot 14H_2O + 2H_3PO_4 \rightarrow 2AlPO_4 + 3H_2SO_4 + 18H_2O \quad [7]$$

The use of alum is safer, easier to handle and less corrosive than ferric chloride. In addition, alum is the most efficient chemical to use because phosphorous is not released during storage, recycling, the point of addition is flexible, low sludge volumes are produced, no pH adjustments are necessary, and it helps improve clarifier performance. Yeoman et al. (1988) found that oxidation ditches used in conjunction with chemical treatment, can also remove phosphorous, producing effluent phosphorous concentrations of <1.0 mg-P/L.

The required alum dose depends on influent concentrations of soluble phosphate, effluent requirements, pH, total organic carbon (TOC), hardness, temperature, flow rate, the point of addition, loading rates, frequency of dosing, engineered systems and SRT (a longer SRT does not allow sludge to absorb phosphorous as well and is more difficult to dewater) (Yeoman et al., 1988). Longer SRT leads to the cell mass no longer having the ability to uptake phosphorus causing the growth rate to gradually decline and continue to reduce until cell death occurs. pH is important for efficient removal using alum with the most efficient pH being 5-7 (Jeyanayagam, 2005).

2.2.4 Combined Chemical Biological Phosphorus Removal

EBPR can be combined with chemical phosphorus removal to achieve stringent discharge limits (Goel and Motlagh, 2013). Chemical addition in a combined removal process is often used as a polishing step in secondary treatment. This allows EBPR to provide the substantial phosphorus removal and cost savings, while the chemical addition to help meet regulations.

2.3 Process Control for Biological Nutrient Removal

Variability in wastewater treatment comes from variations in influent wastewater flow rates and characteristics, processes and that caused be mechanical breakdown and operational

failures. The variability in wastewater treatment depends on factors such as time of day, season, size and characterization of population and the collection system (WSBC, 1986).

2.3.1 Wasting Control

All processes within the treatment process are interrelated and one adjustment will lead to a change in other variables. Adjustments in RAS rate and wasted activated sludge (WAS) wasting rate will produce changes in aeration requirements, sludge settleability, SRT, F/M ratio and the concentrations of nutrients (WSBC, 1986).

It is important to note if sludge aggregates well, settles uniformly, leaves a clear supernatant, floats or remains settled. All of these factors will help determine sludge age. Rising sludge/ splitting sludge is caused by endogenous decay of organic matter in the biomass accompanied by gas release. Additionally, settling tests can be used to decide if wasting needs to be increased (WEF, ASCE, EWRI, 2006). Lack of settleability may also indicate sludge bulking conditions. Sludge bulking is caused by the growth of filamentous bacteria, which inhibits settling. This is caused by either a low DO concentration, a low F/M ratio or nutrient deficiency. The sludge volume index (SVI) is determined by MLSS settling test results and is used as a measure of sludge settleability (WEF, ASCE, EWRI, 2006).

Two types of wasting exist: controlled wasting and uncontrolled wasting (self wasting). Controlled wasting uses a control method to determine how much WAS to purposely waste based on settleability tests, centrifuging and gravimeter testing. It is important to calculate how much to waste from the system using F/M ratio and SRT. Uncontrolled wasting occurs when the amount of biomass exceeds the solids loading rates of the unit processes and results in solids washout of clarifiers (Pellegrin, 2013). F/M ratio is the amount of food available to VSS. A high

ratio indicates young sludge meaning the wasting rate should be reduced and a low ratio indicates old sludge meaning the wasting rate must be increased.

Frequently, wasting is calculated based on a targeted mixed liquor suspended solids (MLSS) concentration and the amount of space in the WAS storage tank. Operators are beginning to move away from this technique as wasting based on MLSS concentration can cause unnecessarily high SRTs and too high of a concentration of MLSS will increase solids in the system and this leads to over loading of the clarifiers, which has a negative effect on sludge quality. If the SRT is too low, bacteria will be washed out of the system and ammonia concentrations will increase in the effluent. Thus, wasting should be based on SRT, not MLSS concentrations (WEF, 2002).

2.3.2 Observation and Nutrient Loading

By simply observing the treatment processes, observations can be made which can provide information on how the process is doing. Observation of surface turbulence and foam in aeration tanks, surface scum, floc, clouds and sludge clumps in the final clarifier can lead to crucial information.

Microscopic observations, using a microscope, can be helpful in looking for key floc observations. The floc shape and density, filament presence/ abundance, protozoa/ metazoan abundance and activity, and quality of liquid around floc are important factors. A round floc shape indicates immature floc particle, an irregular floc shape indicates a mature floc, and an oval indicates a congealed floc, which means a presence of metals and a dispersed floc (which is irregular in shape) indicates mechanical sheering.

Observing foam can indicate sludge age, nutrient deficiency, conditions and bacterial processes. A white/ light loose foam indicates surfactants or young sludge age, a white heavy

foam indicates nutrient deficiency, a heavy chocolate brown foam indicates a presence of *Nocardia*, and a dark brown/ black foams indicates anaerobic conditions (WEF, 2002).

2.3.3 Aeration Control

The respiration rate (RR) can be used to measure the microbiological activity of microorganisms in a process and can help to determine if treatment is complete once the oxygen uptake rate (OUR) is determined. A high RR indicates an under oxidized sludge, organic overloading, too short of an aeration time, undertreated waste or a high F/M ratio. It will lead to sludge that will settle slowly and not compact well. The effluent will be high in TSS, BOD and NH_4^+ and will indicate young sludge or under oxidized conditions. A low RR indicates an over oxidized sludge, completely treated waste or low F/M ratio. The sludge will settle and compact rapidly and pin floc will be left behind. This means that the plant is producing an effluent above optimum BOD and TSS and has old sludge or over oxidized conditions. A very low or zero RR indicates an inhibitory or toxic influent.

The oxidation reduction potential (ORP) measures the reduced versus oxidized species present, shown in mV. Aerobic conditions (+50- +250 mV) indicate the presence of free DO or a higher presence of oxidizers than reducers and the oxidation of carbon compounds and conversion of ammonia to nitrate. Anoxic conditions (+50- -100 mV) indicate the presence of nitrate but no free DO (this is a good range for denitrification). Anaerobic conditions indicate no nitrate or free DO present (-100 to -250mV (indicates volatile acid production) -175- -350 mV (indicates methane gas production)). These conditions are is very important for biological phosphorus removal (WEF, ASCE, EWRI, 2006).

CHAPTER 3: MATERIALS AND METHODS

This chapter describes the wastewater treatment plant that was the site of this research, materials and methods used in each experiment.

3.1 Site Description

The Falkenburg AWWTP (Fig. 3.1), is a biological nutrient removal (BNR) facility located on N. Falkenburg Road, in Tampa, Florida. The average influent flow rate is 9.27 MGD, with a permitted annual average daily flow rate of 12.0 MGD. The plant receives domestic wastewater and a small fraction of industrial wastewater. The plant's National NPDES permit requires the removal of cBOD5, TSS, TN and TP to levels of 5, 5, 3, and 1 mg/L (annual averages), respectively. The plant must also meet Florida public access reuse standards.

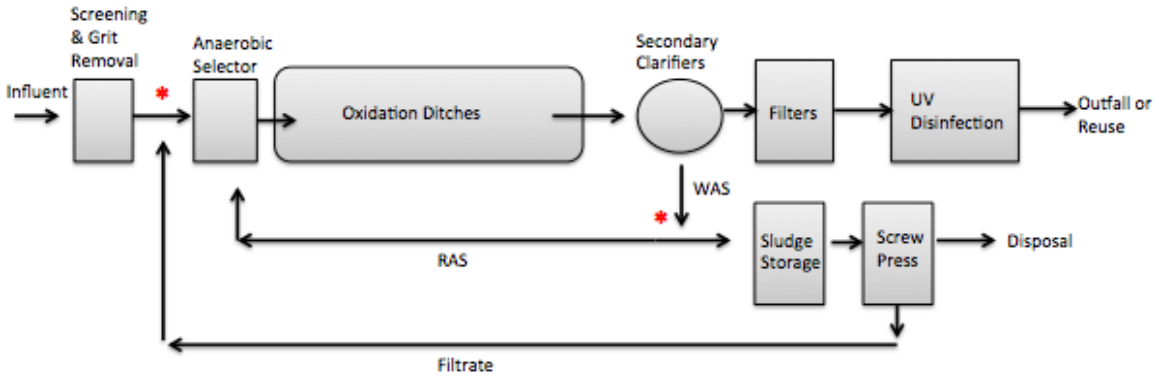

Figure 3.1 Layout of the Falkenburg AWWTP. (* Indicates sampling locations for bioreactor experiments)

In the liquid train, wastewater first passes through screening and grit removal in the head works and then travels through an anaerobic selector (used to promote EBPR) and then to Carrousel® oxidation ditches for BOD removal, nitrification and denitrification. Aeration in the

oxidation ditches is provided by mechanical aerators with variable frequency drives (VFDs) that are controlled manually. Mixed liquor then is divided between circular secondary clarifiers, where alum ($Al_2(SO_4)_3$) is dosed from spitter boxes for chemical phosphorus removal. The clarified effluent travels through deep bed filters and ultra violet (UV) disinfection before the effluent is used for reclaimed water or discharged to the Palm River/ Hillsborough River Bypass Canal. Solids from the clarifiers are returned to the anaerobic selector as screened influent RAS, or it is wasted and sent to a holding tank before a screw press is used for dewatering and disposal to a landfill. The reject water from sludge dewatering at the screw press is returned to the influent for treatment. Dimensions of the anaerobic basins, oxidation ditches and clarifiers are provided in Table 3.1 and were obtained from the Falkenburg Operations and Maintenance (O & M) Manual.

Table 3.1 Physical WWTP Data (Falkenburg Operations and Maintenance Manual)

Tank	**Dimensions**			**Number of Tanks**	**Total Volume (gallons)**
Anaerobic	Length: 48 ft	Width: 51 ft	Depth: 16.6 ft	4	1,215,800
Oxidation Ditch	Area: 15,890 ft^2	Width of Pass: 30 ft	Depth: 15 ft	4	7,130,000
Clarifier	Diameter: 100 ft		Depth: 14 ft	5	4,112,300

3.2 Bench Scale Bioreactor Tests

3.2.1 Experimental Set Up

To mimic an oxidation ditch at Falkenburg AWWTP, bench scale bioreactor experiments were set up in 4-liter glass beakers in a 22° C constant temperature room in the Environmental Engineering laboratories at University of South Florida (USF) (Figures 3.2 and 3.3). Experiments at 29.5 °C were maintained using a water bath. Influent wastewater and RAS for these experiments were collected from the Falkenburg AWWTP at sampling locations noted by

an asterisk (*) in Figure 3.1. Bioreactors were constantly mixed using magnetic stirrers (Nuova II Stir Plate, SP18425, Thermo Scientific, Waltham, MA) or an overhead mixer (Arrow Engineering 1750, Hillside, NJ) at a speed where complete mixing was observed without creating a vortex. Beakers were aerated using two aquarium pumps (Whisper 10 Air Pump, Tetra, Blacksburg, VA) and diffusers to maintain a target DO concentration based on measurements of DO at the facility. DO was controlled manually. Conditions for each experiment are shown in Table 3.2. The procedures used were based on methods described by Jimenez et al. (2014).

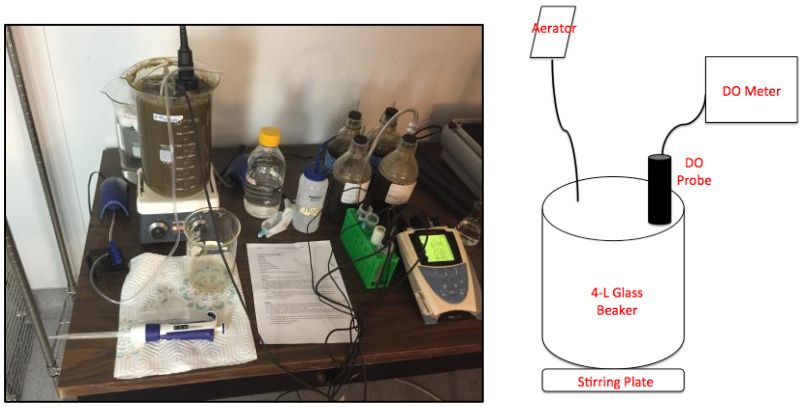

Figure 3.2 Typical Nitrification and Denitrification Bench Scale Bioreactor Set Up

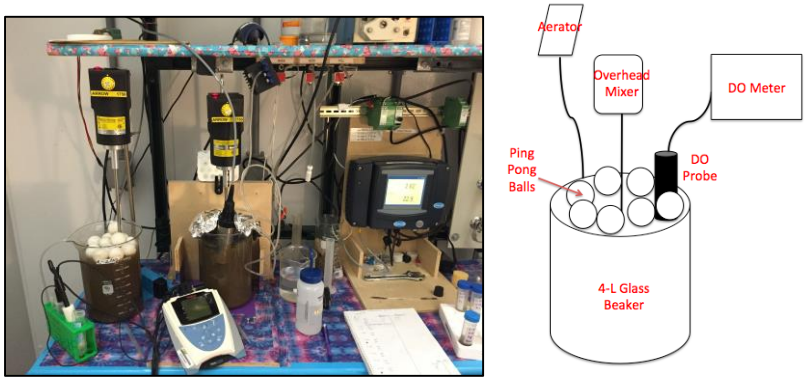

Figure 3.3 SND Bench Scale Bioreactor Set Up

Table 3.2 Specifics of Each Bench Scale Bioreactor Test

Experiment	Influent (L)	RAS (L)	DO (mg/L O_2)	Temp. (° C)	Ping Pong Balls Added?	Aeration	Mixing	Total duration of Experiment (hrs)
Nitrification	2	2	1.5- 5.0	22	No	Yes	Yes	4
Denitrification	0	3	< 0.5	22	Yes	No	Yes	6
SND	2	2	Alternated (see Table 3.5)	22, 29.5	Yes	Yes	Yes	16

3.2.2 Experimental Procedures

During the bench-scale nitrification experiment, ammonium chloride was dosed into the reactor to achieve an initial NH_4^+-N concentration of 25 mg/L. Samples (25mL) were collected in duplicate at the start of the experiment (T=0h) for analysis of TSS, VSS, ammonium, nitrate, nitrite and soluble COD (sCOD). Subsequent samples (25mL) were collected every 20 minutes for the first two hours and every 40 minutes for the next two hours. DO and pH were measured hourly throughout the experiment. A final sample (10mL) was collected at the end of the experiment (t=16h) to measure TSS and VSS. Sampling specifics are shown in Table 3.3. This experiment was a preliminary experiment and a full data set was not collected.

