

INVESTIGATION OF BIOLOGICAL NITROGEN  
REMOVAL FOR AN INDUSTRIAL WASTEWATER  
TREATMENT PLANT

By

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INVESTIGATION OF BIOLOGICAL NITROGEN  
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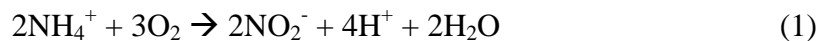
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## CHAPTER I

### INTRODUCTION

Nitrogen is an important element in all biological process. Nitrogen is found in important cell building blocks such as amino acids, proteins, and nucleic acids. In wastewater treatment systems, nitrogen can appear in a variety of compounds ranging from basic forms such as ammonia or nitrate, to complex organic compounds. The major nitrogen forms of concern when discussing wastewater treatment plants are ammonia and organic nitrogen. Complex organic nitrogen compounds are often broken down during cellular metabolism into simpler forms such as dissolved ammonia (Stover, 1974).

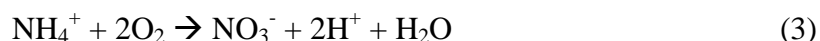
Biological nitrification is a two step process in which ammonia is oxidized and ultimately converted to nitrate. The first oxidation step is facilitated by the bacterial group *Nitrosomonas* in which ammonia is converted to nitrite as shown by the following reaction:



The second oxidation step is facilitated by the bacterial group *Nitrobacter* in which nitrite is further oxidized to nitrate as shown by the following reaction:



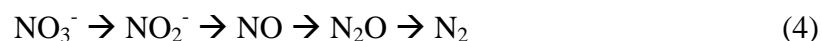
The total oxidation reaction for the conversion of ammonia to nitrate is as follows:





The bacterial groups *Nitrosomonas* and *Nitrobacter* are both of the autotrophic bacterial genera which means that they can harness energy, the release of electrons, from the oxidation of inorganic compounds, in this case ammonia, in the form of adenosine triphosphate (ATP) for energy storage or use (Stover, 1974). It should be noted that not all ammonia taken up by nitrifying organisms is directly entered into the nitrification process. Some of the ammonia taken up by nitrifying organisms may enter into maintenance or synthesis pathways instead.

Biological denitrification is the oxidation of organic compounds in a wastewater treatment system where nitrite or nitrate is used as the final electron acceptors in the place of oxygen and is referred to as anoxic respiration. This process occurs in the absence of oxygen, or under very low dissolved oxygen concentrations. The denitrification process is facilitated by facultative aerobic bacteria, which can switch between final electron acceptors depending on conditions. In the presence of DO, facultative aerobic bacteria will use oxygen as the final electron acceptor, which provides the most possible ATP production from organics degradation. In the absence of DO, or very low DO conditions, the facultative aerobic bacteria will switch nitrite or nitrate for the final electron acceptor, which produces the most possible ATP production from organics degradation, second to using oxygen as the final electron acceptor. The denitrification process reduces nitrate to nitrite, to nitric oxide, to nitrous oxide, and ultimately to nitrogen gas, as follows:



A portion of the total nitrogen found in a system is removed through normal bacterial functions such as maintenance and synthesis, and sludge wasting. Any nitrogen

present in a treatment system above the maintenance and synthesis requirements of the mixed liquor suspended solids must be in the ammonia form or converted to an ammonia form to be removed through the nitrification process.

It is beneficial to remove nitrogen from wastewater using biological treatment for several reasons. First, ammonia is readily taken up by many types of bacteria as ammonia is one of the most basic and useable forms of nitrogen for bacteria. Nitrogen compounds can be removed by physical/chemical treatment, but it is often much more cost effective to use biological treatment as many of the bacterial types needed for nitrogen removal are readily found in industrial and municipal wastewaters. It is well known that ammonia exhibits toxicity to many species of fish, even at fairly low concentrations. Ammonia and nitrate can also provide the nitrogen needed for algal blooms, which can result in extreme drops in DO concentrations as well as nutrient concentrations, resulting in the death of many aquatic species.

The research and analysis that follows were performed to determine whether or not biological nitrogen removal is occurring at the industrial wastewater treatment plant (IWTP) of interest. Samples were collected from the industrial wastewater treatment plant and transported back to the Oklahoma State University campus for analysis of biochemical oxygen demand (BOD), chemical oxygen demand (COD), ammonia in terms of ammonia-nitrogen, nitrate, in terms of nitrate-nitrogen, total suspended solids (TSS), volatile suspended solids (VSS), and total nitrogen (T-N). The T-N analyses were performed by the Oklahoma State University Soils Laboratory.

## CHAPTER II

### REVIEW OF LITERATURE

#### Nitrification Process

Biological nitrogen removal is a two-stage process facilitated by autotrophic bacteria. The first step in the process is nitrification in which ammonia is converted to nitrate. The nitrate is then converted to nitrogen gas in a denitrification step.

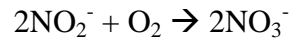
Nitrification is a two-step biological process in which ammonia ( $\text{NH}_4^+$ ) is converted to nitrite ( $\text{NO}_2^-$ ), then ultimately to nitrate ( $\text{NO}_3^-$ ) by two separate oxidation reactions. There are several benefits associated with the nitrification treatment process itself. Nitrification basically converts ammonia to nitrate. This removes the effect of ammonia on receiving streams as far as dissolved oxygen depletions and fish toxicities are concerned. Ammonia is a well known nitrogen source for many naturally occurring microorganisms, such as algae. In the presence of usable phosphorus, ammonia can promote undesirable eutrophication scenarios such as algal blooms in which rapid dissolved oxygen depletion can occur resulting in deadly conditions for organisms that rely on dissolved oxygen for respiration. Ammonia itself is also known, even in fairly low to moderate concentrations, to be toxic to many fish species.

The first oxidation step is an energy-yielding step in which ammonia is oxidized to nitrite is facilitated by the bacterial group *Nitrosomonas*. Other autotrophic bacterial

groups are also capable of completing the energy-yielding first-stage oxidation process, such as *Nitrosococcus*, *Nitrospira*, *Nitrosolobus*, and *Nitrosorobrio* (Painter, 1970). The first oxidation reaction is as follows:



The second oxidation step is also an energy-yielding step in which the nitrite produced from the first reaction is further oxidized to nitrate by the bacterial group *Nitrobacter*. Again, other genera are known to be capable of this process, such as *Nitrococcus*, *Nitrospira*, *Nitrospina*, and *Nitrooestis* (Metcalf and Eddy, 2003). These bacteria, as well as the bacteria listed in the above paragraph are known to be commonly found in activated-sludge wastewater systems. The second oxidation reaction is as follows:



The total oxidation reaction for conversion of ammonia to nitrate is as follows:



When looking at the total oxidation reaction, it becomes apparent that for every mole of ammonia oxidized, one mole of nitrate is produced. The complete oxidation of ammonia as nitrogen to nitrate consumes 4.57 grams of oxygen per gram of ammonia as nitrogen. Nitrite production consumes 3.43 grams of the 4.57 grams of oxygen, while the conversion of nitrite to nitrate consumes 1.14 grams of oxygen. The complete oxidation process also consumes 7.14 grams of alkalinity as  $\text{CaCO}_3$  for every gram of ammonia as nitrogen oxidized (Metcalf and Eddy, 2003).

### Nitrification Inhibition

The nitrification process is susceptible to inhibition from a variety of factors, such as pH, toxicity from chemical compounds, toxicity from metals, and un-ionized ammonia.

A study performed by the U.S. EPA demonstrated that the nitrification rates decline considerably below a pH value of 6.8, and at pH values near 5.8 to 6.0, the nitrification rate may be only 10 to 20 percent of the nitrification rate at a pH value of 7.0 (U.S. EPA, 1993). Nitrification rates tend to reach a maximum at pH values near 7.5 to 8.0, while nitrification systems are typically operated within a pH range of 7.0 to 7.2. Alkalinity is consumed during the nitrification process, which lowers the pH of the wastewater to be treated. Therefore, if the water does not possess sufficient alkalinity, or alkalinity addition is not performed, the desired nitrification process could result in the lowering of pH, and hence become self-inhibiting. If alkalinity addition is needed, alkalinity is typically added in the form of lime, soda ash, sodium bicarbonate, or magnesium hydroxide (Metcalf and Eddy, 2003).

Nitrifying organisms are known to be susceptible to numerous types of organic and inorganic compounds. Organic compounds that are known to be toxic to nitrifying bacteria are solvent grade chemicals, amines, proteins, tannins, phenolic compounds, alcohols, cyanates, ethers, carbamates, and benzene (Hockenbury and Grady, 1977, and Sharma and Ahlert, 1977). Due to the numerous types of chemicals often found in a wastewater treatment system, it can be very difficult to identify the source of toxicity if chemical toxicity is believed to be occurring. Metals have also exhibited toxicity to nitrifying bacteria, as exhibited in a study by Skinner and Walker (1961) where complete

inhibition of ammonia oxidation occurred at concentrations of 0.25 mg/L of nickel, 0.25 mg/L of chromium, and 0.10 mg/L of copper. The nitrification process can also be inhibited by un-ionized or free ammonia (NH<sub>3</sub>) and un-ionized nitrous acid (HNO<sub>2</sub>) (Metcalf and Eddy, 2003).

### Denitrification Process

Denitrification takes place over a series of steps in which nitrate is reduced to nitrite, to nitric oxide, to nitrous oxide, and ultimately to nitrogen gas as follows:



Nitrogen gas is an inert gas that is insoluble in water and comes out of solution once produced. The nitrogen gas is typically vented to the atmosphere, which is comprised of approximately 78 percent nitrogen gas.

Bacteria capable of denitrifying are both heterotrophic, and autotrophic, facultative aerobes, meaning that the bacteria capable of this process can switch between oxygen and nitrate or nitrite as final electron acceptors, while some can produce energy from organic compounds where others can produce energy from the oxidation of inorganic compounds. Denitrification can only occur in the absence of DO, or the presence of DO in very low concentrations.

Using the term C<sub>10</sub>H<sub>19</sub>O<sub>3</sub>N to represent biodegradable organic matter in wastewater, the heterotrophic denitrification reaction is as follows:



(U.S. EPA, 1993). As illustrated in the above equation, for every equivalent of nitrate as nitrogen reduced during denitrification, one equivalent of alkalinity is produced. This turns out to be 3.57 grams of alkalinity produced for every gram of nitrate as nitrogen

reduced. For this reason, it is beneficial to accompany nitrification with denitrification as the denitrification process can return approximately one-half the alkalinity consumed during the nitrification process.

