5-1-2015

The Use of COD, TOC, Fluorescence, and Absorbance Spectroscopy to Estimate Biochemical Oxygen Demand in Wastewater

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THE USE OF COD, TOC, FLUORESCENCE, AND ABSORBANCE SPECTROSCOPY TO ESTIMATE BIOCHEMICAL OXYGEN DEMAND IN WASTEWATER

By

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Bachelor of Science in Civil Engineering
University of Nevada, Las Vegas
2012

A thesis submitted in partial fulfillment
of the requirements for the

Masters of Science in Engineering – Civil and Environmental Engineering

Department of Civil and Environmental Engineering and Construction
Howard R Hughes College of Engineering
The Graduate College

University of Nevada, Las Vegas
May 2015

UNLV | GRADUATE COLLEGE

We recommend the thesis prepared under our supervision by

Evelyn Aramaine Christian

entitled

The Use of COD, TOC, Flourescence, and Absorbance Spectroscopy to Estimate Biochemical Oxygen Demand in Wastewater

is approved in partial fulfillment of the requirements for the degree of

Master of Science in Engineering -- Civil and Environmental Engineering

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May 2015

ABSTRACT

The Use of COD, TOC, Fluorescence, and Absorbance Spectroscopy to Estimate Biochemical Oxygen Demand in Wastewater

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All wastewater treatment facilities must obtain a National Pollution Discharge Elimination System (NPDES) permit, which regulates the quality of water that is discharged. Common to all NPDES permits is a limit on organic matter, as determined by the five-day biochemical oxygen demand (BOD_5) test. More rapid methods, such as chemical oxygen demand (COD), total organic carbon (TOC), fluorescence, and absorbance spectroscopy are also capable of quantifying organic matter. Previous studies indicate it is possible to develop correlations between these parameters. This study explored the correlations using influent, primary clarifier effluent, and finished effluent samples from an operational wastewater treatment plant located in Nevada. It was concluded that COD could be used to estimate BOD_5 for influent, primary clarifier effluent, and finished effluent. TOC could be used as a surrogate for finished effluent, but it was not suitable for influent or primary clarifier effluent. The relationship developed for fluorescence and BOD_5 was nonlinear, presumably due to inner filter effect (IFE) interference. Power functions were developed for region I of the excitation emission matrix (EEM) and peak T (excitation=275 nm, emission=340 nm) that could be used to estimate BOD_5 for finished effluent and primary clarifier effluent, but they were poor estimators for plant influent. Comparison of removal efficiencies indicated that TOC and fulvic-like fluorescence peaks increased in the primary clarifier, presumably due to the return of centrate that is sent back to the primary clarifier. The fluorescence removals indicated that over 80% of protein-like (typically associated with BOD_5) fluorescence was removed during treatment and approximately half of humic- and fulvic-like fluorescence was removed during treatment. Quality control experiments indicated that holding the samples overnight biased the test results low, which was more pronounced when samples were held with headspace. Degradation likely occurs due to the biological consumption of organic matter that is occurring within the sample during the holding time.

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CHAPTER 1

PROBLEM STATEMENT AND OBJECTIVES

In accordance with the United States (US) Clean Water Act (CWA), wastewater treatment plants must obtain a National Pollution Discharge Elimination System (NPDES) permit to discharge treated domestic wastewater into a receiving water body (EPA, 2014). In Nevada, NPDES permits are issued and enforced by the Nevada Division of Environmental Protection (NDEP) (NDEP, 2014). Common to all NPDES permits for treated domestic wastewater is a limit on the biochemical oxygen demand (BOD) of the treated wastewater, as determined by the five-day BOD test (BOD_5).

BOD is a surrogate parameter used to determine the content of biodegradable organic matter present in water. In the BOD test, the depletion of dissolved oxygen utilized by bacteria as they consume the organic matter is measured. Therefore, the BOD_5 test indirectly quantifies the biodegradable organic matter based on the level of oxygen depletion over the 5-day test period (Standard Methods, 2005). The presence of BOD in wastewater discharge can impact receiving waters because the water body cannot absorb oxygen from the atmosphere as quickly as the bacteria are utilizing it, which leads to an oxygen deficit in the water that can potentially cause the deaths of aquatic organisms.

As implied by the subscript in its name, the BOD_5 test takes five days to complete, which is a major drawback. A treatment facility that experiences operational problems may unintentionally discharge polluted water for up to five days before plant staff become aware of the issue. The long time needed to complete the BOD test also delays adjustments by plant staff during operational upsets.

Although the BOD_5 test is a Standard Method approved by the EPA to quantify organic discharge from wastewater treatment, it has become desirable to seek alternative test procedures to quantify organic matter more quickly than five days (40CFR136, 2012). Alternative test methods, such as chemical oxygen demand (COD), total organic carbon (TOC), fluorescence, and absorbance can potentially be used as a more direct measure of the organic content of waters. Each method offers distinct advantages and limitations based on the manner in which the organic matter is quantified. The results from all four of the aforementioned tests can be obtained within minutes or hours or even with on-line TOC, fluorescence, or absorbance instruments. Currently, the only approved analytical method to measure the organic content of treated wastewater is the BOD test. However, some treatment facilities in Nevada have started using TOC measurements to estimate the BOD_5 of both raw influent wastewater and the effluent discharged to receiving waters (Drury, 2014). In these instances, upon establishing a correlation between BOD_5 and TOC, the plants run TOC daily, convert the TOC to BOD_5, and then report the BOD_5 value to the to comply with permit requirements.

1.1 Objectives and Hypothesis

This study explores the relationships between various organic matter quantification and characterization methods using samples collected from an operating wastewater treatment facility located in Nevada.

1. The primary objective of this research was to develop relationships between BOD_5 and alternate test methods that quantify organic matter. Literature review indicates that COD, TOC, fluorescence, and absorbance can be used to predict BOD_5 in wastewater samples. It was hypothesized that independent correlations can be made for BOD_5 to COD, BOD_5 to TOC, BOD_5 to fluorescence, and BOD_5 to absorbance. The basis for the hypothesis is that all of the methods quantify the organic matter in water, albeit by targeting different organic matter components. The BOD_5 method indirectly quantifies the biodegradable organic matter based on the amount of oxygen depleted during an incubation period, the COD method quantifies the organic matter that can be chemically oxidized, and the TOC method quantifies the organic carbon that can be mineralized to carbon dioxide (Standard Methods, 2005). Fluorescence quantifies organic matter that contains fluorophores, which have unique fluorescence properties that make it possible to identify them in water. Fluorophores are not characteristic of all organic molecules so fluorescence is only linked to a portion of the overall organic matter in an environmental water sample. The amount of fluorescence is assumed to be proportionate to the organic matter contained in the sample (Locowicz, 1999). Absorbance is a measure of how much incident light is absorbed by a sample. Specifically, chromophore molecules in the water sample will absorb light at unique wavelengths, and this absorbance is

proportionate to the chromophore concentration according to Beer's Law. The amount of light absorbed is assumed to be proportionate to the concentration of organic matter (Locowicz, 1999).

2. The second objective of this research was to determine how sample collection, holding times, and storage techniques impact the results of the methods currently used to measure organic content in wastewater. It is well known that the BOD_5 test should be performed as quickly as possible, because biological degradation can occur in the sample with time, which will bias the test results low. It was hypothesized that the TOC, BOD_5, COD, fluorescence, and absorbance would be significantly affected by sampling methodology. The basis for the hypothesis is that the organic matter will be consumed by microorganisms during the holding time.

CHAPTER 2

LITERATURE REVIEW

Part A: Permits, Organics, and BOD

2.1 Permits

2.1.1 NPDES Permits

The United States (US) Environmental Protection Agency (EPA) was given the ability to control pollution in 1972 through the Clean Water Act (CWA) (*33 U.S.C. §1251 et seq.,* 2002). Specifically, section 402 of the CWA allowed the EPA to develop and implement the National Pollutant Discharge Elimination System (NPDES) program. In some cases, the EPA issues permits directly, but the EPA has also granted certain states the ability to issue permits (EPA, 2014).

Entities or persons who wish to discharge water or wastewater into a receiving body of water must first obtain a NPDES permit that restricts the quality of the effluent by establishing minimum water quality standards. Restrictions are placed on a number of water quality characteristics including, but not limited to, total suspended solids (TSS), biochemical oxygen demand (BOD), fecal coliforms, ammonia, and nitrogen. Permits can vary

depending on the municipality or entity that has obtained a permit; one can contact the EPA or appropriate issuing state to request copies of NPDES permits.

The EPA has given the Nevada Division of Environmental Protection (NDEP) the authority to issue NPDES permits in Nevada, with the exception of tribal lands (EPA, 2014). NDEP is a broad organization that regulates many environmental laws including air quality, greenhouse gas emissions, and water quality. The subdivision of the NDEP that is responsible for regulating water quality is the Bureau of Water Pollution Control (BWPC). The purpose of the BWPC is to protect all water sources by permitting discharges to surface water, groundwater, and underground injection wells (NDEP, 2014).

Samples collected in this research were collected from an operational WWTP in Nevada. At the time of this writing, the NPDES limited the 30-day average uninhibited BOD_5 to 30 mg/L and the 7-day average limit was limited to 45 mg/L. The influent uninhibited BOD_5 must be monitored and reported, but influent water quality is not directly regulated. For both the influent and effluent samples, daily composite samples are required for permit compliance. The uninhibited BOD_5 and total TSS must be calculated monthly and demonstrate that the 30-day average percentage removal is at least 85%. The uninhibited BOD_5 test means the test accounts for oxygen demand due to the presence of both organics and ammonium in the wastewater.

NDEP has allowed the Nevada plant to use an alternate test procedure, specifically TOC, to estimate their BOD_5 for plant influent and for treated effluent. TOC at plant influent and treated effluent are converted to a BOD_5 equivalent, and this number is reported to NDEP.

2.1.2 Alternate Test Procedures for NPDES Permit Compliance

The Clean Water Alternate Test Procedure (ATP) is a program offered by the EPA that allows persons or entities with NPDES permits to apply for permission to use alternate test methods in lieu of the method required by their permit. This allows WWTPs greater flexibility to modify their current methods to overcome matrix interference and also to explore alternate test procedures in lieu of their current methods.

Alternate test procedures are addressed in section 40 CFR Part 136 of the Code of Federal Regulations. A person or agency with an NPDES permit can apply for an ATP by submitting an application or letter to the Regional Administrator through the Director of the State agency that issued the NPDES permit. The application or letter should provide the identity of the person applying for the permit and the permit number, identify the pollutant, provide justification as to why test procedures identified in CFR Table I were not used, and provide information on the proposed alternate test procedure. The code specifies that the procedure should include a detailed description of the alternate test and also include references to published studies that support that applicability of the procedure.

The applicant sends the application or letter to the Regional Administrator, which is then forwarded to the Director of the State who has the final approval of any alternate test procedure application. The Director of the State will review the information and then forward their recommendation to the Regional Administrator within 30 days of receipt.

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the Director of the State recommends denial of the application based on technical and scientific reasons, then the Regional Administrator must deny the application. The Regional Administrator has 90 days from the time the application is received to contact and inform the applicant as to whether the application has been approved or denied. The Regional Administrator may request more information or provide technical and scientific recommendations. The Regional Administrator must forward all approved and denied applications to the Director of the Analytical Methods Staff in Washington, DC.

The CFR does not directly address the minimum testing requirements when submitting an application. It would appear that the applicant, Director of the State, and Regional Administrator make technical judgments based on the goals of the alternate test procedures. This allows the applicant some leeway based on available resources and the water matrix in question.

2.2 Organic Compounds

2.2.1 Organic Compounds and Their Presence in Wastewater

Organic compounds are composed of carbon (C), hydrogen (H), oxygen (O), and nitrogen (N) (Tchobanoglous et al., 2003; Crittenden et al., 2012). Certain compounds such as carbon monoxide, carbon dioxide, carbonate, bicarbonate, and cyanide are also composed of C, H, O, and N, but these behave as inorganic compounds and they are classified as such (Crittenden et al., 2012). Organic compounds have strong carbon-carbon bonds, whereas inorganic compounds do not (Crittenden et al., 2012). Organic matter in wastewater typically consists of proteins (40 to 60%), carbohydrates (25 to 50%), and oils and fats (8 to 12%), but it can also contain urea (from urine) and synthetic organic compounds (Tchobanoglous et al., 2003).

Treated wastewater effluent will typically contain organic compounds that are classified as naturally occurring, anthropogenic, or as soluble microbial products. These three classes are collectively referred to as effluent organic matter (EfOM) when referring to treated wastewater effluent (Shon et al., 2007). Naturally occurring organics come from nature (e.g., humic and fulvic acids), whereas anthropogenic organic matter comes from human activities; both of these will be present in WWTP influent. Soluble microbial products are created during biological treatment process at the WWTP; these are typically not present in the influent, but they are present in the effluent (Barker and Stuckey, 1999).

Natural organic matter (NOM) is found in natural water bodies and is composed of the secretions and excretions of living organisms such as algae and fish. Terrestrial sources can also contribute to NOM in the water when vegetation and other debris are washed into the water source and decompose. When a natural water body is used as a drinking water source, NOM is particularly important because the chlorine used in the disinfection process will convert the humic substances in NOM to certain disinfection byproducts, such as trihalomethanes, which are harmful to human health (Crittenden et al., 2012).

Anthropogenic organic matter originates from human activities and includes a myriad of compounds, including synthetic organic compounds used for industrial purposes, pesticides, herbicides, and other contaminants of emerging concern. Contaminants of emerging concern are contaminants that have recently been identified and are being investigated to understand how they impact human health and the environment. Many of these compounds are not yet regulated by the EPA and may never be regulated by the EPA if they do not pose a significant risk to human or environmental health. These contaminants are only partially removed by drinking water and wastewater treatment processes, and it is often

cost prohibitive to test and/or treat specifically for these contaminants. This category includes medications (e.g., antibiotics, antidepressants, antacids), industrial and household wastes (fragrances, antioxidants, pesticides, insecticides, detergents, fire retardants), and steroid hormones (cholesterol, mestranol, estrone, estradiol, ethinylestradiol, progesterone, testosterone) (Tchobanoglous et al., 2003). Contaminants of emerging concern are a current topic of interest with extensive literature exploring their impacts (Baker et al., 2014; Houtman et al., 2014; Jarvis et al., 2014; Taylor and Senac, 2014; Wu et al., 2014).

Soluble microbial products (SMPs) are primarily created during the biological treatment process within a wastewater treatment plant. In a review by Barker and Stuckey (1999), they found that the definition of SMPs was ambiguous among the literature, but the generally accepted definition is " the pool of organic compounds that result from substrate metabolism (usually with biomass growth) and biomass decay." SMPs associated with substrate metabolism and biomass growth are categorized as utilization associated products (UAP), whereas SMPs associated with biomass decay are categorized as biomass associated product (BAP). UAP is proportional to the rate of substrate utilization and BAP is proportional to the concentration of biomass (Barker and Stuckey, 1999).

Organic compounds can be classified as either particulate organic carbon (POC) or dissolved organic carbon (DOC). POC, such as bacteria, algae, and protozoa, are compounds greater than 0.45 μm. DOC is smaller than 0.45 μm and can be classified as either hydrophobic (humic, fulvic, alkyl monocarboxylic, dicarboxylic), transphilic (hydroxyl, sugar acids, sulfonic acid), or hydrophilic (polysaccharide, alkyl alcohol, amide, alkyl amine, amino acids) (Shon et al., 2007). DOC can also be classified as biodegradable or recalcitrant. Biodegradable refers to the labile organic matter that is easily broken down by

microorganisms. Recalcitrant matter is very small compared with other DOM (<0.1 nm) and is resistant to biodegradation (Shon et al., 2007; Lu and Speitel, 1991)

2.2.2 Organic Compounds Removal in Activated Sludge Systems

Biodegradable organic matter can be removed successfully using activated sludge systems. Conventional wastewater treatment trains typically consist of primary clarifiers followed by an activated sludge system with aeration basins and secondary clarifiers (Figure 2.1). The primary clarifier is used for initial sedimentation, the aeration basin is used for biological treatment, and the secondary clarifier is used for secondary sedimentation and biomass collection. The solids settled from the clarifiers are called sludge. The sludge from the secondary clarifier has high concentrations of microbes; therefore, a portion of the sludge is sent back to the aeration basin to 'seed' the biological treatment process.

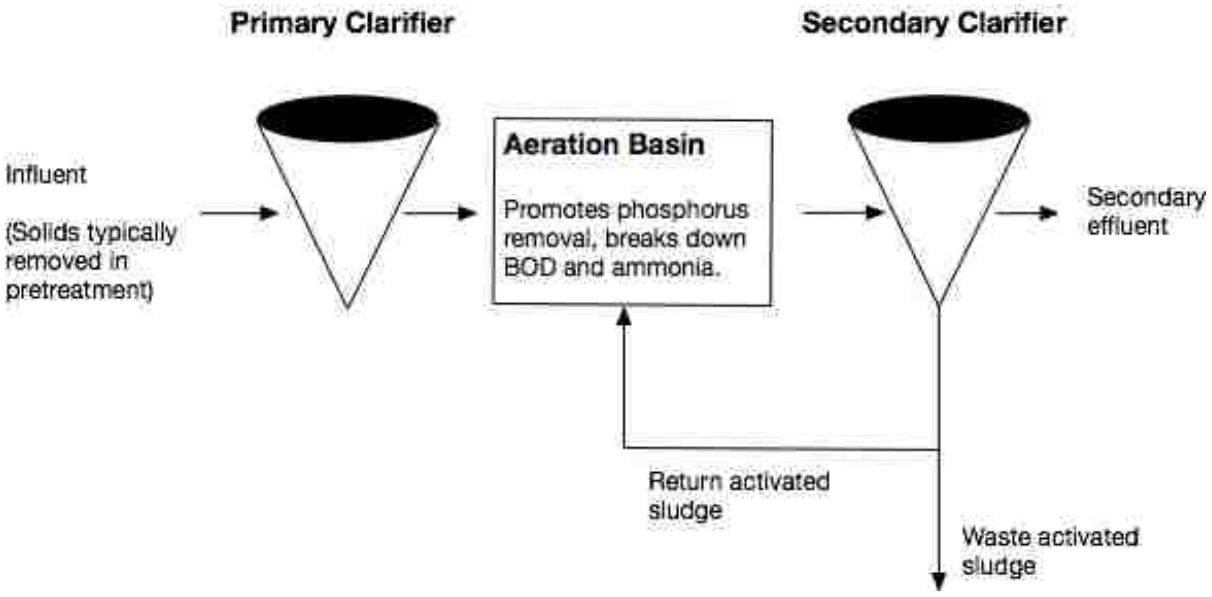

Figure 2.1: Schematic of an activated sludge system.

It is estimated that 25 to 40% of the biodegradable organic matter and 50 to 70% of the TSS can be removed in the primary clarifier without chemical addition (i.e., coagulants such as alum or ferric chloride), but this can be increased to 50 to 80% removal of organics and 80 to 90% removal of TSS with chemical addition (Tchobanoglous et al., 2003).

The purpose of the aeration basin is primarily to remove biodegradable organic matter, but ammonia, nitrates, and phosphorous can also be removed depending on the configuration. The design of the aeration basin can vary based on the treatment facility, but the aeration basin is typically divided into zones that either have dissolved oxygen (aerobic) or do not have dissolved oxygen (anoxic). In anoxic zones, dissolved oxygen is generally unavailable, which causes microbes to use nitrate as an electron acceptor (i.e., conversion of nitrate to nitrogen gas). In the aerobic zones, pure oxygen or compressed air is injected into the water to increase the efficiency of biodegradation, and oxygen is used as the electron acceptor (i.e., conversion of organic molecules to carbon dioxide). The specific number of zones will depend on the design, but each zone is designed to promote specific microorganism growth (Tchobanoglous et al., 2003). It should be noted that the competition of the microorganisms varies depending on the environmental conditions (e.g., seasonal differences); therefore, their activity can change throughout the year (Esener et al., 1981).

Biodegradable organic matter is primarily removed in the aerobic zones. In the first aerobic zone, return activated sludge (RAS) with high concentrations of microorganisms, particularly bacteria, is pumped in. These microorganisms utilize organic matter, air, ammonia, and phosphate to produce simple byproducts. One of the principal byproducts is biomass, but other byproducts such as carbon dioxide, water, and SMPs are also generated.

In the aeration basins, it is assumed that organic nitrogen is converted to ammonia (Tchobanoglous et al., 2003). Assuming the appropriate bacteria are present, the ammonia is then converted to nitrite and then nitrate via the nitrification process. When the nitrate-containing RAS is recycled to the head of the activated sludge process (i.e., the anoxic zones), denitrification, or the conversion of nitrate to nitrogen gas, will occur. The presence of specific bacteria facilitates the transformations during nitrification and denitrification. Additional bacteria are capable of converting nitrogen directly to gaseous nitrogen in the aeration basins as well (Tchobanoglous et al., 2003).

Phosphorous removal can be achieved in advanced biological treatment plants using various combinations of activated sludge, chemical addition, sedimentation, and filtration. Certain bacteria in the aeration basin are capable of the uptake and storage of inorganic phosphorous. In the aeration basin, these bacteria grow and increase their storage capacity. This biomass is then settled in the secondary clarifier. Phosphorous can be further removed by adding chemical coagulants, which will chemically transform the soluble orthophosphate into a precipitate, which can be removed from the water by filtration (Tchobanoglous et al., 2003).

Following the aeration basin, water is sent through a secondary clarifier, which is similar to the primary clarifier, but the water is allowed to settle for a longer period of time. The SMPs that were created in the aeration basin in addition to recalcitrant organic matter that could not be removed in the aeration basin will be present in the secondary clarifier effluent.

2.2.3 Characterization of Organics in the WWTP

Based on the prior discussion, it is clear that influent water consists of NOM and anthropogenic organic matter, and only a portion of the overall organic matter is biodegradable. During the treatment process, much of the biodegradable organic matter is removed, but residuals and recalcitrant organic matter remain. SMPs are also created in the biological treatment process; therefore, SMPs will be present in the effluent water, but generally not in the influent water. Effluent water can thus be characterized as the sum of the residual biodegradable organic matter, recalcitrant NOM, recalcitrant anthropogenic matter, and SMPs.

2.3 Biochemical Oxygen Demand

2.3.1 BOD_5 and Biodegradable Organics

Biodegradable organic matter is quantified using the BOD test. There are different approved methods for the BOD test, but the most common is the 5-day BOD test, also referred to as BOD_5. In this specific method, a sample is prepared and incubated for five days. The change in oxygen is quantified and expressed in mg/L. The BOD_5 test directly measures the oxygen demand of the sample, which is used as a surrogate to quantify the biodegradable organic matter in the sample. BOD_5 and biodegradable organics are assumed to be proportional; therefore, increases in BOD_5 are associated with higher biodegradable organic content. Ideally, the BOD_5 method would only measure the carbonaceous demand (i.e., the demand from biodegradable organic matter). In practice, interference can occur due to reduced forms of ammonia (nitrogenous demand) and inorganic matter (iron and sulfur compounds).

2.3.2 Why is BOD_5 a 5-day test?

As mentioned, BOD_5 is the most common of the BOD methods, but a common question is why five days was selected as the test period. The common belief is that the five day test period was based on a report by the Royal Commission on Sewage Disposal in the United Kingdom (UK), which concluded that the maximum time it took water to travel through British rivers to the open sea was five days, and the maximum temperature of the water in the river was 18.3°C. The incubation period has remained at 5 days, but the incubation temperature has been rounded up to 20°C (Royal Commission on Sewage Disposal, 1915; Delzer and McKenzie, 2003)

Indeed, it is true that the UK published a report in 1915 that established the five-day incubation period, but it is not a reasonable explanation as to why this is still a standard in the 20^{th} century. The incubation period is still true today for two important reasons. First, approximately 60-70% of the organics are degraded within the first five days. Approximately 95% of the organics will be consumed around day 20 of incubation, and it will slowly continue to degrade thereafter. The second reason is that nitrifying bacteria are slow to reproduce, and they will not become active until five to eight days after the start of the incubation test. It therefore seems unnecessary to continue the test after five days when it is approximately known what percentage of organics will degrade and also because nitrogenous demand will become an issue after five days (Tchobanoglous et al., 2003; Siwiec et al., 2011; Riffat, 2012).

2.3.3 Importance of BOD_5

BOD_5 is one of the parameters used to size a wastewater treatment plant when it is being designed. After a WWTP is built, the BOD influent waste loadings can be compared to

the effluent waste loadings to determine the removal efficiency of the treatment plant. Treatment plants that use activated sludge processes can benefit from knowing the BOD_5 because it will aid them in determining the oxygen required to stabilize the organic matter.

As mentioned, the BOD test is a direct measurement of the oxygen demand of the water, but it is also a surrogate test for the organic compounds present in the water. The amount of organic matter in the water can be used as an indicator of the degree of pollution of the water. The discharge of organic compounds into receiving waters can impact the ecological system and cause distress or death to different aquatic species. When organic compounds are introduced, the native microorganisms will utilize the oxygen naturally present in the water to metabolize the organics. If the water body cannot absorb oxygen from the atmosphere faster than the microorganisms are utilizing the oxygen within the water body, then there will be an oxygen deficit in the water. Lack of sufficient oxygen can lead to fish kills.

2.3.4 Limitations to the BOD_5 test

The BOD_5 test is a relatively straightforward test procedure, but it has some major limitations. The most obvious drawback to the five-day BOD test is that it takes five days to obtain the results of the test. By the time the results are obtained, the water has completed the treatment process and if treatment is not adequate, polluted water may be unintentionally discharged into receiving waters.

An additional limitation to the test is that it only measures biodegradable organics (Tchobanoglous et al., 2003). As such, the BOD test is not a reliable indicator of nonbiodegradable organic matter, which can also be harmful to the environment.

The BOD test can be limited by certain industries and toxic wastes, thus pretreatment may be necessary (Tchobanoglous et al., 2003). For instance, paper mills use hydrogen peroxide as a bleaching process so it is necessary to quench the peroxide (a disinfectant) prior to conducting the BOD test (Standard Methods, 2005). It is important for the laboratory analyst to have a thorough understanding of the wastewater influent, and the possible pollutants, contaminants, and toxic wastes that may be added by nearby industries and commercial activities.

Part B: Test Procedures for Organic Content Determination in Water

BOD_5 is important to wastewater treatment plants for many reasons such as treatment plant adjustments, permit compliance, and to protect the environment, but the time that it takes to obtain the test results is a major limitation. As such, it has become desirable to explore alternate test methods that produce results much faster, usually within a matter of hours as opposed to days. Alternate test methods can be used for faster response to treatment plant adjustments. Additionally, it may be possible for WWTPs to get approval for alternate test methods to estimate their BOD_5.

The Standard Methods for the Examination of Water and Wastewater 21st Edition (commonly referred to as Standard Methods, 2005) has approved several methods for the determination of aggregate organic constituents; these methods are discussed in Standard Methods Part 5000. The three approved standard methods that are included in this research include Biochemical Oxygen Demand (BOD), Chemical Oxygen Demand (COD), and Total Organic Carbon (TOC) analysis. An alternate method for determining organic compounds is fluorescence, but this is not an approved Standard Method. Each of these methods quantifies

organic material in different ways, but the literature has shown that each can be correlated with BOD_5.

The following discussion provides information on the five methods (BOD_5, COD, TOC, fluorescence, and absorbance) such as the background, selection of a method, interferences, and sample preservation. A side-by-side comparison of each of the methods is then provided to show the advantages and disadvantages of each method and how each quantifies organic matter.

2.4 Biochemical Oxygen Demand

2.4.1 BOD Principle

BOD is an empirical test that measures the oxygen requirements of the water during a specified incubation period. During the incubation period microorganisms consume the biodegradable organic matter in the sample. The oxygen utilized is affected by the pH and temperature of the water; therefore, the Standard Methods specifies that the pH of the water should be between 6 and 8. The BOD results can be used to directly determine the oxygen requirements of the water, but the results are also used as a surrogate to determine how much biodegradable organic matter is present in the water (Standard Methods, 2005).

During the incubation period, the sample undergoes three distinct processes: oxidation, synthesis, and endogenous respiration. Energy is obtained by oxidizing the waste (oxidation); the energy is then used during the oxidation process and for the production of cell tissue (synthesis). Once the energy is exhausted from the organic matter, the cells begin to consume themselves (endogenous respiration). The chemical processes are described below. The term "COHNS" represents the organic waste, which is composed of carbon,

oxygen, hydrogen, nitrogen, and sulfur. The new cell tissue is represented by $C_5H_7NO_2$ (Tchobanoglous et al., 2003).

Oxidation:

$$COHNS + O_2 + bacteria \rightarrow CO_2 + H_20 + NH_3 + other\ end\ products + energy$$

Synthesis:

$$COHNS + O_2 + bacteria + energy \rightarrow C_5H_7NO_2$$

Endogenous respiration:

$$C_5H_7NO_2 + 5O_2 \rightarrow 5CO_2 + 2H_20 + NH_3$$

2.4.2 Selection of a BOD Method

There are three approved Standard Methods for determining BOD: the five-day BOD test (BOD_5), the ultimate BOD test (BOD_U), and the respirometric method. The most common method is the five-day BOD test, which is commonly referred to as BOD_5; the subscript is a reference to the incubation time frame. This is the required method for the NPDES permit; therefore, this is the method adopted for this research.

Modifications to the BOD_5 test can be made to parameters such as the incubation period, the incubation temperature, dilution, and seeding to provide similar conditions to receiving waters, which is useful when evaluating potential environmental effects. As mentioned, a five-day incubation period at 20°C is the most common test parameter, but a seven-day incubation period (i.e., BOD_7) may be useful for working schedules, where results cannot be read on the weekends. The ultimate BOD (BOD_U, UBOD, or Method 5210C) test is conducted over an incubation period (20°C) period of 30 to 60 days, and possibly longer. This method is similar to BOD_5, with some modifications, but the purpose of this method is to determine the oxygen required to degrade all of the organic matter. The respirometric

method directly and continuously measures the oxygen uptake of the microorganisms during the incubation period. The method has use for assessing how chemicals degrade over time and the effects of toxic wastes on oxygen uptake (Standard Methods, 2005).

2.4.3 Interferences

Samples that contain chlorine, toxic substances, or hydrogen peroxide can interfere with the BOD analysis; therefore, these samples require either pretreatment or a special study (Standard Methods, 2005). Samples used in this research were purely domestic wastewater, so special considerations were not necessary.

When inhibitors are not used, the oxygen demand is the sum of the carbonaceous demand, the nitrogenous demand, and the demand from inorganic matter such as sulfides and ferrous iron. The carbonaceous demand is the oxygen demand of biodegradable organic matter in the water, and it is the BOD value that is typically desired. Inorganic matter can affect the results of the BOD test, but there are not inhibitors available or adjustments that can be made to the results to account for inorganic demand (Standard Methods, 2005).

Nitrogenous demand is the oxygen consumed when oxidizing reduced forms of nitrogen, such as ammonia. The types of microorganisms in the water will affect how and if reduced forms of nitrogen will be oxidized. If nitrogenous oxidation will occur in the water, then inhibitors can be used to prevent oxidation (Tchobanoglous et al., 2003; Standard Methods, 2005).

When nitrogenous inhibitors are not used, then both the carbonaceous and nitrogenous demands are included in the BOD results. Methods are available that estimate the theoretical nitrogen demand, which can be subtracted from the test results to obtain the carbonaceous demand (Tchobanoglous et al., 2003; Standard Methods, 2005). This method

is not recommended by the Standard Methods (2005) because it is subject to error and it is cumbersome. The use of inhibitors is more direct and reliable; therefore, the Standard Methods recommends using inhibitors when the carbonaceous demand is desired. NPDES permits typically specify that the total BOD_5 be reported; therefore, nitrogenous inhibitors were not used for this research and the procedure does not reflect their use.

2.4.4 Sample Handling and Preservation

The Standard Methods (2005) specifies that samples that cannot immediately be analyzed should be stored at 4 ± 1°C for up to 24 hours following the end of the 24 hour composite period or up to 48 hours after a grab sample.

2.4.5 Sample Pretreatment

Samples temperature should be 20 ± 3°C with a pH between 6 and 8 before analysis. If necessary, the pH of the sample should be adjusted to 7.0 to 7.2 using either sulfuric acid or sodium hydroxide. Ideally, samples should be collected ahead of chlorination; if chlorine is present then the sample must be dechlorinated (Standard Methods, 2005). Sample temperature and chlorine residuals were checked in this research.

2.4.6 Quality Assurance/ Quality Control

The Standard Methods (2005) outlines four quality controls for the BOD_5 method: a) samples must meet oxygen depletion requirements (referred to as qualified samples), b) seed controls, c) a glucose glutamic acid check and d) a dilution water blank. Qualified samples are used to verify that the microorganisms are effectively uptaking the oxygen. The GGA sample is used to verify the seed source is adequate and it is used as a benchmark for the precision and accuracy of the test. The dilution water blank is used to verify the dilution

water is free from contaminants (organics and/or microorganisms) and to verify the cleanliness of the glassware. The following discussion provides additional information regarding each of these quality controls. A calculation flowchart addressing each of these controls is provided in Chapter 3 to guide the analyst with validation of their test results.

2.4.7 Qualified Samples (BOD_5)

Samples must meet two requirements in order for them to be qualified 1) the sample must deplete a minimum of 2 mg/L of oxygen during the incubation period and 2) the sample must have a minimum residual dissolved oxygen (DO) reading of 1 mg/L.

The minimum depletion requirement is a minimum tolerance to ensure that the results are valid. A minimum 1 mg/L residual DO is required to ensure that there was enough oxygen available during the incubation period. Since the DO is only being recorded at the beginning and end of the incubation period, there is no way of knowing when the oxygen was depleted. It is assumed that if there were more oxygen present, then the microorganisms would have continued oxidizing the biodegradable organic matter. When all dilutions have a residual DO of $\leq$ 1 mg/L, the results are reported as "greater than."