Table 3.3 Nitrification Test Details

Hour	0	0.33	0.67	1.0	1.33	1.67	2.0	2.67	3.33	4.0
Aeration	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Mixing	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Dose	Ammonium chloride to 25 mg-N/L									
Sample for	Cations & Anions									
Measure	pH and DO									

During the bench-scale denitrification experiment, ping pong balls were added to the top of the beaker to limit the oxygen input to the system. Samples (25mL) were collected in

duplicate prior to dosing for analyses of TSS, VSS, COD, nitrate, nitrite, phosphate and ammonium. After dosing, samples (25mL) were collected every 30 minutes for the first two hours and every hour for the next four hours and analyzed for nitrate, nitrite, ammonium and phosphorous. pH and DO were measured hourly throughout the experiment. Sampling specifics are shown in Table 3.4.

Table 3.4 Denitrification Test Details

Hour	0	0.5	1.0	1.5	2.0	3.0	4.0	5.0	6.0
Aeration	No	No	No	No	No	No	No	No	No
Mixing	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Dose	Nitrite to 25 mg-N /L, Nitrate to 5 mg-N/L								
Sample for	TSS, VSS, COD, Cations, Anions	Cations & Anions							TSS, VSS, COD, Cations, Anions
Measure	pH and DO								

During the bench-scale SND experiment, ammonium chloride was dosed into the reactor to achieve an initial ammonium concentration of 25 mg NH_4^+- N/ L. Aeration was adjusted to achieve a DO concentration of approximately 1.0 mg/L. Ping pong balls were placed on top of the reactor to reduce the oxygen input into the system. An initial sample was collected and analyzed for cations, anions, COD, TSS, VSS and alkalinity. Every hour, pH and DO were measured. Every other hour, a sample was taken and analyzed for cations and anions. At hour four, the aeration was turned down or off to achieve a target DO concentration of approximately 0.3 mg/L. At hour eight, the aeration was turned back on to achieve a target DO concentration of about 1.0 mg/L and turned back down or off at hour 12. This experiment was repeated with the

beakers in an ISO Temp 220 water bath, which was used to maintain a reactor temperature of 29.5°C. Sampling specifics are shown in Table 3.5.

Table 3.5 SND Test Details

Hour	0	0.5	1.0	1.5	2.0	3.0	4.0	5.0	6.0
Aeration	1.0 (mg/L-O_2)	1.0 (mg/L-O_2)	1.0 (mg/L-O_2)	1.0 (mg/L-O_2)	1.0 (mg/L-O_2)	1.0 (mg/L-O_2)	1.0 (mg/L-O_2)	0.3 (mg/L-O_2)	0.3 (mg/L-O_2)
Dose	Ammonium chloride to 30 mg/L-N								
Sample for	Cat, An, COD, TSS, VSS, Alkalinity	Cations & Anions					Cations & Anions		Cations & Anions
Measure	DO, pH								

Hour	7.0	8.0	9.0	10.0	11.0	12.0	13.0	14.0	15.0	16.0
Aeration	0.3 (mg/L-O_2)	1.0 (mg/L-O_2)	1.0 (mg/L-O_2)	1.0 (mg/L-O_2)	1.0 (mg/L-O_2)	0.3 (mg/L-O_2)	0.3 (mg/L-O_2)	0.3 (mg/L-O_2)	0.3 (mg/L-O_2)	0.3 (mg/L-O_2)
Sample for		Cations & Anions		Cations & Anions		Cations & Anions		Cations & Anions		Cations & Anions, COD, TSS, VSS, Alkalinity
Measure	DO, pH									

3.3 Full-Scale Plant Performance

Three phosphorous sampling campaigns (October, November and December) were carried out and samples were taken at six locations at Falkenburg AWWTP, shown in Figure 3.4. Samples were collected in 1-liter acid bath washed containers, transported to the USF Environmental Engineering laboratories on ice and analyzed within 2 hours of collection. Samples from each location were analyzed for filtered and unfiltered total phosphorus, orthophosphate and polyphosphates, filtered and unfiltered total nitrogen, soluble, total and

rbCOD, volatile acids, cations, anions, alkalinity, TSS and VSS. pH and DO were measured on site.

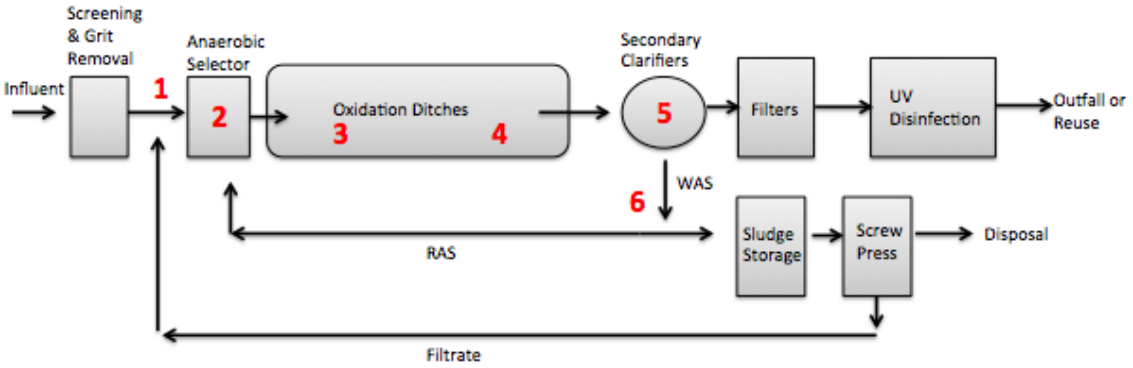

Figure 3.4 Full- Scale Plant Investigation Sampling Locations. (Location 1 includes filtrate and location 5 is a clarifier Full- Scale Plant Investigation Sampling grab sample)

3.4 Analytical Methods

Samples were collected in 50 mL centrifuge tubes and immediately centrifuged at 8.5 r/ min for 10 minutes. Samples were subsequently filtered using 0.45µm HA filter paper and refrigerated to prevent sample degradation. Samples were subsequently analyzed for anions (NO_3^-, NO_2^-, PO_4^{3-}) and cations (NH_4^+) via Ion Chromatography with chemical suppression of eluent conductivities (Dionex, 2001) using a Metrohn 850, Professional Ion Chromatograph [Method Detection Limits (mg/L): NH_4^+, 0.20; NO_3^-, 0.21; NO_2^-, 0.01; PO_4^{3-},0.02]. Total N concentrations were measured using Hach TNT plus 827 test kits [Method Detection Limits (mg/L): (LR) 1.0, (HR) 5.0] and Total P was measured using Hach TNT plus 845 test kits [Method Detection Limits (mg/L): (LR) 0.5, (HR) 1.5, (UHR) 6.0]. VFA concentrations were measured by the esterification method using Hach TNT plus 872 test kits [Method Detection Limits (mg/L): 50.0]. Results are reported as the equivalent concentration of COD, assuming that all VFAs were acetic acid. rbCOD was measured using the method of Mamais et al. (1993).

Hydrolyzable Phosphorus was measured using EPA Method 365.3. rbCOD and Hydrolyzable Phosphorus methodology can be found in Appendix D.

All other water quality measurements were performed using *Standard Methods* (APHA et al., 2012): COD (5220B) [Orbeco mid-range (0-1500 mg/L)], TSS (2540-D), and VSS (2540-E). pH (4500-H+B) was measured with an Orion 5 Star Meter Probe. DO (4500-O G) was measured with a Hach SC1000 Controller. A YSI 556 Handheld Multiparameter Instrument (Yellow Springs, OH) was used to measure DO and pH at the treatment plant. Alkalinity (2320 B) measurements were performed with a Metrohm Dosimat Plus multipurpose dispensing unit. Details of analytical chemistry methods, instruments and max daily loads (MDLs) are listed in Appendix B. Standard calibrations were prepared when appropriate and duplicates were analyzed for all samples. Propagation of error is shown in Appendix E.

CHAPTER 4: RESULTS AND DISCUSSION

To investigate SND kinetics, five bench-scale nitrification, five denitrification and four SND studies were carried out at 22°C using influent and RAS from the Falkenburg AWWTP. Four additional SND tests were carried out at 29.5°C to investigate the effect of seasonal temperature changes on SND at the facility. To characterize the removal of phosphorus at the Falkenburg AWWTP, three extensive sampling campaigns were performed at the treatment plant. Note that additional trial results not shown in this chapter are shown in Appendix C.

4.1 Bench Scale Bioreactor Study

4.1.1 Nitrification Study

Results from one of the nitrification studies are shown in Figure 4.1. Approximately 50% of ammonia was removed during the four-hour experiment, with a nitrification removal rate of 6.31 mg-N/L/hour. Nitrite and nitrate accumulation were observed after t=1hr. Nitrate and nitrite formation was slightly lower than ammonia consumption, most likely due to biosynthesis or SND. Even at a bulk DO concentration between 3.5 and 5.0, SND could still occur because of possible different zones in the floc or reactor (Metcalf and Eddy, 2014). Four hours was not sufficient time for complete nitrification to occur therefore SND experiments were carried out for a longer time period.

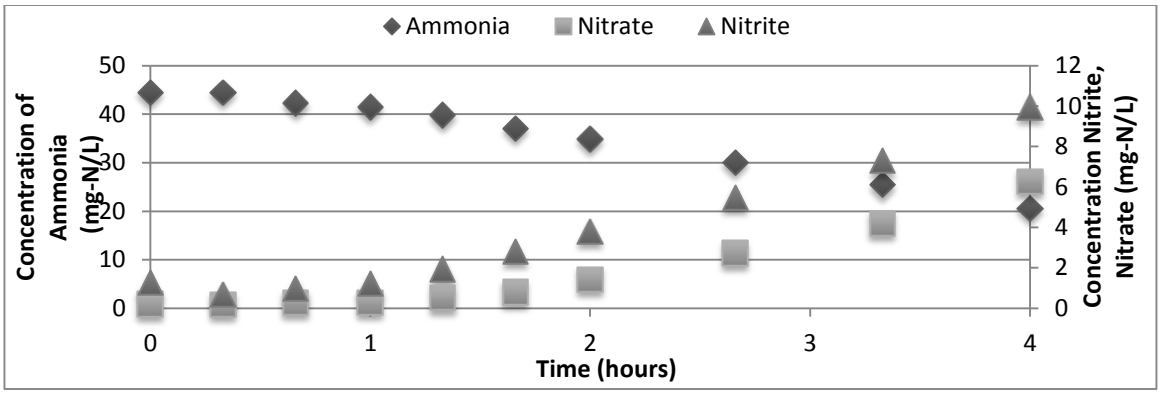

Figure 4.1 Nitrogen Species Concentrations Over t=4 hrs during a Typical Batch Nitrification Test. (Initial TSS= 800 mg/L, VSS=600 mg/L, average DO= 3.5 mg-O_2/L, Temp = 22°C)

4.1.2 Denitrification Study

Results for the denitrification study are shown in Figure 4.2. Complete removal of nitrate was observed after four hours under anoxic conditions. Nitrite was observed until t= 4hr, but complete removal of both nitrate and nitrite was achieved by t=6hr. Denitrification successfully occurred at a removal rate of 23.7 mg-NO_3^-/L/hour and 3.6 mg-NO_2^-/L/hour. COD was produced at a rate of 123 mg- COD/L/hour leading to more than a sufficient amount of COD present to drive denitrification. COD production was unexpected in this experiment, but may have been due to endogenous decay of MLSS, which can be seen in Fig. 4.3. This allowed for successful denitrification to occur (only 81.2 mg-COD/L was needed, leading to an excess of 123 mg-COD/L).