One concern during the denitrification process is to ensure adequate BOD is present to provide for a sufficient amount of electron donor. As a general rule, 4 grams of BOD is needed for every gram of nitrate reduced (Barth *et al.*, 1968). A sufficient amount of electron donor is important as the nitrate present will serve as the final electron acceptor, thus being reduced ultimately to nitrogen gas.

### Denitrification Inhibition

Dissolved oxygen is known to inhibit nitrate reduction by suppressing the nitrate reduction enzyme in facultative aerobic bacteria; however, denitrification can continue to occur in low bulk liquid DO concentrations up to 0.13 mg/L for a highly dispersed culture as demonstrated by Nelson and Knowles (1978).

As mentioned previously, the denitrification process produces alkalinity, thus elevating the pH of the wastewater. Nitrification systems operate well within a pH range of 7.0 to 8.0. This pH range shows no significant effects on the rate of denitrification, although the rate of denitrification decreases as the pH range drops to a pH value of 6.0 (Dawson and Murphy, 1972).

### Types of Biological Nitrogen Removal Systems

#### Preanoxic Denitrification

There are two typical types of biological nitrogen removal systems. The first type of system is termed a preanoxic denitrification system. This type of system is named accordingly as the anoxic process precedes the aeration basin. In this type of system, raw

influent flows into an anoxic reactor which then flows to an aeration basin. The aeration basin overflows to a clarifier for settling and sludge thickening. This type of system involves two separate recycle lines. The first recycle line is termed an internal recycle line which recycles the reactor contents of the aeration basin back to the anoxic basin. The purpose of the internal recycle is to return any nitrate produced in the aeration basin back to anoxic basin for denitrification. The second recycle line is the external recycle line which recycles thickened sludge back from the clarifier to the anoxic basin to maintain the mixed liquor suspended solids concentration. The anoxic reactor is named such as it operates in the absence of oxygen to promote the reduction of nitrate.

#### Postanoxic Denitrification

The second type of biological nitrogen removal system is termed a postanoxic denitrification system in that the anoxic process follows after the aeration basin. Raw influent flows into the aeration basin first, then to the anoxic basin before overflowing to the clarifier. In this type of system, only one recycle line is used. An external recycle line is routed from the clarifier to the aeration basin for the return of thickened activated sludge to maintain the mixed liquor suspended solids concentration. Postanoxic systems often require addition of an exogenous carbon source such as methanol or acetate to provide sufficient BOD for nitrate reduction and to increase the rate of denitrification (Metcalf and Eddy, 2003).

#### Simultaneous Nitrification/Denitrification

In activated sludge systems, it is often assumed that the bulk liquid DO concentration is equal throughout the entire basin. In reality, both aerobic and anoxic zones can exist due to aeration diffuser spacing and the bulk liquid DO concentration



does not represent the actual sludge-floc concentration. Under low DO conditions with a sufficient solids retention time, denitrification can occur within the interior of the sludge-floc while nitrification occurs at the exterior of the floc. Overall nitrogen removal by simultaneous nitrification/denitrification systems has shown to produce more than 90 percent nitrogen removal in a municipal activated sludge system at DO concentrations below 0.50 mg/L with a hydraulic retention time greater than 25 hours (Rittman and Langeland, 1985).

Several articles were discovered that focused on the improvement of nitrification and nitrogen removal for wastewater treatment plants similar in nature to the IWTP. The focus of these studies was to improve biological nitrogen removal in the facilities of interest. It was noted in these articles that nitrification rates were very low due to substances present in the waste stream from the various production facilities exhibiting toxicity to the bacteria responsible for nitrification. Studies such as Furtado *et al.* (1998) and Stenstrom and Adam (1984) displayed through numerous studies that the addition of activated carbon significantly increased biological nitrification reaction rates by as much as 900%.

During the course of this study, the activated sludge units 1 and 2 were operated in parallel, while the activated sludge unit clarifiers were operated in series.

## CHAPTER III

### METHODOLOGY

Samples were collected from the IWTP at numerous locations to allow for a comprehensive view of the treatment processes occurring across the treatment plant. Samples were collected by OSU personnel under the supervision of an IWTP contract operator.

The sampling location names used for identification were given to the OSU personnel by the IWTP staff and carried over for clarification purposes. The following sample locations are listed as follows:

<u>Sample Location</u>	<u>Sample Location</u>
PTU	ASU 2 Head
GW Recovery	ASU 2 Exit
GW ASU Middle	Clarifier 1 Effluent
GW ASU Exit	Clarifier 2 Effluent
GW ASU Clar. Pump	Lagoon 1
Lift Station	Lagoon 2
External Line	Lagoon 3
ASU Feed	Lagoon 4
ASU 1 Head	Lagoon 5
ASU 1 Exit	Lagoon 6

The PTU line and GW Recovery line are influent lines flowing into the GW ASU (Ground Water Activated Sludge Unit). The GW ASU Middle and GW ASU Exit are locations across the unit itself. The Lift Station sample location receives influent from the GW ASU as well as other influent from other plant processes outside the scope of this

study. The External Line also receives numerous influents that are outside the scope of this study. It is understood that the Lift Station and External Line flow to the ASU Feed location which serves as an equalization basin. The ASU Exit and Head locations are locations across the ASUs themselves. The ASUs were operated in parallel during the scope of this study and fed Clarifier 1 and Clarifier 2 which were operated in series during the scope of this study. It is also understood that effluent from the clarifier units then flows to the lagoon system. It should be noted that Lagoon 3 and Lagoon 4 were off-line during the scope of this study. A simple flow chart of the plant during the sampling events can be viewed in Figure 1.

Samples were collected in a grab sample manner into one-liter plastic bottles. Two to four liters were collected from each sample location to allow for plenty of sample for analysis. Samples were immediately analyzed for pH, temperature, dissolved oxygen and conductivity. Samples were then transported back to the OSU-Stillwater campus in ice chests for further testing and analysis at the OSU environmental engineering laboratories.

Samples were collected directly into the sample container where possible. For situations where the sample could not be collected directly into the bottle, a sampler (sample cup on an extendable handle) was used for long reaches.

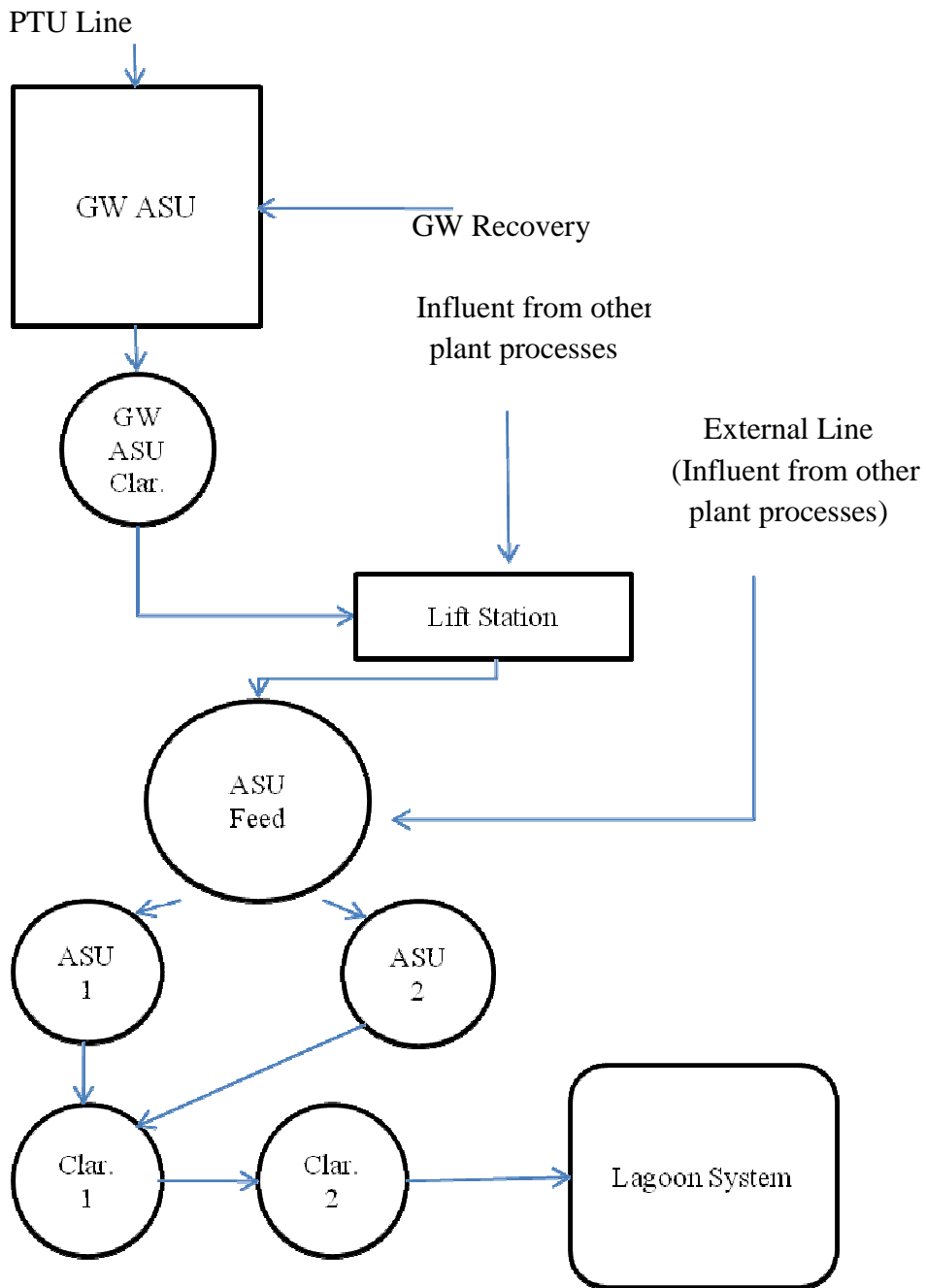


Figure 1. Plant Flow Chart

Samples were analyzed at the OSU laboratories for parameters as follows:

<u>Parameter</u>	<u>Method</u>
BOD	Method 5210B (Standard Methods)
COD	Hach Colorimetric COD Method 8000
TSS	Method 2540D (Standard Methods)
VSS	Method 2540E (Standard Methods)
NH <sub>3</sub> -N	Hach NH <sub>3</sub> -N Colorimetric Method 10031
NO <sub>3</sub> -N	Hach NO <sub>3</sub> -N Colorimetric Method 10020
T-N	Leeco Method (Performed by OSU Soils Laboratory)

### BOD Test Procedure

BOD tests were performed by following the methods outlined in the 21<sup>st</sup> edition of Standard Methods. BOD dilution water was prepared using Hach Company (Hach) nutrient buffer pillows and de-ionized water. The volume of de-ionized water used was determined based on the size of the Hach nutrient buffer pillow used for each batch of BOD dilution water prepared. The BOD dilution water was then aerated for two hours and allowed to stabilize for a period of 24 hours before use. A diffuser stone attached to an aquarium pump was used for aeration and also provided mixing for the BOD dilution water.