2.4.8 Dilution Water Source and Control

The Standard Methods (2005) specifies that the dilution water cannot exert an oxygen demand, because it will bias the results. Specifically, dilution water should not deplete more than 0.20 mg/L of dissolved oxygen during the five-day incubation period, but it is preferable that depletion is limited to under 0.10 mg/L.

The Standard Methods (2005) allows flexibility for the dilution water source, as long as it does not exceed the minimum depletion requirements. Deionized water often contains organics and microorganisms that can bias the test results high; therefore, traditional

laboratory deionized water is not always a suitable dilution water source and additional water treatment may be necessary (Standard Methods, 2005). Treated tap water was used as dilution water in this research, as described in Chapter 3.

Reagents are added to the dilution water to stabilize the pH and provide the necessary nutrients for the microorganisms. The Standard Methods (2005) provides a detailed description of how to prepare the reagents. Alternatively, prepared buffer pillow packs containing the reagents are commercially available from companies such as Hach (Loveland, CO). Prior to use, the dilution water should be at 20 ± 3°C. The water should have a minimum initial DO of 7.5 mg/L to ensure there is enough oxygen available. In addition, the initial DO should be below 9.0 mg/L to prevent supersaturated water from degassing during the incubation period, which can bias the results high. If an aerator is used, it should be free from contaminants (Standard Methods, 2005).

2.4.9 Seed Source

Seed is a concentrated population of microorganisms. The seed source can either come from within the wastewater treatment process or it can be purchased commercially. The seed sample strength is determined by the GGA sample, but it should generally be between 0.6 and 1 mg/L (Standard Methods, 2005). The GGA sample will be discussed in further detail in the next section. The supernatant from settled domestic wastewater is a viable seed source, and preferable because the microorganisms are acclimated to the sample matrix (Standard Methods, 2005). If the seed source is too strong it may be helpful to let the sample sit at room temperature for some time, or store the sample overnight at 4 ± 1°C. Alternatively, the supernatant from a settled primary clarifier influent may be used (Standard Methods, 2005). Commercial seed sources are also available, but they are not ideal because

the microorganisms may not be acclimated to the sample matrix and also there can be variability in the manufacturing process that can cause issues with GGA controls.

2.4.10 Glucose Glutamic Check (GGA)

The GGA is a solution made of 150 mg of glucose and 150 mg of glutamic acid dissolved in 1-liter of distilled water (Standard Methods, 2005). Glucose is a simple sugar that is easily oxidized and glutamic acid is an amino acid that stabilizes the sample results. The GGA can be prepared before use, or commercial GGA standards (Hach, Loveland, CO) are available that can be stored at 4°C or lower.

GGA samples are prepared by adding 6 mL of GGA per 300 mL BOD bottle, and then the sample is seeded with the same volume of seed that is added to the seeded samples. The seed should result in a final GGA BOD_5 of 198±30.5 mg/L (Standard Methods, 2005). The seed strength should be adjusted to meet the GGA BOD_5 requirement. Judgment should be used to determine if an alternate sample site or adjusting the seed volume is more appropriate to change the seed strength.

Once the seed strength consistently meets the GGA requirements, the method for collecting the seed and seed volume should remain the same for future testing. If seeded samples are not meeting the qualified sample criteria, then the volume of sample (not seed) should be adjusted accordingly.

2.4.11 Seed Controls

A minimum of three seed control blanks with different dilutions should be prepared with each BOD_5 setup. The seed control samples are used to determine the seed strength either by the ratio method or by the slope method. For the ratio method, the seed strength is ratio of the oxygen depletion to the volume of seed. For the slope method, the seed strength

is determined by the slope of the plotted data where x=volume and y=DO uptake. The y-axis intersection represents the demand of the dilution water, which should be less than 0.2 mg/L (Standard Methods, 2005).

It should be noted that seed controls might also contain organic matter. Any oxygen demand associated with this organic matter is inherently included in the seed strength calculation. The oxygen demand of the seeded sample is corrected for in the final BOD_5 calculation; therefore, if there is organic matter in the seed source it is corrected for as well.

2.4.12 Sample Preparation

Samples can either be seeded or unseeded. Samples, such as treated effluent, do not have sufficient microorganisms to deplete enough oxygen to meet the minimum qualified criteria requirements; therefore, it is necessary to seed these samples. Influent samples impacted by certain industries may also require seeding. For instance, hydrogen peroxide is used as a bleaching agent in paper mills, but peroxide is also a disinfectant that will kill the influent microbe population, thus influent samples may need to be seeded. The volume of seed is determined based on the GGA requirements. If the seeded samples are not meeting the qualified sample criteria, then the sample volume should be adjusted accordingly.

The volume of sample added to the BOD bottle will depend on the oxygen demands of the sample. Samples with a high BOD_5 will require less sample, and samples with a low BOD_5 will require more sample (and possibly seeding). Ideally, one would want to add enough sample volume such that 50% of the oxygen is depleted. There are several resources available that suggest how to dilute the sample (Delzer and McKenzie, 2003; Standard Methods, 2005).

2.4.13 BOD Calculations

Equation 2.1 is used to calculate the BOD_5. The seed strength is determined based on the average strength of the seed controls, as determined in section 2.4.10.

$$\frac{DO_{Initial} - DO_{Final} - \text{Seed Strength} * \text{Volume seed}}{\frac{\text{Volume of Sample}}{\text{Volume of BOD bottle}}} = BOD_5 \qquad \text{Eqn. 2.1}$$

2.4.14 Interpretation of the BOD_5 Results

As previously mentioned, BOD_5 is a measure of the oxygen demand of the water, which is used as a surrogate for biodegradable organic matter; the oxygen demand is assumed to be proportionate to the biodegradable organic matter. Table 2.1 is a summary of the pollutants in the sample (either microorganisms or organic matter) and how this impacts the BOD_5 results. Table 2.2 shows some common issues encountered during the BOD_5 test and possible solutions.

The amount of oxygen that the microorganisms deplete is what is measured during the BOD_5 test. If there is not a presence of microorganisms, the organic matter will not have an impact on the oxygen demand, and the BOD_5 cannot be measured. If there are microorganisms present, but no biodegradable organic matter, the bacteria will still consume oxygen proportionate to their population; this is represented by the seed samples. Ideally, dilution water will not have microorganisms or organic material; therefore, there will not be a BOD_5 result. Some samples (e.g., plant influent and primary clarifier effluent), naturally will contain both organics and microorganisms, therefore seeding is not necessary to obtain a BOD_5 result. Samples such as effluent typically have limited microorganisms and organics; therefore, they must be seeded with microorganisms to utilize the organics (if they are present) and provide a useful BOD_5 result (i.e., qualified sample).

Table 2.1 Interpretation of the BOD_5 Results for Each of the BOD Samples Adapted from Standard Methods (2005)

Sample	Expected sample pollutants	Expected outcome	BOD_5 result?	Interpretation
Blank	No Organics + No microorganisms	Will not deplete oxygen	No	Good quality dilution water will not exert a demand. If demand occurs it could indicate presence of microorganisms.
Seed	No organics + Microorganisms	Will deplete oxygen	Yes	This is the oxygen demand attributed to the microorganisms. The seed sample could contain organics, but these are not important. All oxygen demand (including its organic matter) is corrected for in seeded samples.
Influent and Primary Clarifier Effluent	Organics + Microorganisms	Will deplete oxygen	Yes	Sufficient microorganisms are naturally present. These will oxidize the organics and exert an oxygen demand.
Advanced treatment Effluent (unseeded)	Organics + None or small population of microorganisms	Will not deplete oxygen	No	Organic matter cannot change the oxygen demand. The sample must be seeded to obtain a BOD_5 result.
Advanced treatment Effluent (seeded)	Organics + Seed (microorganisms)	Will deplete oxygen	Yes	The added presence of microorganisms will consume the organic matter, and give a BOD_5 result. The oxygen demand from the seed is corrected for in the calculations, so the results represent the demand of the effluent sample.

Table 2.2 Common Issues Encountered in the BOD_5 Test Adapted Standard Methods (2005)

Issue	Possible reasons	Possible solutions
Initial dilution water DO reading is below 7.5 mg/L	Check temperature (too warm)	Adjust the temperature. If the problem persists, consider using an aerator.
Initial dilution water DO reading is above 9 mg/L	Check temperature (too cold)	Adjust the temperature. Let the water degas for a period-of-time.
Dilution water depletes more the 0.2 mg/L	Water source has microorganisms or glassware is dirty	Check the water source. Consider an alternate source or further water treatment. If the water source is reliable, check the cleaning procedure.
Sample does not deplete 2 mg/L	Not enough sample volume was added or seeding may be necessary	If this occasionally occurs with the smallest sample volume, then adjustments may not be necessary. If this occurs on a regular basis and with all samples try the following: Increase the sample volumes. Also, verify that the sample has sufficient microbes to oxidize the organics. If not, consider seeding the sample.
Final DO reading is less than 1 mg/L for unseeded samples	Too much sample	If this occasionally occurs with the largest sample volume, then adjustments may not be necessary. If this occurs often to multiple sample dilutions, then decrease the sample volume.
Final DO reading is less than 1 mg/L for seeded samples	Too much sample	See comments for the unseeded sample. If the seed is meeting the GGA requirements, then DO NOT adjust the seed volume. Decrease the sample volume.
Seed is too weak	Seed is not strong enough	Try an alternate seed source. If the seed source is reliable, check the accuracy of pipettor.
Seed is too strong	Seed is too strong	Let the sample settle longer. If this does not work, try a more treated seed source such as primary effluent.
GGA falls below acceptable range	Seed may be too weak	See issue "seed is too weak"
GGA Is above acceptable range	Seed may be too strong or glassware is dirty	See issue "seed is too strong." If the dilution blank also fails, check the cleanliness of the glassware.

2.5 Chemical Oxygen Demand

2.5.1 COD Principle

The COD method determines both the inorganic and organic oxygen demand of water based on the quantity of oxidant (expressed in oxygen equivalence) that is oxidized within a specified time frame (Standard Methods, 2005). The Standard Methods (2005) specifies that dichromate ($Cr_2O_7^{2-}$) be used as the oxidant because of its unique chemical properties. The following chemical equation shows that the dichromate ion is reduced to the chromate ion (Cr^{3+}) during the oxidation process (Tchobanoglous et al., 2003).

$$C_N H_a O_b N_c + dCr_2O_7^{2-} + (8d + c)H^+ \rightarrow nCO_2 + \frac{a + 8d - 3c}{2} H_2O + cNH_4^+ + 2dCr^{3+}$$

$$where\ d = \frac{2n}{3} + \frac{a}{6} - \frac{b}{3} - \frac{c}{2}$$

Digestion time, reagent strength, and aliquot COD concentration can affect the extent of oxidation in the sample. During the COD test, hazardous waste such as mercury, hexavalent chromium, sulfuric acid, silvers, and acids are generated (Standard Methods, 2005).

Table 2.3: Comparison of COD Methods Adapted from Standard Methods (2005)

COD Method Selection			
	Open Reflux Method (Method 5220B)	Closed Reflux Titrimetric Method (Method 5220C)	Closed Reflux Colorimetric Method (Method 5220D)
Use	Use Method 5220B.4a for sample with COD >50 mg O_2/L. Use Method 5220B.4b for sample with COD from 5 to 50 mg O_2/L.	Use Method 5220C.4 for samples with COD from 40 to 400 mg O_2/L. Greater concentrations can be determined by dilution.	Use Method 5220D.4 for samples with COD>50 mg O_2/L. Greater concentrations can be determined by dilution.
Advantages	Useful for several types of water. More accurate and representative then Method 5220C and 5220D.	Produces less hazardous waste and it is more economical then 5220B. Premeasured reagents in ampules and cultured tubes are commercially available.	Produces less hazardous waste and it is more economical then 5220B. Premeasured reagents in ampules and cultured tubes are commercially available.
Disadvantages	Produces more hazardous waste then 5220C and 5220D. Method 5220B requires a blender and reflux apparatus, which can occupy considerable lab space. Procedure 5220B4.b is highly sensitive to organic matter on the glassware.	Less accurate and less representative than 5220B. Requires more homogenous samples to reproduce results.	Less accurate and less representative than 5220B. Requires more homogenous samples to reproduce results. Light absorbing interferents must be removed from the sample.
Principle	The aliquot is boiled with a mixture of potassium dichromate and sulfuric acid, which chemically oxidize the organic matter. After a 2-hr digestion period ferrous ammonium sulfate is used to titrate the remaining potassium dichromate, from which the quantity of oxidizable material can be calculated in oxygen equivalence.	See Principle for Method 5220B	See Principle for Method 5220B. This method uses light absorption to determine oxygen demand. The dichromate ion absorbs light in the 400-nm region and the chromic ion absorbs light in the 600-nm region.

2.5.2 Selection of a COD Method

Table 2.3 compares the uses, advantages, and disadvantages of the three types of COD tests. The closed reflux methods are more common than the open reflux method because they are economical, require less equipment space, and produce less hazardous waste. The caveat is that the closed reflux methods are also less accurate and less representative then the open reflux method (Standard Methods, 2005). The closed reflux colorimetric method was chosen for this research (Method 5220D).

2.5.3 Principle for the COD Closed Reflux Colorimetric Method

As previously discussed, the COD method determines the oxygen demand by reducing the dichromate ion to the chromic ion through oxidation. Each of these ions has a unique color and a unique absorption. Dichromate absorbs light in the 400-nm region and chromic absorbs light in the 600-nm region (Standard Methods, 2005). For this test, light absorbance is used to determine how much COD is oxidized by the dichromate ion, therefore it is crucial that the sample is free from light-absorbing interferents or compensated for in the testing methods.

Hach (Loveland, CO) is company that manufactures laboratory equipment and chemistries that can be used for water and wastewater analysis. They have chemistries to determine the concentrations of contaminants such as ammonia, phosphorus, COD, and many others.

For the COD test, there are different reagent ampules available to accommodate different COD sample concentrations. These fall into four categories: ultra low range (0 to 50 mg/L), low range (0 to 150 mg/L), high range (0 to 1,500 mg/L) and high range plus (0 to 15,000 mg/L).

2.5.4 Principle for the Hach Method

The Hach COD chemistry used in this research is based on the dichromate reactor digestion method. Hach provides ampules that contain premeasured reagents. A specified volume of sample (2 mL) is added to the Hach ampule, and then it is incubated at 150 °C for 2 hours to allow the sample to digest. When the sample has cooled to room temperature, a spectrophotometer is used to measure the COD using the colorimetric method.

Potassium dichromate is the reagent in the ampule, which oxidizes the organic matter during the digestion period. The chromium ion is transformed from a hexavalent to a trivalent state during the digestion period. The ion absorbs light at different wavelengths, dependent on the concentration of COD in the sample. Additional reagents in the ampule include mercuric sulfate to eliminate chloride interference and silver is used as a catalyst (Hach, 1999).

The spectrophotometer is programmed using potassium hydrogen phthalate (KHP) at different concentrations to develop a concentration curve (Hach, 1999; Standard Methods, 2005). There are different methods programmed in the Hach spectrophotometers for each of the Hach COD concentration chemistries; each chemistry has its own wavelength and calibration curve (e.g., the low range COD chemistry has a unique wavelength and calibration curve programmed into the spectrophotometer). When the sample is put into the spectrophotometer, the amount of light absorbed by the sample at a predetermined wavelength is converted to an equivalent COD measurement based on the calibration curve.

The Standard Methods and the Hach method provide instructions for creating KHP standards to verify the equipment is working correctly (Hach, 1999; Standard Methods, 2005). The KHP control should be made from a different stock than the KHP used to

calibrate the spectrophotometer so that it can serve as an independent control to validate analytical technique and to verify the equipment is holding its calibration (Standard Methods, 2005).

2.5.5 Interferences

The COD test is effective at oxidizing 95-100% of the organic compounds, but certain volatile organic compounds may resist oxidation. A silver sulfate catalyst can be used to oxidize straight-chain aliphatic compounds more effectively. Chloride ion, bromide, iodide, and other similar ions inhibit the oxidation of the dichromate ion. The chemical reaction between chloride and dichromate results in halogen and the chromic ion, which will error the oxygen demand on the high side. The interference due to chloride can be overcome in waters where the concentration is less than 2,000 mg/L by adding mercury sulfate ($HgSO_4$) prior to refluxing the sample; if the chloride concentration is greater than 2,000 mg/L, then alternate COD testing techniques specific to saline water must be used. Ammonia and its derivatives are not oxidized in the COD method. Nitrite interference is usually negligible, but significant interference can be eliminated with sulfamic acid. Inorganics compounds (e.g., ferrous iron, sulfide, manganous manganese, etc.) are also oxidized during the test procedure. Suspended matter and color absorbing compounds will impact the results by interfering with the amount of light absorbed; if this occurs, then it may be possible to determine the COD based on the titrimetric method (Standard Methods, 2005).

2.5.6 Sample Collection and Preservation

It is preferable that the sample is collected in a glass bottle to minimize interference. Samples that are unstable should be tested immediately. If the sample cannot be analyzed immediately, then the sample can be preserved by acidification to $pH \leq 2$ using sulfuric acid

(H_2SO_4.) and stored at 4°C±2°C for up to 28 days (EPA, 1999; Standard Methods, 2005). Samples with suspended solids should be blended before analysis to homogenize the sample

2.5.7 Quality Control/Quality Assurance

Standard Methods (2005) recommends that samples be collected in a glass bottle. In the event that the concentration of COD is high, preliminary dilutions can be made to reduce the error associated with small volume size.

A potassium hydrogen phthalate control should be prepared from a different stock solution used to make the spectrophotometer calibration curve. This control sample can be used to validate analytical technique, identify potential contamination, and to verify the calibration curve is valid, and ensure the digester and spectrophotometer equipment are working properly.

2.5.8 Disposal of Hazardous Waste

Mercury, silver, and chromium reagents are used in the analysis, which are considered hazardous waste. As such, the samples need to be disposed of accordingly to local regulations.

2.6 Total Organic Carbon

2.6.1 TOC Principle

The total organic carbon (TOC) method is a more direct way to quantify the organic compounds in water or wastewater. In TOC analysis, organically bound carbon is converted to carbon dioxide (CO_2), which is directly measured. Each of the methods breaks down the organic molecules in slightly different ways. High temperature methods use catalysts and oxygen, whereas lower temperature methods use ultraviolet irradiation or chemical oxidants.

The CO_2 can be quantified using an infrared analyzer, a colorimetric titrator, or by separating the CO_2 from the liquid with a membrane (Standard Methods, 2005).

Table 2.4: Fractions of Total Carbon

Name	Definition*
Inorganic carbon	Carbonate, bicarbonate, and dissolved CO_2
Total organic carbon (TOC)	Carbon atoms covalently bonded in organic molecules
Dissolved organic carbon (DOC)	The fraction of TOC that passes through a 0.45µm filter
Suspended organic carbon	The fraction of TOC that is retained on a 0.45µm filter
Purgeable organic carbon (aka volatile organic carbon)	The fraction of TOC that is removed from an aqueous solution by gas stripping
Nonpurgeable organic carbon	The fraction of TOC that is not removed from an aqueous solution by gas stripping
*Adapted from the Standards Methods (2005)	

Fractions of the total carbon can be determined as well; Table 2.4 summarizes the different fractions and their definitions. Inorganic interference can skew the results because it may be greater than the TOC fraction; interference can be eliminated by acidifying the sample to ≤pH 2 to convert the carbonate and bicarbonate to CO_2. The carbonate CO_2 can then be removed by volatilization by purging the sample with purified gas or vacuum degassing. The purging process will also remove purgeable organic compounds (Standard Methods, 2005).

2.6.2 Method Selection

There are three approved Standard Methods to determine TOC. The high temperature method uses high temperatures to combust the organic matter into CO_2, which can be measured with a nondispersive infrared analyzer or through colorimetric titration. This

method is best for samples that have TOC > 1mg/L. The persulfate method utilizes persulfate to oxidize the organic matter to CO_2 and it is capable of determining TOC concentration < 1mg/L as well as higher concentrations. The wet oxidation method is slightly different than the persulfate method, but it also uses persulfate to oxidize organic matter. This method is more sensitive at determining the concentration of TOC < 1mg/L (Standard Methods, 2005).

Based on historical TOC data, the concentration in the treated WWTP effluent is always above 1 mg/L. Given the levels of TOC (>1 mg/L) of wastewater in this research, the purgeable organic carbon was determined with the high temperature combustion method.

2.6.3 Interferences

As previously discussed, inorganic compounds can be greater than the total organic carbon. This interference can be eliminated by acidifying the sample to less than 2 pH to convert the inorganics to CO_2, and then volatizing the sample to remove the CO_2. Volatile organic substances are removed during this process as well. Misrepresentative samples can be analyzed due to particulate occlusion when the particles do not enter the needle used for injection. Filters used to determine DOC can either adsorb or desorb carbon material, resulting in either a higher or lower DOC value; analysis of a blank will help identify if filters are contributing to the DOC. Finally, contaminated or dirty glassware, containers, and tubing will impact the final TOC results (Standard Methods, 2005).

2.6.4 Sample Preservation and Storage

Samples can be preserved by acidification to a pH less than 2 and stored at 4°C with minimal exposure to light and the atmosphere. The acidification will also eliminate the inorganic carbon-containing compounds, so this should be taken into consideration (Standard

Methods, 2005). The Standard Methods specifies that samples should be collected and stored in in glass bottles that are protected from sunlight and sealed with TFE-backed septa.

2.6.5 Quality Control/Quality Assurance

The instrument detection limit should be determined based on part 1000 section 1030 of the Standard Methods. A control should be prepared from a source of material separate from the calibration standards and at a concentration similar to the samples being tested to verify the equipment is operating correctly and there are no sources of contamination (Standard Methods, 2005).

2.7 Fluorescence and Absorbance Method

At the time of this writing, fluorescence spectroscopy was not an approved Standard Method for quantifying organic matter in water or wastewater (Standard Methods, 2005). Despite this, fluorescence is a method that can be used to identify organic matter (Henderson et al., 2009; Vasel and Praet, 2002; Baker and Inverarity, 2004). The most similar method in the Standard Methods is the Ultraviolet (UV) Absorption Method (Method 5910 B), which has some similarity to fluorescence, but they are not the same.

Fluorescence spectroscopy is a method that that can be used to identify analytes based on their molecular properties. Certain molecules have a molecular component called fluorophores that will absorb energy and reemit energy at unique wavelength. These molecules are sometimes generally referred to as fluorophores, but it is important to realize that fluorophore is a characteristic of certain molecules and that not all molecules have this characteristic. As a consequence, some molecules cannot be identified using fluorescence (Sauer et al., 2011; Lakowicz, 2011).

Chromophores are molecules that absorb energy, but they do not reemit energy. These are distinctly different from fluorophores that absorb light and emit fluorescence (Lakowicz, 2011). Therefore, chromophores are responsible for the absorbance characteristics of a sample and fluorophores are responsible for the fluorescence characteristics of a sample. It is possible for a sample to have chromophores and be void of fluorophores thus exhibiting absorbance properties, but not fluorescence.

2.7.1 Fluorescence Theory

Molecules are typically in a ground state. When a fluorophore molecule absorbs energy at a specific wavelength, it is no longer in its ground state. The molecule will excite and will undergo different states to relax back to its ground state.

Ground State → Higher Vibrational State → Lower Vibrational State →Ground State

Once a molecule absorbs energy it excites into a higher vibrational state and then relaxes into a lower vibrational state. A molecule will transition from the lower vibrational state to the relaxed state via several mechanisms, but the one that is the most important to this research is fluorescence. The molecule will release energy in the form of fluorescence at a unique wavelength to relax from its excited state back to its ground state (Sauer et al., 2011; Lakowicz, 2011).

2.7.2 Spectrofluorometer

Figure 2.2 is a typical spectrofluorometer, such as the Horiba AquaLog ® (Edison, NJ) equipment used for this research. This type of equipment can quantify both the absorbance and fluorescence properties of the sample. A lamp is used as the energy source to excite the molecules in the sample. The light passes through a mirror, which is used to focus the light into the monochromator. Internal components in the monochromator are used

to adjust the wavelength of the light being sent through the sample and also to minimize light scattering. The reference detector is used to check the wavelength being sent through the sample and make internal adjustments to the monochromator if necessary. Two detectors measure the wavelengths after the light is passed through the sample. The intensity detector measures the light that passes through the sample, which is used to determine absorbance. The fluorescence detector is perpendicular to the lamp; this measures the fluorescence emitted by the fluorophore molecules in the sample (Horiba Scientific, 2012).

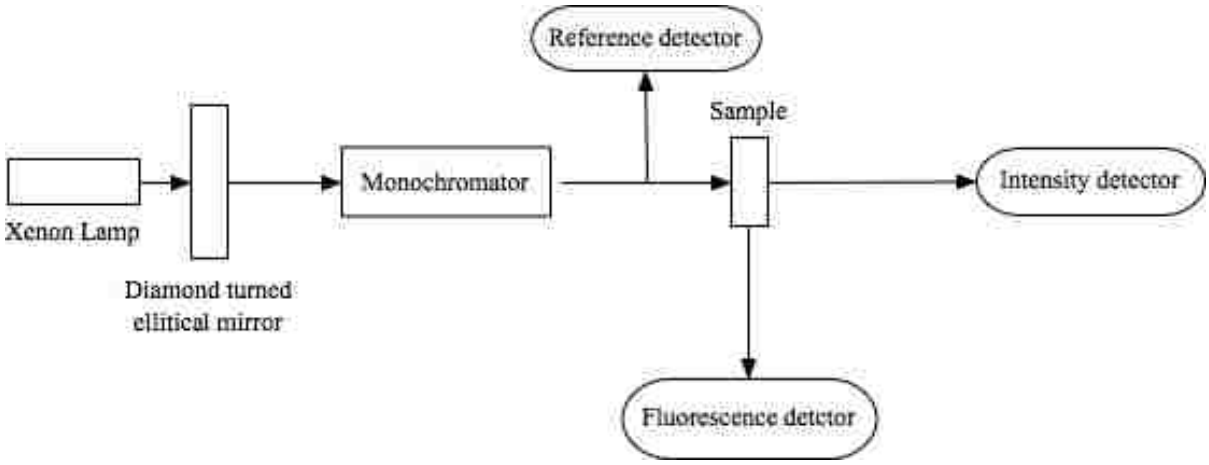

Figure 2.2: Schematic of a typical spectrofluorometer arrangement adapted from Horiba Scientific (2012).

The incident light that is sent through the sample is called the excitation wavelength when referring to fluorescence and it is called the incident wavelength when referring to absorbance; this is the wavelength that the reference detector measures. The light captured by the fluorescence detector is the emission wavelength; this is the fluorescence energy emitted by the fluorophores. Because absorbance and fluorescence are independent of one another, the emission wavelength will never be used to describe absorbance properties.

Spectrofluorometers are capable of analyzing the fluorescence of a sample across a range of excitation and emission wavelengths. An excitation-emission matrix (EEM) is commonly developed for a sample, which captures the fluorescence at a range of paired excitation and emission wavelengths. The results are reported on a 3D plot or a contour plot and are typically represented as (emission, excitation, fluorescence intensity) (Zhou et al., 2013; Henderson et al., 2009).

The spectrofluorometer equipment will typically have a user interface so that the user can adjust the settings (Lakowicz, 2001; Horiba Scientific, 2012). The user can set an excitation range and determine the increments that they want to use, as well as the emission wavelength increments that they desire. Another spectrofluorometer setting is the integration time. Longer integration times will reduce the signal to noise ratio (S/N). The signal is the fluorescence intensity corresponding to the relevant emission wavelength and the noise is the background illumination (Ahmad and Reynolds, 1999). Noise is reduced proportionate to the square root of the lamp pulses (i.e., 60 pulses per second). Longer integration times will increase the number of lamp pulses, and thus decrease the S/N ratio (Ahmad and Reynolds, 1999; University of Washington, 1999). Consider the integration times for 1 second to 5 seconds (Table 2.5). It becomes clear that after 3 seconds the impact of longer integration times plateaus; therefore, it becomes unreasonable to use integration times that exceed 3 seconds (University of Washington, 1999). Longer integration times will decrease the S/N ratio, but it will also increase the amount of time that it takes to analyze a sample; therefore, the minimum integration time should be chosen such that it meets the S/N ratio requirements (Ahmad and Reynolds, 1999).

Table 2.5: Integration Times and the Impact on S/N for Fluorescence Spectroscopy

Integration time	**Noise** $= \sqrt{\frac{\mathbf{60\ pulses}}{\mathbf{sec}} * \mathbf{Int\ time\ (sec)}}$	**1/Noise (e.g., S/N)**
1s	7.75	0.13
2s	10.95	0.09
3s	13.42	0.07
4s	15.49	0.06
5s	17.32	0.06

2.7.3 Interferences

Quenching interference is a broad term used to describe any process that will bias the fluorescence results low. Increases in sample temperature and pH can quench the fluorescence of the sample (Henderson et al., 2009; Hudson et al., 2007). Specifically, when the pH and temperature fall outside of the ranges of 3 – 7 and 10 – 80°C, the results of the fluorescence can be impacted (Ahmad and Reynolds, 1999). Temperature can change the mechanisms (e.g., fluorescence) that molecules use to transition from an excited to a relaxed state (Henderson et al., 2009). The pH impacts the molecular orbits and shape, and also changes the competition between the metal ions and hydrogen ion (Henderson et al., 2009).

An interference known as the inner filter effect (IFE) can occur in samples with high absorbance. When light passes through the sample, it is disproportionately absorbed on the front face of the sample and inhibits the amount of light sent through the remainder of the sample, which will quench the fluorescence. The sample will also reabsorb some of the fluorescence light. This type of interference can be overcome by diluting the sample (Lakowicz, 2011; Henderson et al., 2009; Hudson et al., 2007).

Raman and Rayleigh interference are caused by stray and scattered light that occurs during analysis (Lakowicz, 2011). Spectrophotometers typically have built in functions that

can correct for Rayleigh and IFE interference. The user can normalize the samples to Raman peaks or Raman peak areas. The Raman peak should be identified based on observed peaks in the sample (Yu et al., 2014).

Inorganic compounds (e.g., ferrous iron, iron, nitrite, nitrate, and bromide), oxidants, and reducing agents (e.g., ozone, chlorate, chloramines, and thiosulfate) will absorb light, thus interfering with the absorbance of a sample (Standard Methods, 2005). Chlorination and ozonation have also been found to interfere with fluorescence (Henderson et al., 2009). Different metal ions may interfere with fluorescence, but the results were variable in the literature; however, copper and aluminum are the most likely ions to cause quenching and impact the relationship of BOD_5 to fluorescence (Henderson et al., 2009; Reynolds and Ahmad, 1995).

2.7.4 Sample Preservation

Fluorescence is not yet an approved method for the quantification of organic matter in water and wastewater. It would be prudent to follow sample perseveration techniques outlined for the other methods. The sample should be refrigerated and stored in containers that will minimize light interference and the storage time should be limited to 24 hours after the composite period and 48 hours after a grab sample (Standard Methods, 2005).

2.7.5 Quality Control/Quality Assurance

At the time of this writing, there were not any standardized quality controls for fluorescence. Literature suggests that quinine sulfate may be a potential fluorophore to use as a quality control (Chen et al., 2003; Fletcher, 1969; Drobnik and Yeargers, 1966).

2.7.6 How Organics Are Quantified With Fluorescence

The organic matter quantified in this research is limited to dissolved organic matter (DOM), because samples were filtered prior to analysis to prevent light scattering interference. There are multiple ways to identify dissolved organic compounds based on the results of the spectrophotometer.

1) EEM peaks – these correspond to fluorescent peaks at their respective excitation and emission wavelengths. Organic matter that fluoresces is called fluorescent dissolved organic matter (FDOM) (Zhou et al., 2013).

2) EEM ratios – a ratio between two points on the EEM graph.

3) Regions – the EEM graph can be broken into regions and the fluorescence is determined based on the integrated area of that region.

EEM peaks can be identified on the graph by the level of fluorescence. Peaks can be associated with several compounds including humic acids, fulvic acids, and tyrosine (Chen et al., 2003). Peaks are referred to as protein-like, fulvic-like, and humic-like because it is difficult to identify specific fluorophores (Hudson et al., 2007).

Protein and carbohydrates contribute significantly to the organic matter in wastewater (Tchobanoglous et al., 2003). Three aromatic fluorophore molecules are common to proteins: phenylalanine, tyrosine, and tryptophan; their respective excitation and emission wavelengths are summarized in Table 2.6. Carbohydrates do not fluoresce; therefore, it is not possible to identify them using an EEM (Lakowicz, 2011). Coble et al. (1996) identified the peaks A, C, and T (Table 2.6) using seawater. Nam and Amy (2008) reported that the first maxima humic peaks occur near the excitation/emission wavelengths 280 nm/450 nm, which is consistent with the A and C peaks reported by Coble et al. (1996). Second maxima

humic peaks occur near 340 nm excitation and 420 nm emission wavelengths; second maxima humic peaks were chosen to be 342 nm and 436 nm for this research and labeled as humic (Nam and Amy, 2008).

The second method of quantification is with EEM peak ratios. One such peak is the fluorescence index (FI), which was defined by McKnight et al. (2001). The FI is defined as the ratio of the fluorescence at the excitation/emission wavelengths of 370nm/450nm to 370nm/500nm. The researchers concluded that the FI was higher (1.7 to 2.0) for microbial organics and lower (1.3 to 1.4) for terrestrial organics.

Fluorescence regional integration (FRI) was first introduced by Chen et al. (2003) as a means to simplify the interpretation of the EEM graphs. This technique breaks the EEM graph into unique regions and integrates the area under the spectra; the results are reported as arbitrary fluorescence units (AFU). Figure 2.3 is an adapted version of their defined regions and Table 2.7 summarizes the organics present in each region. Modified regions were adopted for this research, as shown in Figure 2.4.