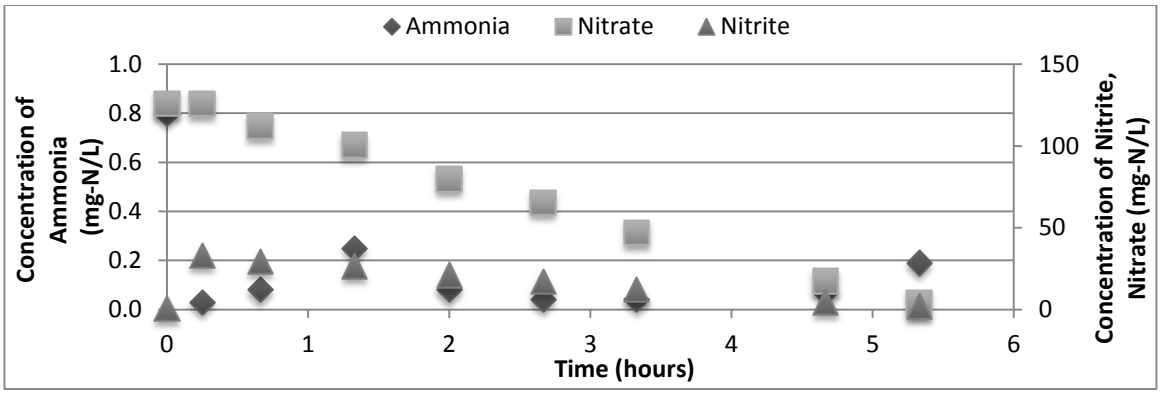

Figure 4.2 Nitrogen Species Concentrations Over t=6 hrs during Typical Batch Denitrification Test. (Initial TSS= 1800 mg/L, VSS= 1700 mg/L, average DO= 0.42 mg- O_2/L, Temp =22°C)

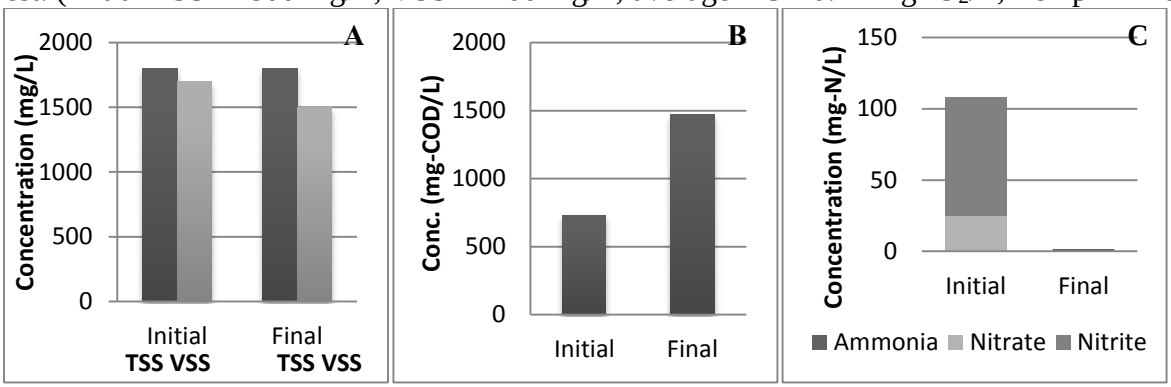

Figure 4.3 Initial and Final TSS and VSS (A), sCOD (B) and Nitrogen Species (C) Concentrations for Typical Batch Denitrification Test

4.1.3 SND Studies at 22°C

Multiple SND experiments were carried out at a temperature of 22°C without cyclically operating the aeration, but because the small-scale size of the beaker, the formation of different zones within the reactor were impossible to create. After failing to achieve SND, aeration was cyclically turned up and down to create different aerobic and anoxic periods within the reactor. Ju et al. (2007) saw similar results, where SND did not occur without the cyclic aeration. These failed results indicate that it is likely zones within the reactor that allow the occurrence of SND, not necessarily zones within the floc.

Results from one SND trial are shown in Fig. 4.4. TSS, VSS and COD results are shown in Figure 4.5. At the conclusion of SND trial 1 at 22°C, NH_4^+ was removed to below detection

limits by t=12hr, NO_3^- was never completely removed and NO_2^- was removed to below detection limits. Nitrification was successful, with a slight accumulation of ammonia occurring during the first anoxic period, which may have been due to endogenous decay of the MLSS. Complete ammonia removal was observed in the second aerobic period. Nitrate accumulation occurred in the aerobic period, with removal occurring in the anoxic period and accumulating again in the aerobic period to a concentration of 10 mg/L by t=16hr. Denitrification was most likely inhibited by a lack of COD, as shown in Figure 4.5.

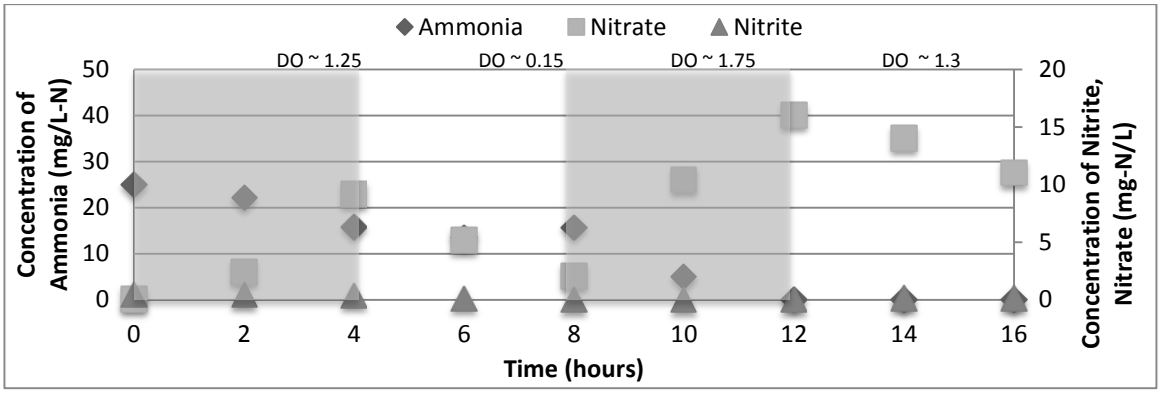

Figure 4.4 Nitrogen Species Concentrations Over t=16 hrs during Trial 1 Batch SND Test. (Initial TSS= 600 mg/L, VSS= 600 mg/L, Temp = 22°). Average DO Concentrations are Shown in the Figure.

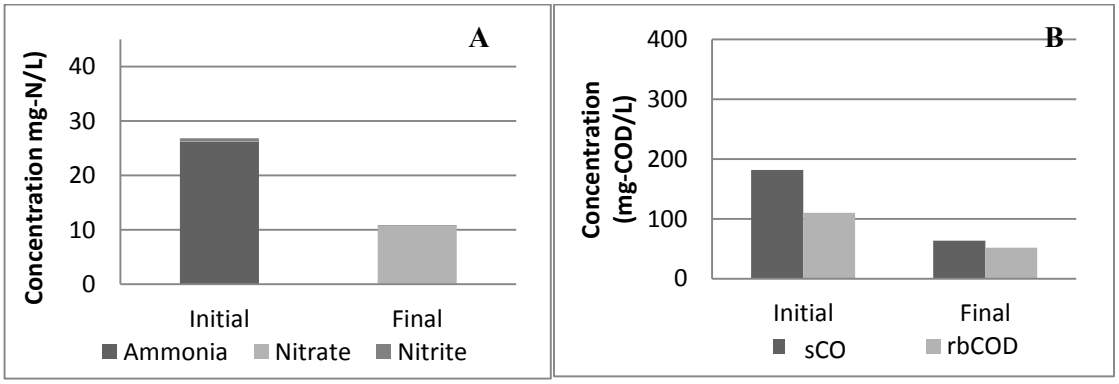

Figure 4.5 Trial 1 Initial and Final Nitrogen (A) and COD (B) Species at 22°C

TSS and VSS results are shown in Figure 4.6. At a temperature of 22°C, there was no change in concentration of TSS and a slight decrease in VSS concentration. It was not expected that TSS concentrations would change significantly during the short period of time of the batch

test. A small decrease in VSS could be the result of endogenous decay, which contributed to denitrification as discussed previously. Alkalinity results are shown in Figure 4.6 and are discussed below.

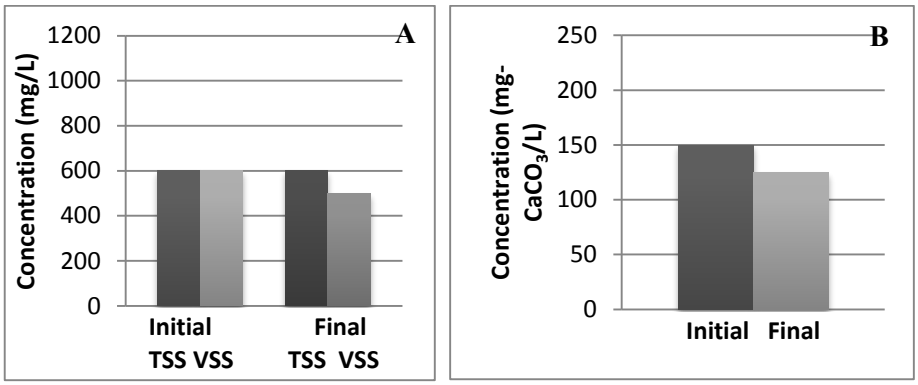

Figure 4.6 Trial 1 Initial and Final TSS and VSS (A) and Initial and Final Alkalinity (B) at 22°C

Average concentrations of DO over the length of trial 1 are shown in Figure 4.7. DO concentrations were consistent with those mentioned in the experimental methods and were fairly easy to maintain during the experiment. Note that at t= 12 hrs a sharp increase in DO was observed, which corresponds with complete ammonia removal (and therefor decreased oxygen demand by nitrifying bacteria). This phenomenon was also observed by Holman and Wareham (2004).

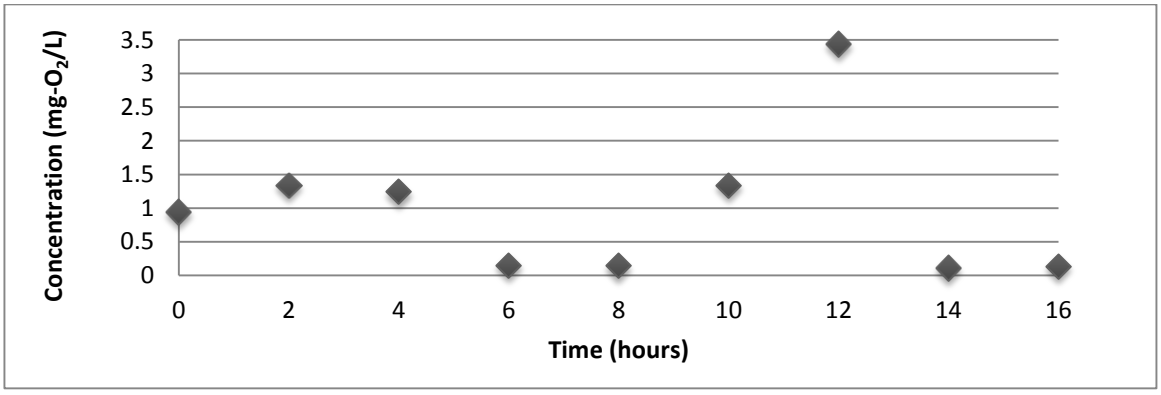

Figure 4.7 Trial 1 DO Concentrations vs. Time at 22°C

Results from a second SND trial are shown in Fig. 4.8. TSS, VSS and COD results are shown in Figure 4.9. At the conclusion of SND trial 2 at 22°C, NH_4^+ was removed to 30 mg/L from an initial concentration of 50 mg/L. A high COD during this test likely inhibited nitrification due to competition between heterotrophic and nitrifying bacteria for DO. NO_3^- accumulated in the aerobic period and decreased in the anoxic, indicating that successful denitrification occurred. NO_2^- stayed below detection limits for the duration of the experiment also indicating successful denitrification and no inhibition from a lack of COD (Fig. 4.9).

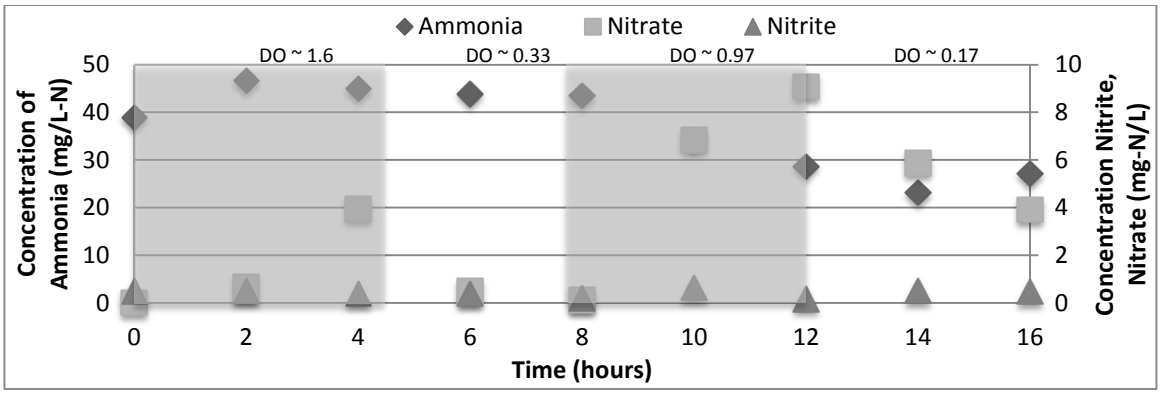

Figure 4.8 Nitrogen Species Concentrations Over t=16 hrs During Trial 2 Batch SND Test. (Initial TSS= 900 mg/L, VSS=800 mg/L, Temp = 22°). Average DO Concentrations are Shown in the Figure.