Bacterial seed solution was also prepared using Hach Polyseed BOD Inoculum according to the included instructions for the BOD tests performed. A one-liter beaker was filled to the 500mL mark with prepared BOD dilution water. One scoop of the Polyseed BOD Inoculum was then added to the beaker and the beaker was mixed using a magnetic stir bar and base as well as a diffuser stone attached to an aquarium pump. The magnetic stir bar provided mixing while the diffuser stone provided aeration as well as additional mixing. The bacterial seed solution was mixed and aerated for one hour. After the one hour mixing period, the diffuser stone was removed and the magnetic stirrer was turned off. The seed solution was allowed to settle before use.

BOD tests were performed using 300 milliliter glass bottles. The sample volumes used for the BOD tests were determined by using the BOD equation listed below. The BOD concentration was estimated by multiplying the corresponding COD concentration by 0.5. Once estimated, the BOD concentration was placed into the BOD equation and the equation was solved backwards for DO depletions of 2, 4, and 6 mg/L, respectively. This provided a dilution factor which was then used to solve for the sample volumes to be added to each BOD bottle. Each BOD bottle was then filled to a level slightly above the bottom of the neck using BOD dilution water and an initial DO measurement was taken. After the initial DO measurement was taken, each BOD bottle received a glass stopper. By filling the BOD bottle to a level slightly above the neck of the bottle, a water seal was produced above the glass stopper, once inserted, which aids in prevention of air infiltration into the bottle during the test period. After the glass stopper was inserted, a plastic cap was placed over the top of the bottle to prevent evaporation of the water seal. The BOD bottles were then incubated in a BOD incubation room for 5 days at 20°C in the absence of light to prevent oxygen release from photosynthesis which would give false low BOD results. At the end of the incubation period, a final DO reading was taken for each BOD bottle (Standard Methods, 2005).

Each set of BOD tests performed included two BOD dilution water blanks and three seed solution blanks. The BOD dilution water blanks are prepared by adding BOD dilution water only to a BOD test bottle. This is done to account for DO depletion (if any) in the BOD dilution water itself. Also, BOD dilution water blanks can indicate any possible contamination in the BOD dilution water as little to no DO depletion should occur. Seed blanks were also prepared for each set of BOD tests performed using

varying seed concentrations of 2, 5, and 10 milliliters per seed blank, respectively. Seed blanks are prepared to account for DO depletion as a result of seed addition alone. The average DO depletion of the BOD dilution water blanks and the seed solution blank DO depletions were then used to determine a seed correction factor for final calculation of BOD concentrations (Standard Methods, 2005).

BOD samples were analyzed for total and soluble BOD, as well as carbonaceous BOD. Total BOD tests were performed using a well mixed sample added directly to the BOD bottle itself. Soluble BOD tests were performed using sample filtered through a 0.45 micron glass-fiber filter to remove any suspended solids before addition to the BOD bottle. All samples were analyzed for total and soluble carbonaceous BOD, as well. Carbonaceous BOD is the occurring BOD in a sample minus any nitrogenous BOD. Nitrogenous BOD is any BOD that occurs from the oxidation of forms of nitrogen, such as ammonia or organic nitrogen. Nitrogenous BOD was prevented from occurring by use of a nitrification inhibitor chemical placed into the corresponding BOD bottles during the setup process. Two shots (approximately 0.16 grams) of the dry nitrification inhibitor were placed into the BOD bottles of interest. The nitrification inhibitor chemical used for this study was provided by Hach.

BOD concentrations were then calculated using the following equation:

$$\text{BOD, mg/L} = \frac{(D1 - D2) - (S)V_s}{P} \quad (6)$$

where:

BOD = BOD<sub>5</sub>

D1 = DO of diluted sample immediately after preparation, mg/L

D2 = DO of diluted sample after 5 day incubation period at 20°C, mg/L

S = oxygen uptake of seed, ΔDO/mL seed suspension added per bottle

V<sub>s</sub> = volume of seed in the respective test bottle, mL, and

P = decimal volumetric fraction of sample used; 1/P = dilution factor (Standard Methods, 2005).

### COD Test Procedure

COD tests were performed using a colorimetric method with pre-prepared vials from Hach that contain the digestion solution. The primary reagent of concern in the digestion solution is the dichromate ion ( $\text{Cr}_2\text{O}_7^{2-}$ ). During the digestion process, the dichromate ion oxidizes COD material in the sample, changing the dichromate ion from a hexavalent state to a chromic ion in the trivalent state. Each of these chromium species has a unique color within the visible range of the color spectrum.

As with the BOD test, COD tests are prepared with a blank. The blank is used for calibrating the spectrophotometer to a level of 0 mg/L COD. The blank is prepared by adding de-ionized water only to the Hach COD vial which already contains the digestion solution.

All samples were tested for total and soluble COD. Total COD tests were performed by adding well mixed sample directly to the COD vial. Soluble COD tests were performed by filtering the sample through 0.45 micron glass-fiber filters before addition to the COD vials. All COD tests were performed using high range (0 – 1500 ppm) COD prepared by Hach. Each test vial received 2 milliliters of sample, except in the case of the blank where de-ionized water was used. In some cases where sample CODs were outside of the vial range, pre-dilutions were used to achieve COD ranges within the test range of the vial. All COD test vials were read using a Hach DR 2010 spectrophotometer at a wavelength of 620 nm.



## TSS Test Procedure

Total suspended solids test were performed according to the methods outlined in the 21<sup>st</sup> edition of Standard Methods. TSS tests were performed for all samples collected. First, filters were prepped ahead of time for TSS analysis. The filters used for TSS test were 0.45 micron glass-fiber filters. All filters received three successive 20-mL rinses with de-ionized water under suction from a vacuum pump. After the three rinse steps, the filters were placed into an aluminum weighing dish and fired in a 550°C furnace for 15 minutes, since the samples were also being analyzed for volatile suspended solids. The filters were then removed and placed in a desiccator to cool to room temperature. Once cooled, all filters and weighing dishes were pre-weighed to provide an initial weight (Standard Methods, 2005).

After the initial filter preparation, the filters were pre-wetted with a small amount of de-ionized water. Next, a known and recorded volume of well mixed sample was passed through each filter under suction. The filters were then washed with three successive 10-mL de-ionized water rinses. After filtration, the glass-fiber filters were transferred back to the aluminum weighing dishes and placed in a 103°C oven for drying with a minimum drying time of one hour, the filtrate can either be discarded or used for other soluble based tests. After a minimum one hour drying time, the weighing dish and filter were placed in a desiccator to cool. Once cooled to room temperature, the filters and weighing dishes were reweighed. Once initial and final weights were collected, TSS concentrations were calculated using the following equation:

$$\text{TSS, mg/L} = \frac{(A - B) * 1000}{\text{sample volume, mL}} \quad (7)$$

where:

A = weight of filter + dried residue, mg, and

B = weight of filter, mg (Standard Methods, 2005).

### VSS Test Procedure

For this study, samples were also tested for volatile suspended solids. Since the filters were already prepared and passed through the TSS testing procedure, no additional setup was necessary for VSS testing.

After the final weighing for the TSS testing, the filters are placed into a 550°C furnace where any volatile compounds (organics) are ignited and vaporized. The filters remained in the furnace for 15 – 30 minutes. The filters were then removed from the furnace and allowed to cool for a minimum of four hours in desiccators. Once cooled to room temperature, the filters and weighing dishes were reweighed. Once all final weights were collected, the VSS concentrations were calculated using the following equation:

$$\text{VSS, mg/L} = ((A - B) * \frac{1000}{\text{sample volume, mL}}) \quad (8)$$

where:

A = weight of residue + dish before ignition, mg, and

B = weight of residue + dish after ignition, mg (Standard Methods, 2005).

It is important to ensure that all filters and weighing dish are cool before weighing due to heat convection that can result in false low weights.

### NH<sub>3</sub>-N Test Procedure

Ammonia-nitrogen tests were performed using a colorimetric method with pre-prepared vials from Hach. All ammonia-nitrogen tests were performed using high range (0 – 50 mg/L) AmVer High Range Ammonia Test 'N Tube vials. The ammonia-nitrogen test requires that all samples be filtered before sample addition to the vial. For the filtering step, 0.45 micron glass-fiber filters were used to filter the samples. After

filtering, 0.1 mL of each sample was added to the corresponding test vial. The reagent powder pillows (ammonia salicylate and ammonia cyanurate pillows) were added to the vials, mixed, and allowed a twenty minute reaction period. As mentioned in some of the previous tests, a blank was prepared and used to calibrate the spectrophotometer to a level of 0 mg/L  $\text{NH}_3\text{-N}$ . All ammonia-nitrogen vials were read using a Hach DR 2010 spectrophotometer at a wavelength of 655 nm.

#### $\text{NO}_3\text{-N}$ Test Procedure

Nitrate-nitrogen tests were performed using a colorimetric method with pre-prepared vials from Hach. All nitrate-nitrogen tests were performed using NitraVer X Test 'N Tube Reagent vials. The nitrate-nitrogen test requires that all samples be filtered before sample addition to the vial. For the filtering step, 0.45 micron glass-fiber filters were used to filter the samples. After filtering, 1.0 mL of each sample was added to the corresponding test vial. Each individual sample was added to a corresponding nitrate-nitrogen vial. The vial was then blanked to account for any color that the existing sample may possess. After the spectrophotometer was calibrated to a level of 0 mg/L  $\text{NO}_3\text{-N}$ , the reagent powder pillow (nitrate chromatropic powder pillows) was added to the vial, mixed, and allowed a five minute reaction period. After the reaction period, the same vial was returned to the spectrophotometer for analysis. This step was repeated for every sample. A de-ionized water blank was also prepared for this test. The de-ionized blank was used to produce a baseline nitrate-nitrogen value that was later subtracted from all of the recorded values to produce a final result for all samples. All nitrate-nitrogen vials were read using a Hach DR 2010 spectrophotometer at a wavelength of 410 nm.