Other methods for interpreting the EEMs include principal component analysis, partial least squares regression, principal factor analysis, and parallel factor analysis (PARAFAC), generalized rank annihilation models, Tucker models, multiway PLS, and multivariate curve resolution (Henderson et al., 2009). A review of each of these methods is outside of the scope of this project. The methods chosen for this research were based on successful approaches in the literature that were able to relate BOD_5 and fluorescence.

Table 2.6: Fluorescence EEM Peaks Associated With Organics

Peak	Excitation (nm)/ Emission (nm) As defined in the literature	Ex. (nm)/ Em. (nm) Adopted for this research	Description	Reference
Proteins	Phenylalanine 260/282 Tyrosine 275/304 Tryptophan 295/353	280/331	The three mentioned fluorophores are molecules are characteristic of proteins.	Lakowicz (2011)
Humic	340/420	342/436	Humic	Nam and Amy (2008)
A	260/380-460	260/450	Humic-like	Coble et al. (1996)
C	350/420-480	330/450	Humic-like	Coble et al. (1996)
T	275/340	275/340	Tryptophan-like, protein-like	Coble et al. (1996)

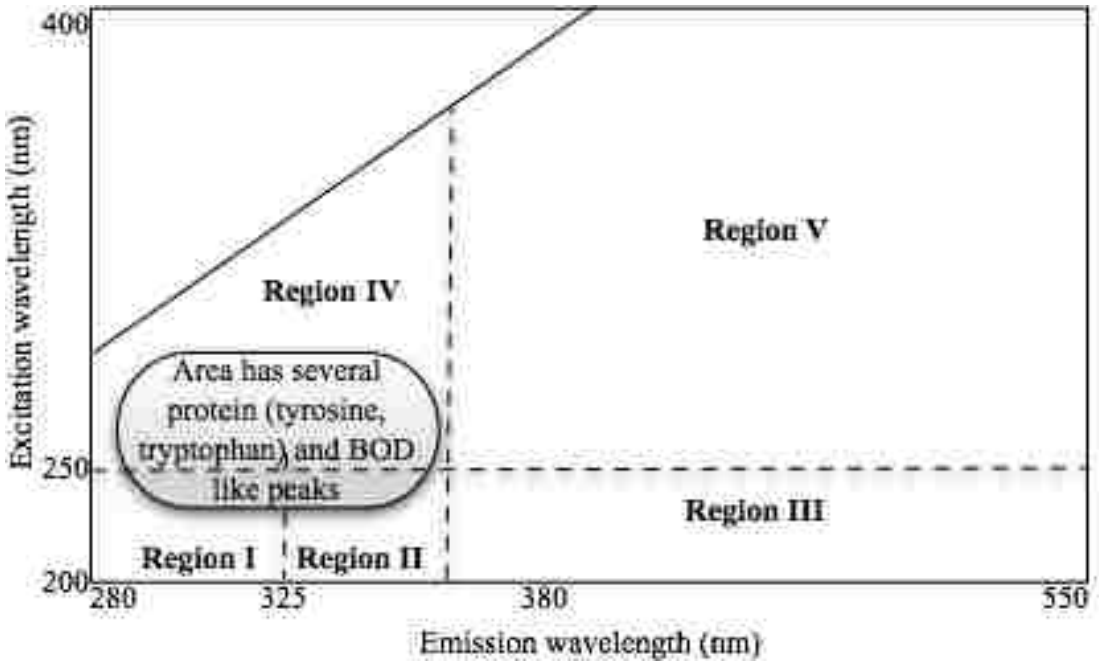

Figure 2.3: Fluorescence EEM regions defined by Chen et al. (2003).

Table 2.7: Fluorescence EEM Peaks for Each Region adapted from Chen et al. (2003)

Region	**Quantification**	**EEM Peaks**
Region I	Aromatic proteins I	Tyrosine
Region II	Aromatic proteins II	BOD_5
Region III	Fulvic-acid-like	Hydrophobic acid & fulvic acid
Region IV	Soluble-microbial-byproduct-like	Tyrosine, protein-like, and tryptophan
Region V	Humic-acid-like	Marine humic acids, hydrophobic acids, humic-like acids, model humic, acid polymers, hydrophobic acids

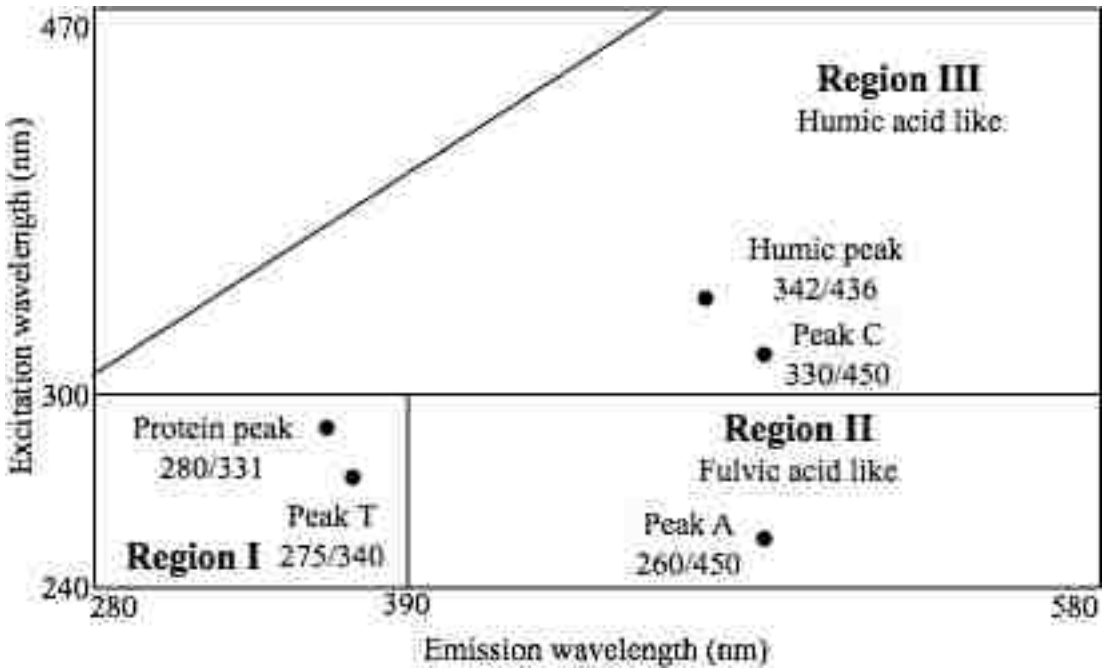

Figure 2.4: Fluorescence EEM regions and peaks that were adopted for this research.

2.7.7 Fluorescence Reporting Units

Fluorescence is unitless and it is reported as "arbitrary fluorescence units" (AFU) or "fluorescence units" (FU). Some researchers may also prefer to normalize their fluorescence results to quinine sulfate and report their results as quinine sulfate units (QSU) or quinine sulfate equivalent (QSE). All results in this report are given in AFU.

Fluorescence is reported with the excitation and emission wavelengths. The excitation wavelength refers to the energy that is sent through the sample, and the emission wavelength is the energy that is reemitted from the samples through fluorescence. The excitation wavelength will always be less than the emission wavelength.

2.7.8 How Fluorescence Can Be Used

Fluorescence can be used to identify organics based on their unique characteristics, such as humic-like, fulvic-like, or protein-like, which is a major advantage over alternate organic quantification methods such as COD, TOC, and BOD_5 (Chen et al., 2003; Coble et al., 1996; Nam and Amy, 2008; Hudson et al., 2007). Fluorescence has been used to characterize a variety of water sources, some of which include groundwater, wastewater, drinking water, river water, and reclaimed water (Albrektiene et al., 2012; Vasel and Praet, 2002; Park et al., 2010; Bridgeman et al., 2011, Baker and Inverarity, 2004; Laws et al., 2011).

It has been discussed that the characteristics of wastewater changes as treatment progresses. Specifically, biodegradable organic matter is removed and SMPs are formed. Fluorescence can be used in a number of ways to quantify the changes. The EEM peak T (tryptophan-like compounds) will decrease in a WWTP as treatment progresses, which can be associated with BOD_5 removal (Hudson et al., 2007; Henderson et al., 2009). All

fluorescence will decrease as a result of filtration (Henderson et al., 2009). The change in SMPs and biodegradable can be further quantified with the fluorescence index. The findings of McKnight et al. (2001) were that the fluorescence index was greater for water with microbial contaminants (SMPs) and less for terrestrial contaminant. It would be reasonable to assume that a higher FI will be observed in the effluent due to the formation of SMPs in the biological treatment, and a lower FI will be observed in the influent.

Furthermore, anthropogenic compounds can be identified using fluorescence (Laws et al., 2011). WWTPs are designed to remove biodegradable organic matter, but fluorescence can be used to quantify the oxidization of anthropogenic organics during the treatment process. This is beneficial for treatment plants that are designed for water reuse. In a closed loop water reuse system, organics that are not removed in the treatment processes will inevitably increase in concentration and thus increase the loadings on the treatment facilities.

2.7.9 How Organics Are Quantified With Absorbance

The absorbance of a sample is independent of the fluorescence properties; therefore, it is possible for a sample to absorb light in the absence of fluorophores. Specifically, molecules that absorb light are chromophores; it is possible for a sample to have chromophores and lack fluorophores (Hudson et al., 2007). Absorbance is related to the amount of light that is transmitted through the sample. Equation 2.2 shows the relationship between the light intensity that enters the sample (I_0) and the light that passes through the sample (I_T). The amount of absorbance (A) is directly related to the path length (d) that the light follows, the molar absorptivity (ϵ), and the concentration in the sample, as described by Beer's Law (Sauer et al., 2011).

$$A = -\log_{10}\frac{I_0}{I_T} = \epsilon cd \qquad \text{Eqn 2.2}$$

$$A_{spectrophotometer} = \sum A = (\epsilon cd)_1 \ldots (\epsilon cd)_2 + (\epsilon cd)_n \qquad \text{Eqn 2.3}$$

The spectrophotometer gives the absorbance property of the entire sample (Equation 2.3). Absorbance alone cannot identify the particular contaminants in the sample. Without information regarding the contaminants and their relative contribution in the sample, it is not possible to determine their respective concentrations. The absorbance results can give a relative understanding of the concentration of contaminants in the sample, and therefore be used as surrogate for the degree of contamination in the sample. The absorbance of the sample is assumed to be proportionate to the organic matter in the sample. Single incident wavelengths can arbitrarily be chosen to determine the absorbance properties of a sample, but they must be used consistently because the absorbance will change at different wavelengths. Absorbance is typically reported in cm^{-1} at a specific incident wavelength.

2.8 Comparison of the Methods

Table 2.8 provides a comparison of each of the methods, including the advantages and disadvantages. It is clear that each method quantifies organic matter differently; therefore, the results of the methods are not interchangeable. It is possible, however, to develop relationships between each of the methods. A clear understanding of how each method quantifies organic matter and the removal of organic matter in the WWTP is crucial to interpreting relationships between the methods.

Organic matter that is easily biodegradable is quantified in the BOD_5 method. The potassium dichromate used in the COD test will oxidize biodegradable matter and inorganics,

as well as recalcitrant matter; therefore, the results of the COD test will always be higher than the results of the BOD_5 test. The absorbance of a sample is assumed to be proportionate to the organic concentration of the sample, based on the presence of chromophores.

The TOC test measures the total organic carbon based on the amount of carbon that is converted to CO_2. The type of organic matter quantified can vary depending on how the analyst chooses to perform the test. For instance, dissolved organic carbon and suspended organic carbon can be determined by filtering the sample. Alternatively, a sample can be analyzed to determine both organic and inorganic (bicarbonate and carbonate). Inorganic interference due to the carbonates can be eliminated when the sample is acidified and sparged, but some organics may be purged during this process that can cause a low bias in the TOC results.

Fluorescence quantifies the dissolved organic matter (DOM) based on its molecular properties. This test has the distinct advantage over the methods because it can discretely identify the organics present. This can be used to identify recalcitrant and SMPs in the effluent water. Fluorescent DOM (FDOM) can be identified as peaks in the EEM, which can include humic-like acids, fulvic-like acids, and proteins (mostly in the form of the amino acid tryptophan, which is a building block for proteins). Sometimes it is useful to consider a range of excitation-emission wavelengths; in this case, the EEM can be broken into regions. Reynolds and Ahmad (1997) have also shown that biodegradable organics and nonbiodegradable organics can be distinguished from one another, which makes it more comparable to the BOD_5 test.

The fate of organic matter in the treatment system is important to the relationships between the methods. Particularly, biodegradable organic matter is easily removed during

biological treatment and recalcitrant organic matter can build up in the process. SMPs are created during the treatment process as well. The BOD_5 and fluorescence methods can quantify biodegradable organic matter in the sample. The TOC, COD, and fluorescence will quantify the recalcitrant and SMPs in the effluent samples, whereas BOD_5 method will not. Fluorescence is the only method that can differentiate between biodegradable and nonbiodegradable organics, humic-like acids, fulvic-like acids, and proteins.

The fact that each of these methods quantifies different types of organic matter is particularly important. The organic matrix evolves as treatment progresses, specifically labile organic matter will decompose, but the recalcitrant matter will remain. Thus, the BOD_5 will decrease at a different rate than the TOC and COD as treatment progresses, which will impact the ratios between these methods. The ratios will be discussed in further detail later in the thesis.

Table 2.8: Comparison of TOC, BOD_5, TOC, and Fluorescence Methodologies

	BOD_5	**COD**	**TOC**	**Fluorescence**
Organics	Biodegradable organics	Organics	Total organic carbon	Dissolved organic matter (DOM)
Interference	Inorganics (sulfur and iron) and nitrogenous demand (unless it is inhibited)	Inorganics are prone to chemical oxidation	Inorganics can bias results high, unless they are acidified and sparged prior to TOC analysis. Some of the organics may be lost during this process.	Inorganics and chemical reducing compounds can absorb light
Advantages	✓ Mimics the biological process of the treatment plant ✓ Approved by the Standard Methods Method ✓ Required by NPDES permits	✓ Quick and easy to perform ✓ Hach equipment is available that nearly anyone (non lab personal) can be trained to use. ✓ Some labs already have the spectrophotometer that they use for other purposes ✓ Effective at oxidizing 95-100% of the organic compounds	✓ Fractions of TOC can be determined ✓ Some TOC analyzers can also determine total Nitrogen (TN) while running the TOC test ✓ Inorganic interference can be eliminated	✓ Can uniquely identify organics based on their fluorescence properties ✓ Can distinguish between NOM, SMPs, and anthropogenic organic matter ✓ Can distinguish between biodegradable and nonbiodegradable
Disadvantages	▪ 5-days to obtain results ▪ Sensitive to organic and bacterial contamination ▪ Cannot reanalyze sample if test fails ▪ Labor intensive	▪ Chemicals used in test are hazardous waste ▪ Unrepresentative samples (e.g., particulate) can impact the quality of results ▪ Potassium dichromate will also oxidize inorganic compounds	▪ Sensitive to organic contamination ▪ Injection points can get clogged ▪ Expensive equipment	▪ Not an approved Standard Method ▪ Does not have a standardized control ▪ Some organics do not fluoresce (e.g., carbohydrates)

Part C: Existing Literature on Alternative Correlations

2.9 Existing BOD_5 and COD Correlations

Several studies have concluded that BOD_5 and COD can be correlated for different water sources (Table 2.9), but samples with minimal organic pollution (e.g., BOD_5 <3 mg/L) may be more difficult to correlate (Aziz and Tebbutt, 1979; Jin et al., 2009). This is likely due to the reporting limits of the BOD_5 method, although the authors did not conclude this. A report by Esener et al. (1981) concluded that the ratio between BOD and COD is reliant on the efficiency of the microbial growth, and that microbial growth is a function of environmental conditions. Both microbes and environmental conditions will vary among water sources; therefore, the correlations may not be consistent among water sources, as suggested by the literature.

A study by Rowe (1968) compared the relationship between synthetic sewage and influent domestic sewage from a WWTP in Louisiana; he concluded that both had strong linear relationships that could be used to estimate BOD_5 within 5%, but correlations were different because the synthetic water responded better to biodegradation compared with the sewage from the WWTP. The samples were diluted to three concentrations to create the correlations. The correlation equations are reported in Table 2.9.

A study conducted by Jin et al. (2009) explored the relationship BOD_5 and COD in coastal seawaters. This study was particularly interesting because the BOD_5 was not determined using the traditional Standard Method, but instead using a "BOD fast measurement system" that estimated the BOD_5 results within thirty minutes. The system consisted of a BOD sensor film that was composed of a dissolved oxygen membrane and

aerobic bacteria. An LED light emits light at 495 nm that excites the BOD sensor film, which will emit fluorescence at 580 nm for detection. The depletion of dissolved oxygen in the water is quantified using an oxygen sensor. This type of monitoring system may be of interest to WWTPs as an alternate test procedure for determining their BOD_5. The results of the study showed that a significant relationship exists for polluted (i.e., $BOD_5 > 3$ mg/L) brackish water ($r = 0.983$), but relatively clean coastal water (i.e., $BOD_5 < 3$ mg/L) did not have a significant relationship.

Domestic wastewater was collected and treated with a continuous flow activated sludge plant (i.e., primary clarifier →aeration basin → secondary clarifier) in the research conducted by Aziz and Tebbutt (1979). A statistically significant linear relationship was established for settled influent, but they concluded that a relationship could not be established for treated secondary clarifier effluent, as shown in Table 2.9. The COD/BOD_5 ratio was approximately 2.412 in the influent and decreased to 1.438 in the secondary clarifier effluent, due to biological treatments. The ratio is somewhat higher compared with other literature that reported a ratio of 1.1 to 2.4 and 1.85 for influent samples (Dazae, 1974; Eckenfelder and Ford, 1970)

The conclusions of Dubber and Gray (2010) were that settled domestic wastewater influent correlated well, but the effluent samples did not have a significant relationship based on samples collected from 11 WWTPs in Ireland. Their influent model was similar to Ademoroti's (1986) generic model. They concluded that their effluent samples did not correlate well because the ratio of BOD to COD varied too much among the WWTPs, which was expected due to the different treatment efficiencies.

Table 2.9: Existing BOD_5 and COD Correlations

BOD-COD Conclusion	r	Statistically significant	Source	Reference
BOD_5=0.46*(COD) - 20	NR	Yes	Domestic WW Harahan, LA	Rowe (1968)
BOD_5=1.03*(COD) - 58	NR	Yes	Synthetic WW	Rowe (1968)
COD=3.54*(BOD_5) + 2.38	r=0.98	Yes	Brackish coastal seawater BOD_5 > 3mg/L	Jin et al. (2009)
NR	r=0.34	No	Brackish coastal seawater BOD_5 < 3mg/L	Jin et al. (2009)
BOD_5=0.413*(COD) + 1.22	r^2=0.75	Yes	Settled domestic WW	Aziz and Tebbutt (1979)
NR	NR	No	Domestic WW secondary treatment	Aziz and Tebbutt (1979)
BOD_5=0.4259*(COD) + 50.99	NR	Yes	WWTP Tehran, Iran	Abyaneh (2014)
BOD_5=0.589*(COD) – 11.3	r^2=0.98	Yes	Settled domestic WW 11 WWTPs in Ireland	Dubber and Gray (2010)
NR	r^2=0.49	No	Final effluent WW from 11 WWTPs in Ireland	Dubber and Gray (2010)
COD=1.64*(BOD_5) + 11.36	NR	Yes	Domestic influent WW	Ademoroti (1986)
COD=1.27*(BOD5) - 134.5	r=0.96	Yes	Sewage	Wagh and Shrivastava (2007)
COD=0.87*(BOD5) - 3.99	r=0.96	Yes	Groundwater	Wagh and Shrivastava (2007)

2.10 Existing BOD_5 and TOC Correlations

There are inconsistencies in the literature regarding the ability to correlate BOD_5 and TOC among a variety of water sources. Some of the literature suggests that the relationship between BOD_5 and TOC is insignificant, while others suggest that correlations exist, as summarized in Table 2.10.

Viraraghavan (1976) reported that the relationship between TOC and BOD_5 was insignificant for raw sewage, septic tank effluent, and polluted groundwater. Aziz and Tebbutt (1979) found a weak relationship for domestic wastewater. Relationships between TOC and BOD_5 did not have a significant relationship for surface water collected from different water sources in Minnesota (Maier and McConnell, 1974). Dubber and Gray (2010) reported insignificant relationships between TOC and BOD_5 for eleven wastewater treatment plant effluents, which was likely due to variations in the treatment processes and due to the buildup of recalcitrant matter during biological treatment.

There have been many successful correlations made between BOD_5 and TOC. For instance, Schaffer et al. (1965) developed a relationship for the combined sample points of influent, primary effluent, and secondary effluent. Dubber and Gray (2010) concluded that a significant model for settled domestic influent existed for samples collected from 11 WWTPs in Ireland. It should be noted that Dubber and Gray (2010) also concluded that the collective effluent samples from the 11 WWTPs could not be correlated well because of the variability in the treatment processes.

Ostendorf and Byrd (1969) did not report a linear equation, but they concluded that the relationship between TOC and BOD_5 was significant at a 95% confidence interval for effluent at a paper mill plant. The work of Emery et al. (1971) concluded that TOC and

BOD_5 relationships were significant for water samples collected from the Cherokee Reservoir in Tennessee.

Chandler et al. (1976) successfully developed relationships for BOD_5 and TOC for six WWTPs in Massachusetts for both influent and effluent water. Five of the plants were grouped together because they discharge into the same receiving water, but the treatment trains were not all the same. The treatment train of one of the plants consisted of an extended aeration facility without primary clarification, one of the plants used conventional activated sludge system followed by an a nitrifying activated sludge system, and the other three plants were trickling filter facilities. Despite the differences in treatment efficiencies, the researchers were able to develop models for both inhibited and uninhibited BOD_5 for the combined treatment plant effluent. This is contradictory to Dubber and Gray (2010), who concluded effluent could not be correlated with different combined effluents due to treatment efficiencies. A correlation for the sixth treatment plant (conventional activated sludge) was developed independently; decent correlations were developed for both raw influent and secondary effluent.

It was found that the BOD_5/TOC ratio was approximately 2.0 for the raw wastewater and decreased to 0.53 for the secondary effluent for the combined treatment plants. The ratios for the conventional activated sludge plants varied between 0.67 and 2.53 for the influent and varied between 0.22 and 0.78 for the effluent water (Chandler et al., 1976). Other literature have reported ratios from 1.3 to 1.9 and 1.35 to 2.62 for influent wastewaters (Ford, 1968; Eckenfelder, 1970)

Santa Cruz Wastewater Treatment Facility currently uses TOC to estimate BOD_5 for their NPDES permit reporting. The plant uses an exponential relationship to estimate their

influent BOD_5 loadings and a linear relationship to estimate their effluent BOD_5. Several data points collected over a year showed that the ratio for influent ranged (TOC/BOD_5) from 0.31 to 0.58 for the influent, indicating little variability (Babatola and Xu, 2010). Indeed, other wastewater plants such as Clark County Water Reclamation District and Inland Empire Utilities Agency also use TOC to estimate their BOD_5 (Drury, 2014).

Constable and McBean (1979) explored the use of different statistical models to relate BOD_5 and TOC. Specifically, they used more complex models that account for errors to determine if they were more suitable than the traditional linear model. It was shown that the traditional linear model had the highest correlation coefficient, so more complex models were not necessary. The correlation coefficient for primary influent and primary effluent was 0.901 and 0.743, respectively, showing significant relationships for both samples. It was shown that the model behaved well for the sampling period in which it was derived, but the model performed poorly during different sampling periods.

The literature indicates that some studies were able to correlate TOC to BOD_5, but others were not. It can be speculated that the differences in the methodologies or the difference in water matrices could have contributed to poor correlations. The unsuccessful studies should not overshadow the successful correlations by other researchers. The fact that three operating WWTPs are currently allowed to use TOC to estimate BOD_5 is encouraging considering the potential time and cost savings associated with such change.

Table 2.10: Existing BOD_5 and TOC Correlations

BOD-TOC Conclusion	r	Statistically Significant	Source	Reference
BOD_5=0.031*(TOC)+554	r=0.02	No	Raw sewage	Viraraghavan (1976)
BOD_5=1.20*(TOC)+48	r=0.34	No	Polluted groundwater	Viraraghavan (1976)
NR	NR	No	Influent domestic wastewater	Aziz and Tebbutt (1979)
NR	r=0.24	No	Surface Water MN	Maier and McConnell (1974)
BOD_5=1.875*(TOC)-11.6	r^2=0.94	Yes	Domestic WW combined at 3 sites	Schaffer et al. (1965)
BOD_5=1.68*(TOC)+23.7	r^2=0.96	Yes	Settled domestic WW 11 WWTPs	Dubber and Gray (2010)
NR	r^2=0.33	No	Final effluent WW from 11 WWTPs	Dubber and Gray (2010)
BOD_5=1.507*(TOC)-55.43	r=0.90	Yes	Primary influent from WWTP	Constable and McBean (1979)
BOD_5=1.336*(TOC)+2.544	r=0.74	Yes	Primary effluent from WWTP	Constable and McBean (1979)
$TOC=0.479*(BOD)^{0.98}$	r^2=0.82	Yes	Primary influent from WWTP	Babatola and Xu (2010)
$TOC=9.052*(BOD)^{-0.9409}$	r^2=0.93	Yes	Effluent from WWTP	Babatola and Xu (2010)
NR	NR	Yes	Effluent of a paper mill plant	Ostendorf and Byrd (1969)
$BOD_{5\ uninhibited}$ =0.64*(TOC)+19.6	r=0.71	Yes	Effluent of 5 WWTPs	Chandler et al. (1976)
$BOD_{5\ inhibited}$=0.84*(TOC)-1.9	r=0.81	Yes	Effluent of 5 WWTPs	Chandler et al. (1976)
BOD_5=0.84*(TOC)+86.15	r=0.79	Yes	Raw influent of 1 WWTP	Chandler et al. (1976)
BOD_5=0.57*(TOC)-0.58	r=0.76	Yes	Secondary effluent of 1 WWTP	Chandler et al. (1976)
BOD_5=0.0708*(TOC)+0.2470	r=0.69	Yes	Reservoir	Emery et al. (1971)

2.11 Existing BOD_5 and Absorbance and Fluorescence Correlations

Absorbance is typically reported at 254 nm wavelengths, but other wavelengths can be adopted to reduce interference (Standard Methods, 2005). Studies have shown that absorbance can be successfully correlated at BOD_5 at several different wavelengths including 250 nm, 254 nm, 260 nm 280 nm, and 650 nm (Comber et al., 1996; Wu et al., 2006; Chevakidagarn, 2007; Natarja et al., 2006; Michail and Idelvoitch, 1981). Investigation of fluorescence has been gaining attention over absorbance because it has the potential to be used for real time monitoring and it can be used to identify organics based on their fluorescent properties (Reynolds and Ahmad, 1997; Ahmad and Reynolds, 1999; Reynolds, 2002; Hudson et al., 2007).

In the 1990s, Ahmad and Reynolds began to explore fluorescence as a way to identify organic compounds in wastewater and relate it to BOD_5. When their research began, fluorescence was not a novel technique for organic quantification, but it was a novel technique for organic quantification in wastewater (Reynolds and Ahmad, 1995). These researchers have made significant contributions to the characterization of organic matter in wastewater through the use of fluorescence. This literature review begins with the work of Ahmad and Reynolds and progresses to current studies that have used fluorescence to characterize organic matter in wastewater and as a predictor for BOD_5.

A preliminary study (Ahmad and Reynolds 1995) was conducted using 24-hour composite wastewater samples from three wastewater treatment plants. It was shown that the amino acids tryptophan, lignin, and humic were highly fluorescent. Two major peaks occurred near 280 nm and 390 nm that correspond with biodegradable and humic substances, respectively. A comparison between the influent and the effluent samples showed a decrease

in fluorescence that ranged from 50 to 85%. The samples were filtered with 0.45-µm filters to determine the impact of suspended solids on the fluorescence. It was concluded that filtering did not impact the peak associated with the biodegradable matter, but the humic peak was substantially reduced.

The researchers expanded their work by exploring the relationship between inhibited BOD_5 and absorbance/fluorescence using real time monitoring (Reynolds and Ahmad, 1997). To simulate in situ monitoring effect, samples were magnetically stirred during the fluorescence analysis to simulate turbidity. Samples were collected from three WWTPs that were from a) industrial and domestic origins, b) industrial origins, and c) domestic origins. It was observed that significant linear relationships exist between absorbance at 254 nm and fluorescence intensity (excitation=280 nm, emission= 340 nm). The absorbance and fluorescence peaks occurred in the same location for each of the sample sites, but the magnitude of the peaks differed. Table 2.11 summarizes the ratios determined in this study, which indicate that the ratio decreases as treatment progresses; this is expected because BOD_5 is removed during the treatment process and it is consistent with the findings reported in the COD and TOC literature reviews. A comparison between influent and effluent samples indicated that there was a reduction in fluorescence across the entire matrix.

Ahmad and Reynolds (1999) concluded that biodegradable organic matter occurs near emission bands at 350 nm and nonbiodegradable organic matter occurs near emission bands at 440 nm. It was observed that the fluorescence decreased nearly 90% near the 350 nm emission band after wastewater treatment, which is indicative of biodegradable organic removal. Furthermore, there was approximately 60% reduction after treatment near the 440

nm emission band, which is indicative of nonbiodegradable organic matter that was not easily removed during the treatment process.

Table 2.11: BOD_5 ratios for Absorbance and Fluorescence adapted from Reynolds and Ahmad (1997)

Source	Water type	BOD_5/Abs λ_{ex}=254 nm	BOD_5 / Fl λ_{ex}=340 nm	BOD_5 / Fl λ_{ex}=450 nm
Domestic + Industrial	Raw settled WW	162.11	8.23	22.56
	Treated effluent	16.84	1.15	1.14
Industrial	Raw settled WW	113.28	6.94	21.74
	Treated effluents	30.19	1.84	2.34
Domestic	Raw settled WW	129.11	8.85	23.32
	Treated effluent	16.51	1.04	1.97

Reynolds (2002) compared synthetic and wastewater samples; the wastewater samples were grab samples from a treatment facility in England. It was shown that BOD_5 could be correlated to fluorescence intensities for both sample types, but the synthetic samples correlated better than the wastewater samples (Table 2.12). Furthermore, the wastewater fluorescence intensities were up to five times higher compared with the synthetic samples.

Table 2.12 summarizes the work of Ahmad and Reynolds. The fluorescence intensities (reported in AFU) were normalized to their respective Raman peaks at a specific emission/excitation wavelength. Fluorescence intensity in the 1997 study was reported at excitation and emission wavelengths of 280 nm and 340 nm, respectively. The emission wavelength was reduced from 280 nm to 248 in the subsequent 1999 study, but all other parameters remained the same. Relationships for the study conducted in 2002 show that synthetic sewage and actual sewage have similar slopes.

Table 2.12: Early BOD_5 and Fluorescence Relationships

BOD-Fluorescence Conclusion	Sig.	Source	Reference
Biodegradable OM fluoresces at λ_{ex} 280 nm	-	WW	Ahmad and Reynolds (1995)
Humic OM fluoresces at λ_{ex} 390 nm	-	WW	Ahmad and Reynolds (1995)
Eqn NR Abs λ_{254} =f(BOD_5)	r^2=0.97	Industrial and domestic WW	Reynolds and Ahmad (1997)
Eqn NR Abs λ_{254} =f(BOD_5)	r^2=0.95	Industrial WW	Reynolds and Ahmad (1997)
Eqn NR Abs λ_{254} =f(BOD_5)	r^2=0.87	Domestic WW	Reynolds and Ahmad (1997)
Eqn NR AFU = f(BOD_5) [Ex=280 nm, Em=340 nm]	r^2=0.93	Industrial and domestic WW	Reynolds and Ahmad (1997)
Eqn NR AFU = f(BOD_5) [Ex=280 nm, Em=340 nm]	r^2=0.94	Industrial WW	Reynolds and Ahmad (1997)
Eqn NR AFU = f(BOD_5) [Ex=280 nm, Em=340 nm]	r^2=0.89	Domestic WW	Reynolds and Ahmad (1997)
Eqn NR AFU = f(BOD_5) [Ex=248 nm, Em=340 nm]	r^2=0.97	Domestic WW	Reynolds and Ahmad (1999)
Biodegradable OM fluoresces at em. bands near 340 nm	-	Domestic WW	Reynolds and Ahmad (1999)
Nonbiodegradable OM fluoresces at em. bands near 440 nm	-	Domestic WW	Reynolds and Ahmad (1999)
AFU = 0.073(BOD_5) + 0.0061 [Ex=280 nm, Em=350 nm]	r^2=0.89	Synthetic WW (Total relative normalized fl. from 300 to 540 nm)	Reynolds (2002)
AFU = 0.070(BOD_5) +1.606e^{-4} [Ex=280 nm, Em=350 nm]	r^2=0.79	WW (Total relative normalized fl. from 300 to 540 nm)	Reynolds (2002)
AFU = 0.024(BOD_5) + 0.002 [Ex=280 nm, Em=350 nm]	r^2=0.98	Synthetic WW (Relative normalized fl. at 350 nm)	Reynolds (2002)
AFU = 0.025(BOD_5) +6.05e^{-4} [Ex=280 nm, Em=350 nm]	r^2=0.93	WW (Relative normalized fl. at 350 nm)	Reynolds (2002)
Biodegradable OM fluoresces at Ex=280 nm Em =350 nm	-	WW	Reynolds (2002)
Nonbiodegradable OM fluoresces at Ex=280 nm Em =440 nm	-	WW	Reynolds (2002)

A study (Baker and Invarartity, 2004) of sixty-two sites along a river impacted with sewage discharge concluded that BOD_5 correlated well (r=0.85) to tryptophan-like peaks at 220 nm excitation and 350 nm emission wavelengths. Furthermore, the relationship between BOD_5 and humic and fulvic peaks were found to be statistically significant (r=NR). It was found that BOD_5 can be predicted as a function of tyrosine-like fluorescence and fulvic fluorescence. An examination of the data revealed that some of the sample sites were considered outliers because their pollution loads were different from the other sites. When these outlier sample sites were excluded from the dataset, the relationship changed and became a function of tryptophan and fulvic fluorescence. The implications of this are that BOD_5 can be related to fluorescence parameters, but the relationship is most likely site specific due to the water matrix.