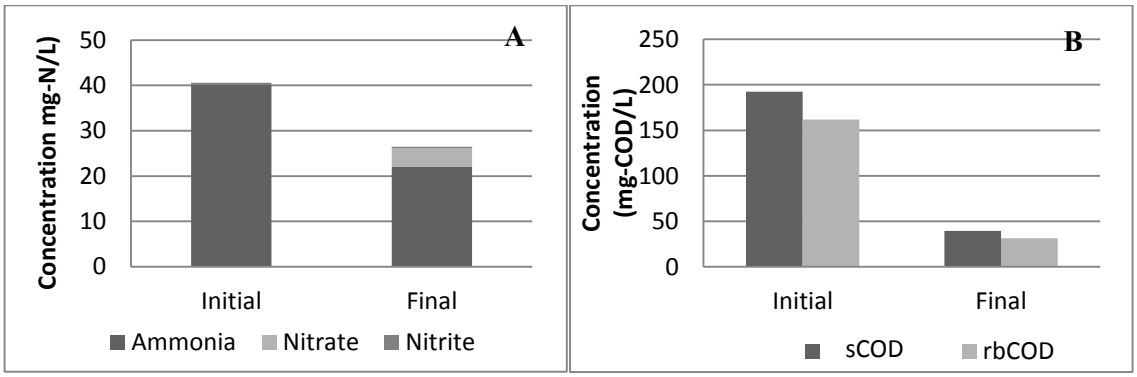

Figure 4.9 Trial 2 Initial and Final Nitrogen (A) and COD (B) Species at 22°C

TSS and VSS results are shown in 4.10. The concentrations of TSS and VSS differed greatly between trial 1 (Figure 4.3) and trial 2 (Figure 4.10), indicating variations in the treatment plant, where more removal occurred in trial 2. Trial 2 had a higher influent TSS and

VSS concentration, which lead to less nitrate accumulation occurrence than trial 1. It would be expected that trial 1 would have a lower rate of SND due to the lower biomass concentration, which was observed. Alkalinity consumption (Figure 4.6 and 4.10) did not vary much between both trials conducted at 22°C. In a conventional system, at a temperature of 22°C, 138 mg-$CaCO_3^-$/L would be consumed in Trial 1; however, during Trial 1 only 25 mg-$CaCO_3^-$/L was consumed. During Trial 2, 82.09 mg-$CaCO_3^-$/L would be expected to be consumed, while only 45 mg-$CaCO_3^-$/L was consumed. The pH remained between 6.7 and 7.3, an ideal range for nitrification and denitrification (Metcalf and Eddy, 2014). pH and alkalinity results support the idea that an advantage of SND is supplemental alkalinity is not required.

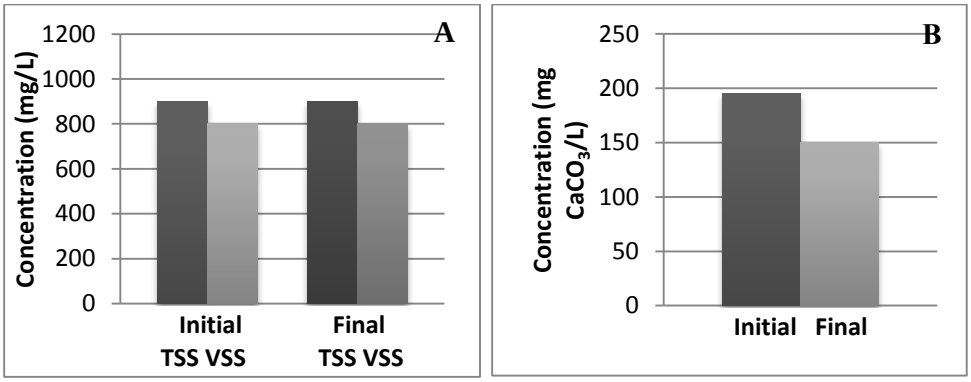

Figure 4.10 Trial 2 Initial and Final TSS and VSS (A) and Initial and Final Alkalinity (B) at 22°C

Average concentrations of DO over the length of trial 2 are shown in Figure 4.11. Concentrations were consistent with those mentioned in the experimental methods and were fairly easy to maintain during the experiment.

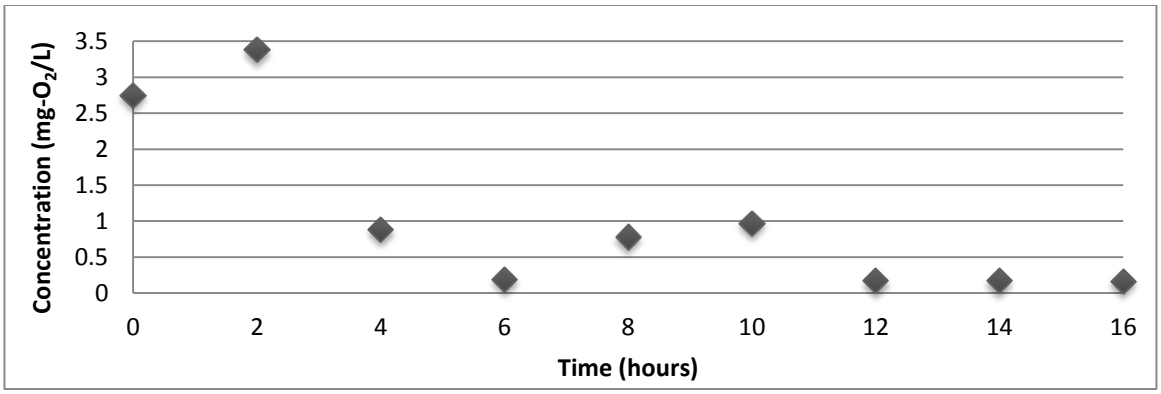

Figure 4.11 Trial 2 DO Concentration vs Time at 22°C

4.1.3 SND Studies at 29.5°C

Results from Trial 1 of the 29.5°C SND experiments are shown in Figure 4.12. COD results are shown in Figure 4.13. During Trial 1 of the 29.5°C experiments, successful nitrification occurred, with ammonia oxidation from 20 to 5 mg/L. A small increase in ammonia was observed in the second anoxic period. There was no accumulation of nitrate or nitrite, indicating successful SND. An accumulation of COD was observed during the experiment, thus there was an excess amount of COD and denitrification was able to occur fully.

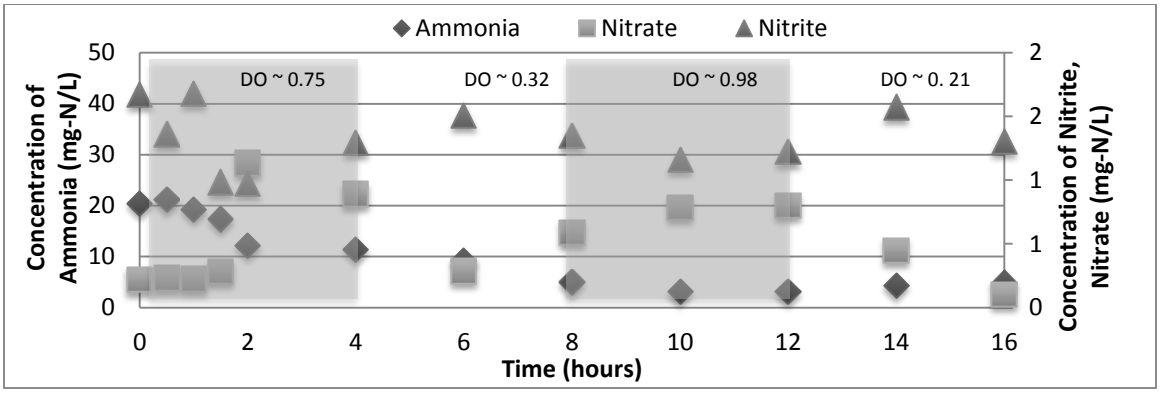

Figure 4.12 Nitrogen Species Concentrations Over t=16 hrs during Trial 1 Batch SND Test. (Initial TSS= 1,100 mg/L, VSS= 700 mg/L, Temp = 29.5°). Average DO Concentrations are Shown in the Figure.

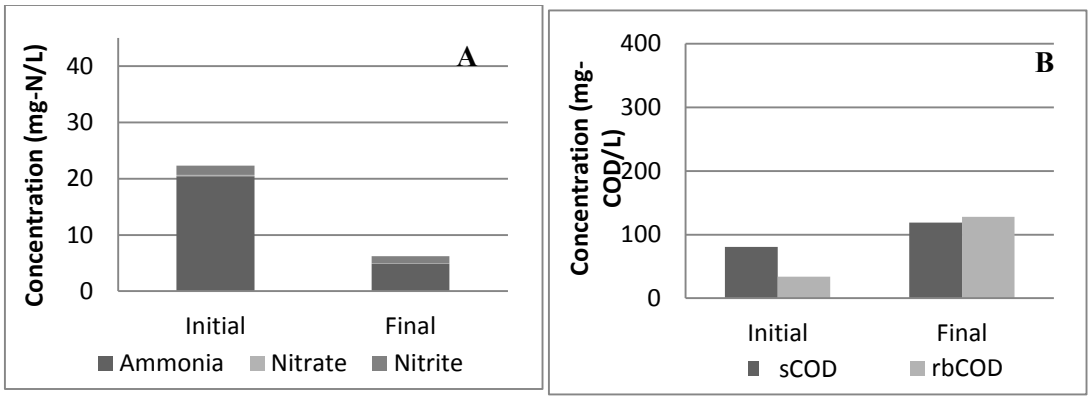

Figure 4.13 Trial 1 Initial and Final Nitrogen (A) and COD (B) Species at 29.5°

Initial and final alkalinity results are shown in Figure 4.14. In a conventional system, 152 mg-$CaCO_3^-$/L would be expected to be consumed for the nitrogen removal that occurred in trial 1, but only 77.5 mg-$CaCO_3^-$/L was consumed. These results are discussed further below. At t=10 hrs, an increase in DO is observed when ammonia is fully removed. Average DO concentrations for trial 1 are shown in Figure 4.15.

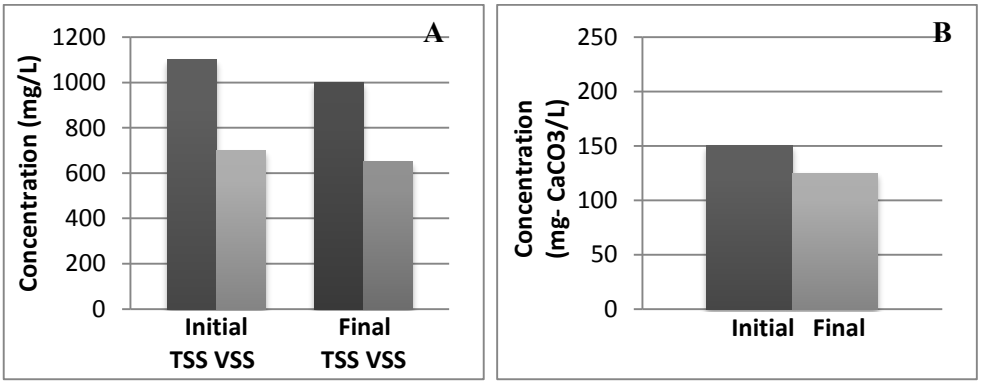

Figure 4.14 Trial 1 Initial and Final TSS and VSS (A) and Initial and Final Alkalinity (B) at 29.5°C

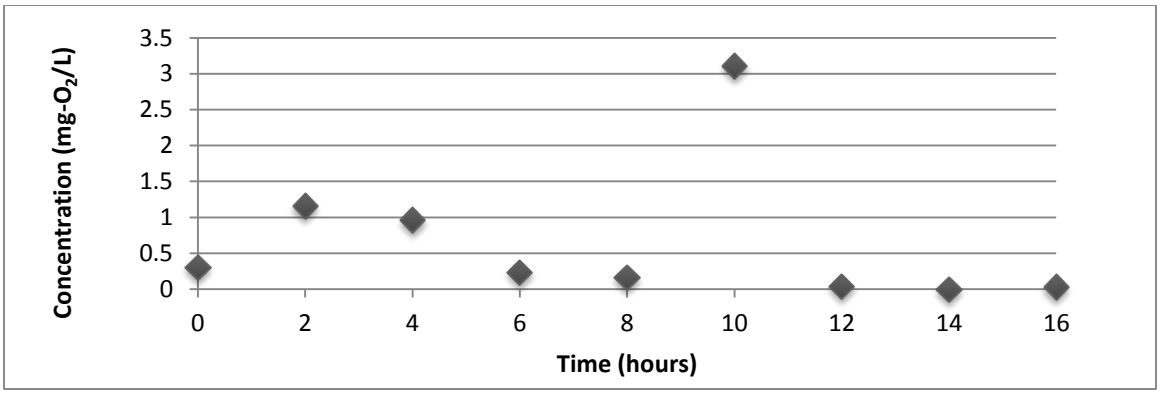

Figure 4.15 Trial 1 DO Concentration vs Time at 29.5°C

Results of Trial 2 of the 29.5°C SND experiments are shown in Figure 4.16. COD results are shown in Figure 4.17. During Trial 2 at 29.5°C, complete ammonia removal occurred by t=8 hr, indicating complete nitrification. An increase of nitrate to 15 mg/L during the first aerobic and anoxic phases and a lack of nitrite accumulation indicates a lack of complete denitrification. Only half of the required COD was consumed, thus denitrification was inhibited slightly leading to a small accumulation of nitrite. There was plenty of rbCOD yet denitrification did not occur. This will be discussed below (Fig. 4.17).