## CHAPTER IV

### FINDINGS

It should be noted when viewing the findings that during the time period of the sampling events, Lagoons 3 and 4 were off-line. For this reason, data for Lagoons 3 and 4 have not been included in the data tables that follow. Data collected immediately during the sampling data can be viewed in Appendix A.

#### BOD Test Results

Samples were analyzed for total and soluble BOD, as well as total carbonaceous and soluble carbonaceous BOD. Samples were analyzed according to these different methods to determine the BOD imparted by solids as well as soluble material, and to determine the nitrogenous oxygen demand, if any, that was occurring. As expected the majority of the BOD removal occurs across the aeration basins. The GW ASU exhibited a total BOD removal efficiency of almost 95% and 93% for the winter and summer sampling events, respectively. ASU 1 and ASU 2 exhibited 76% and 63% total BOD removal during the winter sampling event, respectively, while removing almost no total BOD during the summer sampling event. This may be due to the slightly increased temperature during the summer conditions influencing mixing or short-circuiting. BOD data can be viewed in Table 1. BOD trends for the winter and summer events can be viewed in Figures 2 and 3, respectively. Through the GW ASU and ASU units, the

carbonaceous BOD is lower than the overall BOD exerted, indicating nitrogenous oxygen demand from the nitrification process.

**Table 1****BOD<sub>5</sub> Results**

Sample	Winter tBOD mg/L	Winter tcBOD mg/L	Winter sBOD mg/L	Winter scBOD mg/L	Summer tBOD mg/L	Summer tcBOD mg/L	Summer sBOD mg/L	Summer scBOD mg/L
PTU	572	455	410	320	129	98	52	46
GW Recovery	10	5	<PQL	<PQL	8	<PQL	<PQL	<PQL
GW ASU Mid	132	80	48	36	82	74	30	22
GW ASU Eff. Main Lift Station	7	5	4	<PQL	6	<PQL	<PQL	<PQL
External Line	12	1	<PQL	<PQL	16	<PQL	<PQL	<PQL
ASU Feed	204	162	22	16	203	183	37	25
ASU 1 Inlet	57	55	8	<PQL	147	106	68	52
ASU 1 Exit	400	147	54	39	144	103	60	46
ASU 2 Inlet	98	27	10	4	132	99	22	15
ASU 2 Exit	467	132	50	42	140	111	48	40
ASU Clar. 1 Eff.	175	42	13	8	119	86	9	<PQL
ASU Clar. 2 Eff.	325	102	33	24	318	223	15	7
Lagoon 1	17	5	<PQL	<PQL	10	10	<PQL	<PQL
Lagoon 2	15	14	<PQL	<PQL	8	8	<PQL	<PQL
Lagoon 5	8	8	<PQL	<PQL	5	5	<PQL	<PQL
Lagoon 6	8	8	<PQL	<PQL	5	5	<PQL	<PQL
	9	9	<PQL	<PQL	4	4	<PQL	<PQL

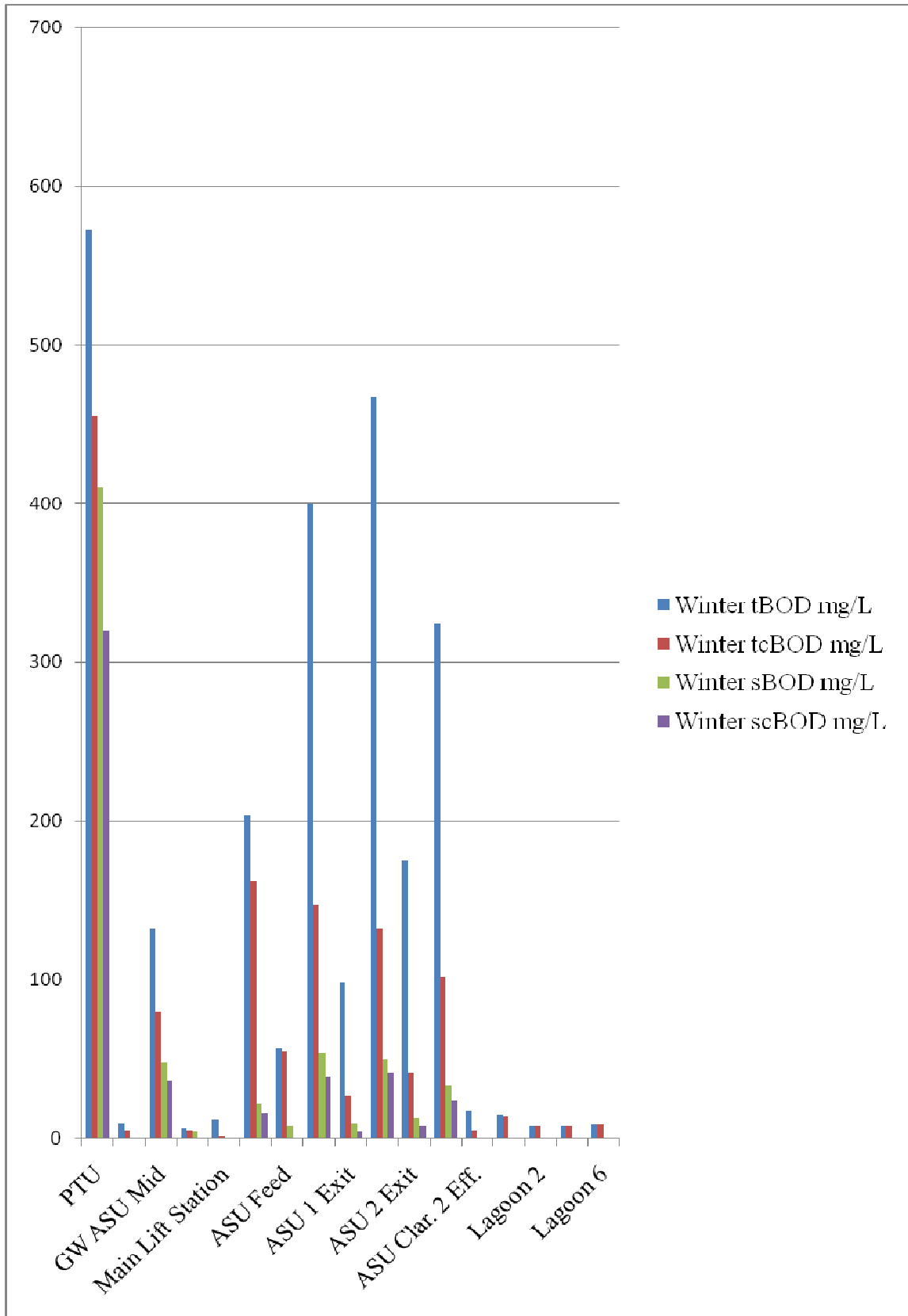


Figure 2. Winter BOD

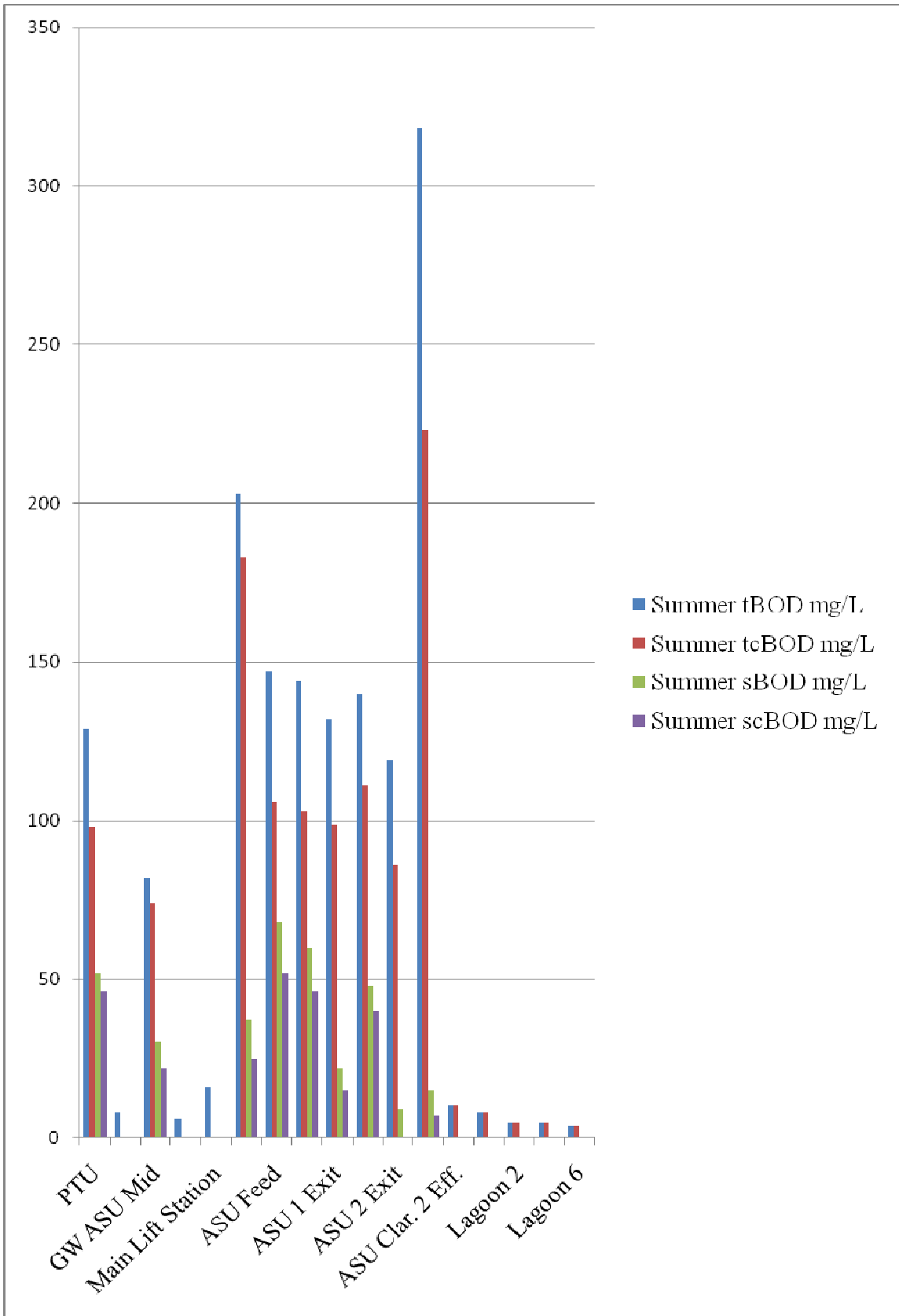


Figure 3. Summer BOD



From Table 1, nitrogenous BOD was determined and compared against the ammonia-nitrogen concentrations for winter and summer conditions. The data comparison can be viewed in Table 1 (A).