Chevakidagarn (2007) studied the relationship between BOD_5 and fluorescence in industrial wastewaters (i.e., Para rubber and seafood industries). They found the correlations to be variable and concluded that BOD_5 would be better correlated as a function of COD and fluorescence to obtain more consistent results.

A study by Hur et al. (2008) conducted on a river that was impacted with sewage discharge concluded that BOD_5 could be correlated to absorbance at 254 nm, and three EEM peaks. Three sample sites were included in the study that included sample sites both upstream and downstream from the plant. It was shown that the strongest correlations were developed when the sites were combined. The peaks could still be correlated for the upstream and downstream sites individually, but the relationship was weaker than when they were combined. Table 2.13 summarizes the correlations; fluorescence was normalized to the

Raman peak. Peak I corresponds to a 280-300 nm wavelength at $\Delta\lambda$=30 nm and peak A corresponds to a 285 nm wavelength at $\Delta\lambda$=60 nm.

Table 2.13: BOD_5 and Fluorescence Correlations adapted from Hur et al. (2008)

Sample point	Peak I	Peak A	UV_{254}
All 3 sample points	BOD_5=2.79x – 2.03	BOD_5=6.52x + 2.93	BOD_5=0.004x + 0.03
Upstream of WWTP	BOD_5=1.67x + 1.80	BOD_5=6.77x + 1.80	BOD_5=0.001x + 0.16
Downstream of WWTP	BOD_5=2.73x – 0.84	BOD_5=6.21x + 8.20	BOD_5=0.003x + 0.04

Hudson et al. (2008) conducted a study that included 469 sample sites that included industrial effluent, surface waters, and pollution sites. Corrections to the unfiltered samples were not made in the fluorescence analysis. The correlations were strongest for the combined sample sites and weakened when surface water and effluent were considered independently, which is consistent with the finding of Hur et al. (2008). The authors segregated the tryptophan peak into T_1 (excitation=280 nm and emission=350 nm) and T_2 (excitation=225-237 nm and emission=340 – 380 nm). It was determined that T_1 had a higher correlation than T_2, but they were both significant. The fluorescence associated with peak T_1 is believed to be related to microbial activity either because of the bioavailable substrate available or because it is the result of SMPs. Their conclusions were that BOD_5 correlated well with fluorescence at an excitation of 280 nm and an emission of 350 nm, which was consistent with the findings of the aforementioned studies (Reynolds and Ahmad, 1997; Ahmad and Reynolds, 1999; Baker and Invararity, 2004).

An investigation of domestic wastewater was conducted using grab samples collected via manholes in a sewage system (Hur et al., 2010). It was observed that BOD_5 could be

estimated by 0.397*PLF+11.2. A protein-like fluorescence (PLF) occurred between 250 and 300 nm, with corresponding BOD_5 values ranged from 5.2 to 208 mg/L. A similar PLF peak occurred in domestic sewage influent samples collected from six wastewater treatment plants (Lai et al., 2011). Tryptophan can be categorized as protein-like fluorescence; therefore, this study is consistent with previously mentioned studies. Lai et al. (2011) observed that the PLF was reduced in effluent matter, indicative of biodegradable organic removal during the biological treatment at the wastewater facility.

An early study (Comber et al., 1996) suggested that fluorescence could not be successfully correlated to BOD_5 in sewage effluents and rivers. That particular study was conducted at a fixed emission wavelength of 430 nm and two excitation wavelengths at 250 nm and 350 nm. Several studies have since contradicted these finding, with the general conclusion that BOD_5 can be related to fluorescence at specific excitation/emission (i.e., 280 nm and 350 nm) wavelengths associated with tryptophan (Reynolds and Ahmad, 1997; Ahmad and Reynolds, 1999; Baker and Invararity, 2004). It can thus be concluded that relationships cannot be developed at arbitrary points on the EEM graph.

All of the literature discussed until this point has reported peaks based on known protein, humic, and fulvic-like peaks (e.g., T peak, A peak, etc.) that were visually observed (Stedmon et al., 2003; Hur and Cho, 2012). Stedmon et al. (2003) introduced the idea of using parallel factor analysis (PARAFAC) to characterize organic matter in surface water. This method is preferred because it mathematically interprets the multiple EEM data points, rather than by human interpretation. Data are decomposed into trilinear components (i.e., three linear components that represent fluorescence, excitation, and emission wavelengths). The components are analyzed using statistical algorithms, which identify significant

components (e.g., EEM peaks associated with fluorescence of fluorophores). The reader is referred to Bro (1997), Stedmon et at. (2003), and Stedmon and Bro (2008) for more information regarding the principles of the PARAFAC model, which include a tutorial and a specific application to EEM fluorescence.

A PARAFAC model was used to identify EEM peak components in surface water samples collected over three days. Four of the components were related to previously identified humic-like peaks and one of the peaks was related to a tryptophan peak (Stedmon et al., 2003). Stedmon and Markager (2005) expanded on this research by collecting surface water samples for a year and then creating a new PARAFAC model. Eight components were identified in this research. Four components had terrestrial origins, two components had anthropogenic origins, and two components had protein-like fractions. Both of these studies showed that PARAFAC models are capable of discretely identify EEM peaks associated with organics from different origins.

Several studies have followed that utilize PARAFAC for water characterization, water quality monitoring throughout the wastewater treatment plant, and as a means to determine removal efficiency (Miettinen et al., 2004; Wang et al., 2007; Singh et al., 2009; Yu et al., 2010; Yu et al., 2013; Cohen et al., 2014; Li et al., 2014). Very few studies have implemented PARAFAC modeling to estimate BOD_5.

Hur and Cho (2012) used PARAFAC analysis to identify components in an urban river that is the receiving body for a wastewater treatment plant. Sample were collected from eight locations along the river (BOD_5< 25 mg/L) and filtered prior to analysis. Sites that were close to the treatment plant had the highest BOD_5 concentrations, which were reduced at sampling points downstream. The authors visually identified three peaks on the EEM. A

tryptophan-like peak (Ex=275 nm, Em=340 nm) was most prominent in sample sites near the treatment plant. Peak A (Ex=250 nm, Em=400-450 nm) and Peak C (Ex=330-340 nm, Em=350-400 nm) were observed in all of the samples. Each of these peaks increased as organic pollution increased. The PARAFAC model identified three components, which were consistent with the authors visual observations. The components were identified as C1 (Ex=250 nm, Em=405 nm), C2 (Ex=250 and 350 nm, Em=450 nm), and C3 (Ex=275 nm, Em=340 nm). BOD_5 could be correlated well to all three components, as well as absorbance at 220 nm and 254 nm. The authors did not consider regression analysis with multiple components to estimate BOD_5.

A study (Yang et al., 2014) of 22 wastewater treatment plants in Korea concluded that PARAFAC models can be used to identify EEM peaks and estimate BOD_5. Humic-like, fulvic-like, and protein-like peaks were identified in the PARAFAC model. The protein-like peak was the best parameter to estimate BOD_5. The correlation model was strengthened when suspended solids were included in the stepwise regression. The study also concluded that protein-like organic matter is most readily removed in wastewater treatment plants, followed by fulvic-like organic matter. Humic-like organic matter accumulated during the biological process, thus causing an increase to the humic fluorescence peaks.

This literature review on fluorescence has shown that BOD_5 correlates well with protein-like peaks that are associated with tryptophan fluorescence. Traditionally, peaks have been identified visually, but the use of PARAFAC modeling has been proven to be a useful tool that can identify these peaks through statistical algorithms. Several studies have investigated the use of PARAFAC to characterize water quality, but only two studies were identified that used PARAFAC modeling to estimate BOD_5. The PARAFAC models were

used to identify humic-like, fulvic-like, and protein-like peaks in the EEM, which is consistent with visual observations previously made. The protein-like peaks were shown to correlate well to BOD_5, which was consistent with previous literature. The consistency between the visualization of peaks and those found by statistical algorithms may suggest that PARAFAC analysis is not always necessary. It can be a useful tool when precision is needed, but visual observations may be sufficient. Furthermore, PARAFAC can be a useful tool to identify peaks that are not visually obvious. For the purposes of this research PARAFAC modeling was not used, because the literature has suggested that BOD_5 correlates well to specific excitation and emission wavelengths.

Alternate analytical methods, such as fluorescence regional integration (FRI), capture a range of excitation emission wavelengths that are associated with fluorescence peaks. FRI is useful because it combines several peaks into a single value. The advantage is that it is less information to process (e.g., five regions versus thousands of peaks). It is important to realize that FRI represents fluorescence that is integrated over an area of excitation/emission wavelengths, whereas PARAFAC and visual observation identify fluorescence at specific excitation/emission wavelengths. As such, these methods are not interchangeable, but rather represent the fluorescence peaks in different ways (i.e., at a single point or across a specified area).

There is a deficit in the literature regarding the use of FRI and BOD_5. The idea was first introduced in 2003, but no studies were identified that used the regions as estimators for BOD_5. Chen et al. (2003) proposed that the EEM could be broken intro five regions represented by humic-like acids, fulvic-like acids, soluble-microbial-product-like, aromatic (tyrosine), and aromatics (BOD_5). The region proposed by Chen et al. (2003) were modified

for this research to better represent the observed peaks of the samples. Region I of this research is associated with protein-like fluorescence. Based on the previous literature that correlated BOD_5 to protein-like fluorescence, it is hypothesized that BOD_5 can be correlated to the FRI region associated with protein-like fluorescence (i.e., region I). This hypothesis will be explored in this research.

Chapter 3

METHODOLOGY

Part A: Sample Collection

3.1 Sample Source, Collection, and Labeling

Samples used in this research were collected from an operating wastewater treatment plant (WWTP) located in Las Vegas, Nevada; the treatment train is depicted in Figure 3.1. The primary industry is tourism and the wastewater treated is purely domestic; there are no known industries contributing toxic wastes or hydrogen peroxide, which could interfere with the BOD_5 method. The treatment plants discharge into the Las Vegas Wash, which eventually flows into Lake Mead, which is the primary drinking water source for the entire Las Vegas Valley.

The three sample sites chosen for this research were the plant influent, primary clarifier effluent (hereafter referred to as "CABI") and finished effluent (referred to as effluent), (Figure 3.1).

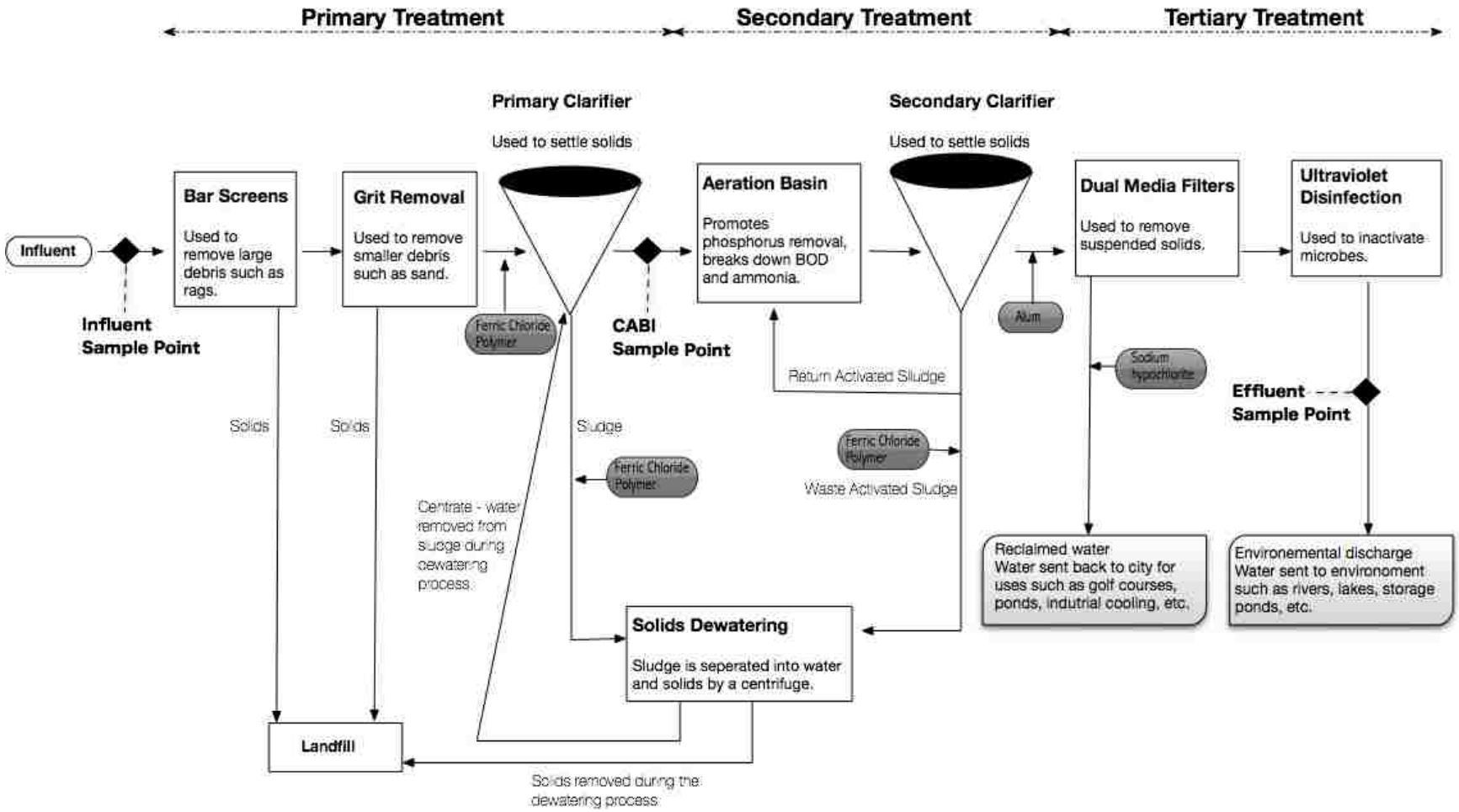

Figure 3.1: Schematic diagram of the wastewater treatment facility used as sample source for this research showing the three sample points used.

Samples were collected on Tuesdays, Wednesdays, Thursdays and Fridays from the middle of June 2014 through the end of September 2014 (Table 3.1 and Table 3.2). They were collected over a 24-hour period using a refrigerated compositor (Hach, Loveland, CO) and stored at 4 ± 1°C. During the composite period, the samples were collected in a 5-liter polyethylene cubitainer. Influent and effluent samples were composited proportional to flow. CABI samples were composited with time using a set volume aliquot. Generally, the composite samples were collected from approximately 7AM to 7AM. Sample labeling throughout this research refers to the date at the end of the composite time. If a sample was collected from 7AM July 1st to 7AM July 2nd, then the sample was labeled as July 2nd.

3.2 Sample Handling

Immediately following the composite period, the samples were transported in ice coolers to the onsite laboratory facility. The samples were immediately divided into their respective containers (Table 3.3) upon arriving at the laboratory. TOC samples were transferred to a 125 mL polypropylene bottle and preserved with hydrochloric acid at pH=2. TOC analysis was typically conducted the same day that the sample was collected; otherwise, the sample was analyzed the following day. Samples can be preserved for the COD test by acidification, but this was not done at any point during data collection because samples were analyzed within five hours.

When BOD_5 analysis was conducted the same day, approximately 500 mL of influent sample was transferred to a single amber glassware container. Similarly, approximately 500 mL of CABI sample was transferred to a single amber glassware container. The sample was analyzed for the COD, BOD_5, and fluorescence the same day as collection. Approximately 300 mL of effluent sample was transferred to an amber glassware container; this sample was

used for COD and fluorescence analysis. Approximately 1-liter of effluent sample was transferred to a polyethylene cubitainer and used for BOD_5 analysis.

When BOD_5 analysis was conducted the following day, samples were transferred into two separate containers, one sample was used for BOD_5 analysis the following day and the other sample was used for COD and fluorescence analysis. Approximately 250 mL of influent sample was refrigerated overnight and used for BOD_5 analysis the following day. Approximately 250 mL of influent sample was transferred into a separate amber glassware container and analyzed for COD and fluorescence the same day as collection. CABI samples were handled the same way as influent samples. Approximately 300 mL of effluent sample was transferred to an amber glassware container and analyzed for COD and fluorescence the same day that the sample was collected. Approximately 1-liter of effluent sample was transferred to a polyethylene cubitainer, refrigerated, and analyzed the following day for BOD_5.

When samples could not be analyzed immediately, they were refrigerated at 4 ± 1°C until analysis could be conducted. Table 3.3 summarizes the time frame within which that analysis typically occurred. All samples and reagents were brought to room temperature prior to analysis, including water samples, standards, dilution water, and sample seeds.

The amber glassware (BOD_5, COD, and fluorescence) and polypropylene containers (TOC) used to store the samples were cleaned by thoroughly rinsing with hot water a minimum of six times, and then it was given three rinses using deionized water. The bottles were stored upside down with their lid off.

Table 3.1: Collection Dates for Correlation Data and BOD_5 Group Holding Time Data

Correlation Samples [a, b]	**Comparative Samples** [a, b]
BOD_5 samples analyzed the same day (Samples used for correlation and for the BOD_5 grouped holding time comparison)	BOD_5 samples held overnight and analyzed the following day (Samples used for BOD_5 grouped holding time comparison)
6/25/14	6/24/14
6/27/14	6/26/14
7/2/14	7/1/14
7/3/14	7/8/14
7/9/14	7/10/14
7/11/14	7/15/14
7/16/14	7/17/14
7/18/14	7/22/14
7/23/14	7/24/14
7/25/14	7/29/14
7/30/14	7/31/14
8/1/14	8/5/14
8/6/14	8/7/14
8/8/14	8/12/14
8/13/14	8/14/14
8/15/14	8/19/14
8/20/14	8/21/14
8/22/14	8/26/14
8/27/14	8/28/14
8/29/14	9/2/14
9/3/14	9/4/14
9/5/14	-
9/9/14	-
9/11/14	-
9/16/14	-

[a]COD, fluorescence, and absorbance were analyzed the same day
[b]TOC was typically analyzed the same day (else the following day)

Table 3.2: Summary of Sample Holding Times for BOD, TOC, COD, and Fluorescence Analysis

Sample schedule for holding tests							
Date	**Holding test** Samples were analyzed the same day, refrigerated overnight, and then reanalyzed the following day	**Comments**	**Test conducted on sample**				**Included in correlation?**
			BOD	**COD**	**TOC**	**Fluorescence**	
9/9/14	Day 1 BOD	Day 2 GGA failed, so trial omitted	✓	✓	✓	✓	✓
9/10/14	Day 2 BOD		✓	×	×	×	×
9/11/14	Day 1 BOD	Trial A BOD hold test	✓	✓	✓	✓	✓
9/12/14	Day 2 BOD		✓	×	×	×	×
9/16/14	Day 1 BOD	Trial B BOD hold test	✓	✓	✓	✓	✓
9/17/14	Day 2 BOD		✓	×	×	×	×
9/30/14	Day 1 COD	Trial A COD hold test	×	✓	×	×	×
10/1/14	Day 2 COD		×	✓	×	×	×
10/1/14	Day 1 COD	Trial B COD hold test	×	✓	×	×	×
10/2/14	Day 2 COD		×	✓	×	×	×
10/6/14	Day 1 Fluorescence	Trial A fluorescence hold test	×	×	×	✓	×
10/7/14	Day 2 Fluorescence		×	×	×	✓	×
10/8/14	Day 1 Fluorescence	Trial B fluorescence hold test	×	×	×	✓	×
10/9/14	Day 2 Fluorescence		×	×	×	✓	×

Table 3.3: Summary of Sample Handling Techniques for TOC, COD, BOD_5, and Fluorescence Analysis of Wastewater Samples

Test Method	Storage Container	Typical time frame that analysis was completed	Chemical preservation available	Additional test information
TOC	125 mL polypropylene container for all samples	Typically the same day sample received, else the next day.	Yes	Samples were immediately preserved with HCl once they were received in the lab.
COD	Amber glassware for all samples.	Same day, usually between 9AM and 12 PM	Yes	Samples were not chemically preserved for this research.
BOD	Amber glassware for influent and CABI samples. Polyethylene cubitainer for effluent.	The BOD analysis was typically performed between 10 AM and 2 PM	No	Samples were analyzed either the same day or the following day, as summarized in Table 3.1.
Fluorescence	Amber glassware for all samples.	Same day, usually between 12 PM and 4PM	No	Sample analyzed the same day it was collected.

3.3 Sample Holding Schedule

Correlation data:

The sampling dates for the correlation data are summarized in Table 3.1 and Table 3.2. Samples that were analyzed the same day for BOD_5, COD, and fluorescence as collection were included in the correlation analysis. Effluent BOD_5 samples that were held overnight were also included in the correlation data, because they were shown not to be statistically different than the samples analyzed the same day. These results are presented and discussed in the Chapter 4 Results. All TOC data was included in the correlation, regardless of the analysis timeframe because the samples were preserved.

BOD_5 grouped data:

Two groups of BOD_5 data were collected during the summer. The first group of data were collected and analyzed the same day they were collected. These are labeled in Table 3.1 as the correlation data. The second group of data, labeled as the comparative data in Table 3.1, is independent of the first group. Samples used for comparison were collected and then refrigerated with headspace overnight. A BOD_5 analysis was performed on the samples the day following collection.

Holding test data:

Samples were collected and analyzed the same day that they were collected. The same samples were held overnight and then reanalyzed the following day. The results of the first day were compared with the results obtained the following day to determine if they were statistically different from one another. Holding tests were conducted on samples for BOD_5, COD, and fluorescence, as summarized in Table 3.2.

3.4 Sample Replicates

The BOD replicate schedule is shown is Table 3.4. It was not possible to have multiple dilutions of the effluent sample due to the sample volume required to conduct the analysis.

Table 3.4: BOD Sample Replicate Schedule

BOD sample replicate schedule	
Tuesday	3 Influent dilutions + 2 replicates of middle dilution
	3 CABI dilutions
	2 to 3 Effluent dilutions
Wednesday	3 Influent dilutions
	3 CABI dilutions + 2 replicates of middle dilution
	2 to 3 Effluent dilutions
Thursday	3 Influent dilutions + 2 replicates of middle dilution
	3 CABI dilutions
	2 to 3 Effluent dilutions
Friday	3 Influent dilutions
	3 CABI dilutions + 2 replicates of middle dilution
	2 to 3 Effluent dilutions

For the COD test, either the CABI or effluent sample was tested in triplicate each analysis day, based on a rotation schedule. The influent sample was always tested in triplicate due to the high standard deviation that was observed with this sample.

One sample site (influent, CABI, or effluent) was analyzed in triplicate each day the fluorescence test was performed, based on a rotation schedule.

Samples for the TOC test were not analyzed in triplicate, but several other QA/QC controls were implemented in the analysis, as discussed in later in this thesis under the TOC methodology.

Part B: Methods

3.5 BOD_5 Methodology

(Adapted from the Standard Methods (2005))

The BOD_5 was determined by diluting the sample and incubating it for five-days. The BOD_5 is calculated based on the initial and final dissolved oxygen readings. Seed blank samples, glucose glutamic acid (GGA) samples, and dilution water blank samples were also prepared with each analysis to validate the test results. For more information regarding background of this method, the importance of the additional samples, and a discussion on the interpretations of the results, refer to Chapter 2 Literature Review.

Wheaton (Millville, NJ) 300 mL glass BOD bottles with glass pennyhead stoppers were used for this research. Polyethylene BOD bottle caps were used to prevent evaporation during the incubation period.

The dissolved oxygen (DO) reading was performed using an LDO (luminescent dissolved oxygen) probe. The specific probe used for this research was the YSI Pro O DO probe (Yellow Springs, OH). When not in use, the probe was stored in a BOD bottle to prevent it from becoming damaged. Daily probe calibration was not necessary (as per the instruction manual). If alternate probe technologies are used, such as a membrane electrode probe, then daily calibration may be necessary to eliminate drift (Standard Methods, 2005).

3.5.1 Quality Control/Quality Assurance

The dilution water blank was used to ensure that the water being used in the test procedure was free from, microorgansisms, organics and metals that can interfere with test procedure and also to verify the cleanliness of the incubation bottles. Glucose glutamic acid (GGA) was used to ensure the seed was effective and ensure proper technique.

Samples that met the minimum depletion requirements were reported and were verified to be within 30% of one another were reported. As discussed in the Chapter 2 literature review, samples must have a residual dissolved oxygen above 1 mg/L to ensure there was enough oxygen available during the incubation period. Additionally, the samples had to deplete a minimum of 2 mg/L to ensure that the results were meaningful.

3.5.2 Sample Preservation

When samples could not be analyzed immediately, they were stored at 4 ± 1°C.

3.5.3 Interferences

Nitrogenous demand can interfere with the test, unless an inhibitor is used. Uninhibited BOD_5 is required on the NPDES permit; therefore, inhibitors were not used in this research. As discussed in the literature review, uninhibited BOD_5 is the sum of the carbonaceous (demand from biodegradable organic matter) and the nitrogenous demand (demand from ammonium). Iron and sulfur compounds can also interfere with the test, but inhibitors or corrections are not available to adjust for these. Additional interferences can occur due to dirty glassware, poor dilution water, inadequate seed source, and poor technique.

3.5.4 Dilution Water Source

Treated tap water, using a pre-filter, a carbon filter, an RO system, and a polishing treatment, was used for dilution water for this study, as illustrated in Figure 3.2. Raw tap water has a chlorine residual, and it contains organics and bacteria that will interfere with the BOD_5 test. The carbon tank and pre-filter carbon filtration remove chlorine and provide preliminary filtration. After the pretreatment, the water goes through an Elix 70 (Darmstadt,

Germany) reverse osmosis (RO) process that produces water quality that has <10 cfu/mL of bacteria, >99.9% silicate removal, and >5 MΩ-cm resistivity. After the water leaves the RO system, it goes through two ion exchange columns that further reduce the ion concentration, resulting in water that has a resistivity of approximately 18.2 MΩ-cm.

The water is then sent into a large storage tank fitted with ultraviolet (UV) disinfection and recirculating pipes designed to keep the water flowing until it is ready to use. The UV prevents bacteria regrowth, and the flow of water prevents water from becoming stagnant.

Before the water is used as a dilution water source for the BOD_5 test, it is passed through a polishing water treatment system (Milli-Q Advantage A10 water purification system, Darmstadt, Germany). The system consists of four cartridges: the first is an activated carbon filter, the second and third contain deionizing ion-exchange resins, and the fourth is a cartridge containing ion exchange designed to remove organic compounds. The water spigot in this system contains a 0.22-μm membrane filter to further remove microorganisms before it is dispensed.

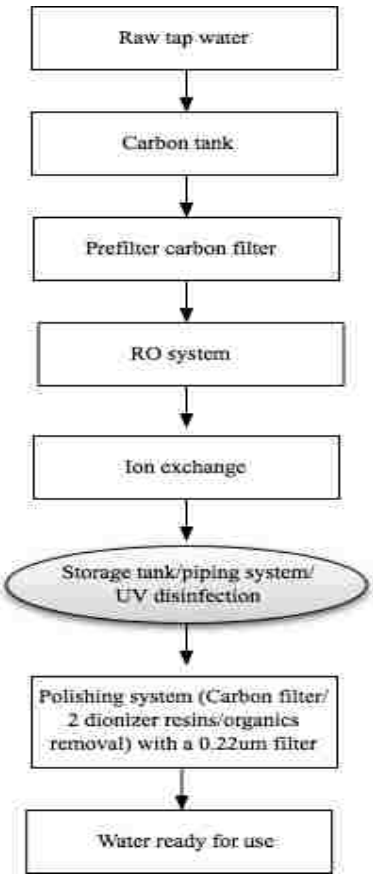

Figure 3.2: BOD dilution water treatment system.

3.5.5 Dilution Water Preparation

The dilution water was collected in a 10-liter polyethylene jug and placed into the BOD incubator overnight. The water jug was removed from the incubator the following day, and a premixed Hach (Loveland, CO) BOD nutrient buffer pillow was added. The buffer pillows are composed of ammonium chloride, calcium chloride, ferric chloride, potassium phosphate monobasic, demineralized water, sodium phosphate dibasic, magnesium sulfate, and potassium phosphate dibasic. The buffer pillow was added to the dilution water, and then the jug was shook for several minutes to thoroughly mix the buffer pillow into the water.

Two dilution water blanks were incubated for five days to verify that the water did not consume more than 0.2 mg/L of dissolved oxygen, as per Standard Methods (2005) requirements. In cases where the dilution water consumed more than 0.2 mg/L of oxygen, the data were reported, but it was not included in the statistical analysis.

3.5.6 Glucose Glutamic Acid

A commercially available glucose glutamic acid (GGA) manufactured by North Central Laboratory (Birnamwood, WI) was used for this research. The GGA standard is designed for a BOD_5 of 198 ± 30.5 mg/L, as per Standard Methods (2005) specifications. The GGA is used to control the strength of the seed and as a quality control to ensure the cleanliness of the glassware and analytical technique.

As per the manufacturer's directions, the GGA sample was prepared with 6 mL of the GGA standard and seeded in a 300 mL BOD bottle. The volume of seed added was the equivalent to the volume of seed used for the samples.

The GGA standard was refrigerated between uses at 4 ± 1°C. Prior to the BOD test, the GGA standard was brought to room temperature.

3.5.7 Seed Preparation

The sample seed was prepared with primary influent from the WWTP. The primary seed sample was refrigerated at 4 ± 1°C overnight. The supernatant was poured off the following day and used as the seed source. The seed was brought to room temperature prior to the BOD test.

3.5.8 Sample Pretreatment

According to the Standard Methods (2005), the pH should be adjusted using sulfuric acid or sodium hydroxide to values between 6-8. For this research, the pH was verified on the day of the BOD test. Each sample fell within this range, so pH adjustments were not necessary.

Chlorine is not added during the treatment process; therefore, a residual is not expected in any of the samples. Potassium iodide was used to verify that chlorine was not present in the sample.

Influent wastewater to this particular WWTP does not contain any toxic metals or hydrogen peroxide; therefore, adjustments were not necessary to accommodate these interferences.

3.5.9 Cleaning Procedure

The dilution water jug was cleaned with three hot water rinses and then three deionized water rinses immediately before it was filled with dilution water.

All containers used for seed or sample storage were rinsed with a minimum of six hot water rinses and then three deionized water rinses. Volumetric containers were rinsed with hot tap water after each use.

BOD bottles, bottle stoppers, and plastic covers were cleaned using a commercial dishwasher (Miele, Auborn Hills, MI) with a disinfection cycle. Dishwasher detergent (Miele, Auborn Hills, MI) was added to the dishwasher as well. After the dishwasher cycle, the bottle stoppers were rinsed with deionized water. Each BOD bottle was rinsed with deionized water three times, and then the stoppers and plastic caps were immediately placed on the bottle to prevent contamination.

The DO and pH probes were rinsed with deionized water prior to taking the reading.

3.5.10 Standard Operating Procedure for the BOD_5 test

Preparation the day immediately preceding the BOD test

1) A polyethylene jug was filled with approximately 7 liters of dilution water. The dilution water was stored in the incubator (20 ± 1°C) overnight with the lid lightly screwed on to allow the water to degas.
2) The sample seed was prepared by obtaining a sample of primary influent. The influent sample was stored in the refrigerator overnight at 4 ± 1°C.

Preparation the day of the BOD test

1) All of the samples were brought to room temperature.
2) The GGA was removed from the refrigerator and brought to room temperature.
3) The seed source was prepared. The primary influent sample was removed from the refrigerator and the supernatant was poured off the sample into a separate container. The supernatant was brought to room temperature and used as the seed source.
4) The dilution water was prepared. The Hach buffer pillow was thoroughly mixed before adding it to the dilution water. The dilution water was removed from incubator and the Hach nutrient buffer pillow was added. The dilution water was mixed for approximately 2 to 3 minutes to ensure the pillow was thoroughly mixed also to release excess dissolved oxygen.

   Note: The dilution water should be at 20 ± 3°C and the initial dissolved oxygen reading of the dilution water should be between 7.5 and 9 mg/L. Adjustments to the temperature were made when necessary.
5) Calibration of the DO probe and pH probe were verified and adjusted when necessary.

BOD test procedure

The test procedure is outlined in Figure 3.3 and the sample/seed volumes used in this research are given in Table 3.5. The effluent volumes were occasionally modified due to limited sample availability; the seed sample remained at 2 mL throughout the data collection. The influent and CABI sample dilutions were not modified. The actual sample volumes used during the experiment are appropriately labeled in the data sheets.