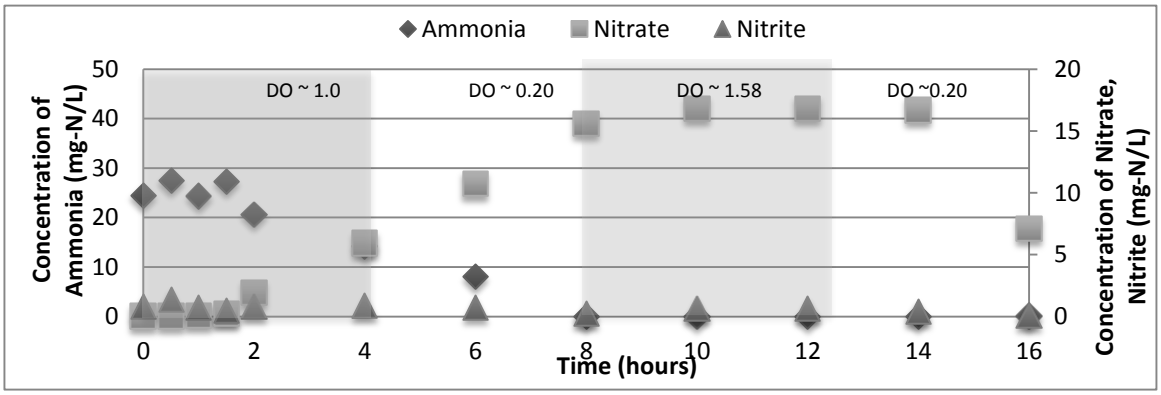

Figure 4.16 Nitrogen Species Concentrations Over t=16 hrs during Trial 2 Batch SND Test. (Initial TSS= 700 mg/L, VSS= 700 mg/L, Temp = 29.5°). Average DO Concentrations are Shown in the Figure.

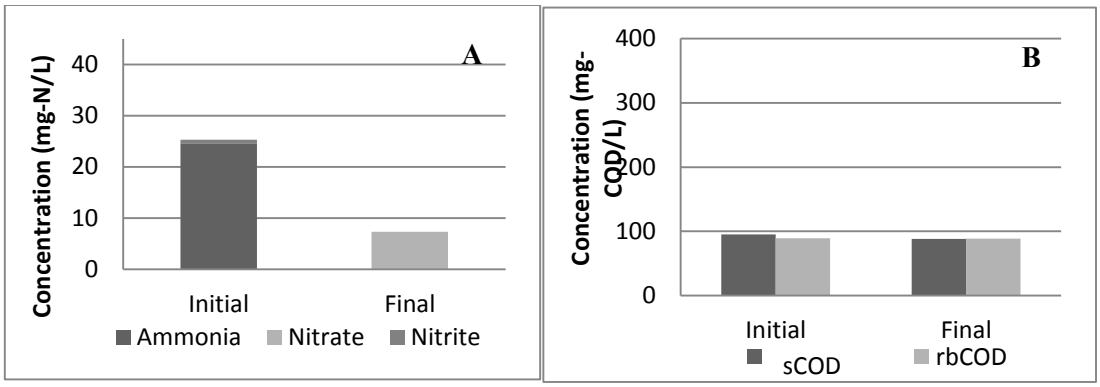

Figure 4.17 Trial 2 Initial and Final Nitrogen (A) and COD (B) Species at 29.5°

Results of initial and final alkalinity results are shown in Figure 4.18. In Trial 2, 111 mg-$CaCO_3^-$/L would be expected to be consumed in a conventional system, but only 25 mg- $CaCO_3^-$/L was consumed. These results are discussed further below. Average DO concentrations for trial 2 are shown in Figure 4.19.

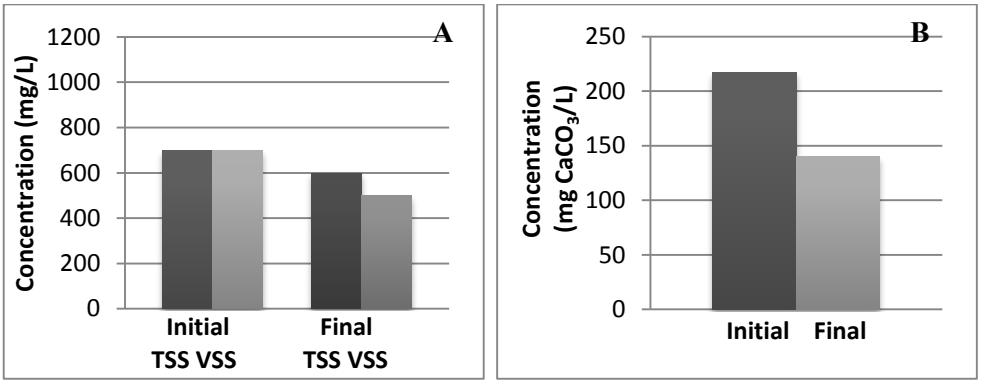

Figure 4.18 Trial 2 Initial and Final TSS and VSS (A) and Initial and Final Alkalinity (B)

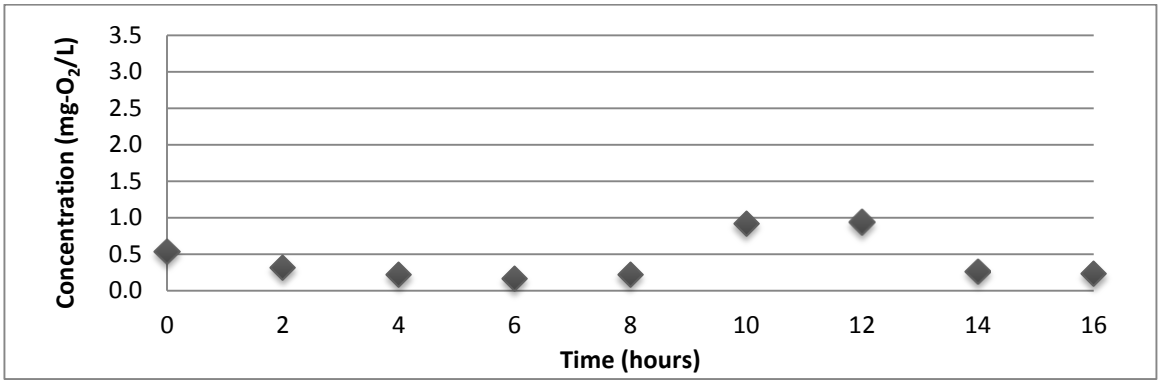

Figure 4.19 Average DO Concentrations vs Time at 29.5°C

Ammonia removal was more efficient at higher temperatures, with a majority of removal occurring by t=8hr. Additionally, at higher temperatures there was not as high of an accumulation of nitrate or nitrite, indicating more successful denitrification. At a higher temperature, SND successfully occurred unlike at the lower one. When nitrification and denitrification occur separately, it is expected that as ammonia oxidation occurs, an increase in nitrate and nitrite concentration occurs but during SND, the ammonia oxidation and nitrate and nitrite reduction occur simultaneously.

At a temperature of 29.5°C, there was a decrease in concentration of TSS and VSS. As with 22°C, it was not expected that there would be a change in TSS and VSS during the 16 hour experiment, but the decrease likely occurred due to bulking (Figure 4.14 and 4.18). As bulking occurred solids accumulated at the surface, which caused variations in biomass concentrations within the reactor. This may have led to the full reactor volume not being available for biodegradation. In addition, some biomass may have been lost from the system, as shown in the TSS and VSS results in Figure 4.14 and 4.18.

Figure 4.20 Bulking in the Reactor at 29.5°C

Alkalinity consumption (Figures 4.14 and 4.18) did not vary between trials conducted at 22°C and 29.5°C. pH remained between 6.7 and 7.3 for all of the trials. pH and alkalinity results further support the idea that an advantage of SND is that supplemental alkalinity is not required.

The initial concentrations of rbCOD during the 22°C experiments were close to twice that of the 29.5°C experiments. The contents of the bioreactor reactor were collected from the treatment plant, which is susceptible to variations concentrations and flows in the influent. This difference in initial concentration likely occurred because 22°C experiments were carried out in July, while the 29.5°C were carried out in late August and early September. During the first three weeks of August, Tampa, FL experienced severe flooding and rainfall, which led to a dilution in wastewater influent concentrations. Comparing Figures 4.5, 4.9 and 4.13, 4.17, it can be seen that there was less rbCOD available to help denitrification occur in the 29.5°C experiments, leading to a higher final nitrate concentrations than in the 22°C experiments.

Throughout 29.5°C trials, DO concentrations (Fig. 4.15 and 4.19) were significantly harder to maintain than 22°C trials. At 29.5°C trials, aeration had to be adjusted manually every 15-20 minutes whereas during the 22°C experiments, aeration had to be adjusted manually every hour. The amount of oxygen in the system is affected by an increase in temperature because the solubility of oxygen changes with temperature. At lower temperatures, DO concentrations can be higher than in warmer temperatures. In addition, at higher temperatures the rate of DO consumption by nitrifying bacteria and aerobic heterotrophs increases, which highlights the need for stringent operator control over DO at high temperature. This is similar to Holman and Wareham (2004) who found that the variations in DO concentrations could be directly related to changes in COD and ammonia concentrations, which could cause an increase in DO. At low DO levels, the decrease in a DO concentration was believed to have been due to microbial activity

causing the DO to be utilized by COD and ammonia oxidation as quickly as it was supplied. The second change in DO concentration (an increase) was believed to have occurred when the COD was depleted. DO was also observed to increase when the ammonia concentrations were depleted. They believe that the lack of nitrate detected could indicate that the oxidation of nitrite to nitrate may not exist in SND or that nitrite could be reduced to nitrogen gas directly from nitrite, thus skipping the oxidation to nitrate and reduction to nitrite. Similar results were seen in Figures 4.15 and 4.19. These relationships can be related to full-scale treatment and at Falkenburg AWWTP, where nutrient removal is required to meet NPDES permits, it is crucial that proper DO control is maintained for those standards to be achieved.

4.2 Full-Scale Plant Performance

The results from analysis of phosphorus species at six locations throughout the treatment train are shown in Figure 4.21. The results indicate that biological phosphorus removal is occurring and can be observed by high phosphorus concentrations in the anaerobic reactor followed by low concentrations in the oxidation ditch. The average concentration of total phosphorus in the anaerobic reactor and oxidation ditch was 40 mg-P/L and 0.9 mg-P/L, respectively. The average concentration of TP in the clarifier was 20 mg-P/L, with 14.6 mg-P/ L in the form of particulate phosphorus and 0.2 mg-P/L in the form of soluble phosphorus. These results indicate that the alum dosed prior to the clarifier successfully precipitated remaining soluble phosphorus.

Total phosphorus is determined by the unfiltered sample that includes all forms of phosphorus. Particulate phosphorus is determined by the digestion of a filtered sample subtracted by an unfiltered sample and is the form of phosphorus expected to be wasted with the WAS and RAS. The results agree that the particulate form is expected to be the second highest

phosphorous species concentration. Soluble phosphorus is the result of filtered phosphorus samples and is the form of phosphorus that exits with the clarifier effluent; this form will continue to be reduced in concentration as it travels through the final filters at the treatment plant. The organic phosphorus is calculated by subtracting polyphosphates by orthophosphate from particulate phosphorus samples. The lowest phosphorus species concentration is expected to be the polyphosphates, which is calculated from subtracting orthophosphates from acid hydrolysable phosphorus. Ideally, throughout the treatment train, soluble phosphorus will decrease and after precipitation with alum, particulate phosphorus will increase which is shown in Figure 4.21.

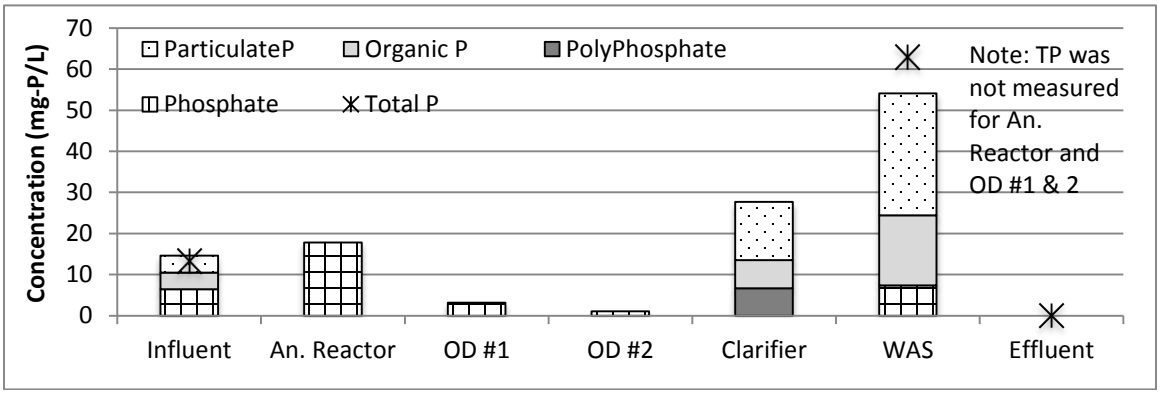

Figure 4.21 Typical Phosphorus Concentrations Profiles from Grab Samples Throughout the Treatment Process. (Effluent Data Obtained from Plant Operators)

Alum dosing at the treatment plant varied during the course of the sampling period (Figure 4.22). For the first sampling campaign, alum was dosed at 100 GPD, 200 GPD for the second and at 300 GPD for the third sampling period. Alum is dosed through a splitter box after the oxidation ditch and before the clarifier. Reduction of flow-pacing of alum at Falkenburg AWWTP (from ~260 gpd) was suggested by Knapp (2014) to reduce the chemical costs, sludge production and possible impacts on the biological process. Alum addition varies at the treatment plant based on a number of factors including pipe clogging, “bad batches”, phosphorus removal

and settling factors. Following the second sampling campaign, a “bad batch” of alum caused alum feed pumps to clog. Prior to this sampling campaign, alum dosing was increased above the needed amount for phosphorus removal due to clogging in the feed pipes from oversized pipes. It is thought that a “bad batch” was received because multiple treatment plants reported the same clogging issue. Operators have tried through trial and error to find an optimum alum dose, though no jar tests have been completed to our knowledge. Throughout this trial and error period, the plant has not failed to meet permit limits for total phosphorus, though further alum reduction is still possible. Operators should be conducting jar tests until an optimum dose is determined and after any large plant shifts or weather changes at the plant. Additional sampling of sulfate and phosphorus species in the clarifier will allow the operators to better understand phosphorus removal and impacts at the treatment plant.