**Table 1 (A)****nBOD vs. NH<sub>3</sub>-N**

Sample	Winter nBOD (mg/L)	Winter NH <sub>3</sub> -N (mg/L)	Summer nBOD (mg/L)	Summer NH <sub>3</sub> -N (mg/L)
PTU	117	53.0	31	26.4
GW Recovery	5	0.1	8	0.1
GW ASU Middle	52	26.3	8	23.8
GW ASU Exit	2	8.8	6	19.0
GW ASU Clar. Pump	11	0.5	16	4.4
Lift Station	42	1.0	20	3.2
External Line	2	11.6	41	6.0
ASU Feed	253	4.5	41	31.0
ASU 1 Head	71	6.1	33	29.3
ASU 1 Exit	335	3.9	29	22.0
ASU 2 Head	133	7.4	33	29.0
ASU 2 Exit	223	4.5	95	22.6
Clar. 1 Effluent	12	4.8	0	2.0
Clar. 2 Effluent	1	0.1	0	1.3
Lagoon 1	0	0.2	0	4.1
Lagoon 2	0	0.1	3	4.1
Lagoon 5	0	0.7	0	1.8
Lagoon 6	0	0.8	0	<PQL

### COD Test Results

Samples were analyzed for total and soluble COD. The winter and summer COD values were, for the most part, fairly similar to each other. It is demonstrated in Table 2 that COD removal is occurring in the treatment plant, with the majority of the removal taking place in the activated sludge units and across the clarifiers, as expected. It is also noticed that the COD present in the wastewater is primarily total COD, meaning that the COD results mostly from the solids present in the wastewater. As seen from Table 2, the COD removal efficiencies are very high, such as 83% - 90% removal efficiency for total COD across the GW ASU and GW ASU clarifier and 96% - 98% removal efficiency for total COD across the ASU units and clarifiers. COD trends can be viewed in Figures 4 and 5.

**Table 2****COD Results**

Sample	Winter tCOD (mg/L)	Winter sCOD (mg/L)	Summer tCOD (mg/L)	Summer sCOD (mg/L)
PTU	1112	492	511	40
GW Recovery	40	34	43	25
GW ASU Middle	2472	69	2211	49
GW ASU Clar. Pump	109	47	85	27
Lift Station	86	77	191	66
External Line	1091	333	1203	527
ASU Feed	527	289	837	306
ASU 1 Head	3732	160	4568	93
ASU 1 Exit	1464	152	3892	89
ASU 2 Head	3206	164	5096	98
ASU 2 Exit	2030	158	3732	97
Clar. 1 Effluent	1954	157	754	84
Clar. 2 Effluent	162	132	104	70
Lagoon 1	156	100	77	74
Lagoon 2	166	106	85	81
Lagoon 5	180	97	84	66
Lagoon 6	182	95	94	73

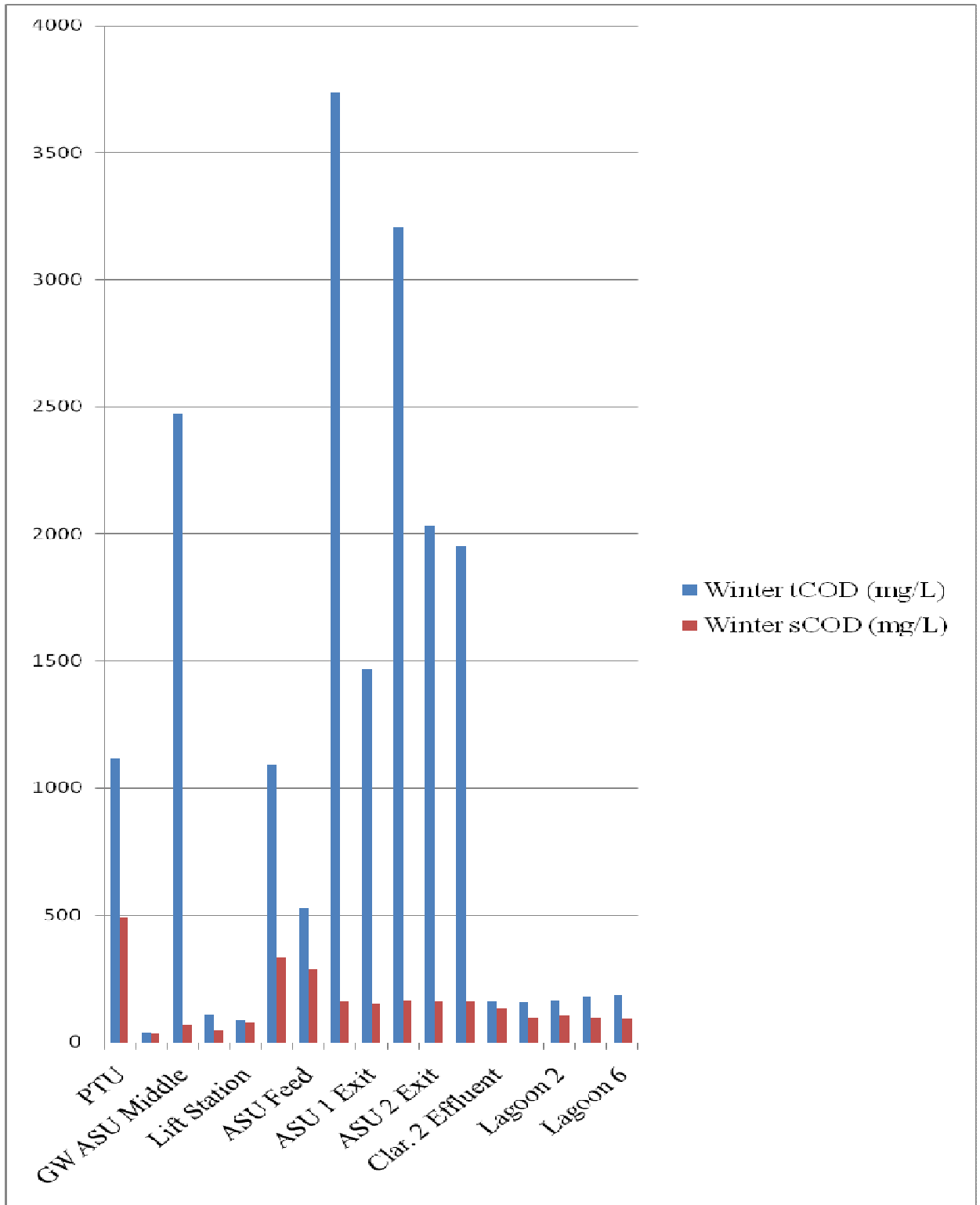


Figure 4. Winter tCOD vs. sCOD

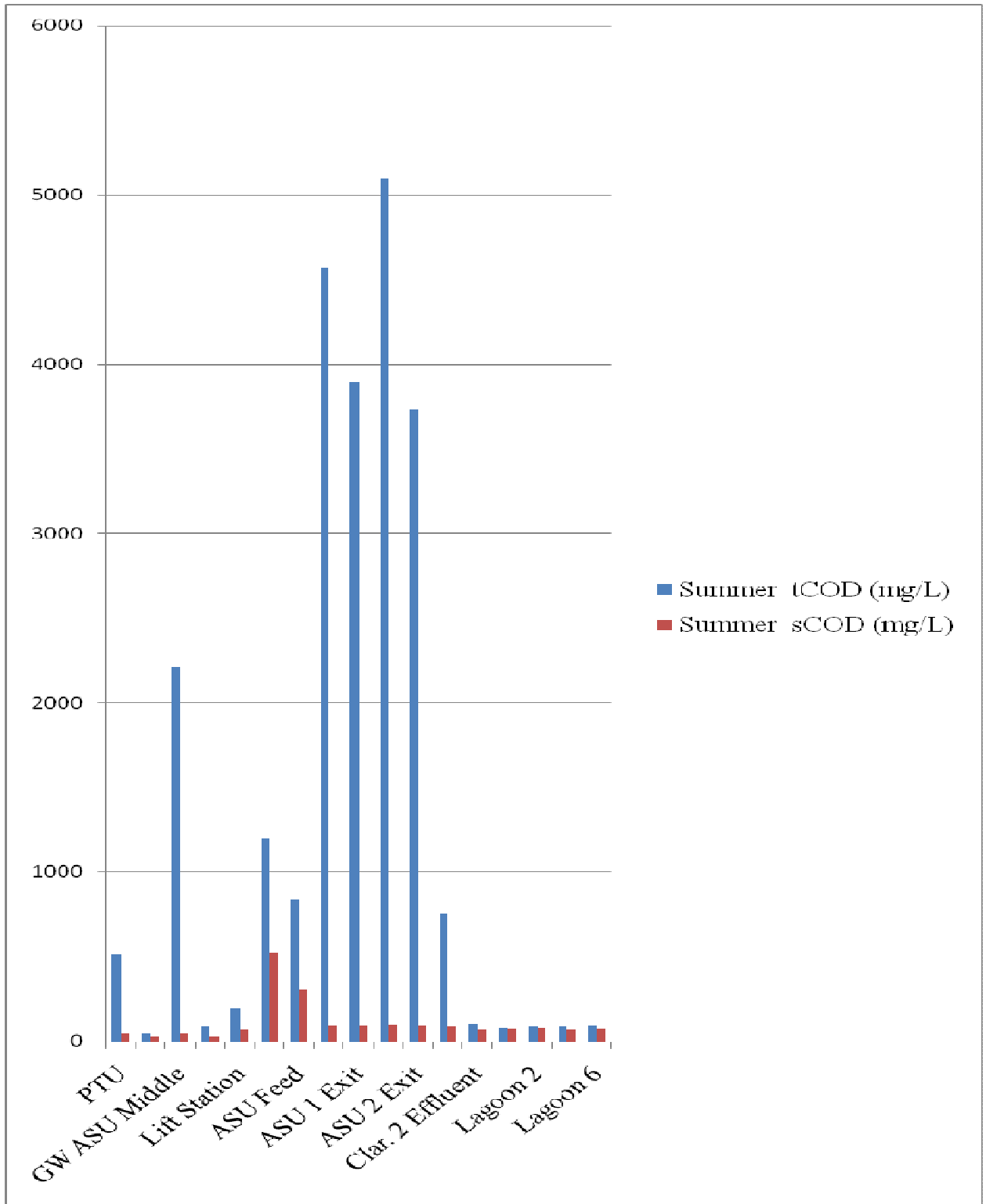


Figure 5. Summer tCOD vs. sCOD

## TSS and VSS Test Results

The samples collected were analyzed for TSS and VSS. The VSS/TSS ratios across the GW ASU are approximately 35% and 32% for the winter and summer sampling events, respectively. During both the winter and summer sample events, the GW ASU Clarifier exhibited very good performance with over 99% solids removal for both seasons.