Table 3.5: BOD_5 Sample Volumes for GGA, Seeds, Influent, CABI, and Effluent

Sample	Sample Volume	Seed volume	Dilution Water
Blank duplicate A & B	0 mL	0 mL	Fill to middle of bottleneck of 300 mL BOD bottle
GGA duplicate A & B	6 mL	2 mL	
Seed dilution 1	0 mL	3 mL	
Seed dilution 2	0 mL	5 mL	
Seed dilution 3	0 mL	7 mL	
Seed dilution 4	0 mL	10 mL	
Influent dilution 1	2 mL	0 mL	
Influent dilution 2	3 mL	0 mL	
Influent dilution 3	5 mL	0 mL	
CABI dilution 1	3 mL	0 mL	
CABI dilution 2	5 mL	0 mL	
CABI dilution 3	10 mL	0 mL	
Effluent dilution 1	100 mL	2 mL	
Effluent dilution 2	150 mL	2 mL	
Effluent dilution 3	200 mL	2 mL	

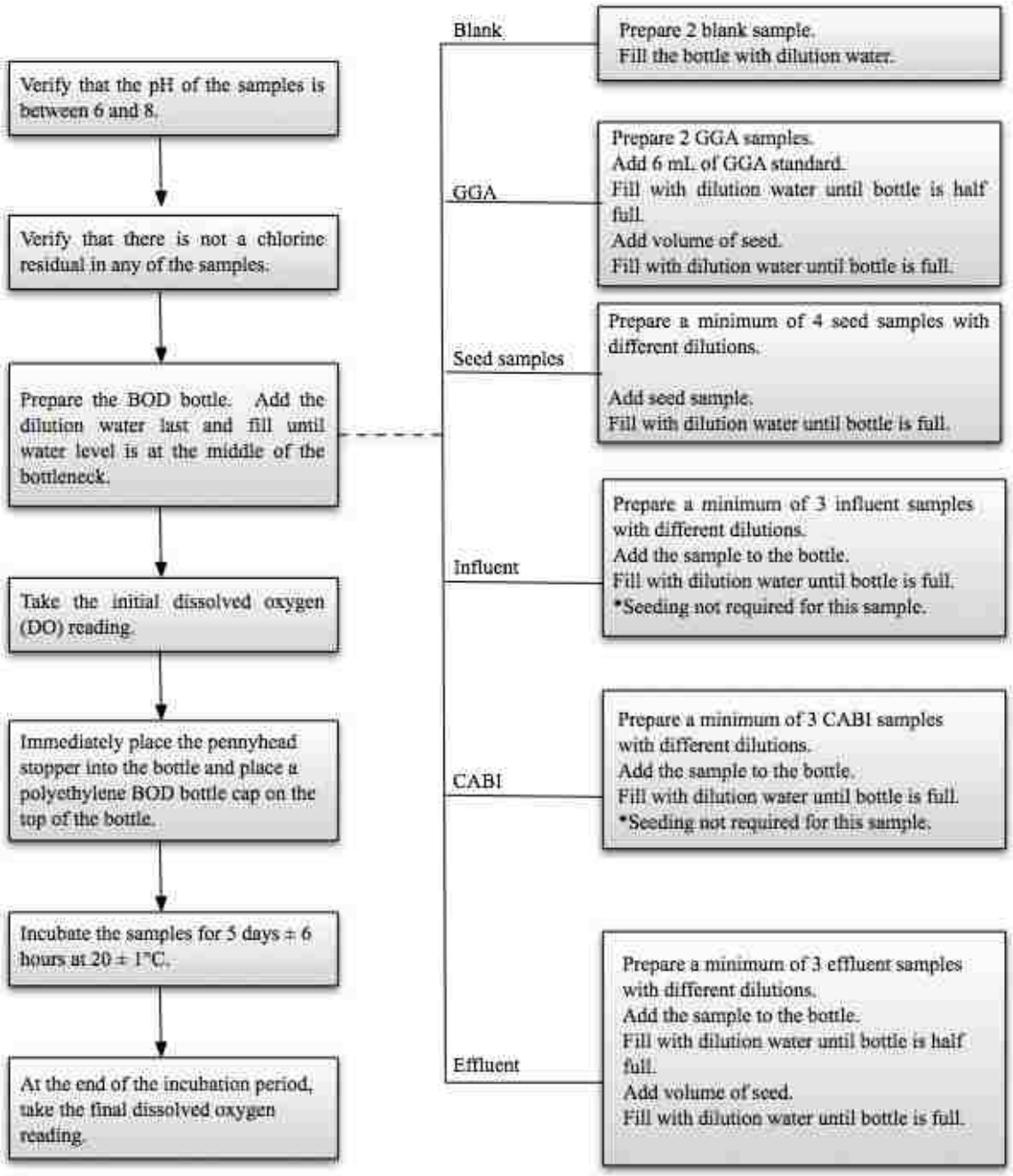

Figure 3.3: Flowchart for the BOD_5 test setup. Table 3.5 provides the sample and seed volumes used in this experiment. Refer to the important guidance section for additional information on sample preparation.

Important Guidance for the BOD Test Procedure

The following tips were implemented during sample preparation to improve the quality of the test results by minimizing errors associated with elapsed time, air bubbles, and overall poor technique.

- ✓ One sample bottle was prepared at a time. The initial DO reading was taken immediately, and then the bottle was placed in the incubator right away. After the bottle was placed in the incubator, then the next sample was prepared. This was repeated until all of the samples were prepared and placed in the incubator.
- ✓ After the incubation period, BOD bottles were removed in the same order that they were prepared. One bottle was removed and the final DO reading was taken. This was repeated until all of the final readings were recorded.
- ✓ The BOD bottles were filled to the top with dilution water. There is not an exact specified volume of water that should be added to the BOD bottle; the rule of thumb adopted in this research was to fill the bottle up to the middle of the bottleneck. This gives some room for the DO probe to be inserted, but the bottle was full enough to prevent air bubbles from being trapped.
- ✓ BOD bottles were filled with dilution water in a way that avoided entraining air. Bottles were slowly filled and the dilution water was poured such that it flowed down the inside of the bottle.
- ✓ Entrapment of air bubbles was avoided during DO readings and also after the bottle stopper was placed into the bottle.
- ✓ The DO probe was rinsed prior to each reading to prevent cross contamination.
- ✓ The bottle stopper was placed gently to avoid displacing additional sample.

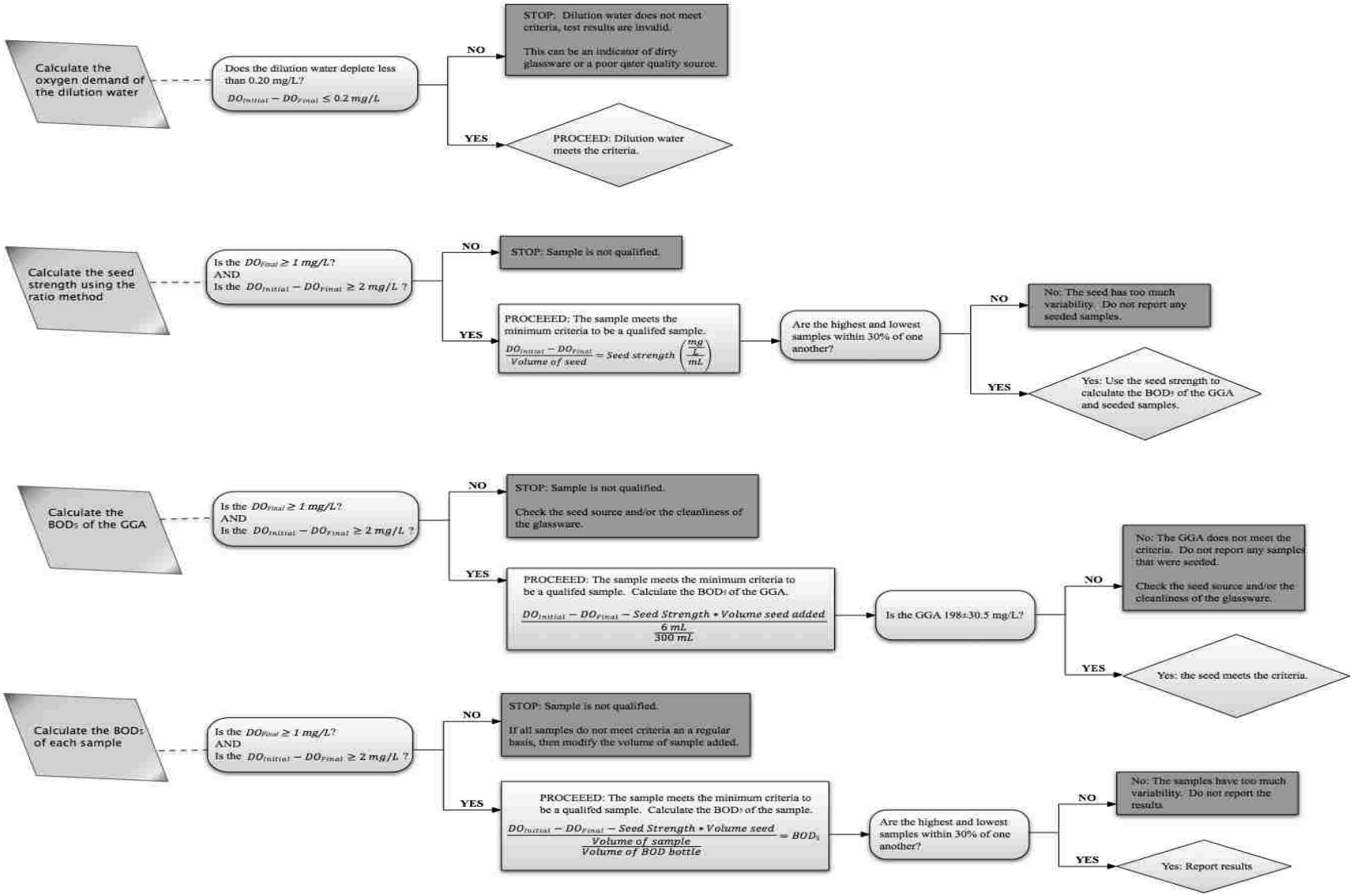

Figure 3.1: Flowchart of the BOD_5 calculation procedure adapted from Standard Methods (2005).

3.5.11 Calculation Procedure

Figure 3.4 provides a flowchart for the calculation procedure.

Definition of "Qualified Samples"

Samples that met the minimum qualification criteria, as defined by the Standard Methods (2005), were used in this research. Qualified samples are samples that deplete a minimum of 2 mg/L and have a final dissolved oxygen reading greater than 1 mg/L. Refer to the Literature Review for additional information regarding qualified samples.

Sample Check:

$$DO_{Final} \geq 1 \text{ mg/L} \qquad \text{Eqn. 3.1}$$

$$DO_{Initial} - DO_{Final} \geq 2 \frac{mg}{L} \qquad \text{Eqn. 3.2}$$

1) It was verified that the dilution water did not deplete more than 0.20 mg/L. If the water has a demand greater than 0.20 mg/L, then the BOD results cannot be used because the water is exerting a demand and can cause inaccurate results (Standard Methods, 2005).

Dilution Water Check:

$$DO_{Initial} - DO_{Final} \leq 0.20 \frac{mg}{L} \qquad \text{Eqn. 3.3}$$

2) The average seed strength of the qualified samples was calculated using the ratio method. It was also verified that the largest and smallest seed strengths were within 30% of one another.

$$\frac{DO_{Initial} - DO_{Final}}{\text{Volume of seed}} = \text{Seed strength} \left(\frac{\frac{mg}{L}}{mL} \right) \qquad \text{Eqn. 3.4}$$

3) The BOD_5 of the GGA was calculated and verified to be 198±30.5 mg/L. The average seed strength calculated in Step 2 was used for the seed strength. If the GGA did not meet the criteria, then seeded samples were not reported.

$$\frac{DO_{Initial} - DO_{Final} - \text{Seed Strength} * \text{Volume seed}}{\frac{\text{Volume of GGA}}{\text{Volume of BOD bottle}}} = BOD_5 \qquad \text{Eqn. 3.5}$$

4) The BOD_5 of the qualified samples was calculated and averaged. The highest and lowest BOD_5 values were verified to be within 30% of one another.

$$\frac{DO_{Initial} - DO_{Final} - \text{Seed Strength} * \text{Volume seed}}{\frac{\text{Volume of sample}}{\text{Volume of BOD bottle}}} = BOD_5 \qquad \text{Eqn. 3.6}$$

Notice that the unseeded samples do not have a volume of seed added; the unseeded BOD_5 equation can be reduced to:

$$\frac{DO_{Initial} - DO_{Final}}{\frac{\text{Volume of sample}}{\text{Volume of BOD bottle}}} = BOD_5 \text{ (Unseeded sample)} \qquad \text{Eqn. 3.7}$$

3.6 COD Methodology

(Adapted from the Hach Wastewater and Biosolids Analysis Manual)

The COD method was performed using Hach (Loveland, CO) chemistries. The Hach method was adapted from the Standard Method's closed reflux colorimetric method. The principles of both the closed reflux method and the Hach method are provided in the Chapter 2 literature review.

Specifically the low range COD chemistry (0 – 150 mg/L) was used for plant effluent and the high range COD chemistry (0 – 1,500 mg/L) was used for plant influent and CABI.

These test chemistries were determined based on preliminary COD testing using different COD range kits.

3.6.1 Quality Control/Quality Assurance

A 500 mg/L COD standard was prepared to check the accuracy of the test. The standard was prepared by dissolving 0.425 g of dried potassium hydrogen phthalate (KHP) in 1,000 mL of deionized water, as per Hach instructions. KHP is used to develop the calibration curves of the spectrophotometer; therefore, it is also used as the standard control to verify the equipment is operating correctly.

A standard control ampule was prepared with each batch of samples and verified to be between 90 – 110% of the true value. The 500 mg/L standard was used with the high range COD test. The prepared 500 mg/L standard was diluted to 50 mg/L to check the accuracy of the low range COD test. Both of the standards were stored in the refrigerator at 4 ± 1°C between uses.

New blank samples were prepared approximately once a week or after pipettor calibration adjustments were made; the blank was stored in the dark. The calibration of the pipettor was periodically verified using an analytical balance.

3.6.2 Sample Preservation

Samples can be preserved by acidification to pH ≤ 2 and stored up to 28 days. During this research, samples were not preserved. This includes samples that were held overnight for the holding time test.

3.6.3 Sample Interferences

Interference related to chloride, bromide, iodide, and other contaminants was discussed in Chapter 2 literature review. Additional interference can happen when the acid etches the COD vials, which will affect the spectrophotometer readings; this is overcome by rinsing the sample with deionized water and drying it prior to digesting the sample. Skewed test results can occur in samples with large particulate content. The Hach method specifies to blend the sample prior to testing, but this step was omitted because it is not the common practice at the laboratory facility at the WWTP studied. Pipettors with small openings can occlude particulate, so a wide mouthed pipettor tip for influent and CABI samples was used in this research.

3.6.4 Standard Operating Procedure for the COD test

1) All of the samples and control standards were bought to room temperature.
2) The Hach DRB200 digestion block was preheated to 150°C.
3) Samples were homogenized by thoroughly mixing the sample (note: samples were not blended).
4) A volume of 2 mL (based on Hach directions) of sample was pipetted into the appropriate Hach ampule. The vial lid was closed tightly and inverted several times to mix the contents of the vial.

    Note: The high range Hach method was used for the influent and CABI samples.

    Note: The low range Hach method was used for the effluent samples.

    Note: A blank and control vial were prepared for each Hach range used.
5) The vial was rinsed with deionized water and dried to prevent acid etching on the vial during the digestion period.

6) The vials were placed into the Hach DRB200 digestion block, the shield was closed, and the samples were digested for 2 hours.
7) After the samples cooled, the vials were removed from the digester, inverted several times, placed into a rack, and stored in a dark drawer until they reached room temperature. The samples were stored in a dark drawer to avoid light interference.
8) After the samples reached room temperature, the COD was measured with the Hach DR2700 spectrophotometer (Program 430 for the low range COD test and Program 435 for the high range COD test). Each of the vials was wiped (Kimtech, Roswell, GA) to remove fingerprints, smudges, etc. The instrument was zeroed with the appropriate blank vial, and then the COD reading of each of the samples (including the controls) was taken using the spectrophotometer.

3.7 TOC Methodology

(Adapted from the Standard Methods)

The high temperature combustion method was used to convert the organics to CO_2, and a non-dispersive infrared (NDIR) gas analyzer measured the CO_2. A Shimadzu TOC-V Auto Analyzer with TNM-1 (Honshu, Japan) with a 50 µL volume injector was used to determine the nonpurgeable organic carbon (NPOC) in the range of 1 – 50 mg/L; greater concentrations were determined by dilution. The sample was acidified, homogenized, sparged and diluted (if necessary) to remove the carbonates and bicarbonates from the sample. The sample was then sent through a combustion tube with a platinum catalyst and heated to 680°C to combust the samples and covert the organic matter to CO_2. The sample then flowed (150 mL/min) through a dehumidifier, a halogen scrubber, and then to the NDIR, which measured the CO_2 with a detection signal. The TOC – Control V Software

interprets the peak area of the NDIR detection signal to a final NDOC value that the user can understand.

3.7.1 Quality Control/Quality Assurance

The 0.06% hydrochloric acid solution used to prepare the controls was prepared by diluting 10 mL of 6N hydrochloric acid into reagent water in a 1-liter volumetric flask. The reagent water is the same water that was used for the BOD dilution water source. The 0.06% HCl reagent was prepared daily.

Equipment Calibration

The equipment was calibrated every three months, when significant changes were made, or when calibration samples failed. A linear curve that was not forced through the origin was used. The correlation coefficient had to be ≥ 0.995; the equipment is capable of a correlation coefficient up to 0.9999. The validity of the calibration curve was based on the correlation coefficient (≥ 0.995), the Y intercept (it should not be too far from the origin or results may be biased), the quality control standards (initial calibration sample and continuing calibration verification sample), and by visual inspection. The TOC standard solution was prepared by dissolving 2.128 grams of potassium hydrogen phthalate into the 0.06% HCl reagent in a 1-liter volumetric flask. The TOC standard was diluted in 0.06% HCl to the following TOC concentrations: 1.0 mg/L, 2.5 mg/L, 5.0 mg/L, 10.0 mg/L, 25.0 mg/L, and 50 mg/L. The calibration curve was developed using a minimum of five standard dilutions and a blank to determine the quantitation range.

The initial calibration verification (ICV) sample was analyzed after the initial calibration and at the beginning of the day. This sample had to be within 10% of the true value for the results to be valid. If issues occurred, the equipment was recalibrated. The ICV

was prepared with a different stock source than the one used for the calibration; the standard was prepared by dissolving 2.128 grams of anhydrous potassium hydrogen phthalate into the 0.06% HCl reagent in a 1-liter volumetric flask on a weekly basis.

The initial calibration blank (ICB) was analyzed immediately following the ICV sample. This sample had to be below the minimum detectable limit for the results to be valid. The ICB is an aliquot of reagent water that was used to verify the equipment was free from contamination after the equipment was calibrated.

Controls

A lab fortified blank (LFB) was analyzed every 20 or fewer samples. The TOC standard was diluted to the desired concentration (20 mg/L) with the 0.06% HCl reagent to be used as the TOC spike. The LFB sample was prepared by diluting the TOC spike with reagent water. The LFB sample was valid if it had a recovery within 10% of its true value.

A method blank was prepared and analyzed after each LFB. This sample was an aliquot of reagent water and treated the same as a sample including being exposed to all glassware, equipment, solvents, reagents, internal standards, and surrogates used with other standards. The sample was valid if it was less than the reporting limit.

Continuing Calibration Controls

A continuing calibration verification (CCV) sample was analyzed between each group of 10 injecting samples. This standard was prepared by diluting the TOC stock standard with 0.06% HCl to the midpoint of the calibration curve. This sample had to be within 10% of the true value to verify that the equipment was maintaining its calibration.

A continuing calibration blank (CCB) was analyzed after the CCV sample to verify that the equipment was not contaminated. This sample was reagent water and the results were valid if the results were less than the reporting limit.

Matrix Spike Sample

A matrix spike sample was analyzed every 20 or less field samples. The TOC standard was diluted to the desired concentration (20 mg/L) with the 0.06% HCl reagent to be used as the TOC spike. A matrix spike sample was prepared by spiking a field sample with the TOC spike. The concentration of the TOC spike was determined in a separate aliquot. The matrix spike sample was then corrected for the known amount of TOC spike. The spiked sample should have a recovery within 20% for the results to be valid. This process was repeated for every sample matrix. The purpose of this control is to determine if the sample matrix is contributing bias to the results.

3.7.2 Interferences

Contamination in the glassware, reagents, reagent water, and other items can impact the TOC results. The reagent water used in this analysis was the same source water that was used as the dilution water for the BOD_5 test. Samples that have large particulate (>0.45-µm) can clog and damage the instruments and flow system; samples were filtered through a 1.2-micron filter to minimize this interference. Halogens can cause interference as well; a halogen scrubber was used to prevent this interference. Interference due to inorganic (carbonate and bicarbonate) compounds was minimized by acidifying the sample and sparging. It should be noted that this process could potentially remove some of the purgeable organic compounds and bias the results low.

3.7.3 Sample Preservation

The samples were collected in a clear polyethylene container during the composite period, and then transferred to a clear 125 mL polypropylene container and acidified with hydrochloric acid (HCl).

3.7.4 Standard Operating Procedure for the TOC analysis

Sample preparation

1) The sample was filtered through a 1.2-micron filter.

2) The pH of the sample was adjusted using HCl drops until the pH was below 2.

3) The sample was then homogenized by inverting the sample several times.

4) When it was necessary, the homogenized sample was diluted with the 0.06% HCl reagent. Dilution is necessary for final TOC values greater than the upper limit of the calibration curve (i.e. 50 mg/L).

Sample analysis

The proper method (either TOC and high level total nitrogen or TOC and low level nitrogen) was chosen. The samples were analyzed in in the following order using the TOC analyzer. The TOC software provided the results, given in mg/L.

a) Initial calibration verification (ICV)

b) Initial calibration blank (ICB)

c) Laboratory fortified blank (LFB)

d) Method Blank (MB)

e) Reporting limiting standard (RL)

f) 9 injecting samples + matrix sample

g) Continuing calibration verification (CCV)

h) Continuing calibration blank (CCB)

i) Up to 10 injecting samples

j) CCV

k) CCB

l) Repeat steps i) through k) until all samples have been analyzed.

3.8 Fluorescence Methodology

The fluorescence test was conducted using a Horiba AquaLog® (Edison, NJ) spectrofluorometer and calculations were executed in Matlab. The instrument was warmed up for at least 20 minutes to allow the xenon lamp to stabilize prior to use. The settings provided in the standard operating procedure reflect the input settings to the Horiba software. The software provided with the equipment automatically subtracts the blanks from the samples, and it is capable of making the inner filter effect corrections and the Rayleigh corrections.

The corrected data were exported from the Horiba software and imported into MatLab to perform the calculations; the calculation procedure is discussed after the standard operating procedure.

Samples were filtered using 0.7-micron glass microfiber GF/F syringe filters with polypropylene housing (Whatman, Pittsburg, PA). A 10-mm path length Spectrosil® rectangular quartz cell (Starna Cells, Inc., Atascadero, CA) was used in the fluorometer.

3.8.1 Quality Control/Quality Assurance

Samples were transferred to amber glassware to minimize light interference. The equipment was given a minimum of 20 minutes to allow the lamp to warm up. New blank

and Raman samples were analyzed each day of analysis. Approximately 20 mL of sample was purged through the syringe filters to minimize leaching interference.

It was observed that when new gloves were used, the variance between the triplicate samples was reduced. Therefore, it was necessary to change gloves between every sample to prevent cross contamination from other samples, clothing, and the lab area.

3.8.2 Interferences

Filters can either adsorb or desorb organic materials into the sample, which will affect the results; a minimum of 20 mL of sample was purged and disposed of to minimize leaching interference. The lamp used in the equipment can become aged and also impact the results; the manufacturer offers recommendations on the frequency required to change the bulbs. The cuvettes were visually inspected prior to each use to verify that there was no damage, such as chips or scratches that could impact the analysis. Suspended materials can impact the test results by either scattering or absorbing light; samples were filtered prior to analysis to minimize interference. Changing gloves prior to each sample analysis reduced interference from cross contamination. Light scattering and reabsorbance can occur during the test procedure, as discussed in the Literature Review; Raman, inner filter effect, and Rayleigh corrections were performed during the analysis to minimize the interference.

3.8.3 Standard Operating Procedure for the Fluorescence Test

1) The fluorometer turned on for at least 20 minutes to allow the xenon lamp to stabilize before analysis was conducted.
2) All samples were brought to room temperature.
3) Three Raman measurements were performed on the same sample.
    a) The cuvette was rinsed with deionized water three times.

b) The cuvette was filled with deionized water and wiped with a Kimtech (Roswell, GA) to removed fingerprints, smudges, etc.

c) Three Raman measurements were taken at a 350 nm excitation wavelength, using the settings in Table 3.6.

Table 3.6: Fluorescence Raman Settings

Raman settings	
Integration	3 seconds
Accumulations	1
Excitation wavelength park	350 nm
Excitation wavelength increment	0.82 nm (2 pixels)
CCD gain speed	Medium

4) A blank sample was analyzed for each analysis event.

a) The sample that was used for the Raman measurements was also used for the blank measurement.

b) The blank measurement was taken with the settings that are summarized in Table 3.7.

Table 3.7: Fluorescence Settings for Blank, Influent, CABI, and Effluent Samples

Sample settings	
Integration	3 seconds
Accumulations	1
Excitation range	High = 470 nm
	Low= 240 nm
	Increments = 1 nm
Excitation wavelength increment	0.82 nm (2 pixels)
CCD gain speed	Medium

5) Each of the samples was prepared for fluorescence analysis.

a) The sample was homogenized by inverting the sample several times, and then a 50 mL aliquot was drawn with a syringe.

b) A 0.7-micron filter was placed onto the syringe.

c) Approximately 20 mL of sample was purged through the filter and disposed of to minimize organic leaching from the filter.

6) Each of the samples was analyzed for fluorescence.

a) The cuvette was rinsed three times with deionized water.

b) The cuvette was then rinsed three times with the filtered sample.

c) The cuvette was filled with filtered sample.

d) The fluorescence of the sample was determined using the settings that are summarized in Table 3.7.

7) Sample corrections were performed using the AquaLog® software.

Note: The Horiba AquaLog® software corrects for blanks, dark offsets, emission corrections, and excitation corrections automatically.

a) An inner filter effect (IFE) correction was made to each sample.

b) A Rayleigh correction with an offset of 10 nm in each direction was made to each sample.

3.8.4 Fluorescence Calculations

Matlab was used to perform Raman corrections and fluorescence regional integration (FRI) based on techniques that were introduced by Chen et al. (2003). The fluorescence data was normalized to the average of three Raman peaks areas at a 350 nm excitation wavelength to allow for direct comparisons with data from other instruments or laboratories.

Figure 3.5 illustrates the EEM regions that were adopted for this research. The units for the regions are reported in arbitrary fluorescence units (AFU). A series of calculations were performed to determine the fluorescence of each region. The projected area is calculated with the excitation and emission axis.

$$\text{Fractional projected area} = \frac{\text{Projected area of region (nm}^2\text{)}}{\sum \text{All projected region areas (nm}^2\text{)}} \quad \text{Eqn. 3.8}$$

$$\text{MF}_i = (\text{Fractional projected area})^{-1} \quad \text{Eqn. 3.9}$$

$$\Phi_{\text{region}} = \sum \text{Fluorescence in region} \quad \text{Eqn. 3.10}$$

$$\text{Total fluorescence of region (AFU)} = \text{MF}_i \Phi_{\text{region}} \quad \text{Eqn. 3.11}$$

$$\text{Total fluorescence (AFU)} = \sum \text{MF}_i \Phi_{\text{region}} \quad \text{Eqn. 3.12}$$

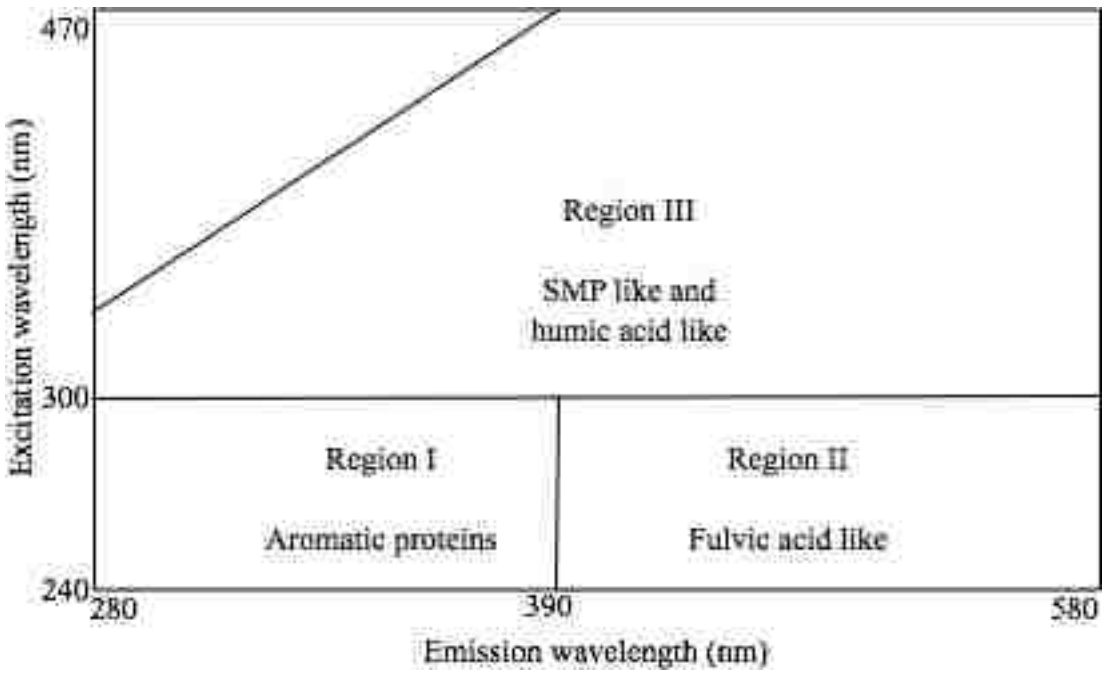

Figure 3.5: EEM regions defined for this research.

Part C: Calculation Methodology

3.9 Correlations

3.9.1 Data Pairing Methods

Bivariate data can be paired either based on their means or the data can be paired based on the replicate samples (Mutulsky and Christpoulos, 2003). BOD_5 data and COD data were collected in uneven replicates. The BOD_5 replicates were paired with the average of the COD replicates for their respective sample days. Similarly, fluorescence data were collected in uneven replicates. The average fluorescence was paired with the each BOD_5 replicate. BOD_5 data were collected in replicates, but the TOC data were not. The BOD_5 replicates were paired with the actual TOC value.

3.9.2 Correlations

All correlations data were analyzed in R statistical software (R Core Team, 2013). Least square regression was used to independently correlate BOD_5 to several parameters including COD, TOC, absorbance, fluorescence at specific excitation/emission wavelengths, and fluorescence of integrated regions. The data were correlated using linear regression and the correlation coefficient was used to determine the goodness of fit. The assumed normality of the data was checked using the Shapiro-Wilk test. It was determined that none of the data were normally distributed.

Due to the violations of the normality assumption, robust linear and nonlinear regressions were implemented. Robust regression methods use an iterative reweighted least squares method to estimate the model. These models are not as susceptible to outliers and heteroscedastic data. Heteroscedasticity refers to the horizontal spread of data. Ideally, the

scatterplot of the residual versus fitted value plot will not have heteroscedastic trends or curves; rather, the scatterplot should be random and show no pattern (Navidi, 2011). The pseudo correlation coefficient was determined by creating a linear model between the true BOD_5 value and the fitted BOD_5 value of the model.

When multiple models were developed, they were compared based on the pseudo correlation coefficient, the residual standard error of the model, and the visual observation of the fit of the data. Robust linear models were fitted using the "rlm" function and nonlinear regression lines were fitted using the "nlrob" function in R statistical software.

3.10 Removal Efficiency

The removal efficiency was calculated for the primary clarifier, biological treatment (i.e., the aeration basin), and the overall treatment train. The three removal efficiency datasets were treated independent from one another. Bootstrapping was used to create a dataset of 1,000 random bootstrapped samples, which were chosen with replacement from the original dataset. The mean and standard deviations of the bootstrap samples were used to construct the 95% confidence interval.

$$\text{Removal efficiency} = \frac{\text{Input} - \text{Output}}{\text{Input}} \text{x}100\% \qquad \text{Eqn. 3.13}$$

$$\text{Removal primary clarifier} = \frac{\text{Influent} - \text{CABI}}{\text{Influent}} \text{x}100\% \qquad \text{Eqn. 3.14}$$

$$\text{Removal aeration basin} = \frac{\text{CABI} - \text{Effluent}}{\text{CABI}} \text{x}100\% \qquad \text{Eqn. 3.15}$$

$$\text{Removal enitre treatment plant} = \frac{\text{Influent} - \text{Effluent}}{\text{Influent}} \text{x}100\% \qquad \text{Eqn. 3.16}$$

$$95\%\ \mathrm{CI} = \overline{\mathrm{X}} \pm 1.96 * \mathrm{SD} \qquad \text{Eqn. 3.17}$$

Method Development

3.11 Two-Sample t-tests For Holding Tests

R statistical software (R Core Team, 2013) was used to conduct the t-test, which was used to compare the means of the samples for the holding tests. The test was conducted at a 95% confidence interval, and it was assumed that the samples had unequal variance and that the samples were normally distributed. The null hypothesis was that the means were the same, and the alternate hypothesis was that the means differed. When the null hypothesis was rejected, it could be interpreted that the means were different and thus holding time had an effect. When the null hypothesis was not rejected, it was interpreted that it was plausible that the means were the same. Notice that it is only possible to say that it is plausible that the means are the same, but it is not the same as saying that it is true that the means are the same.

3.12 Fluorescence Integration Times

The fluorescence integration times were compared using the two-sample t-test discussed in the previous section. The normalized standard deviations were also used to compare the results of the integration times. The normalized standard deviation was calculated by dividing the standard deviation by the mean.