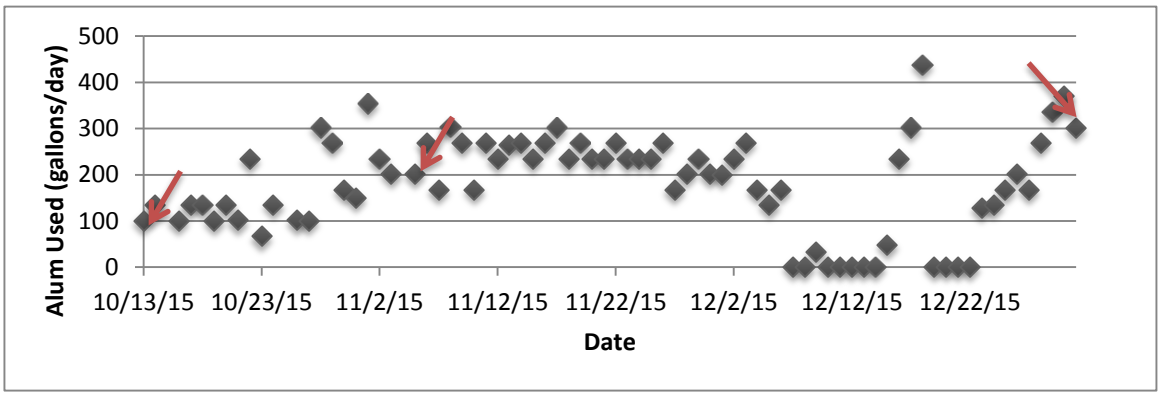

Figure 4.22 Alum Dosing at Falkenburg AWWTP During Sampling Period. (10/13/15-12/31/15) (Arrows note sampling dates)

Additional tests were conducted to fully understand the processes occurring at the treatment plant. Cations, anions, total nitrogen, COD and TSS/VSS results are shown in Figure 4.23, 4.24, 4.25, 4.26 and 4.27, respectively. Additional test results are provided in Appendix C.

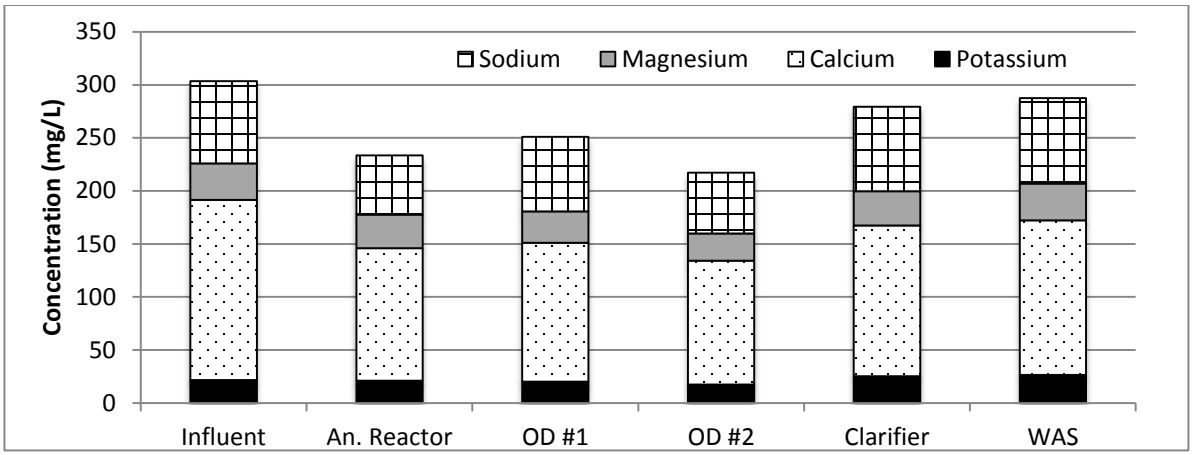

Figure 4.23 Typical Cation Concentration from Grab Samples Throughout the Treatment Process

Sulfur is required for protein synthesis and is reduced biologically in anaerobic conditions. Sulfate concentrations of less than 200 mg/L are required to not upset the biological process. Sulfate concentrations were below 200 mg/L in the influent, anaerobic reactor and oxidation ditch, but at or above in the clarifier and WAS. An increase in sulfate in the clarifier was expected and is shown as alum is dosed prior to the clarifier. Anion results are shown in Figure 4.24.

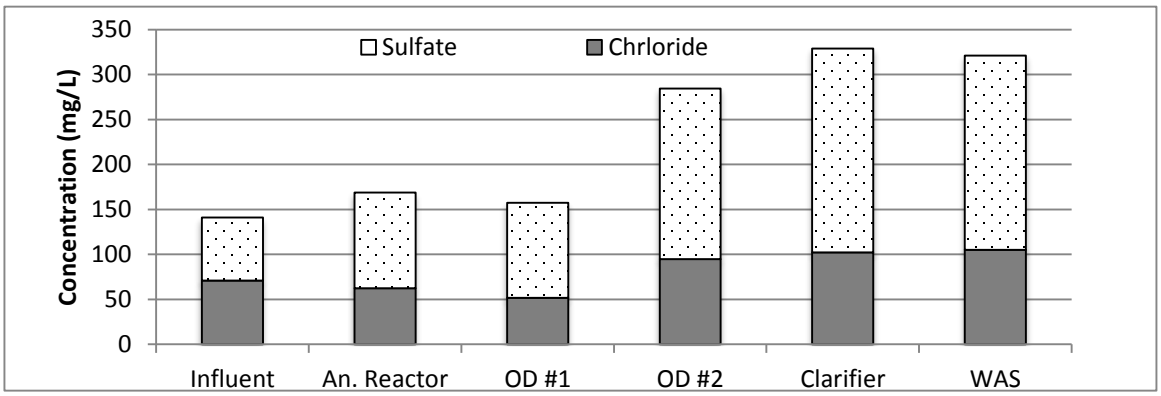

Figure 4.24 Typical Anion Concentrations from Grab Samples Throughout the Treatment Process

Concentrations of nitrogen species during the phosphorous sampling campaign are shown in Figure 4.25. A high TN occurs in the influent, as expected, with a decrease in concentration occurring through the oxidation ditch. The clarifier has a higher concentration of particulate

nitrogen, likely due to poor settling. Soluble nitrogen, in the form of ammonia, nitrate and nitrite, was below permit requirements by this point, as expected. Additional nitrogen removal can be expected in the filters before discharged as effluent.

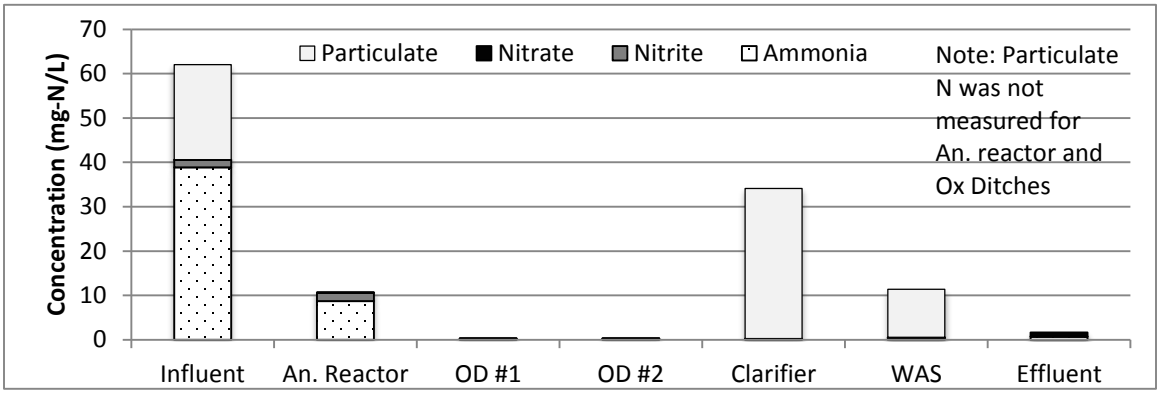

Figure 4.25 Typical Nitrogen Species Concentrations from Grab Samples Throughout the Treatment Process. (Effluent data was obtained from plant operators)

Similar to the SND batch studies, some rbCOD formation occurred throughout the treatment train, likely due to endogenous decay of the MLSS. COD is extremely important for both denitrification and phosphorus removal at the plant. Ideally there will be a decrease in rbCOD in the anaerobic zone as it is consumed for PAO production and phosphorus removal follows in the aerobic zone. The results show rbCOD consumption from influent through the first oxidation ditch sampling point, which corresponds with EBPR but then a production in the oxidation ditch which corresponds with the production and consumption that occurs in different zones and stages in the aerobic zone when SND occurs.

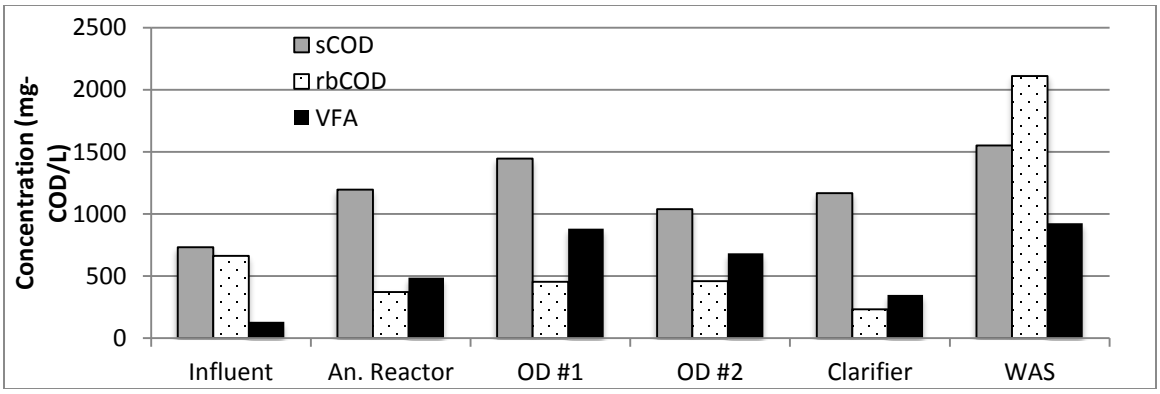

Figure 4.26 Typical COD Analyses from Grab Samples Throughout the Treatment Process

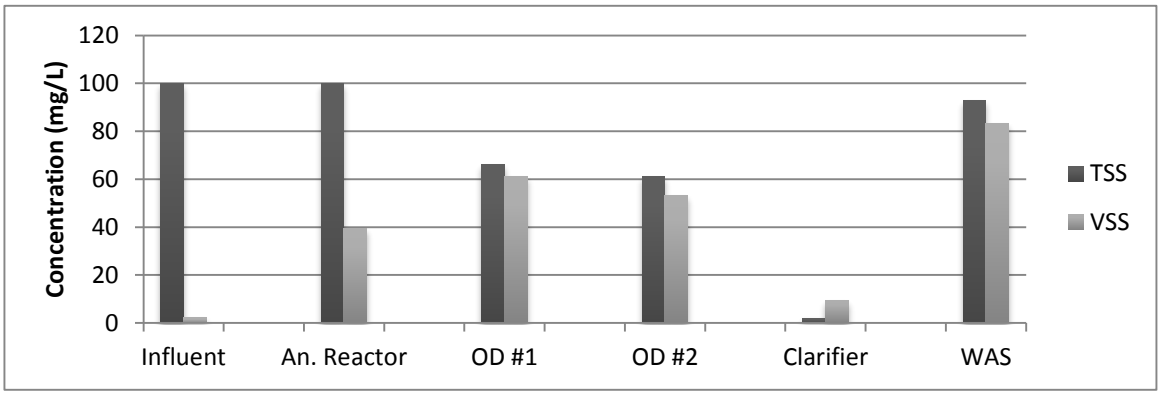

Figure 4.27 Typical TSS and VSS Results from Grab Samples Throughout the Treatment Process

CHAPTER 5: CONCLUSIONS AND RECOMMENDATIONS

The operations staff at Falkenburg AWWTP consistently meets and exceed NPDES permit requirements. In addition to meeting the permits, the operators are willing to adjust plant operations to better optimize the treatment overall. Areas of improvement at Falkenburg AWWTP include decreasing the variations in alum dosing and DO control in warmer temperatures (such as summer months).

A bench top bioreactor was created and successfully mimicked the oxidation ditch at the treatment plant. Nitrification, denitrification and SND were all achieved during different experiments in a batch scale reactor at 22° C and additional SND experiments were conducted 29.5° C to study the impacts of seasonal temperature change on kinetics. Trends in ammonia, nitrate and nitrite concentrations were consistent with SND. Additional sampling was completed to further understand the fate of phosphorus, nitrogen and organic carbon in the treatment train at the plant.