During the winter sampling event, ASU 1 and ASU 2 both exhibited a VSS/TSS ratio of approximately 70%. ASU 1 exhibited a VSS/TSS ratio of approximately 54% while ASU 2 exhibited a VSS/TSS ratio of 70% during the summer sampling event.

It was noted during the winter event that Clarifier 1 was exhibiting poor solids removal efficiency with only about 29% solids removal. Clarifier 2 achieved almost 99% solids removal during the winter event. It should be noted that Clarifier 1 and Clarifier 2 were operated in series during the winter sampling event.

During the summer sampling event, Clarifier 1 achieved a very high solids removal of 99.6% with Clarifier 2 removing a further 43% of the solids overflowing from Clarifier 1. Again, it should be noted that Clarifier 1 and Clarifier 2 were operated in series during this sampling event. All of the above mentioned information was determined using data from Table 3. TSS and VSS trends can be viewed in Figures 6 and 7, respectively.

**Table 3****Winter and Summer TSS and VSS Concentrations**

Sample	Winter TSS (mg/L)	Winter VSS (mg/L)	Summer TSS (mg/L)	Summer VSS (mg/L)
PTU	72	4	60	10
GW Recovery	64	20	44	14
GW ASU Middle	6,100	1,700	8,300	2,800
GW ASU Exit	9,900	3,500	8,800	2,600
GW ASU Clar. Pump	70	14	38	8
Lift Station	40	2	143	33
External Line	80	40	77	43
ASU Feed	106	28	110	40
ASU 1 Head	2,100	1,500	2,800	1,500
ASU 1 Exit	700	540	2,800	1,600
ASU 2 Head	2,150	1,500	3,700	2,600
ASU 2 Exit	800	550	2,300	1,450
Clar. 1 Effluent	1,750	1,250	14	8
Clar. 2 Effluent	20	16	8	2
Lagoon 1	8	8	14	4
Lagoon 2	5.5	5.5	8	2
Lagoon 5	6	2	6	2
Lagoon 6	10	10	4	2



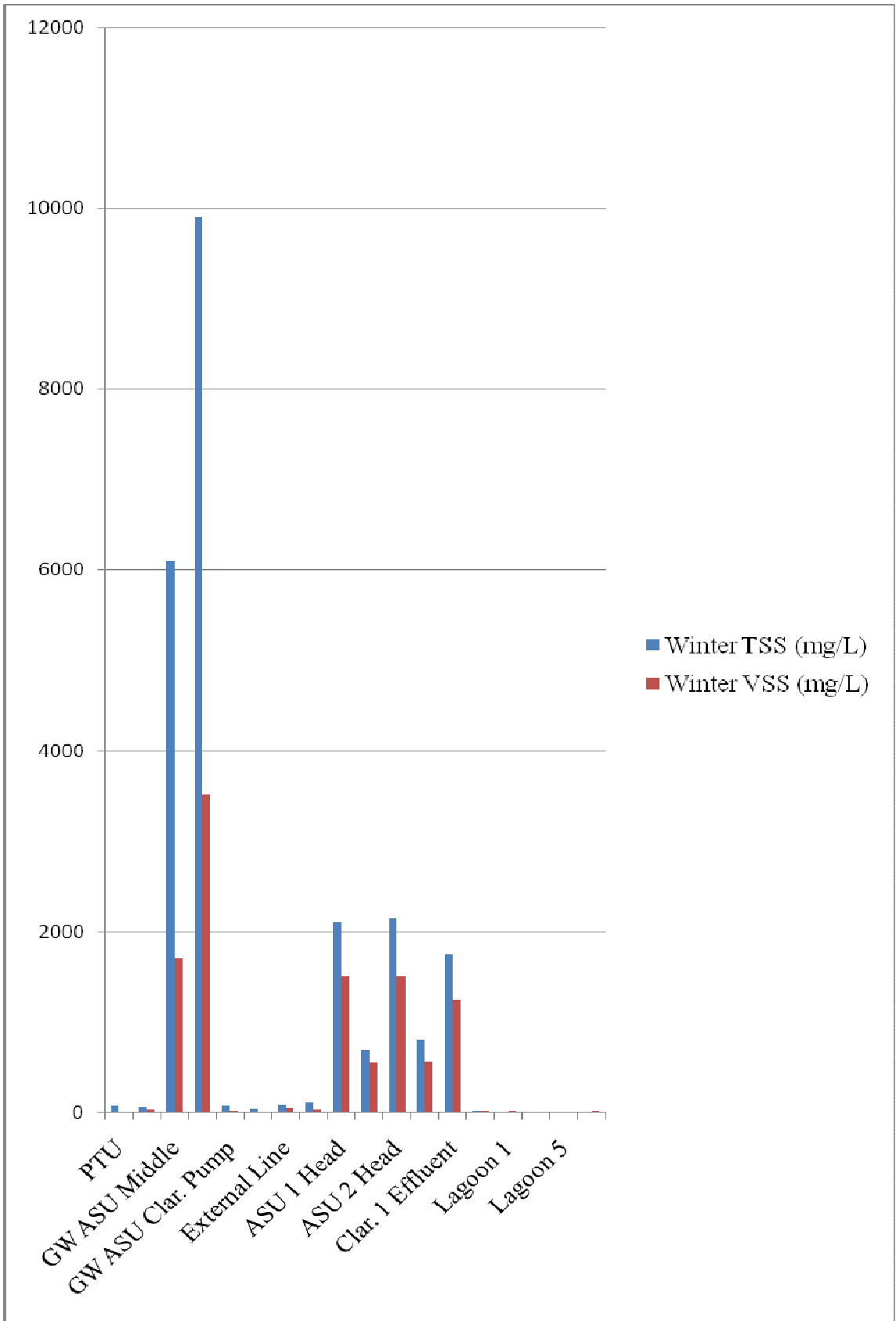


Figure 6. Winter TSS vs. VSS

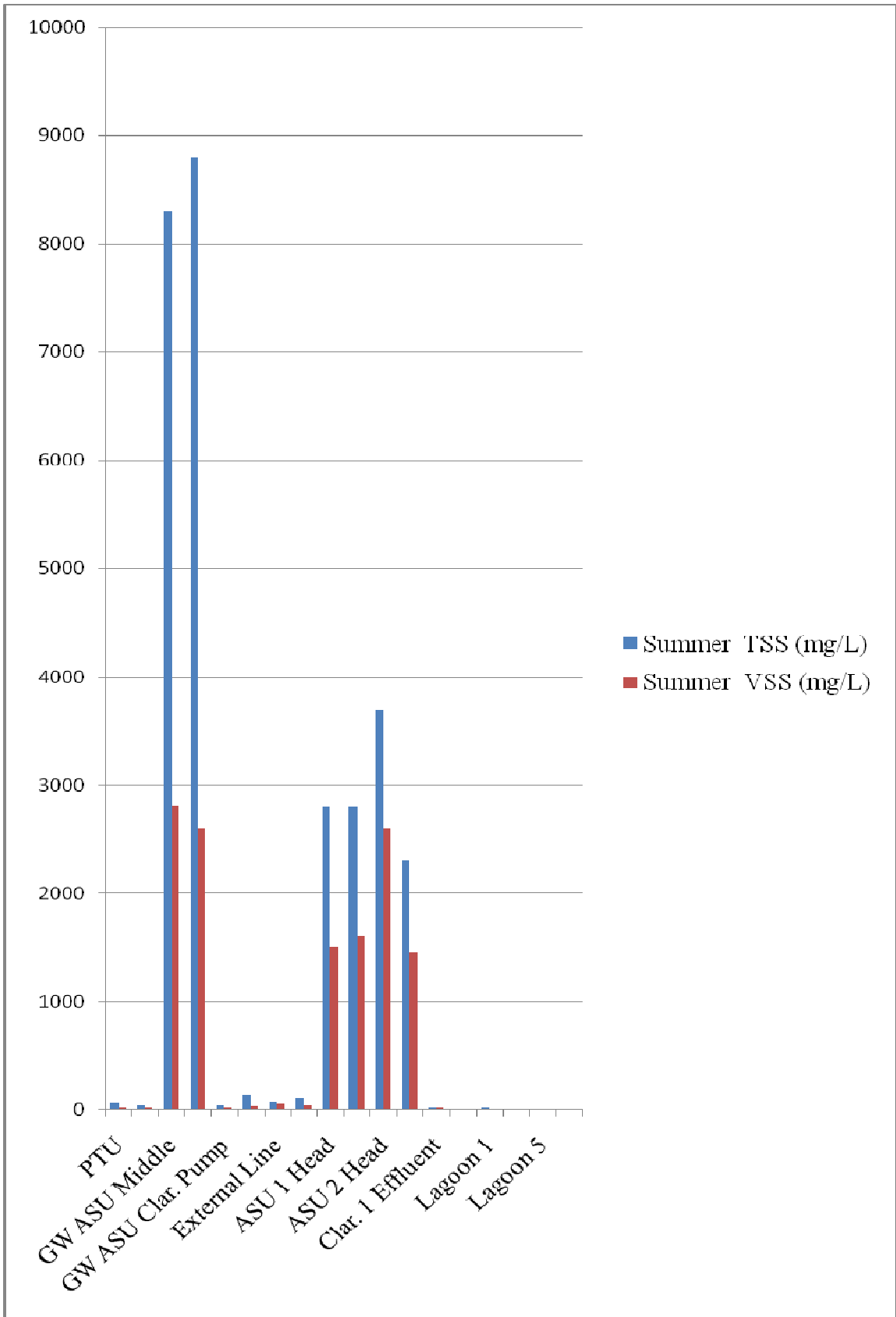


Figure 7. Summer TSS vs. VSS

### NH<sub>3</sub>-N Test Results

The results from the ammonia-nitrogen colorimetric tests indicate that the ammonia-nitrogen present in the waste streams of the industrial wastewater treatment plant is being consumed or converted into other nitrogen forms through the treatment processes taking place.