CHAPTER 4

RESULTS

4.1 Important Terminology

The removal of organics with an activated sludge system was discussed in Chapter 2 Literature Review, and a schematic of the treatment train was presented in Chapter 3 Methodology. This section is intended to clarify the terminology used throughout this chapter. As shown in Figure 3.1 (Methodology Chapter), the treatment process is primary clarifier → aeration basin → secondary clarifier → tertiary treatment (filtration/disinfection) → treated effluent.

The terms influent, raw sewage, and sewage refers to the wastewater that is entering the wastewater treatment plant (WWTP). The terms primary effluent and CABI are used interchangeably to describe the water that is leaving the primary clarifier and entering the aeration basin. Secondary effluent refers to the water that is leaving the secondary clarifier. The terms effluent and treated effluent are used to describe the water that has passed through the entire treatment train and is being discharged into the environment.

Sludge refers to the settled solids from both of the clarifiers. The sludge is sent to solids handling, where the liquid is removed from the solids. The liquid, referred to as centrate, is sent back to the primary clarifiers. The centrate is highly concentrated with

organic matter and suspended solids. The return of centrate to the primary clarifier causes an increase in organic matter loading in the primary clarifier (Tchobanoglous et al., 2003).

Part A: Correlation Results

4.2 Correlations Developed with Sample Site as an Independent Variable

The following discussion includes correlations that were developed as a function of the alternate test procedure (Eqn. 4.1), as well as correlations that were developed as a function of the alternate test procedure and the sample site (Eqn. 4.2). Eqn. 4.1 is straightforward and easily interpreted. Eqn. 4.2 is unique to this research; this section is intended to clarify how the model was developed and explain how the subsequent regression models were derived for each sample site.

$$\mathrm{BOD_5} = \beta_0(\text{Alternate Method}) + \beta_1 \quad \text{Eqn. 4.1}$$

$$\mathrm{BOD_5} = \beta_0(\text{Alternate Method}) + \beta_1(\text{Sample Site}_1) \ldots + \beta_n(\text{Sample Site}_n) \quad \text{Eqn. 4.2}$$

Consider a generic model developed with the independent variables COD, influent, CABI, and effluent (Eqn. 4.3). In the actual model, one of the independent variables will become a coefficient; the CABI sample site typically became the coefficient in this research (Eqn. 4.4), but this may not always be the case.

$$\mathrm{BOD_5} = \beta_0(\mathrm{COD}) + \beta_1(\text{Influent}) + \beta_2(\mathrm{CABI}) + \beta_3(\text{Effluent}) \quad \text{Eqn. 4.3}$$

$$\mathrm{BOD_5} = \beta_0(\mathrm{COD}) + \beta_1(\text{Influent}) + \beta_2 + \beta_3(\text{Effluent}) \quad \text{Eqn. 4.4}$$

BOD_5 (mg/L) is estimated by entering the value for COD (mg/L), entering the value 1 (unitless) for the sample site which COD is known, and entering the value 0 for all other sample sites. For example, influent BOD_5 can be estimated from Eqn. 4.4 as shown in Eqn. 4.4a; the reduced form of the equation is shown in Eqn 4.4b.

$$\text{Influent BOD}_5 = \beta_0(\text{COD}) + \beta_1(1) + \beta_2 + \beta_3(0) \quad \text{Eqn. 4.4a}$$

$$\text{Influent BOD}_5 = \beta_0(\text{COD}) + (\beta_1 + \beta_2) \quad \text{Eqn. 4.4b}$$

Similarly, effluent BOD_5 can be determined from Eqn. 4.4, as shown in Eqn. 4.4c and Eqn. 4.4d. CABI BOD_5 is determined from Eqn. 4.4 by entering 0 for influent and effluent (Eqn. 4.4e and Eqn. 4.4f).

$$\text{Effluent BOD}_5 = \beta_0(\text{COD}) + \beta_1(0) + \beta_2 + \beta_3(1) \quad \text{Eqn. 4.4c}$$

$$\text{Effluent BOD}_5 = \beta_0(\text{COD}) + (\beta_2 + \beta_3) \quad \text{Eqn. 4.4d}$$

$$\text{CABI BOD}_5 = \beta_0(\text{COD}) + \beta_1(0) + \beta_2 + \beta_3(0) \quad \text{Eqn. 4.4e}$$

$$\text{CABI BOD}_5 = \beta_0(\text{COD}) + \beta_2 \quad \text{Eqn. 4.4f}$$

The regression model is valid for the range of COD values which the model was developed, based on the specific sample site. Another limitation to this type of model is that the slope is the same for each of the sample sites considered in the model, but the intercept is different. The literature has shown that biodegradable organic matter is selectively removed during the treatment process, which will cause the ratio of BOD_5 to other methods to change as treatment progresses. This type of regression model assumes that the ratio is the same (i.e., the same slope) throughout the treatment process.

Despite these limitations, the models were developed and they are discussed throughout this chapter. Models were developed that considered influent and CABI sample sites, as well as influent, CABI, and effluent sample sites. These models were developed to determine if considering sample site as an independent variable resulted in a better-fit model, compared with a regression model that considered the data at these sample sites, but did not consider sample site as an independent variable in the model (i.e., Eqn. 4.1).

4.3 BOD_5 and COD Correlations

Figure 4.1 is a plot of the COD and BOD_5 data that were used for the correlations; Figure 4.2 and Figure 4.3 summarize the regression models developed for the data, and Table 4.1 summarizes the regression equations represented in the figures. It is clear that the values obtained are clustered by sampling location. Figure 4.1 shows that the influent data points tend to be more spread out, and then they become more tightly clustered as treatment progresses. The quality of the plant influent is uncontrolled, but the quality of the water as treatment progresses becomes more consistent, because treatment is engineered to generate a certain organic removal level.

Correlation coefficients were determined for each of the sample sites individually, for CABI and influent, and for all three of the sample sites combined, as summarized in Table 4.1. Due to the clustering of data, linear correlations were poor for influent (r^2= 0.03) and CABI (r^2= 0.15), but effluent correlated well when it was considered independently (r^2= 0.77). When influent and CABI are combined, the correlation coefficient (r^2= 0.73) is respectable, and it further increases when effluent is included in the correlation (r^2=0.92).

Initially, the data were analyzed using a traditional least squares model, which assumes that the data are normally distributed. The Shapiro-Wilk test for normality indicated that the data violated this assumption, so a robust regression analysis was used that does not assume that the data are normally distributed. From Table 4.1, one can see that the residual square errors were reduced when the robust regression was used in lieu of the least squares regression, with the exception of effluent, which slightly increased. The r^2 value reported for the robust models were determined by plotting the actual BOD_5 value to the (robust) fitted BOD_5 value.

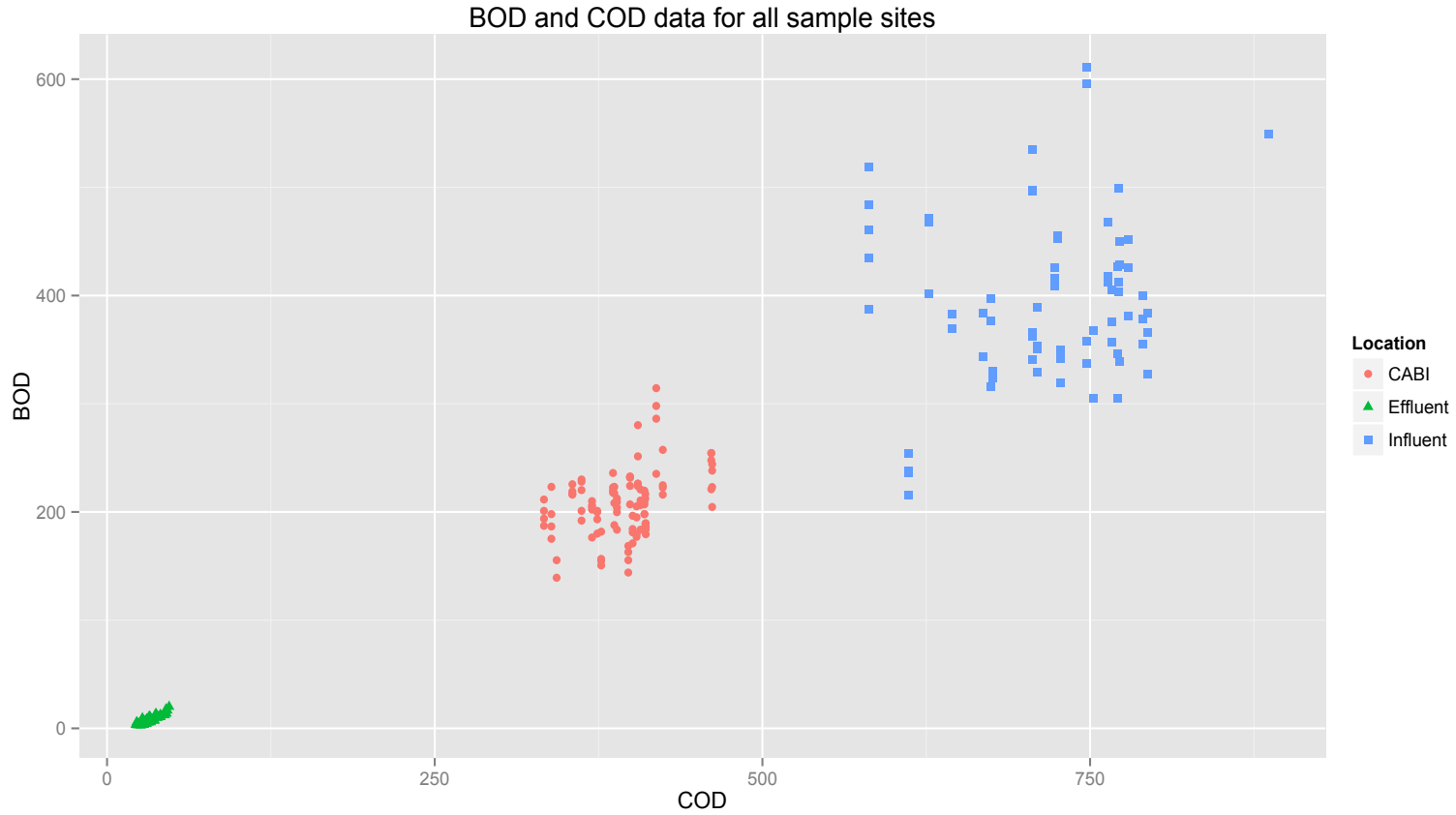

Figure 4.1: Plot of BOD_5 and COD data points used in correlation for influent, CABI, and effluent sample sites.

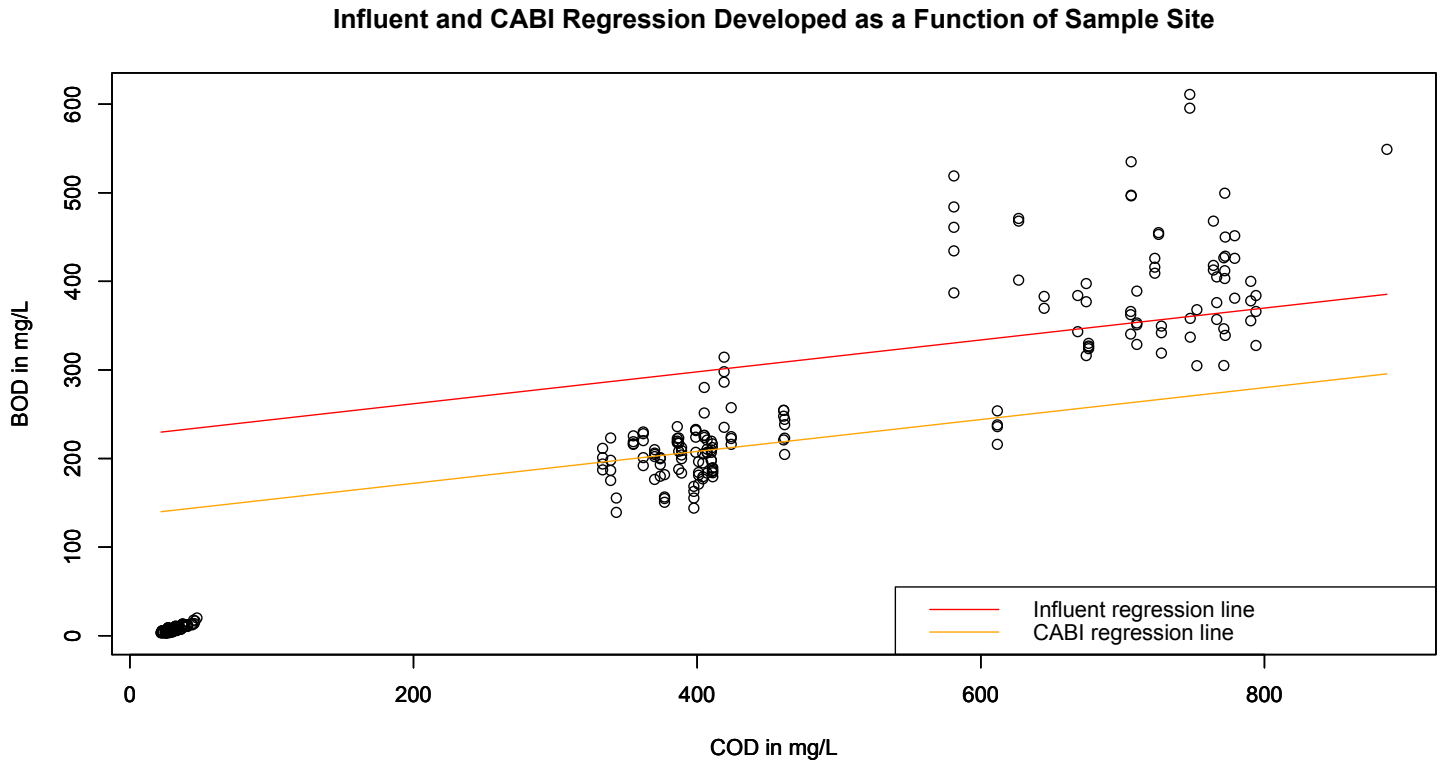

Figure 4.2: Regression lines for BOD_5 and COD correlations that were developed with influent and CABI sample sites as an independent variable (Table 4.1 Eqn. III). The model can be broken into two regression lines that represent influent (Table 4.1 Eqn. IIIa) and CABI (Table 4.1 Eqn. IIIb).

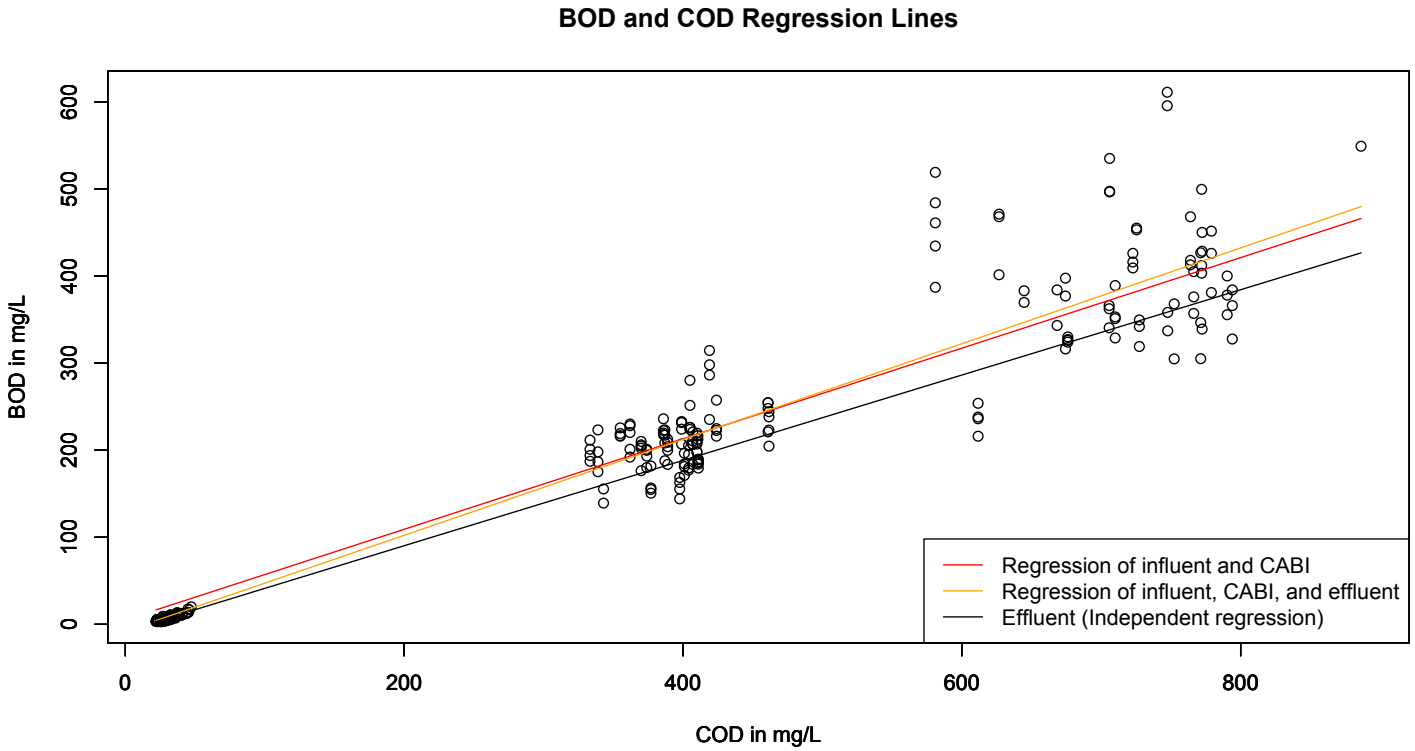

Figure 4.3: Regression lines for BOD_5 and COD correlations; the models do not consider sample site as an independent variable. The regression model that was developed using influent and CABI data (Table 4.2 Eqn. II) is represented by the red line on the graph. The model that considers influent, CABI, and effluent data (Table 4.1 Eqn. IV) is represented by the orange line. A visual comparison of the two trend lines indicates that the models are similar with respect to influent and CABI data. The effluent regression model (Table 4.1 Eqn. I) was developed independent of influent and CABI.

Table 4.1: Summary of BOD_5 and COD Correlations for Influent, CABI, and Effluent Samples

Reference Equation	Sample site(s) included in correlation	r^2 Least squares	Shapiro-Wilk normality test p value (p<0.05 indicates non normal)	Least squares residual standard error	Robust linear model	Robust model residual standard error	r^2 Robust
-	Influent	0.03	-	-	-	-	-
-	CABI	0.15	-	-	-	-	-
Eqn. I	Effluent	0.77	0.0241	1.713	[3]BOD_5 = 0.49*COD-7.78 (Graph shown in Figure 4.3)	1.931	0.77
Eqn. II	Influent + CABI	0.73	1.01E-08	55.75	[4]BOD_5 = 0.52*COD + 5.09 (Graph shown in Figure 4.3)	41.17	0.73
Eqn. III	[1]Influent + CABI	-	-	-	BOD_5 = 0.18*COD + (119.84* Influent) + 136.01	35.87	0.76
Eqn. IIIa Eqn. IIIb	Derived equations from Eqn. III (Graph shown in Figure 4.2)				BOD_5 Influent = 0.18*COD + 255.85 BOD_5 CABI=0.18*COD + 136.01	-	-
Eqn. IV	Influent + CABI + Effluent	0.92	2.73E-16	44.98	BOD_5 = 0.55*COD - 7.79 (Graph shown in Figure 4.3)	20.16	0.92
Eqn. V	[2]Influent + CABI + Effluent	-	-	-	BOD_5 = 0.20*COD + (110.87*Influent) - (127.73* Effluent) + 129.02	16.21	0.93
Eqn. Va Eqn. Vb Eqn. Vc	Derived equations from Eqn. V (Graph not shown)				BOD_5 Influent = 0.20*COD + 239.89 BOD_5 CABI = 0.20*COD + 129.02 BOD_5 Effluent = 0.20*COD + 1.29	-	-

[1]Influent and CABI sample sites were considered as independent variables in the regression model
[2]Influent, CABI, and effluent sample sites were considered as independent variables in the regression model
[3]Final recommendation for best fit regression model for effluent
[4]Final recommendation for best fit regression model for influent and CABI

Table 4.2: Results for COD to BOD_5 Ratios for Influent, CABI, and Effluent Samples

COD/BOD_5 Ratio	**Influent**	**CABI**	**Effluent**
Minimum	1.12	1.33	2.37
Average ± SD[1]	1.88 ± 0.37	1.93 ± 0.27	4.77 ± 1.57
Maximum	2.83	2.76	8.67
[1]Standard deviation			

One model was developed that considered COD, influent, and CABI as components in the model, and another model was developed that considered COD, influent, CABI, and effluent as components in the model. These models had similar slopes and intercepts; the model that was developed as a function of influent and CABI was plotted, (Figure 4.2) but the other model was not. Although the models are similar, the correlation coefficient of the model that considers all three of the sample sites ($r^2 = 0.93$) is higher than the model that only considers influent and CABI ($r^2 = 0.76$). It appears that effluent is increasing the correlation coefficient, which can mislead someone into believing that this model is a better fit for influent and CABI, even though the regression models are similar in terms of slope and coefficients. Figure 4.2 shows that the models that include sample site as part of the regression model are decent indicators of BOD_5 for CABI, but they underestimate influent BOD_5. As such, this model is not ideal.

Models were also created that only consider COD as the independent variable; they did not consider sample site as part of the regression equation. One of the models considers influent and CABI sample sites together ($r^2 = 0.73$) and the other considers influent, CABI, and effluent sample sites together ($r^2 = 0.92$). Both of the regression models are plotted in Figure 4.3. From the figure, it is clear that both of the models are quite similar for influent and CABI. The model that considers all three of the sample sites has a higher correlation coefficient and lower residual square error, which can be attributed to the effluent sample

being considered as part of the regression equation. Despite the better statistical parameters associated with model, all three sample sites should not be considered as part of the model, primarily due to the changing ratio that occurs as treatment progresses. Table 4.2 shows that the average ratio between influent (1.88±0.37) and CABI (1.93±0.27) is nearly the same, but the ratio increases after biological treatment (4.77±1.57). Because the ratios are similar for influent and CABI, it indicates that the relationship is linear between the two sample sites and they can be combined. Furthermore, effluent correlates well when it is considered independent of the other sites (Table 4.1 and Figure 4.3), so it is not necessary to combine the sample site to strengthen the correlation.

Table 4.2 shows that the ratio between COD and BOD_5 increases as treatment progresses. Colloidal organic matter is removed during primary treatment, and dissolved biodegradable organic matter is removed in the aeration basins. Recall that the BOD_5 test quantifies the portion of biodegradable organic matter that microorganisms consume during the five-day incubation period, but the COD test quantifies all of the biodegradable organic matter as well as some inorganics and recalcitrant organic matter. As such, the COD and BOD_5 will decrease at different rates through the train because conventional wastewater treatment, specifically the activated sludge process, selectively removes the biodegradable fraction. The influent ratio ranged from 1.33 to 2.76 (average=1.93), which is consistent with the findings of other researchers that reported influent ratios of 2.412 (Aziz and Tebbutt, 1979), 1.1 to 2.4 (Dazae, 1974), 1.85 (Eckenfelder and Ford, 1970) and 1.25 to 3.33 (Tchobanoglous et al., 2003). The ratio at CABI ranged from 1.33 to 2.76 (average=1.93), which is also consistent with previous literature that reported a ratio of 1.412 (Aziz and Tebbutt, 1979) and 1.67 to 2.5 (Tchobanoglous et al., 2003). The ratio for treated effluent

was between 2.27 and 8.67 (average=4.77). The most similar ratio identified in the literature for effluent was a range of 3.33 to 10 for COD to carbonaceous BOD (i.e., the BOD demand due to biodegradable organic matter) (Tchobanoglous et al., 2003), which is consistent with this research. Although nitrifying inhibitors (i.e., demand due to ammonia) were not used in this research, it is assumed that the BOD during the first five days can be attributed to carbonaceous demand because nitrifying bacteria are not yet active; therefore, the ratios of this research can be compared with the ratios reported by Tchobanoglous et al. (2003).

The literature focused primarily on correlations developed for specific sample sites. Several researchers were able to correlate BOD with COD in influent wastewater (Rowe, 1968; Aziz and Tebbutt, 1979; Dubber and Gray, 2010; Abyaneh, 2014), which was inconsistent with the findings of this research (when influent was considered independently), presumably due to the variability of COD and BOD_5 influent data that was observed.

It was possible to develop linear regression models for treated effluent in this research, which was consistent with the research of Jin et al. (2009), but inconsistent with other literature (Aziz and Tebbutt, 1979; Jin et al., 2009 Dubber and Gray, 2010). Jin et al. (2009) were able to correlate polluted seawater (BOD_5> 3mg/L), but they were not able to correlate relatively clean seawater (BOD_5< 3mg/L); the researchers were unable to conclude why there was a difference in the relationships, although it may be attributed to the detection limits of the BOD_5 test. Dubber and Gray (2010) were unable to correlate the treated effluent of 11 combined WWTPs, which they attributed to the variability in treatments processes and the differing operational conditions.

The regression models developed in this research indicate that BOD_5 can be correlated to COD, which is consistent with the literature. Also consistent with the literature is the

change in ratios observed as treatment progressed. The changes in ratios are an important indicator that correlations are better when made at site-specific locations, rather than across the entire treatment train, which is consistent with the conclusions of Aziz and Tebbutt (1979). Influent and CABI had similar ratios in this research, but the ratio drastically changes after aeration basin treatment. It can be concluded that the influent and CABI model is the best surrogate for influent and CABI samples because they have similar ratios, and the independent effluent model is the best surrogate for the effluent samples. The linear model that combined the sample sites had the best correlation coefficient, but it is not recommended due to the ratio change from CABI to effluent.

4.4 BOD_5 and TOC Correlation

The data for the BOD_5 and TOC correlations were not normally distributed; therefore, robust regression was performed to develop the models, similar to the COD models developed in the previous section. Figure 4.4 summarizes the data, Figure 4.5 summarizes the regression lines, and Table 4.3 summarizes the models and their respective correlation coefficients. It is clear that influent and CABI do not correlate well when they are considered individually. When influent and CABI are combined, the correlation coefficient (r^2=0.11) is still insignificant; therefore, it is not a good model. Finished effluent (r^2=0.87) correlates well when it is considered independently. When all three of the sample sites are combined (r^2=0.75), it appears that the effluent sample data are significantly contributing to the improved correlation. As shown in Figure 4.5, the model overestimates BOD_5 for CABI samples and underestimates the BOD_5 for influent samples.

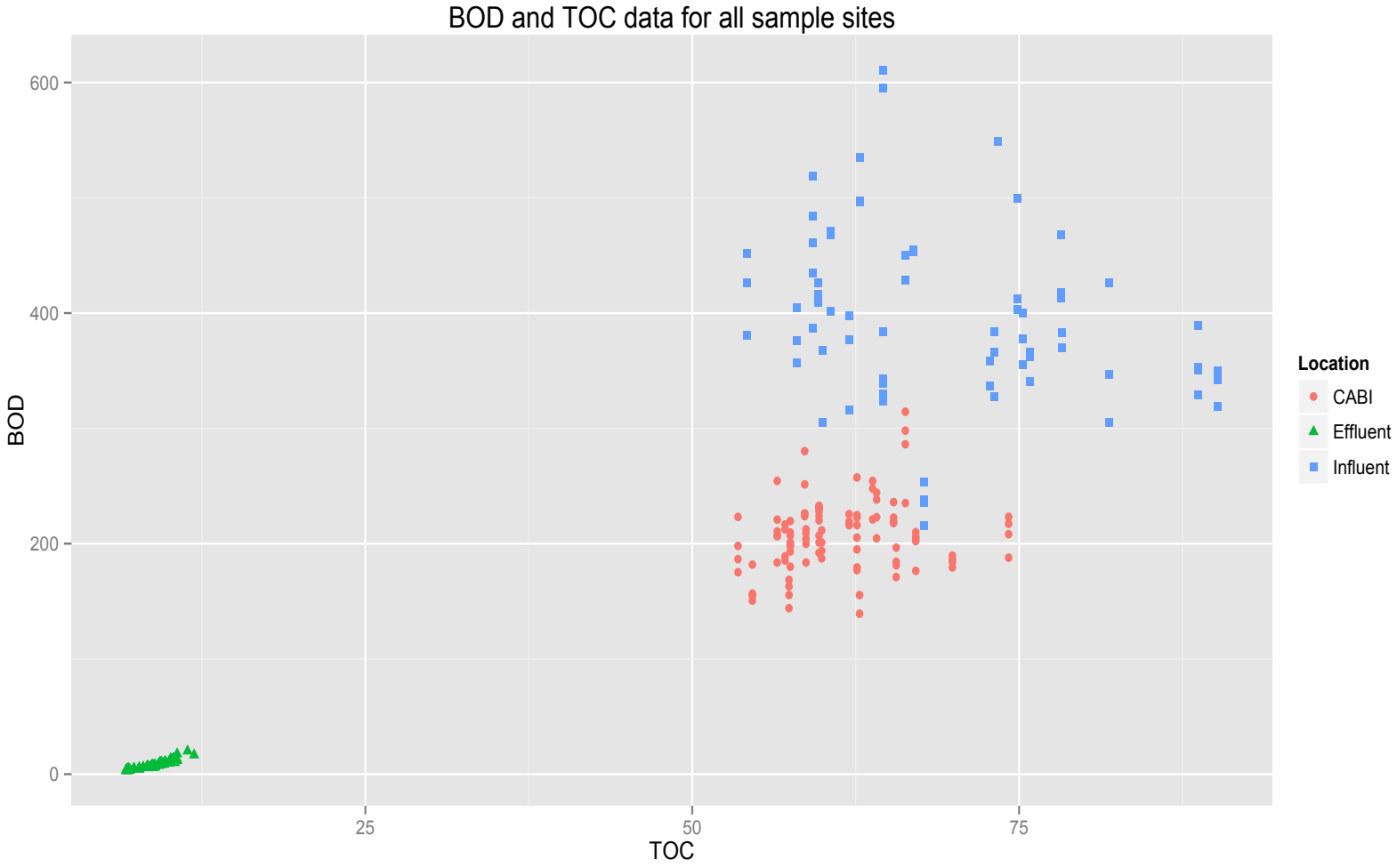

Figure 4.4: Plot of BOD_5 and TOC data points used in correlation for influent, CABI, and effluent sample sites.

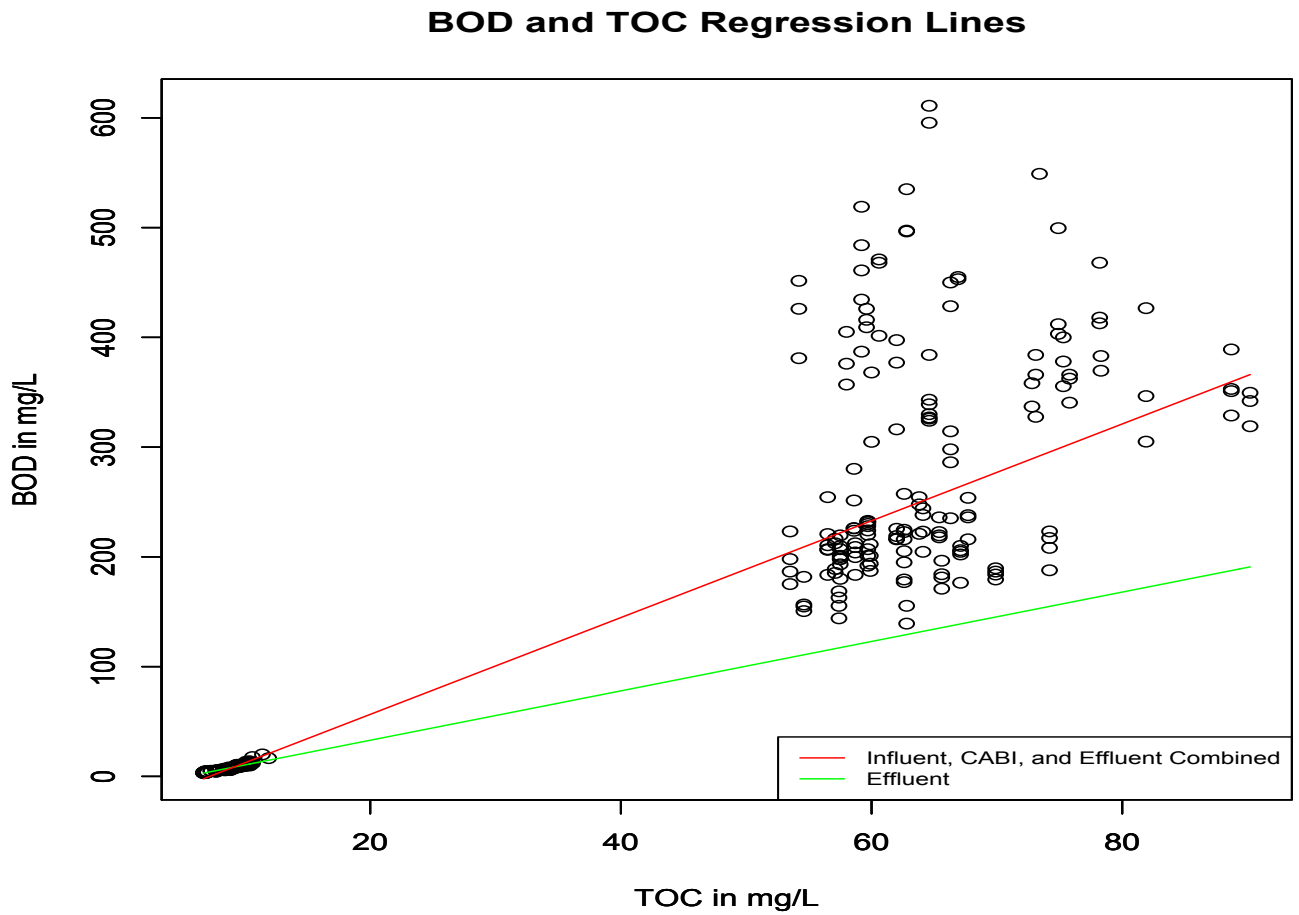

Figure 4.5: Plot of BOD_5 and TOC regression models for influent, CABI, and effluent sample sites. The influent, CABI, and effluent model (Table 4.3 Eqn. II) considered that data points at each of these samples sites, but sample site was not considered an independent variable in the model. From the graph, the model underestimates influent and overestimates CABI BOD_5. The effluent regression model was developed considering only the effluent data points (Table 4.3 Eqn. I) and appears to be a good estimator for effluent BOD_5.