In the nitrification batch reactors, in four hours, 50% of ammonia was successfully removed at a rate of 6.31 mg-N/L/hr indicating that four hours is not sufficient time to achieve complete removal. In the denitrification batch reactors, in six hours there was successful removal of nitrate and nitrite at a rate of 23.7 mg-NO_3^-/L/hr and 3.6 mg-NO_2^-/L/hr. In an SND batch reactor experiments at 22° C, ammonia oxidation successfully occurred in 12 hours but denitrification was inhibited due to insufficient rbCOD in the reactor. In an SND batch reactor at 29.5° C, no accumulation of nitrate or nitrite was observed, indicating SND successfully

occurred. At a higher temperature, sludge bulking occurred in the reactor resulting in variations in TSS and VSS concentrations.

The results of the bench-scale experiments showed that the SND was successfully achieved. The microbial community in the MLSS from Falkenburg AWWTP is capable of SND under the right environmental conditions, such as temperature, DO and rbCOD. DO is much more difficult to maintain and control at a higher temperature, further supporting the idea that stricter operator control is needed in warmer months. Additionally, because SND removal still occurred with poor DO control at 29.5°C, it further supports the idea that SND occurs because of zones within the floc or the reactor or that novel microorganisms exist that allow denitrification to occur above ideal DO concentration and nitrification to occur below ideal concentrations of DO. A variation in rbCOD in the influent wastewater at the treatment plant caused nitrification and denitrification to be inhibited in different trials. With too much rbCOD, nitrification was inhibited and with too little rbCOD, denitrification was inhibited. Additionally, alkalinity consumption was minimal which supports the idea that supplemental alkalinity is not needed in SND processes.

Results from the sampling campaigns at the treatment plant indicate that successful phosphorus removal. Alum addition varied before each sampling and a relationship between alum addition and sulfate can be made. rbCOD was consumed throughout the treatment process as expected and noticeable results can be noted when rbCOD was low in terms of phosphorus removal.

The objectives were achieved and overall, the plant is achieving SND and EBPR and the plant is performing as designed. The addition of alum should continue to be studied to determine a better dose and save the county taxpayers money while still meeting permit regulations. Jar

tests should be used to determine the proper dosing that will not hinder the settling properties further in the treatment train. Additionally, alum feed pipe sizes should be investigated at the plant to ensure no clogging occurs with a decrease in alum flow and automated aeration based on ammonia concentrations should be considered to remove the manual operation of aerators.

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APPENDICES

Appendix A List of Acronyms

Alum	Aluminum sulfate
AOB	Ammonia Oxidizing Bacteria
ASM	Activated Sludge Model
AWWTP	Advanced Wastewater Treatment Plant
BOD	Biological Oxygen Demand
BNR	Biological Nutrient Removal
$cBOD_5$	5-day Carbonaceous Biological Oxygen Demand
COD	Chemical Oxygen Demand
CSTR	Continuously Stirred Tank Reactor
DO	Dissolved Oxygen
EBPR	Enhanced Biological Phosphorus Removal
F:M	Food to Microorganism Ratio
GHG	Greenhouse Gases
HRT	Hydraulic Residence Time
MGD	Million Gallons Per Day
MLSS	Mixed Liquor Suspended Solids
MLVSS	Mixed Liquor Volatile Suspended Solids
NOB	Nitrite Oxidizing Bacteria
NPDES	National Pollutant Discharge Elimination System
OHO	Ordinary Heterotrophic Organisms
O&M	Operation and Maintenance
ORP	Oxidation Reduction Potential
PAO	Phosphorus Accumulating Organisms
RAS	Return Activated Sludge
rbCOD	Readily Biodegradable COD
RR	Respiration Rate
SAR	Sum of Absolute Residuals
SND	Simultaneous Nitrification Denitrification
SRT	Solids Retention Time
SSV	Settled Sludge Volume
SVI	Sludge Volume Index
TIN	Total Inorganic Nitrogen
TMDL	Total Max Daily Load
TN	Total Nitrogen
TP	Total Phosphorus
TSS	Total Suspended Solids
VFA	Volatile Fatty Acids
VFD	Variable Frequency Drive
VSS	Volatile Suspended Solids
WAS	Waste Activated Sludge
WEF	Water Environment Federation

WQS	Water Quality Standards
WWTP	Wastewater Treatment Plant

Appendix B Analytical Chemistry Methods and Instruments for Analytical Work

Table B1.1. Analytical Chemistry Methods, Method Detection Limits (MDL) or Range of Test and Instruments Used for Analytical Work

Analyte	Method	Instrument	MDL or Range (mg/L)
Alkalinity	Standard Method 2320 B (APHA, 2012)	Metrohm Dosimat Plus	20
Chemical Oxygen Demand (COD)	Standard Method 5220B (APHA, 2012)	HACH® DR2800 spectrophotometer	0-1,500 (MR)
Readily Biodegradable COD	Mamis et al., 1993	HACH® DR2800 spectrophotometer	0-1,500 (MR)
Cl^-, NO_2^-, NO_3^-, PO_4^{3-} SO_4^{2-}	Ion chromatography with chemical suppression USEPA 1997	Metrohm 850 Ion Chromatograph	0.1, 0.04, 0.01, 0.02, 0.01
DO	Standard Method 4500-O G (APHA, 2012)	HACH® SC1000 Controller	0.00-90.00
Na^+, K^+, Mg^{2+}, Ca^{2+}, NH_4^+	Ion chromatography with chemical suppression USEPA 1997	Metrohm 850 Professional IC	0.50, 0.07, 0.09, 0.27, 0.20
Acid Hydrolyzable Phosphorus	EPA Method 365.3	HACH® DR2800 spectrophotometer	0.5- 1.5 (LR) 1.5- 15.0 (HR)
pH	Standard Method 4500-H+ B (APHA, 2012)	Orion 5 Star meter and probe	0-14
Temp.	Standard Method 2550 B (APHA, 2012)	NA	-5° - 105°C
Total Nitrogen (TN)	Standard Method 4500-N (Per Sulfate) (APHA, 2012)	HACH® DR2800 spectrophotometer	1-16 (LR) 5-40 (HR)
Volatile Acids (VFA)	Esterification method using Hach TNT plus 872 test kits	HACH® DR2800 spectrophotometer	50- 2,500
TP/PO_4^{3-}	Standard Method 4500-P E (APHA, 2012)	HACH® DR2800 spectrophotometer	0.5- 1.5 (LR) 1.5- 15.0 (HR) 6-60 (UHR)
TSS/ VSS	Standard Method 2540-D and Standard Method 2540-E (APHA, 2012)	Fisher Drying Oven and Fisher Muffle Furnace	Up to 1L sample volume to yield min of 200 mg dried residue

Appendix C Sampling Data

Table C1.1 Results of the Full- Scale Plant Investigation Sampling Campaign on October 13, 2015. (Ambient Air Temperature: 85°F)

Location	**Influent**	**Anaerobic Reactor**	**Oxidation Ditch #1**	**Oxidation Ditch #2**	**Clarifier**	**WAS**
sCOD	206.50	49.50	231.00	39.00	60.00	20.50
rbCOD	300.00	716.00	272.00	310.50	11.50	549.00
VFA (mg COD/L)	19.91	89.62	24.43	18.30	10.29	23.31
Ammonia	40.91	17.58	0.51	0.42	0.45	1.44
Nitrate	0.03	0.02	0.00	0.02	0.00	0.02
Nitrite	0.95	1.01	0.60	1.11	1.12	0.79
Potassium	15.07	31.06	24.19	24.96	26.68	36.37
Calcium	158.24	158.99	137.23	141.36	143.15	157.32
Magnesium	27.03	34.64	21.39	22.41	22.49	31.59
Sodium						
Chloride	61.16	83.45	92.57	88.43	90.63	93.01
Sulfate	26.17	96.59	147.70	140.70	145.72	151.76
Alkalinity	185.00	210.00	152.20	170.00	152.50	227.50
pH	7.39	7.23	7.51	7.37	7.49	-
DO	-	0.13	0.27	1.26	0.20	-
TSS	NM	689.00	1544.00	439.00	0.00	1139.00
VSS	NM	628.00	1383.00	372.00	6.00	983.00
TSS/VSS Ratio	0.00	1.09	1.12	1.18	0.00	1.16
Total P	8.44	40.42	0.83	1.36	0.43	30.63
Soluble P	0.80	8.05	0.28	0.19	0.21	.588
Particulate P	7.64	32.37	0.55	1.18	0.22	30.04
Alum used	100 gal/day					

Table C1.2 Results of the Full- Scale Plant Investigation Sampling Campaign on November 3, 2015. (Ambient Air Temperature: 80.6°F)

Location	**Influent**	**Anaerobic Reactor**	**Oxidation Ditch #1**	**Oxidation Ditch #2**	**Clarifier**	**WAS**
sCOD	1465.00	4786.00	5790.00	4156.00	2334.00	6212.00
rbCOD	663.50	372.00	455.00	4602.00	2312.00	2112.00
VFA (mg COD/L)	132.55	486.32	1761.32	683.02	347.64	926.89
Ammonia	35.25	17.46	0.21	0.12	0.07	0.25
Nitrate	0.05	0.04	0.24	0.37	0.47	0.32
Nitrite	0.07	0.05	0.33	0.52	0.66	0.44
Potassium	12.90	28.57	26.05	22.36	28.94	28.81
Calcium	147.05	150.50	137.93	121.89	141.99	141.02
Magnesium	32.10	38.09	27.10	22.50	27.26	31.10
Sodium	68.80	69.12	66.14	55.02	73.39	67.73
Chloride	91.48	78.95	67.37	70.97	70.93	76.83
Sulfate	54.68	92.05	109.24	108.18	134.67	129.64
Alkalinity	230.00	245.00	311.50	192.50	150.00	272.50
pH	7.32	7.05	7.38	7.29	7.19	-
DO	-	2.60	3.60	4.30	4.40	-
TSS (g/day)	60.00	860.00	6780.00	1040.00	530.00	1770.00
VSS (g/day)	80.00	730.00	1490.00	870.00	0.00	149.00
TSS/VSS Ratio	0.75	1.18	4.55	1.20	0.00	1.19
Total P	13.64	17.69	22.98	18.77	13.96	16.11
Phosphate	6.22	38.52	1.50	0.17	0.00	12.86
Poly P	-	-	-	10.13	6.53	0.19
Organic P	-	-	-	0.00	0.17	0.00
Particulate P	5.74	0.445	10.75	78.83	6.50	7.15
Alum Used	264 gal/ day					

Table C1.3 Results of the Full- Scale Plant Investigation Sampling Campaign on December 30, 2015. (Ambient Air Temperature: 70°F)

Location	**Influent**	**Anaerobic Reactor**	**Oxidation Ditch #1**	**Oxidation Ditch #2**	**Clarifier**	**WAS**
sCOD	414.50	325.50	173.50	249.50	223.50	171.00
rbCOD	471.00	347.00	187.50	181.50	204.50	274.50
VFA (mg COD/L)	158.49	665.09	609.43	586.79	126.42	951.42
Ammonia	38.85	8.68	0.43	0.23	0.09	0.20
Nitrate	0.06	0.06	0.10	0.02	0.22	0.04
Nitrite	1.66	1.87	1.56	1.82	2.20	2.22
Soluble N	27.65	15.65	3.795	4.69	3.06	14.16
Total N	49.10	-	-	-	36.85	25.00
Particulate N	21.45	-	-	-	33.79	10.85
Potassium	21.60	21.08	20.19	17.53	25.38	26.49
Calcium	169.79	124.92	130.91	116.58	142.01	145.88
Magnesium	34.44	31.60	29.72	25.87	32.24	34.52
Sodium	77.65	55.91	70.03	57.11	79.68	80.69
Chloride	70.84	62.37	51.68	94.55	102.31	105.06
Sulfate	70.15	106.37	105.65	189.77	226.64	216.03
Alkalinity	230.00	245.00	311.50	192.50	150.00	272.50
pH	-	7.12	7.38	7.48	7.56	7.42
DO	-	0.34	0.22	0.56	0.21	-
TSS (mg/L)	-	-	660.00	510.00	200.00	940.00
VSS(mg/L)	220.00	390.00	610.00	530.00	940.00	830.00
TSS/VSS Ratio	n/a	n/a	1.08	0.96	0.24	1.13
Total P	13.30	-	-	-	29.80	62.92
Phosphate	6.41	17.78	3.13	1.08	0.00	7.26
Poly P	0.00	-	-	-	5.30	0.00
Organic P	2.35	-	-	-	6.75	17.08
Particulate P	5.59	-	-	-	14.12	29.61
Soluble P	0.80	8.05	0.28	0.19	0.21	0.59
Alum Used	370					

Table C1.4 Results for Nitrification Studies. (Trial 1 run on March 25, 2015; Trial 2 run on April 1, 2015)

Time (hrs)	Trial	Ammonia	Nitrate	Nitrite
0	1	44.5	0.203	1.268
	2	40	1.7	0.456
	Avg	42.25	0.95	0.86
	St. Dev.	3.18	1.06	0.57
0.33	1	44.488	0.1809	0.679
	2	56.43	0.823	0.754
	Avg	50.46	0.50	0.72
	St. Dev.	8.44	0.45	0.05
0.66	1	42.32	0.28	0.975
	2	56.53	0.9705	0.732
	Avg	49.43	0.63	0.85
	St. Dev.	10.05	0.49	0.17
1	1	41.4477	0.283	1.216
	2	54.08	2.26	0.99
	Avg	47.76	1.27	1.10
	St. Dev.	8.93	1.40	0.16
1.33	1	39.83	0.519	1.935
	2	53.69	0.918	0.92
	Avg	46.76	0.72	1.43
	St. Dev.	9.80	0.28	0.72
1.66	1	37.03	0.7808	2.811
	2	52.23	1.465	0.998
	Avg	44.63	1.12	1.90
	St. Dev.	10.75	0.48	1.28
2	1	34.9	1.416	3.789
	2	50.15	1.663	1.07
	Avg	42.53	1.54	2.43
	St. Dev.	10.78	0.17	1.92
2.66	1	30.0522	2.72	5.49
	2	48.61	1.551	1.066
	Avg	39.33	2.14	3.278
	St. Dev.	13.12	0.83	3.13
3.33	1	25.45	4.189	7.29
	2	45.62	0	0
	Avg	35.54	2.09	3.65
	St. Dev.	14.26	2.96	5.15