As can be seen in Table 4, the winter ammonia-nitrogen concentrations are highest at the activated sludge unit inlets. The ammonia-nitrogen concentrations are considerably lower at the activated sludge unit outlets. The ground water activated sludge unit (GW ASU) is fed by the PTU line. The ammonia-nitrogen concentration at the middle and exit of the GW ASU is 26.3 mg/L and 8.8 mg/L respectively. This corresponds to a treatment removal efficiency of approximately 67% under winter conditions. Activated sludge units 1 and 2 (ASU 1 and ASU 2) received inlet ammonia-nitrogen concentrations of 6.1 mg/L and 7.4 mg/L, respectively, with outlet concentrations of 3.9 mg/L and 4.5 mg/L, respectively. This corresponds to treatment removal efficiencies of approximately 36% and 39% for ASU 1 and ASU 2, respectively, with ammonia-nitrogen removal continuing through the ASU clarifiers. It is also noted that the ammonia-nitrogen concentrations throughout the lagoon system remain very low.

It was also observed from the data in Table 4 that during the summer months, ammonia-nitrogen removal efficiencies decreased. The GW ASU treatment removal efficiency decrease from 67% under winter conditions to approximately 20% during summer conditions, removing 4.8 mg/L ammonia-nitrogen across the unit. ASU 1 provided a treatment removal efficiency of approximately 25% by removing 7.3 mg/L ammonia-nitrogen across the unit, while ASU 2 removed 22% or 6.4 mg/L of the

**Table 4****Winter vs. Summer Ammonia-Nitrogen Concentrations**

Sample	Winter NH <sub>3</sub> -N (mg/L)	Summer NH <sub>3</sub> -N (mg/L)
PTU	53.0	26.4
GW Recovery	0.1	0.1
GW ASU Middle	26.3	23.8
GW ASU Exit	8.8	19.0
GW ASU Clar. Pump	0.5	4.4
Lift Station	1.0	3.2
External Line	11.6	6.0
ASU Feed	4.5	31.0
ASU 1 Head	6.1	29.3
ASU 1 Exit	3.9	22.0
ASU 2 Head	7.4	29.0
ASU 2 Exit	4.5	22.6
Clar. 1 Effluent	4.8	2.0
Clar. 2 Effluent	0.1	1.3
Lagoon 1	0.2	4.1
Lagoon 2	0.1	4.1
Lagoon 5	0.7	1.8
Lagoon 6	0.8	<PQL

ammonia-nitrogen present. Figures 8 and 9 below compare the winter and summer values of ammonia-nitrogen versus nitrate-nitrogen.

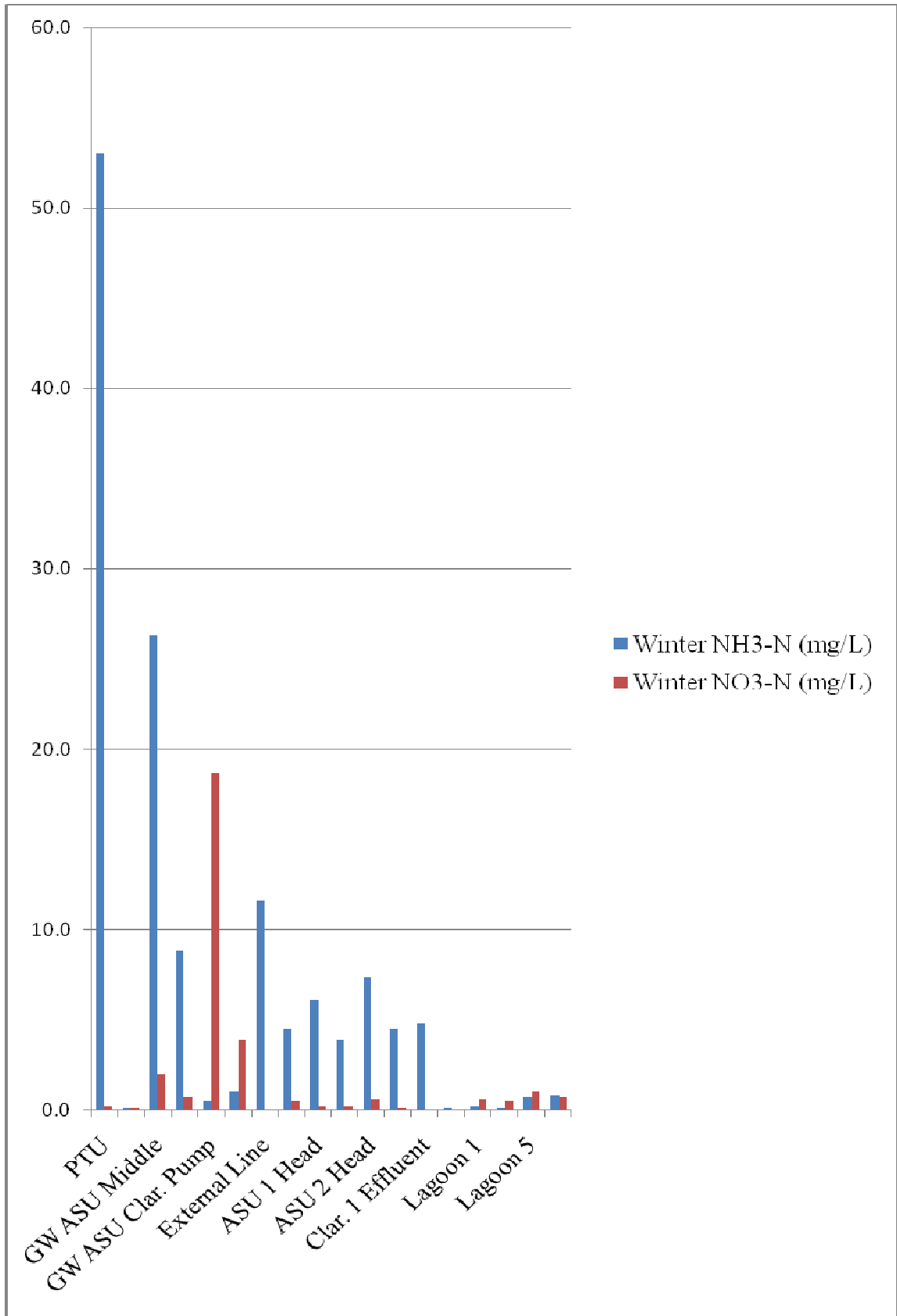


Figure 8. Winter NH<sub>3</sub>-N vs NO<sub>3</sub>-N

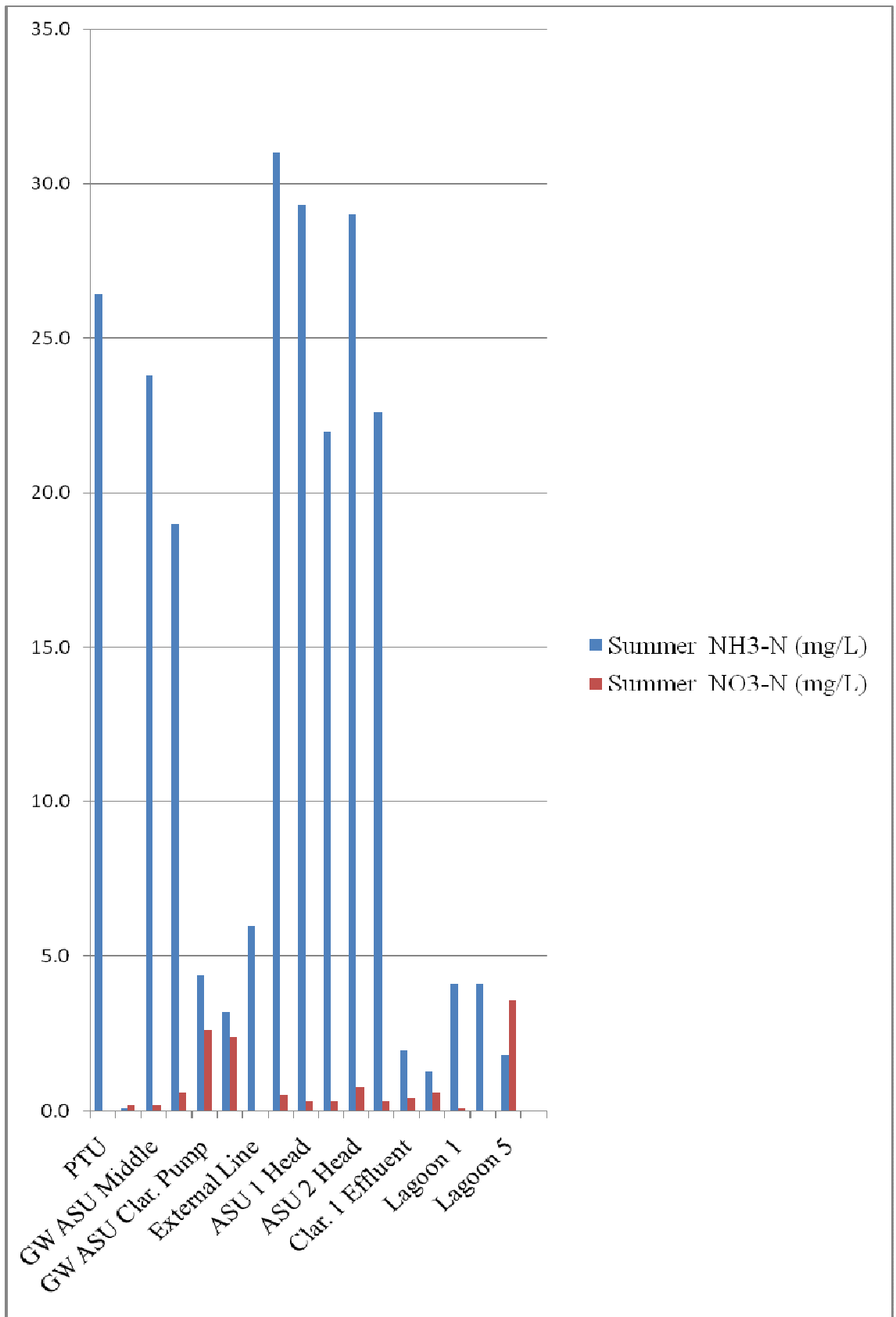


Figure 9. Summer NH<sub>3</sub>-N vs NO<sub>3</sub>-N

### NO<sub>3</sub>-N Test Results

Samples collected were analyzed for nitrate-nitrogen to determine if ammonia present in the waste stream is being oxidized biologically and converted to nitrate. As seen in Table 5 and Figure 8 during the winter sampling event, a spike in nitrate-nitrogen concentration of 18.7 mg/L is seen at the GW ASU Clarifier Pump with very low nitrate-nitrogen concentrations present before this point, indicating conversion of ammonia-nitrogen to nitrate-nitrogen. After the GW ASU, nitrate-nitrogen concentrations remain low in the waste stream (below 1.0 mg/L).