Table 4.3: Summary of BOD_5 and TOC Correlations for Influent, CABI, and Effluent Sample Sites

Reference Equation	Sample site(s) included in correlation	r^2 Least squares	Shapiro Wilks normality test p value (p<0.05 indicates non normal)	Least squares residual standard error	Robust model	Robust model residual standard error	r^2 Robust
-	Influent	0.06	-	-	-	-	-
-	CABI	0.03	-	-	-	-	-
Eqn. I	Effluent	0.87	5.90E-08	1.237	[2]BOD_5 = 2.25*TOC - 12.07 (Graph shown in Fig 4.5)	0.8721	0.87
-	Influent + CABI	0.11	-	-	-	-	-
Eqn. II	Influent + CABI + Effluent	0.75	2.85E-15	79.54	BOD_5 = 4.41*TOC - 31.71 (Graph shown in Fig 4.5)	27.92	0.75
Eqn. III	[1]Influent + CABI + Effluent	-	-	-	BOD_5= -1.11*TOC + (193.75 *Influent) - (258.93*Effluent) +275.45 (Graph not shown)	42.82	0.93

[1]Influent, CABI, and effluent sample sites were considered as an independent variable in the regression model
[2]Final recommendation for best fit regression model for effluent
No final recommendations for best fit regression model influent or CABI

A model was also developed that considered all three of the sample sites as independent variables in the regression model. The model is shown in Table 4.3 (Eqn. III), but it was not plotted. The slope of the model is -1.11, which indicates that BOD_5 is decreasing when TOC is increasing. This is inconsistent with the physical relationship between TOC and BOD_5; therefore, this is not a practical model.

Viraghaven (1976) and Aziz and Tebbutt (1979) reported insignificant correlations for domestic wastewater influent samples, which is consistent with the findings in this research. Despite these findings, other researchers have successfully developed influent correlations (Chandler et al., 1976; Constable and McBean, 1979; Dubber and Gray, 2010). Dubber and Gray (2010) developed their correlations using influent samples from eleven WWTPs in Ireland that received wastewater from domestic and industrial sources. Chandler et al. (1976) developed a correlation for domestic wastewater influent from five municipal WWTPs combined and a correlation for the domestic wastewater influent from a single municipal WWTP. These two studies are notably different from this research because influent from one treatment facility was used in this research. The data collected in this study was clustered, which is indicative of consistent influent wastewater and a reliable treatment process. It may be possible that the success of the other researchers was because they were able to create a larger spread of data (i.e., no clustering) by collecting samples form a series of WWTPs and thus they could develop site-specific correlations. This type of correlation may not be reasonable though, because the treatment efficiencies of the WWTPSs can differ, thus creating differences in the ratios among the different facilities, which will lead to different linear regressions for each of the treatment plants. Constable and McBean (1979) developed their regression model using domestic wastewater influent collected over a

two-day sampling period from a single WWTP, but they also concluded that the model did not adequately predict BOD_5 outside of the sampling period for which the model was developed; therefore, the model was of little practical use.

Despite the failures of the influent and CABI correlations, effluent proved to correlate rather well in this research. This is consistent with the findings of Chandler et al. (1976) who were able to correlate effluent for one WWTP and a combination of five WWTPs, but inconsistent to the findings of Dubber and Gray (2010) who could not develop correlations for eleven combined WWTP effluents. Constable and McBean (1979) developed a statistically significant effluent regression model, but the model was not useful because it could not predict BOD_5 outside of the sampling period for which the model was developed; this is consistent with their conclusions made for their influent regression model.

Interestingly, Schaffer et al. (1965) were able to correlate BOD_5 to TOC by combining influent, secondary effluent (CABI), and effluent samples sites for domestic wastewater collected from a WWTP similar to this research. A similar model was developed in this research, but it was not a good predictor. Their sampling techniques were different from the methods adopted in this research, most notably the sampling period, which may be attributed to the inconsistencies in the results of the studies. Schaffer et al. (1965) collected grab samples by hand every 15 minutes over an eight-hour composite period for four consecutive days. The samples were refrigerated overnight and then analyzed the following day. Samples collected in this research were mechanically sampled proportional to flow and analyzed the day that they were collected over a period of four months. The short sampling duration of Schaffer et al. (1965) is major limitation to their work, particularly because large variations cannot be observed in such a short time frame. Possibly, these researchers may

not have developed significant correlations had the sampling campaign been extended over several months.

Table 4.4: Results for BOD_5 to TOC Ratios for Influent, CABI, and Effluent Sample Sites

BOD_5/TOC ratio	**Influent**	**CABI**	**Effluent**
Minimum	3.19	2.22	0.44
Average ± SD[1]	5.82 ± 1.54	3.40 ± 0.51	0.84 ± 0.26
Maximum	9.46	4.78	1.76
[1]Standard deviation			

Table 4.4 summarizes the ratios between BOD_5 and TOC found in this research. Reported ratios of BOD_5/TOC have been 1.44 (Aziz and Tebbutt, 1979), 2.0 (Chandler et al., 1976), and 1.2 to 2.0 (Tchobanoglous et al., 2003) for domestic wastewater influent. The BOD_5/TOC ratio of influent in this research ranged from 3.19 to 9.46, with an average ratio of 5.82±1.54, indicating a higher influent biodegradable organic content than what has been reported in the literature. The range of BOD_5 values (216 to 611 mg/L) observed in this research were much higher than those reported by Aziz and Tebbutt (1979), which ranged from 136 to 231 mg/L. The TOC values in this research ranged from 54 to 90 mg/L, which was less than the TOC values reported by Aziz and Tebbutt (1979), which ranged from 95 to 163 mg/L. The ratio reported by Viraraghavan (1976) was based on the stoichiometric relationship, not actual wastewater samples, and the ratio range reported by Tchobanoglous et al. (2003) was based on values from various municipal wastewater treatment facilities. The discrepancies between the BOD_5/TOC of this research and the stoichiometric ratio are unclear. Figure 4.4 shows that the relationship between BOD_5 and TOC is highly variable for the influent, which could be an indication that TOC and BOD_5 become less related as BOD_5 increases. Indeed, both of these methods quantify different types of organic matter. It

may be possible that as the BOD_5 increases, the TOC analysis does not quantify the increased biodegradable organic matter, particularly if the increase in organics is associated with the volatile organics removed during the acidification/sparging process in the TOC analysis. This would also explain why there is a difference in the ratios between this research and Aziz and Tebbutt (1979).

The ratio of CABI decreases relative to the influent sample. The average ratio reported in this research (3.40±0.51) was higher than the range (0.8 to 1.2) reported by Tchobanoglous et al. (2003), but the relative change in the ratio from influent to CABI was similar (i.e., the ratio decreases by approximately the same percentage). The decrease in the BOD_5/TOC ratio from influent to CABI is impacted by two things. First, organic matter is selectively removed from the primary clarifier, which will change the ratio. Second, the WWTP in this research has a return flow of centrate into the primary clarifier, which increases the organic loading and suspended solids. The characterization of BOD_5 and TOC of centrate is unknown; however, it is known that TOC has a negative removal in the primary clarifier, whereas BOD_5 does not have a negative removal (this will be discussed later in the thesis). Based on this information it can be concluded that organics are removed by primary clarification, but organics also increase due to the return flow of centrate. A mass balance of the primary clarifier and information regarding the characterization of the return flows would be necessary to fully understand the impacts on the BOD_5/TOC ratio. The data collected in this research show that the ratio decreases, which is indicative that either biodegradable organic matter is removed during primary sedimentation, TOC is increased during primary sedimentation due to return flows, or a combination of both. It is believed that both mechanisms are occurring and contributing to the change in the ratio.

The average ratio between BOD_5/TOC in the effluent (0.84±0.26) decreased from CABI (3.40±0.51), and it is significantly less than the influent ratio (5.82±1.54). Aziz and Tebbutt (1979), Dubber and Gray (2010), Ford (1968), Tchobanoglous et al. (2003) also reported that the BOD_5/TOC ratio decreased from primary influent to treated effluent. The decrease in the ratio is attributed to the removal of biodegradable organic matter and the accumulation of recalcitrant organic matter (Aziz and Tebbutt, 1979; Dubber and Gray, 2010).

The data and ratios suggest that the ratio between TOC and BOD_5 is changing as treatment progresses. This conclusion is important because it signifies that correlations should be developed independently at each sample site (Chandler et al., 1976; Aziz and Tebbutt, 1979; Dubber and Gray, 2010). Most of the literature attempted to correlate data using a linear model. Babatola and Xu (2010) used a power function to fit their model, which may be a better approach when trying to combine several sample sites, due to the change in ratio. The data in this research was fitted using a linear model when all of the sample sites are combined, but it is not a good predictor for CABI or influent BOD_5. Nonlinear regression models were also explored in this research, but these did not provide a good fit for the data; therefore, they were not reported. The linear model developed for effluent was the only correlation that could be developed between TOC and BOD_5. This model appears to be a good predictor, and may be useful for permit compliance.

4.5 BOD_5 and Fluorescence Results

Figure 4.6 illustrates the regions and peaks that were adopted for this research. These were presented and discussed previously in the literature review, but the figure is provided here to provide clarification for the following discussion. Figures 4.7 to 4.15 are the results of BOD_5 and each of the fluorescence parameters (i.e., total, region I, region II, region III, protein peak, humic peak, peak A, peak C, and peak T). Table 4.5 summarizes the non-robust linear correlation coefficients for each of the parameters, and Figure 4.16 provides a ranking of the correlation coefficients.

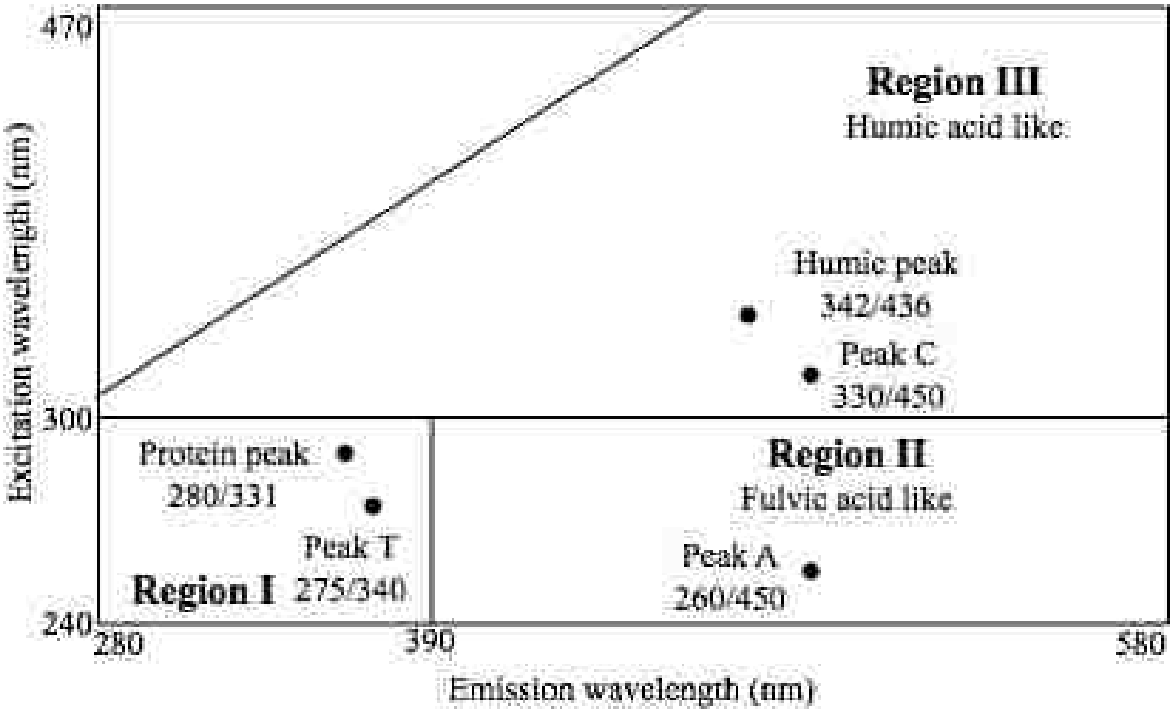

Figure 4.6: Fluorescence regions and peaks adapted for this research.

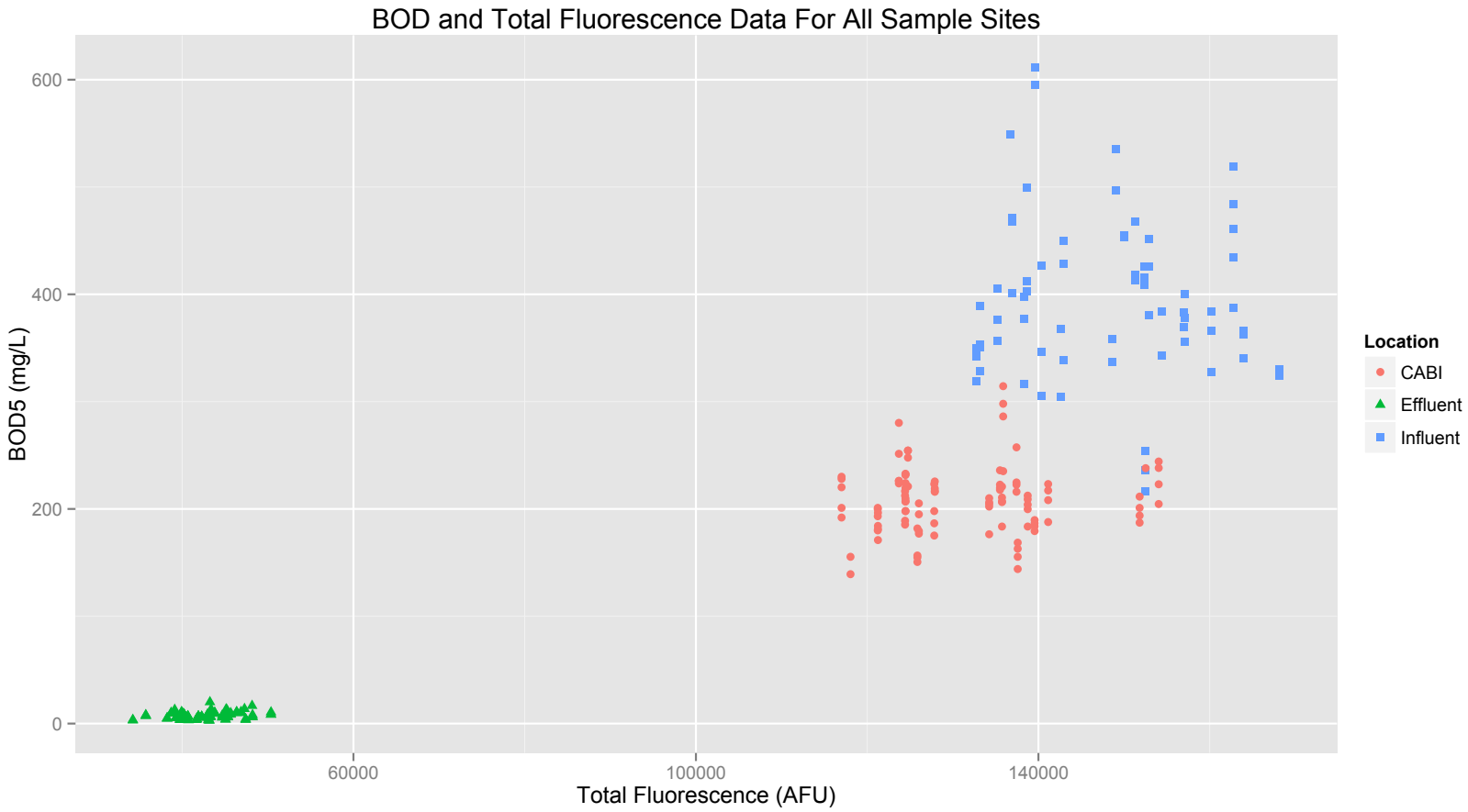

Figure 4.7: Results of BOD_5 and total fluorescence for influent, CABI, and effluent.

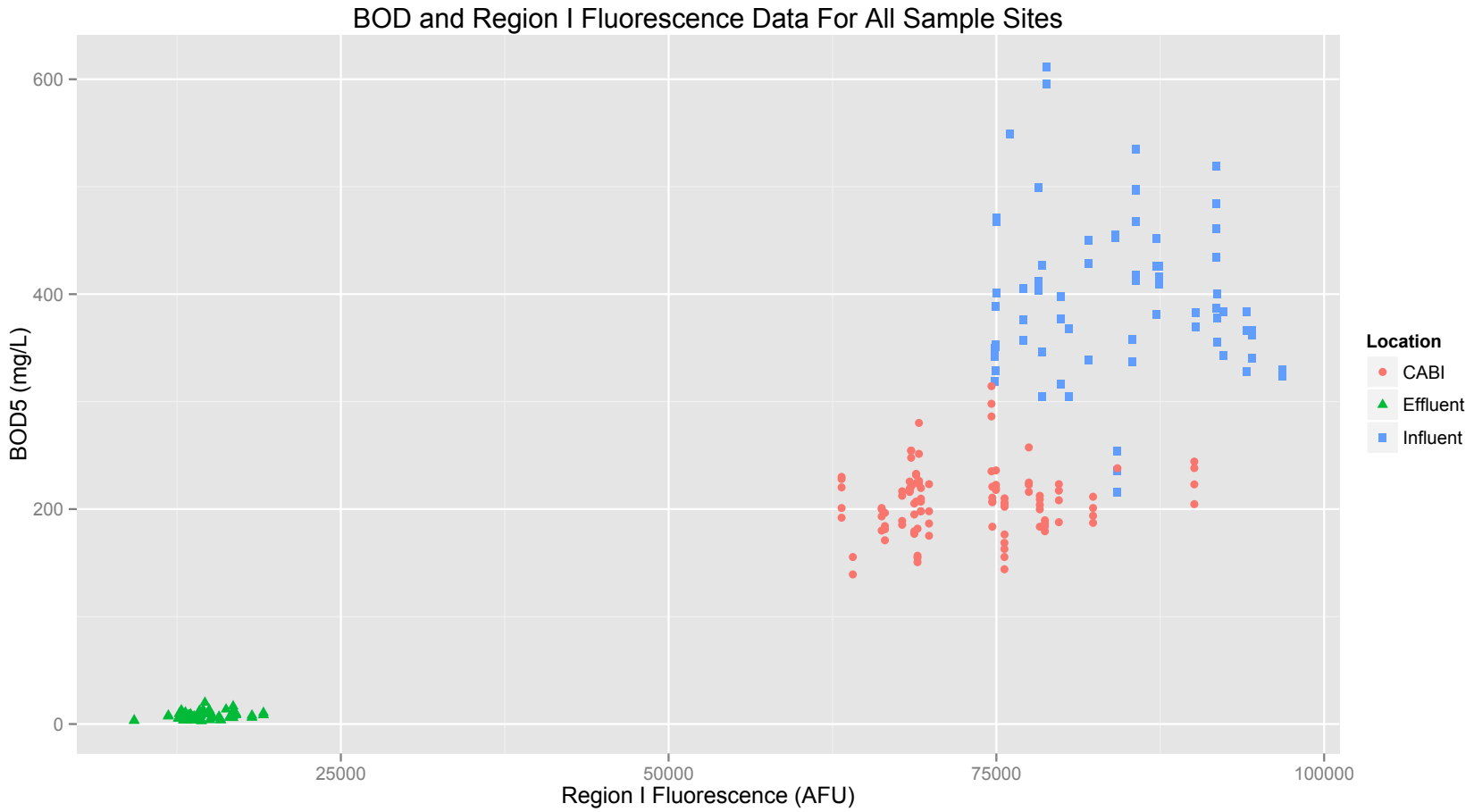

Figure 4.8: Results of BOD_5 and region I fluorescence influent, CABI, and effluent.

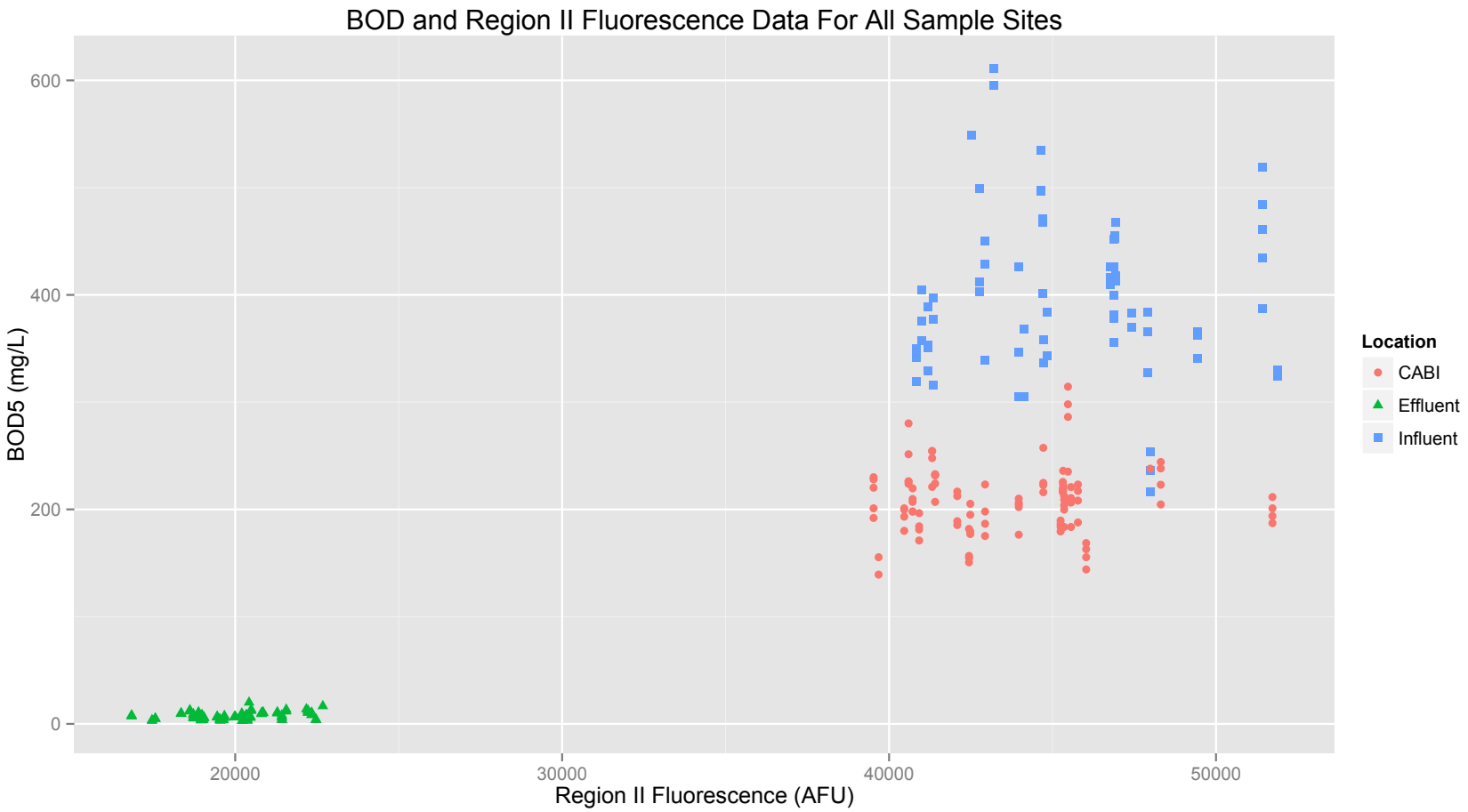

Figure 4.9: Results of BOD_5 and region II fluorescence for influent, CABI, and effluent.

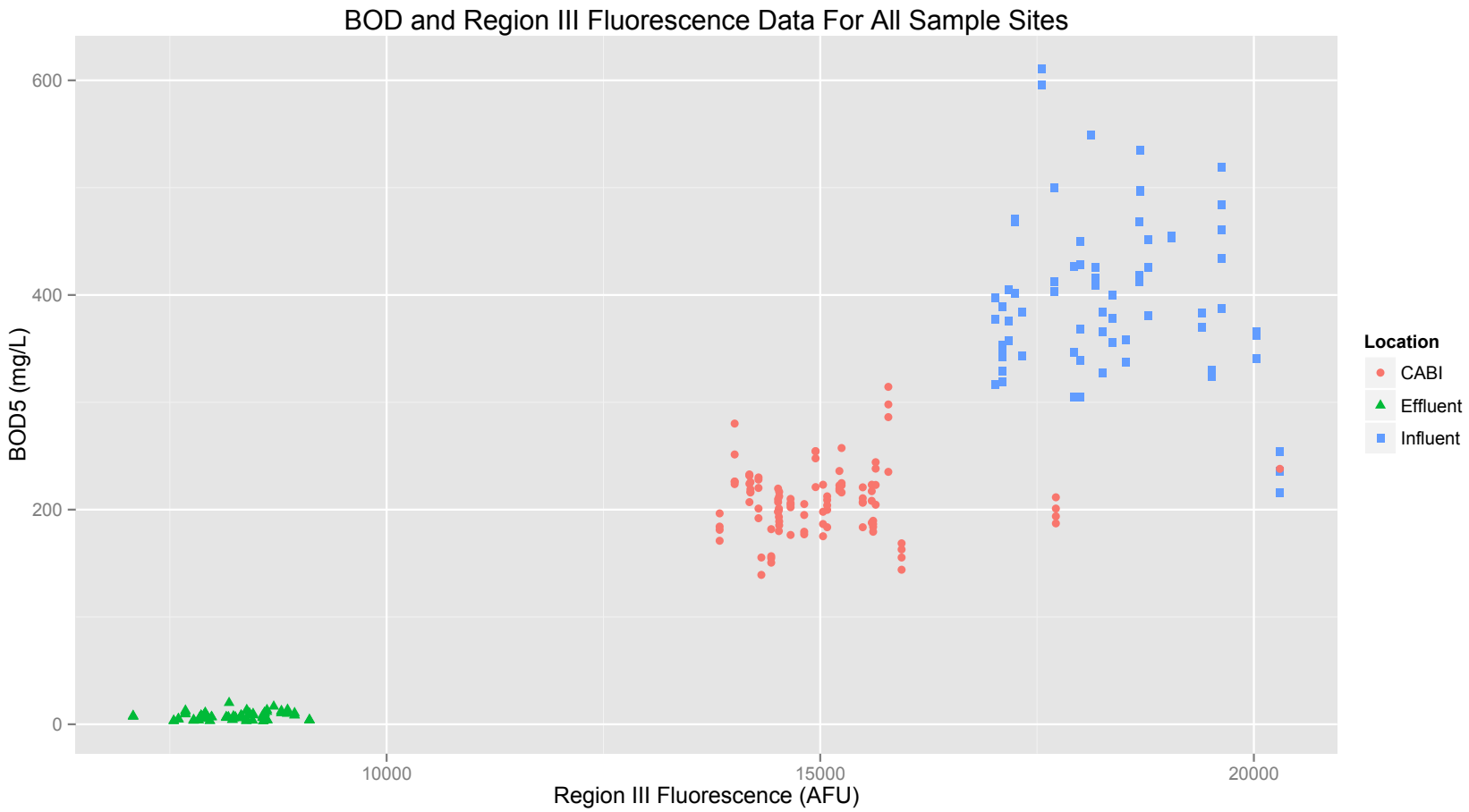

Figure 4.10: Results of BOD_5 and region III fluorescence influent, CABI, and effluent.

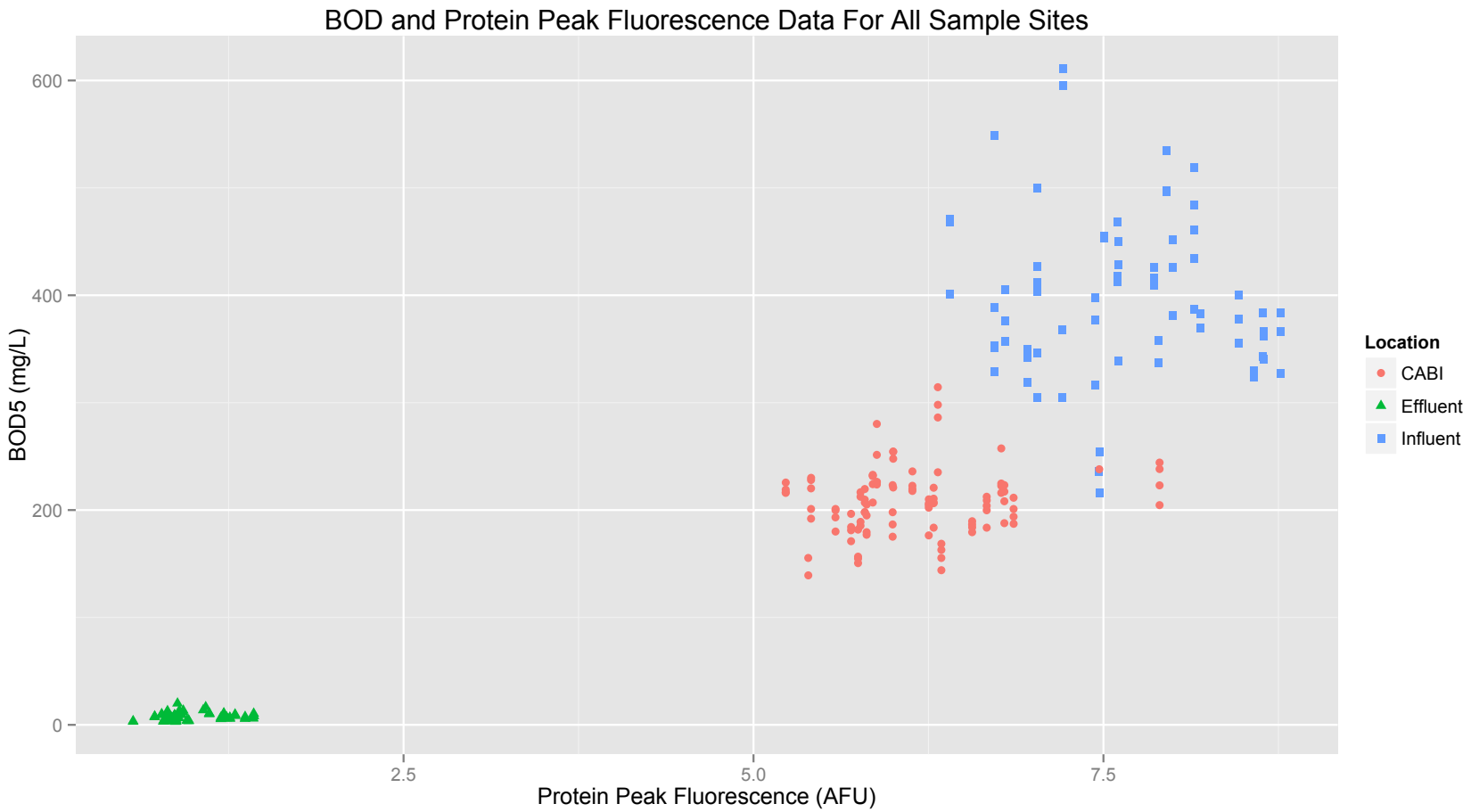

Figure 4.11: Results of BOD_5 and protein peak fluorescence influent, CABI, and effluent.

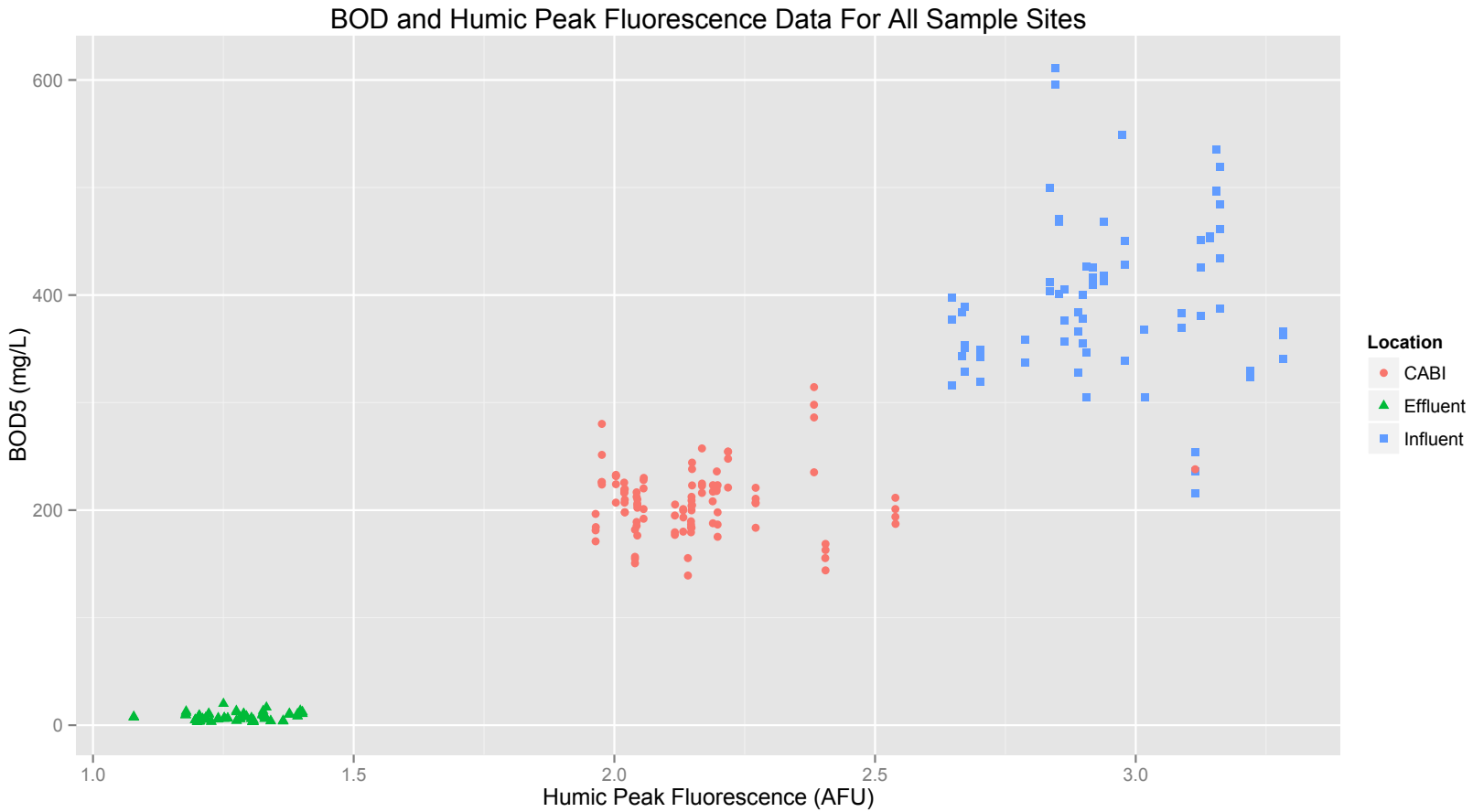

Figure 4.12: Results of BOD_5 and humic peak fluorescence influent, CABI, and effluent.