Table C1.4 (Continued)

4	1	20.59	6.2667	9.954
	2	43.86	0	0
	Avg	32.23	3.13	4.98
	St. Dev.	16.45	4.43	7.04

Table C1.5 Results for Denitrification Studies. (Trial 1 Run on April 27, 2015; Trial 2 Run on May 18, 2015; Trial 3 Run on May 20, 2015; Trial 4 Run on June 4, 2015)

Time (hrs)	Trial	Ammonia	Nitrate	Nitrite
0	1	0.80	125.70	0.55
	2	0.11	1.19	0.31
	3	0.00	0.75	0.17
	Avg	0.30	42.55	0.34
	St. Dev.	0.43	72.01	0.19
0.25	1	0.03	125.72	33.08
	2	0.17	117.14	30.62
	3	0.29	110.45	25.18
	Avg	0.16	117.77	29.63
	St. Dev.	0.13	7.65	4.04
0.66	1	0.08	112.10	29.50
	2	0.41	111.11	29.04
	3	0.29	109.39	24.94
	Avg	0.26	110.87	27.83
	St. Dev.	0.17	1.37	2.51
1.33	1	0.25	100.52	26.45
	2	0.69	105.99	27.71
	3	0.52	105.07	23.96
	Avg	0.49	103.86	26.04
	St. Dev.	0.22	2.93	1.91
2.00	1	0.08	79.84	21.01
	2	0.88	97.45	25.47
	3	0.27	110.70	25.24
	Avg	0.41	96.00	23.91
	St. Dev.	0.42	15.48	2.51
2.67	1	0.04	65.51	17.24
	2	0.76	89.10	23.29
	3	0.52	97.21	22.16

Table C1.5 (Continued)

	Avg.	0.44	83.94	20.90
	St. Dev.	0.37	16.47	3.22
3.33	1	0.04	46.83	12.32
	2	1.44	78.72	20.58
	3	0.13	97.40	22.21
	Avg.	0.54	74.32	18.37
	St. Dev.	0.78	25.57	5.30
	1	0.07	17.25	4.54
4.667	2	0.00	71.22	18.62
	3	0.15	90.53	20.64
	Avg.	0.07	59.67	14.6
	St. Dev.	0.08	37.98	8.77
5.33	1	0.19	4.05	2.16
	2	9.07	1.55	9.37
	3	1.62	0.00	0.00
	Avg.	3.63	1.87	3.84
	St. Dev.	4.77	2.04	4.91

Table C1.6 Results for SND Studies at 22° C. (Trial 1 Run on July 8, 2015; Trial 2 Run on July 15, 2015; Trial 3 Run on July 21, 2015; Trial 4 Run on July 28, 2015)

Time (hrs)	Trial	sCOD	NH_4^+	NO_3^-	NO_2^-	pH	DO	Alk.	TSS	VSS	rbCOD	Temp
0:00	1	62.50	24.82	0.00	0.00	7.16	0.30	195.00	900.00	800.00		22.00
	2	63.50	24.69	0.00	0.00	7.02	0.29	215.00	1000.00	1000.00		22.00
	3	192.50	38.94	0.00	0.00	7.10	0.28	212.50	700.00	600.00	131.00	22.00
	4	55.50	24.90	0.00	0.00	7.45	1.48	197.50	700.00	600.00	48.00	22.00
	Average	93.50	28.34	0.00	0.00	7.18	0.59	205.00	825.00	750.00	89.50	
	Stdev	66.10	7.07	0.00	0.00	0.19	0.60	10.21	150.00	191.49	58.69	
2:00	1					7.06	0.19					22.00
	2					6.92	0.21					22.00
	3		1.20	0.02	0.01	7.46	0.71					22.00
	4		1.88	0.10	0.02	7.55	1.34					22.00
	Average		1.54	0.06	0.01	7.25	0.61					
	Stdev		0.48	0.06	0.00	0.31	0.54					
4:00	1		0.46	0.03	0.00	7.05	0.18					22.00
	2		0.46	0.03	0.00	6.89	0.19					22.00
	3		0.29	0.02	0.00	7.28	0.20					22.00
	4		0.46	0.03	0.00	7.29	1.24					22.00
	Average		0.42	0.02	0.00	7.13	0.45					
	Stdev		0.08	0.00	0.00	0.19	0.53					
6:00	3					7.40	3.30					22.00
	4					7.08	0.50					22.00
	5		1.13	0.01	0.01	7.27	0.17					22.00
	6		1.76	0.02	0.01	7.14	0.15					22.00
	Average		1.44	0.02	0.01	7.22	1.03					
	Stdev		0.45	0.01	0.00	0.14	1.52					
8:00	1		0.27	0.06	0.00	7.35	0.44					22.00
	2		0.27	0.06	0.00	7.26	0.25					22.00
	3		0.17	0.04	0.00	7.25	2.23					22.00

Table C1.6 (Continued)

	4		0.27	0.06	0.00	6.94	0.14					22.00
	Average		0.24	0.05	0.00	7.20	0.77					
	Stdev		0.05	0.01	0.00	0.18	0.98					
10:00	1					7.26	0.16					22.00
	2					7.26	0.16					22.00
	3		0.88	0.18	0.00	7.02	2.81					22.00
	4		1.38	0.28	0.01	6.76	1.34					22.00
	Average		1.13	0.23	0.01	7.08	1.12					
	Stdev		0.35	0.07	0.00	0.24	1.26					
12:00	3		0.39	0.01	0.00	7.23	0.15					22.00
	4		0.40	0.01	0.00	7.12	0.16					22.00
	5		0.25	0.00	0.00	6.95	0.17					22.00
	6		0.39	0.01	0.00	6.82	3.44					22.00
	Average		0.36	0.01	0.00	7.03	0.98					
	Stdev		0.07	0.00	0.00	0.18	1.64					
14:00	1					7.06	0.80					22.00
	2					7.31	0.22					22.00
	3		0.59	0.14	0.01	6.95	0.17					22.00
	4		0.93	0.22	0.02	6.87	0.11					22.00
	Average		0.76	0.18	0.02	7.05	0.33					
	Stdev		0.24	0.06	0.00	0.19	0.32					
16:00	1		0.15	0.44	0.00	6.93	0.27	150.00	900.00	800.00		22.00
	2		0.15	0.44	0.00	7.20	0.55	-	1000.00	900.00		22.00
	3	39.50	0.10	0.28	0.00			202.50	600.00	700.00		22.00
	4	40.50	0.15	0.44	0.00		0.13	142.50	800.00	1000*0.6	22.00	22.00
	Average	40.00	0.14	0.40	0.00	7.07	0.32	165.00	825.00	800.00	22.00	22.00
	Stdev	0.71	0.03	0.08	0.00	0.19	0.21	32.69	170.78	100.00	0.00	

Table C1.7 Results for SND at 29.5° C. (Trial 1 Run on August 25, 2015; Trial 2 Run on September 1, 2015; Trial 3 Run on September 8, 2015; Trial 4 Run on November 25, 2015)

Time (hrs)	Trial	sCOD	NH_4^+	NO_3^-	NO_2^-	pH	DO	Alk.	TSS	VSS	rbCOD	Temp
0:00	1	44.500	24.530	0.330	0.640	7.490	0.450	205	600	700	32.000	28.50
	2	51.000	24.490	0.030	0.800	7.130	0.300	217.5	700	700	55.000	20.50
	3	75.500	27.090	0.110	0.090	7.350	0.250	100	600	600	39.000	19.70
	4	80.500	20.450	0.220	1.670	6.900	0.540	150	1100	700	33.810	16.70
	Avg.	62.875	24.140	0.173	0.800	7.218	0.385	168.125	750	675	39.953	
	Stdev	17.783	2.744	0.131	0.655	0.258	0.134	54.059	238	50	10.461	
2:00	1		1.130	16.990	0.000	7.160	3.100					30.00
	2		20.670	1.920	0.820	7.050	1.160					29.10
	3		17.670	1.440	0.070	7.200	0.120					28.90
			12.130	1.130	0.980	7.290	0.320					29.20
	Avg.		12.900	5.370	0.297	7.175	1.175					
	Stdev		8.607	7.753	0.455	0.099	1.360					
4:00	1		0.090	20.930	0.670	7.050	2.450					29.30
	2		14.290	5.920	0.860	7.120	0.960					29.40
	3		20.790	1.490	0.080	7.140	0.350					29.20
	4		11.410	0.890	1.290	7.220	0.220					29.40
	Avg.		11.723	9.447	0.537	7.133	0.995					
	Stdev		10.586	10.189	0.407	0.070	1.022					
6:00	1		0.120	15.340	0.180	6.930	0.100					29.40
	2		8.030	10.740	0.750	7.110	0.230					29.40
	3		18.310	0.140	0.130	7.020	0.060					29.20
	4		9.190	0.290	1.510	7.170	0.170					29.70
	Avg.		8.820	8.740	0.353	7.058	0.140					
	Stdev		9.121	7.795	0.344	0.105	0.075					
8:00	1		0.120	11.170	0.000	6.980	0.100					29.10

Table C1.7 (Continued)

	2		0.000	15.590	0.230	6.910	0.160					29.30
	3		14.670	0.810	0.030	6.870	0.060					29.20
	4		5.000	0.590	1.350	6.950	0.220					29.60
	Avg.		4.930	9.190	0.087	6.928	0.135					
	Stdev		8.435	7.586	0.125	0.048	0.070					
10:00	1		0.110	10.700	0.650	6.970	1.450					29.50
	2		0.020	16.810	0.650	6.860	3.110					29.40
	3		10.970	1.560	0.020	6.870	0.240					29.20
	4		3.180	0.780	1.160	7.020	0.920					29.60
	Avg.		3.700	9.690	0.440	6.930	1.430					
	Stdev		6.296	7.675	0.364	0.078	1.225					
12:00	1		0.110	11.370	0.670	6.950	1.170					29.70
	2		0.020	16.810	0.650	6.590	0.040					29.30
	3		9.590	3.780	0.030	6.840	0.350					29.20
	4		3.130	0.800	1.220	7.050	0.940					29.60
	Avg.		3.240	10.653	0.450	6.858	0.625					
	Stdev		5.499	6.544	0.364	0.198	0.521					
14:00	1		0.180	8.410	0.190	6.800	0.000					29.70
	2		0.010	16.700	0.430	6.590	0.000					29.30
	3		3.710	8.860	0.090	6.710	0.370					29.20
	4		4.350	0.450	1.570	6.910	0.260					29.60
	Avg.		1.300	11.323	0.237	6.753	0.158					
	Stdev		2.089	4.662	0.175	0.136	0.187					
16:00	1	23.500	0.250	5.300	0.000	6.640	0.000	142.5	0.300	0.300	19.000	29.70
	2	50.000	0.130	7.100	0.070	6.580	0.030	140	0.600	0.500	21.000	29.30
	3	22.500	0.000	13.950	0.150	6.790	3.200	132.5	0.600	0.500	36.000	29.20
	4	18.000	4.830	0.450	1.570	6.880	0.230	125	-	-	28.000	29.60
	Avg.	28.500	0.127	8.783	0.073	6.723	0.865	135.000	0.500	0.433	26.000	29.45
	Stdev	14.532	0.125	4.564	0.075	0.137	1.560	7.906	0.173	0.115	7.703	0.24

Appendix D Step By Step Analytical Methods

- Methodology for rbCOD: The rbCOD was determined using a physical-chemical method developed by Mamais et al. (1993). The sample was flocculated by adding 1mL of a 100 g/L zinc sulfate solution to 100 mL of influent wastewater and rapidly mixing with a magnetic stirrer for 1 min. Then, the pH of the sample was adjusted to 10.5 using a 6M NaOH solution. After stirring was stopped, the sample was allowed to settle for approximately 5 minutes. Forty milliliters of supernatant were removed with a pipette, taking care not to disturb the settled portion of the sample, and vacuum filtered through a 0.45µm membrane filter (Fisherbrand 0.45µm, 47mm, MCE membrane filters). The COD of the sample was determined using Standard Methods 5220D (APHA et al, 2012).
- Methodology for Acid Hydrolyzable Phosphorous: The acid hydrolysable phosphorus method is used to calculate polyphosphates and organic phosphates. Acid hydrolysable phosphorus was determined by a method described by EPA method 365.3. First, all glassware was washed with a 6N hydrochloric acid and rinsed with deionized water. Next, 25-mL of sample was measured into a 50-mL Erlenmeyer flask. Then, 2.0-mL of 5.25 N Sulfuric Acid solution was added and the flask was placed on a hot plate and boiled gently for 30 minutes. Deionized water was added to the flask to maintain a volume near 20-mL throughout the 30 minutes. The sample was cooled to room temperature. After, 2.0-mL of 5.0N NaOH solution was added to the sample and swirled to mix. Hach TNT Plus 845 test kits were used to complete the test following the instructions for measurement of reactive phosphorus. Note that the results included the orthophosphate and the acid- hydrolysable phosphate thus the results were subtracted from a reactive phosphorus test on an untreated sample.