Nitrate-nitrogen tests performed on the samples collected during the summer sampling event indicate conversion of ammonia-nitrogen to nitrate-nitrogen, but at much lower levels as seen in Figure 9. On an interesting note, a small nitrate-nitrogen spike is seen in Lagoon 5, which subsides in Lagoon 6. Again, ammonia-nitrogen and nitrate-nitrogen data were placed in Figures 8 and 9 for side-by-side comparisons of the two data sets for the winter and summer sampling events, respectively.

### T-N Results

The T-N results were found to be inaccurate due to the method used and the low concentrations present. The results for the T-N tests are listed in Appendix A.



**Table 5****Winter vs. Summer Nitrate-Nitrogen**

Sample	Winter NO <sub>3</sub> -N (mg/L)	Summer NO <sub>3</sub> -N (mg/L)
PTU	0.2	<PQL
GW Recovery	0.1	0.2
GW ASU Middle	2.0	0.2
GW ASU Exit	0.7	0.6
GW ASU Clar. Pump	18.7	2.6
Lift Station	3.9	2.4
External Line	<PQL	<PQL
ASU Feed	0.5	0.5
ASU 1 Head	0.2	0.3
ASU 1 Exit	0.2	0.3
ASU 2 Head	0.6	0.8
ASU 2 Exit	0.1	0.3
Clar. 1 Effluent	<PQL	0.4
Clar. 2 Effluent	<PQL	0.6
Lagoon 1	0.6	0.1
Lagoon 2	0.5	<PQL
Lagoon 5	1.0	3.6
Lagoon 6	0.7	<PQL

## CHAPTER V

### CONCLUSION

The IWTP is not designed to operate as a biological nutrient removal plant. Instead, the IWTP is designed to operate as a BOD removal plant with aeration basins and clarifiers for solids removal with a lagoon system to provide effluent polishing. Therefore, the IWTP can facilitate nitrification if ammonia-nitrogen in excess of the biomass requirements for cell maintenance and synthesis is present; however, with no anoxic basins present, it can be more challenging to achieve denitrification.

When considering the ammonia-nitrogen and nitrate-nitrogen data above, it becomes apparent that nitrification is taking place in the GW ASU. As seen in Table 4, ammonia-nitrogen levels decrease significantly after the GW ASU and nitrate-nitrogen levels increase significantly at the GW ASU Clarifier Pump during the winter conditions as seen in Table 5. The nitrate-nitrogen levels also decrease significantly between the GW ASU Clarifier Pump and the Main Lift Station. When considering the DO levels found in Appendix A for the winter conditions, the DO is too high for denitrification to explain the decrease in nitrate-nitrogen levels. Therefore, the explanation comes from the flow chart for the IWTP, as viewed in Figure 1. It is believed that the nitrate-nitrogen levels most likely decrease due to dilution by waste streams from other sections of the production plant. During the summer sampling event, the nitrification across the GW ASU was much lower, with less than 3 mg/L nitrate-nitrogen produced.

When considering both the winter and summer data for ASU 1 and ASU 2, nitrate-nitrogen concentrations remain very low at less than 1 mg/L, while the corresponding ammonia-nitrogen levels decrease significantly across the ASU units and ASU Clarifiers. When compared to the DO data found in Appendix A, it is believed that the ammonia-nitrogen removal is due to simultaneous nitrification/denitrification occurring in both the ASU units. The DO levels across the ASU units, for the most part, remain at 0.5 mg/L or less with aeration still occurring. Operating in this fashion provides oxygen to facilitate nitrification, while keeping DO levels low enough to facilitate denitrification inside the floc where oxygen diffusion may be limited. Ammonia-nitrogen and nitrate-nitrogen levels are very low at Lagoon 6 (less than 1 mg/L each) during both the winter and summer sampling events. The IWTP exhibits very good biological nitrogen removal performance overall for the ammonia-nitrogen levels present.

As noted in the ammonia-nitrogen results above, an ammonia-nitrogen spike occurs in Lagoons 3 and 4. It was noticed during the sampling event that a green chemical substance was present around the edges of these lagoons as well as the surrounding water surface. The sampling apparatus reach was not significant to sample outside of the water surface area with the chemical substance present. It is believed that the chemical substance is responsible for the ammonia-nitrogen spike.

### Recommendations

For the IWTP to continue to perform biological nitrogen removal without the addition of anoxic basins, it is recommended that ASU1 and ASU 2 be operated at a DO level of 0.5 mg/L or slightly less. This will continue to provide oxygen at levels sufficient enough to achieve simultaneous nitrification/denitrification.

Another alternative is to add a pre-anoxic basin(s) between the ASU Feed and the ASU units. An internal recycle line would be required to return nitrate produced in the aerobic basins back to the anoxic basin(s) for denitrification. Internal recycle rates can range from 100% to 400% of the influent flow to the anoxic basins and would have to be determined according to the individual system. An external recycle line would also be required to return mixed liquor volatile suspended solids back to the anoxic basin(s). External recycle rates are typically around 100% of the influent flow to the anoxic basins. The addition of anoxic basins will allow for reduced applied aeration horsepower in the ASU units by facilitating BOD removal in the anoxic basins. The addition of anoxic basins could also be performed to accommodate future plant expansions by adding plant volume and allowing for increased operations flexibility.

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## APPENDIX A

**Sampling Date: February 15, 2008**

Ambient Temp = 29F

**SUMMARY**

No.	Location	Temp	DO	pH	Total N
		oC	mg/L		mg/L
1	Sour water stripper effluent (PTU)	44.4	0.1	8.5	0.0
2	GW recovery wells	16.3	2.7	7.0	30.6
3	GW ASU head	25.4	3.1	7.7	0.0
4	GW ASU middle	24.9	2.3	7.6	0.0
5	GW ASU clarifier	23.8	2.7	7.7	0.0
6	External line effluent	35.0	0.4	6.8	0.0
7	Main Lift station effluent	34.2	3.2	9.2	0.0

8	ASU1 and ASU2 feed line	32.3	0.2	9.4	43.3
9	ASU1 head	31.7	0.8	7.6	0.0
10	ASU2 head	29.9	1.3	7.4	0.0
13	ASU 1 near exit	33.6	0.3	8.6	0.0
14	ASU2 near exit	30.2	0.3	7.7	0.0
15	Clarifier 1 effluent	30.4	0.5	7.4	0.0
16	Clarifier 2 effluent	28.8	0.5	7.4	0.0

17	Lagoon 1 effluent	19.5	3.8	7.9	0.0
18	Lagoon 2 effluent	15.2	4.0	8.0	200.0
19	Lagoon 3 effluent	3.8	10.3	9.2	0.0
20	Lagoon 4 effluent	3.5	10.8	9.2	0.0
21	Lagoon 5 effluent	9.1	5.6	8.4	0.0
22	Lagoon 6 effluent	9.8	6.4	8.3	0.0

\* The Leeco method was used for Total Nitrogen analysis. Its sensitivity at these relatively low levels is unreliable.

An alternate method has been found and is being tested on preserved samples.



**Sampling Date: September 14, 2007**

Ambient Temp = 83F

**SUMMARY**

No.	Location	Temp oC	DO mg/L	pH	Total N* mg/L
1	Sour water stripper effluent (PTU)	47.4	1.2	8.6	53.0
2	GW recovery wells	26.0	2.0	7.2	0.0
3	GW ASU head	51.0	3.0	9.9	17.0
4	GW ASU middle	27.5	5.5	9.2	97.0
5	GW ASU clarifier	26.6	3.6	9.2	0.0
6	External line effluent	40.5	0.0	7.4	193.0
7	Main Lift station effluent	30.9	3.2	9.2	151.0

8	ASU1 and ASU2 feed line	33.0	0.0	10.3	26.0
9	ASU1 head	29.0	4.2	7.8	168.0
10	ASU2 head	34.5	4.0	6.8	15.0
13	ASU 1 near exit	33.0	4.3	7.8	0.0
14	ASU2 near exit	34.5	2.5	9.0	0.0
15	Clarifier 1 effluent	34.2	0.2	7.7	102.0
16	Clarifier 2 effluent	34.9	1.3	7.7	0.0

17	Lagoon 1 effluent	28.5	5.2	8.2	0.0
18	Lagoon 2 effluent	27.8	6.1	8.3	0.0
19	Lagoon 3 effluent	22.2	9.5	8.7	0.0
20	Lagoon 4 effluent	22.3	9.5	8.7	0.0
21	Lagoon 5 effluent	24.6	8.0	8.3	0.0
22	Lagoon 6 effluent	25.1	6.2	8.4	0.0

\* The Leeco method was used for Total Nitrogen analysis. Its sensitivity at these relatively low levels is unreliable.

An alternate method has been found and is being tested on preserved samples.

VITA

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Candidate for the Degree of Master of Science

Major Field: Environmental Engineering

Scope and Method of Study: The purpose of this study was to determine if biological nitrogen removal is occurring at the industrial wastewater treatment plant of interest. Two seasonal sampling events were performed, one winter and one summer event. The samples were then analyzed in the Oklahoma State University Environmental Engineering laboratories for a variety of parameters.

Findings and Conclusions: It was determined from the samples collected that biological nitrogen removal is occurring at the industrial wastewater treatment plant of interest. Ammonia-nitrogen is oxidized biologically by autotrophic bacteria and converted to nitrate-nitrogen. The nitrate-nitrogen is then serves as the final electron acceptor for heterotrophic bacteria under no or low DO concentrations and is reduced to nitrogen gas. The nitrogen gas is insoluble, therefore, coming out of solution and dissipating into the atmosphere. Since the industrial wastewater treatment plant is not designed or operated as a biological nitrogen removal plant, it was determined that nitrogen removal is occurring through simultaneous nitrification/denitrification.

ADVISER'S APPROVAL: Dr. John N. Veenstra

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