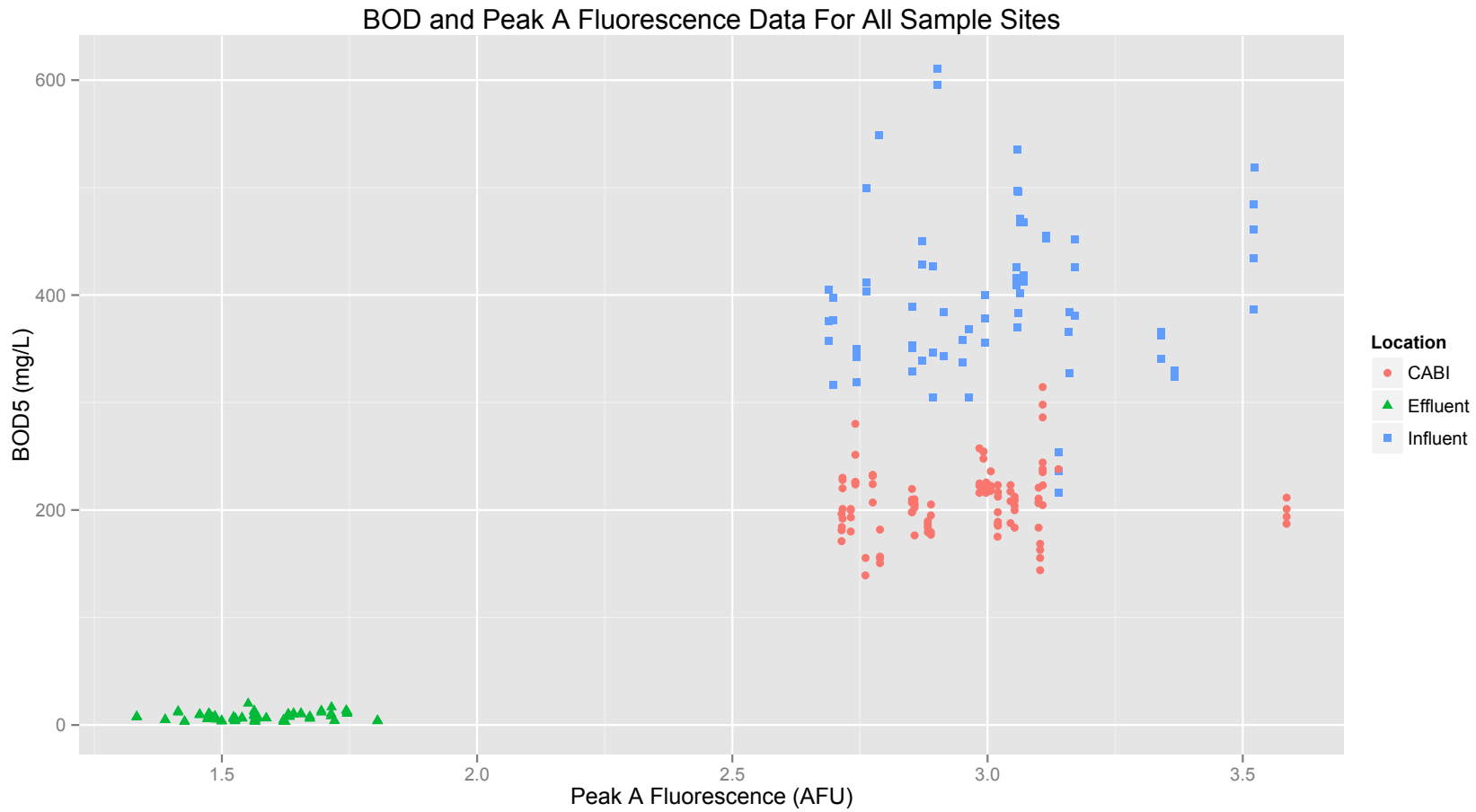

Figure 4.13: Results of BOD_5 and peak A fluorescence for influent, CABI, and effluent.

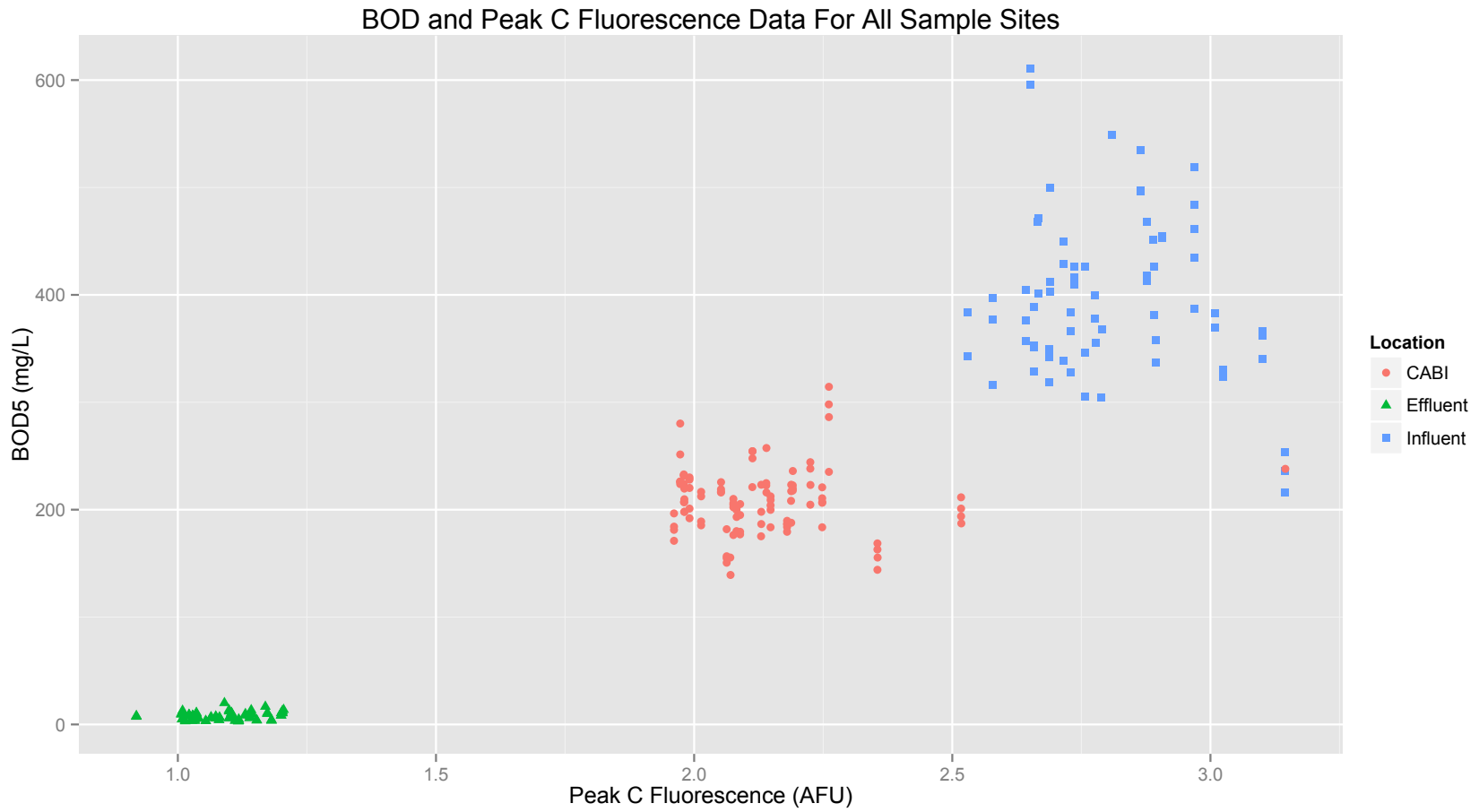

Figure 4.14: Results of BOD_5 and peak C fluorescence for influent, CABI, and effluent.

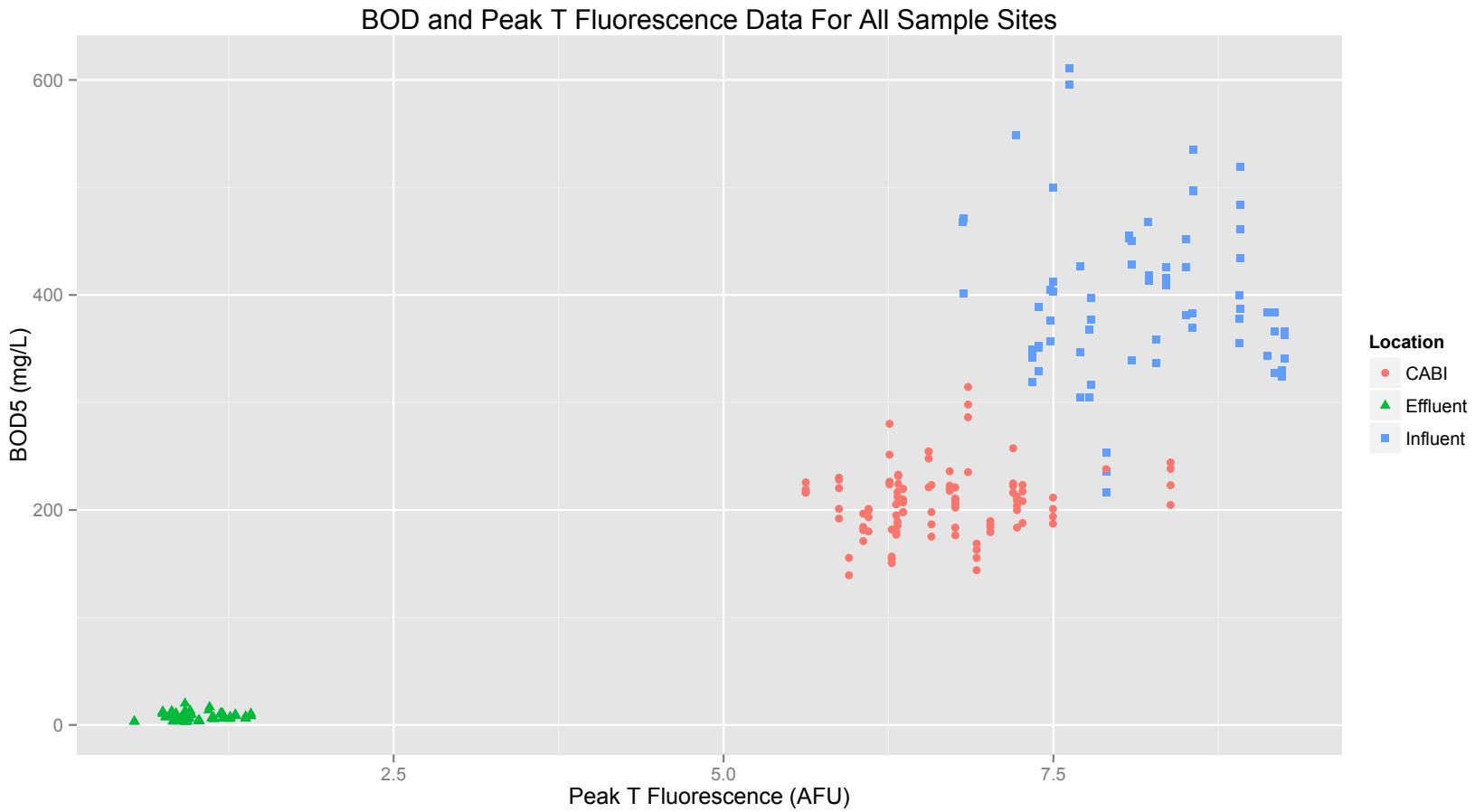

Figure 4.15: Results of BOD_5 and peak T fluorescence for influent, CABI, and effluent.

Table 4.5: Correlation Coefficients for Non-robust Linear Models for Each of the Fluorescence Parameters and Regions for Each Sample Site

BOD~	Influent	CABI	Effluent	Influent + CABI	Influent + CABI + Effluent
Total	0.0139	0.0069	0.1111	0.2953	0.7915
Region I	0.0132	0.0113	0.0758	0.3208	0.7971
Region II	0.0072	0.0016	0.1354	0.0608	0.7303
Region III	0.0354	0.0002	0.068	0.5331	0.8618
Proteins	0.0118	0.0209	0.0262	0.4314	0.8261
Humic	0.0014	0.0015	0.0655	0.6489	0.8946
Peak A	0.0019	0.0097	0.0492	0.0355	0.7128
Peak C	0.0642	0.0015	0.092	0.5699	0.8764
Peak T	0.0068	0.0144	0.0192	0.4322	0.8234

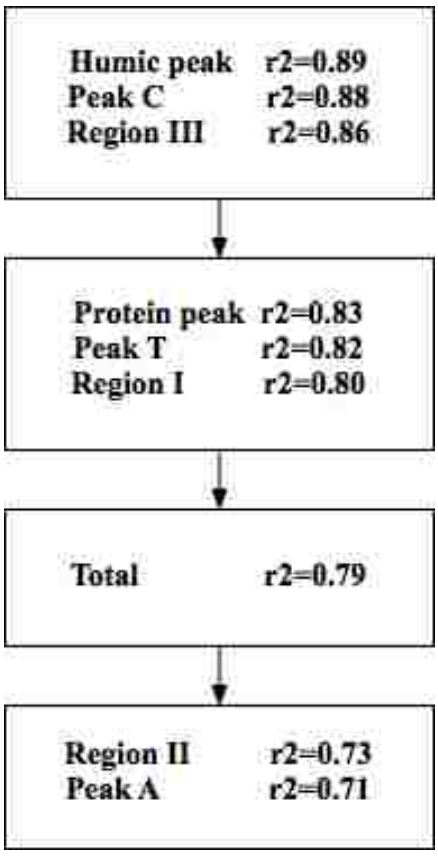

Figure 4.16: Ranking of the correlation coefficients for each of the fluorescence parameters and regions in descending order.

4.5.1 Observed Fluorescence IFE and the Impact on the Results

Figures 4.7 to 4.15 indicate that the trend is not linear; therefore, nonlinear regressions were used to correlate BOD_5 to the fluorescence regions. The literature suggested that the relationship between BOD_5 and fluorescence data were linear, which is contradictory to the results obtained in this research. There are two possible explanations for the result deviations. The first explanation is that the fluorometer reached the maximum fluorescence detection limit. Inspection of the raw uncorrected fluorescence data indicated that the data were not likely surpassing the detector limit; therefore, this was determined an unlikely cause. The second explanation for the nonlinear trend in data is due to inner filter effects (IFE). As discussed in the literature review, IFE occurs in samples that have high fluorescence and it is characteristic of samples that have high absorbance. The excitation wavelength is absorbed primarily on the front face of the sample, which inhibits the energy from penetrating through to the remainder of the sample. The result is that the energy is disproportionally absorbed and the sample absorbs some of the fluorescence, which ultimately quenches the fluorescence (i.e., the observed fluorescence is lower than the true fluorescence).

IFE can be reduced by using equipment corrections, diluting the sample, or by normalizing the sample to an absorbance wavelength (Henderson et al., 2009). The Horiba AquaLog® software was used to make IFE corrections on all samples. Normalizing the fluorescence data to the absorbance at 254 nm did not change the trend of the fluorescence graphs (i.e., they were still nonlinear); since the absorbance correction only changed the magnitude of the graph and not the trend of the graph, it was determined to be an unnecessary step for correlation. Several researchers diluted their samples to minimize IFE;

samples were diluted such that the absorbance at a particular wavelength (most commonly 254 nm) did not exceed a predetermined absorbance value (e.g., 0.05 cm^{-1}, 0.10 cm^{-1}, or 0.30 cm^{-1}) (Hur et al., 2010; Hur et al., 2012; Zhou et al., 2013; Yang et al., 2014). Samples were not diluted for this research, and the observed absorbance at influent and CABI were typically higher than the maximum absorbance criteria mentioned in the literature. The influent and CABI absorbance values obtained in this research were consistent with values reported by Tchobanoglous et al. (2003), which ranged from 0.30 to 0.55 cm^{-1} for primary wastewater and 0.15 to 0.30 cm^{-1} for secondary wastewater. It was concluded that fluorescence of influent and CABI was presumably quenched due to IFE. In addition, it is likely that other researchers will encounter the same issue because the absorbance of a typical characteristic of wastewater is often related to IFE interference.

The literature suggests that correlating the data with uncorrected IFE may be an alternative to implementing IFE corrections, such as normalizing the data to absorbance values or dilution (Henderson et al., 2009). The adopted approach of this study was to correct for IFE through equipment corrections only, and not by dilution or normalization to absorbance. One of the primary reasons this approach was chosen is because diluting the sample is not practical for in situ fluorescence monitoring. Interpretation and use of these models should take into account that fluorescence was being quenched due to IFE.

4.5.2 BOD_5 and Fluorescence Linear Correlation Coefficients

Table 4.5 summarizes the correlation coefficients for each of the parameters, based on non-robust linear correlations. Similar to the previous findings of this research, independent correlations at each sample site were not significant. The correlation coefficients for the three combined sample sites suggest that linear models are a good fit. An interesting observation

can be made when the correlation coefficients are organized in descending order, as shown in Figure 4.16. The correlation coefficients show consistency in their grouping based on regions and the peaks within the region. The humic peak and peak C are contained within region III, and each of these are ranked in the top three for correlation coefficients. The next group of coefficients are peak T and the protein peak, which are contained within region I. The fulvic-like region and peak A have the lowest correlation coefficients.

4.5.3 BOD_5 and Fluorescence Correlation Approach

Figure 4.16 and the literature review were used to determine which peaks and regions should be explored as possible surrogates for the BOD_5 test. The linear correlation coefficients indicate that the humic-like peak appears to be the best linear fit. This peak was chosen as well as region III, which contains the humic-like peak. Several studies have suggested that BOD_5 was most related to tryptophan-like peaks (peak T), which are near 280 nm excitation wavelengths and 340 nm emission wavelengths (Reynolds and Ahmad, 1997; Ahmad and Reynolds, 1999; Baker and Invararity, 2004; Hudson et al., 2008; Hur et al., 2008; Hur et al., 2010; Yang et al., 2014). The T peak adopted in this research was at a 275 nm excitation wavelength and a 340 nm emission wavelength.

As previously mentioned, the relationship between BOD_5 and each of the fluorescence parameters do not appear to have a linear relationship based on the graphs, but the correlation coefficients suggest that linear relationships exist. Robust linear and robust nonlinear regression models were explored for each of the chosen fluorescence parameters (region I, region III, peak T, and the humic-like peak), as summarized in the following equations.

$$BOD_5 = \beta_0 * (Parameter) + \beta_1 \quad \text{Eqn. 4.5}$$

$$BOD_5 = \beta_0 * (Parameter) + \beta_1 * (Influent) + \beta_2 * (CABI) + \beta_3 * (Effluent) \quad \text{Eqn. 4.6}$$

$$BOD_5 = \beta_0 * (Parameter)^{\beta_1} \quad \text{Eqn. 4.7}$$

$$BOD_5 = \beta_0 * (Parameter)^{\beta_1} + \beta_2 \quad \text{Eqn. 4.8}$$

$$BOD_5 = \beta_0 * e^{\beta_1 * (Parameter)} \quad \text{Eqn. 4.9}$$

$$BOD_5 = \beta_0 * e^{\beta_1 * (Parameter)} + \beta_2 \quad \text{Eqn. 4.10}$$

Eqn. 4.5 represents the linear regression model that was developed considering the data from all three of the sample sites (i.e., influent, CABI, and effluent). Eqn. 4.6 represents the linear regression model that considers each of the sample sites as independent variables in the model.

Eqn. 4.7 through Eqn. 4.10 represent the nonlinear regression models that were developed. Eqn. 4.7 and Eqn. 4.8 represent the power model and the power model with a constant, respectively. Eqn. 4.9 and Eqn. 4.10 represent the exponential model and the exponential model with a constant, respectively.

Each of these models were developed and compared using the correlation coefficient, the residual standard error, and by visually comparing the goodness of fit.

4.5.4 BOD_5 and Humic Peak Correlations

The results of the robust linear and nonlinear regressions are shown in Figures 4.17 and 4.18, respectively. The regression lines, pseudo correlation coefficients (i.e., the correlation coefficient of the true BOD_5 plotted against the fitted BOD_5 value), and the residual standard errors are summarized in Table 4.6.

The linear functions had lower residual standard errors when compared with the nonlinear functions, indicating that they were a better fit. Two linear functions were developed: one that estimated the BOD_5 as a function of the humic fluorescence and another that estimated BOD_5 as a function of the humic fluorescence and the sample sites. When the sample sites are considered as part of the function, then independent equations can be developed for each of the sample sites, as shown in Figure 4.17 and summarized in Table 4.6 Eqn. IIa, IIb, and IIc. The figure shows that the independent equations estimate effluent BOD_5 fairly well, but it is not a good fit for CABI or influent. On the other hand, the linear equation that does not include sample sites appears to be a better fit for CABI and influent, but not for effluent.

As mentioned, the nonlinear functions were subpar to the linear models in terms of the residual standard error. Figure 4.18 indicates that the power and exponential functions overestimate effluent BOD_5 and underestimate both CABI and influent BOD_5. The exponential and power models have nearly identical trend lines, as shown in Figure 4.18. Both of these models are poor estimators for effluent BOD_5, but they appear to be reasonable estimators for CABI and influent BOD_5.

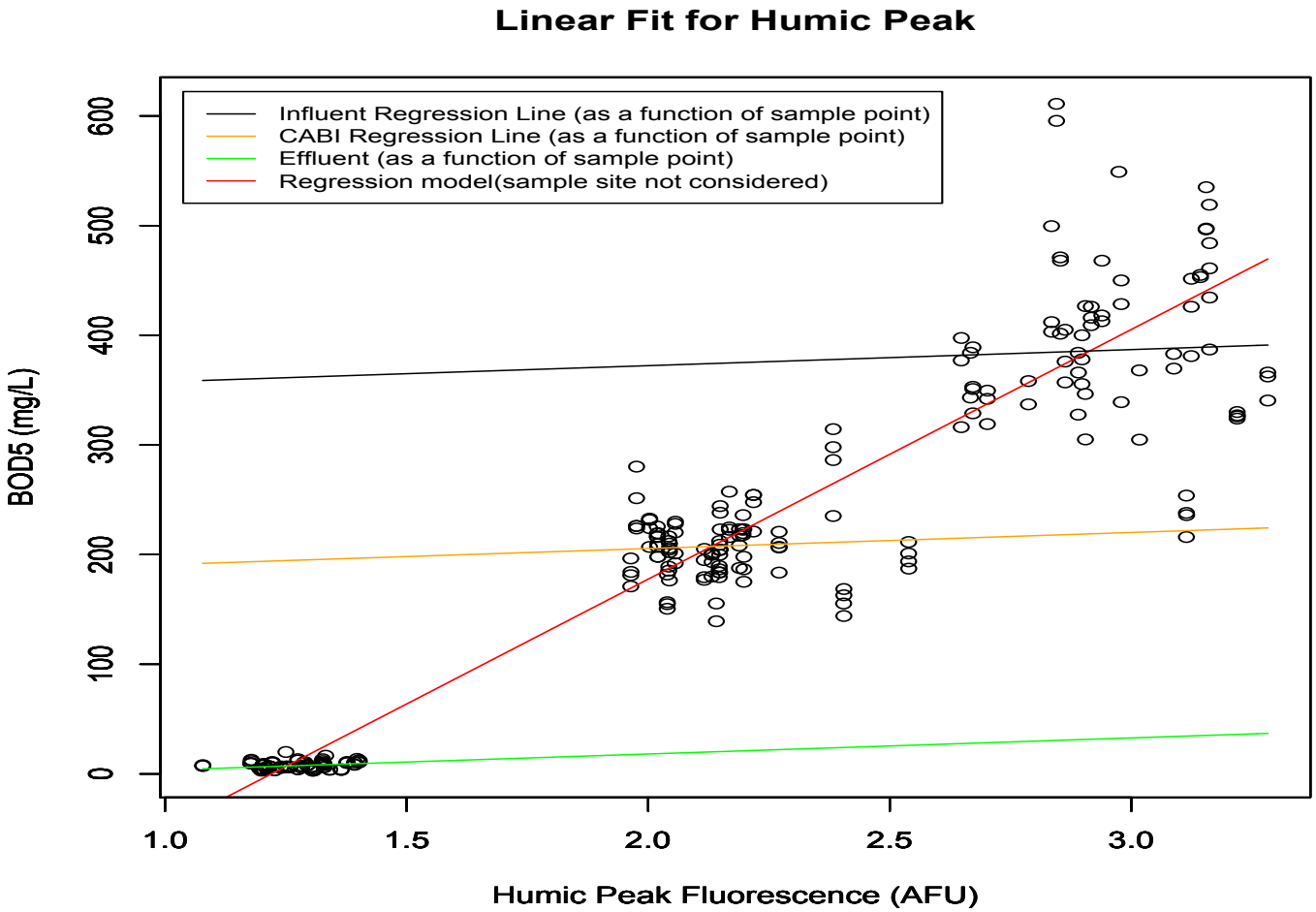

Figure 4.17: Linear regression fits between BOD_5 and humic peak fluorescence for influent, CABI, and effluent samples. The influent, CABI, and effluent regression lines (Table 4.6 Eqn II) that were developed with sample site are not good indicators for CABI or influent. The regression model that was developed with data from each sample site (Table 4.6 Eqn. I), but did not consider sample site as an independent variable, appears to estimate influent and CABI, but it is not a good fit for effluent.

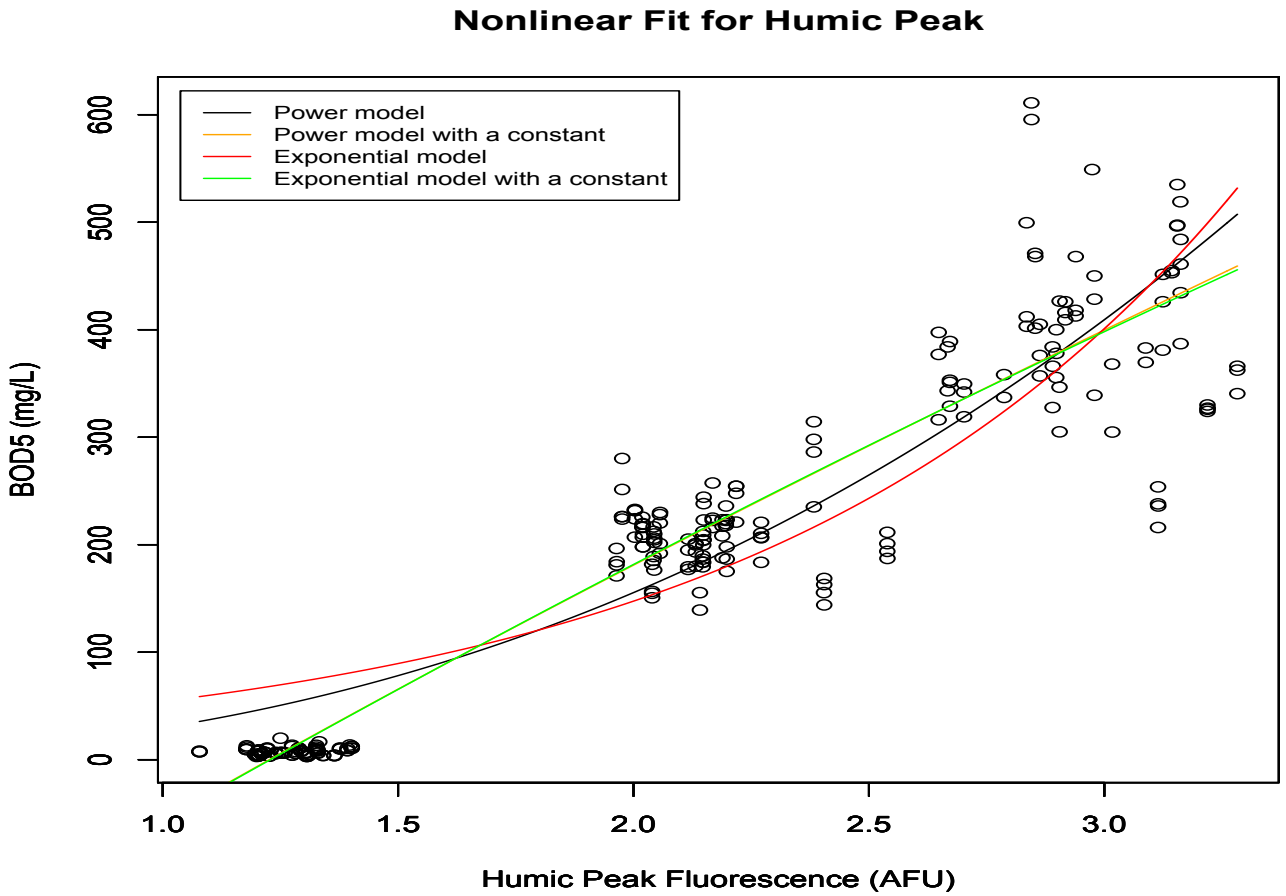

Figure 4.18: Nonlinear regression fits between BOD_5 and humic peak fluorescence for influent, CABI, and effluent samples. The power and exponential models (Table 4.6 Eqn. III and Eqn. V, respectively) are poor estimators for each of the sample sites. The power and exponential models with constants (Table 4.6 Eqn. IV and Eqn. VI, respectively) are poor estimators for effluent BOD_5, but they both appear to be reasonable estimators for CABI and influent BOD_5.

Table 4.6: Linear and Nonlinear Regression Fits for Humic Peak Fluorescence

Humic Peak Regression Models				
Reference Equation	**Generic Model**	**Regression Model**	**r^2**	**Residual Standard Error**
Eqn. I	Eqn. 4.5 – Linear model	BOD_5= 227.68*Humic-277.76	0.89	28.86
Eqn. II	Eqn. 4.6 – Linear model (sample site independent variable)	BOD_5= 14.65*Humic + 166.75*Influent - 187.34*Effluent + 176.22	0.93	13.59
Eqn. IIa	Influent	BOD_5= 14.65*Humic + 342.97	-	-
Eqn. IIb	CABI	BOD_5= 14.65*Humic + 176.22	-	-
Eqn. IIc	Effluent	BOD_5= 14.65*Humic - 11.12	-	-
Eqn. III	Eqn. 4.7 – Power model	$BOD_5 = 29.70*(Humic)^{2.39}$	0.85	64.81
Eqn. IV	Eqn. 4.8 – Power model with a constant	$BOD_5 = 297.28*(Humic)^{0.85} - 353.35$	0.90	31.97
Eqn. V	Eqn. 4.9 – Exponential model	$BOD_5 = 19.96*e^{(Humic)*1.00}$	0.75	90.80
Eqn. VI	Eqn. 4.10 – Exponential model with constant Eqn. 4.10 with a constant	$BOD_5 = 2903*e^{(Humic)*-0.094} + 2585$	0.90	32.09
No final recommendations for a best fit regression model influent, CABI, or effluent				

4.5.5 BOD_5 and Region III Correlations

The results of the robust linear and nonlinear regressions are shown in Figures 4.19 and 4.20, respectively. The regression lines, pseudo correlation coefficients, and the residual standard errors are summarized in Table 4.7. Similar to the humic peak fluorescence findings, the models have high correlation coefficients, but the graph of the models indicate that they are not necessarily a good fit. The model with the lowest residual standard error was the linear model with sample sites included as a function of the model. Figure 4.19 indicates that this is not a good fit for CABI or influent, but it fits the effluent data fairly well. When the model is considered, the slope is negative; in other words, as fluorescence increases, the BOD_5 decreases.

Each of the nonlinear models (Figure 4.20) indicates that they are reasonable estimators for influent and CABI BOD_5, but they are poor estimators for effluent BOD_5. The exponential and power models without constants overestimate effluent BOD_5. The exponential and power models with constants are a poor fit for the effluent data.

It can be concluded that neither the linear or nonlinear models are a good fit for effluent BOD_5, but the nonlinear models can be used to estimate CABI and influent BOD_5. None of the models can be used for permit compliance; therefore, region III fluorescence is not recommended as an alternate test procedure.

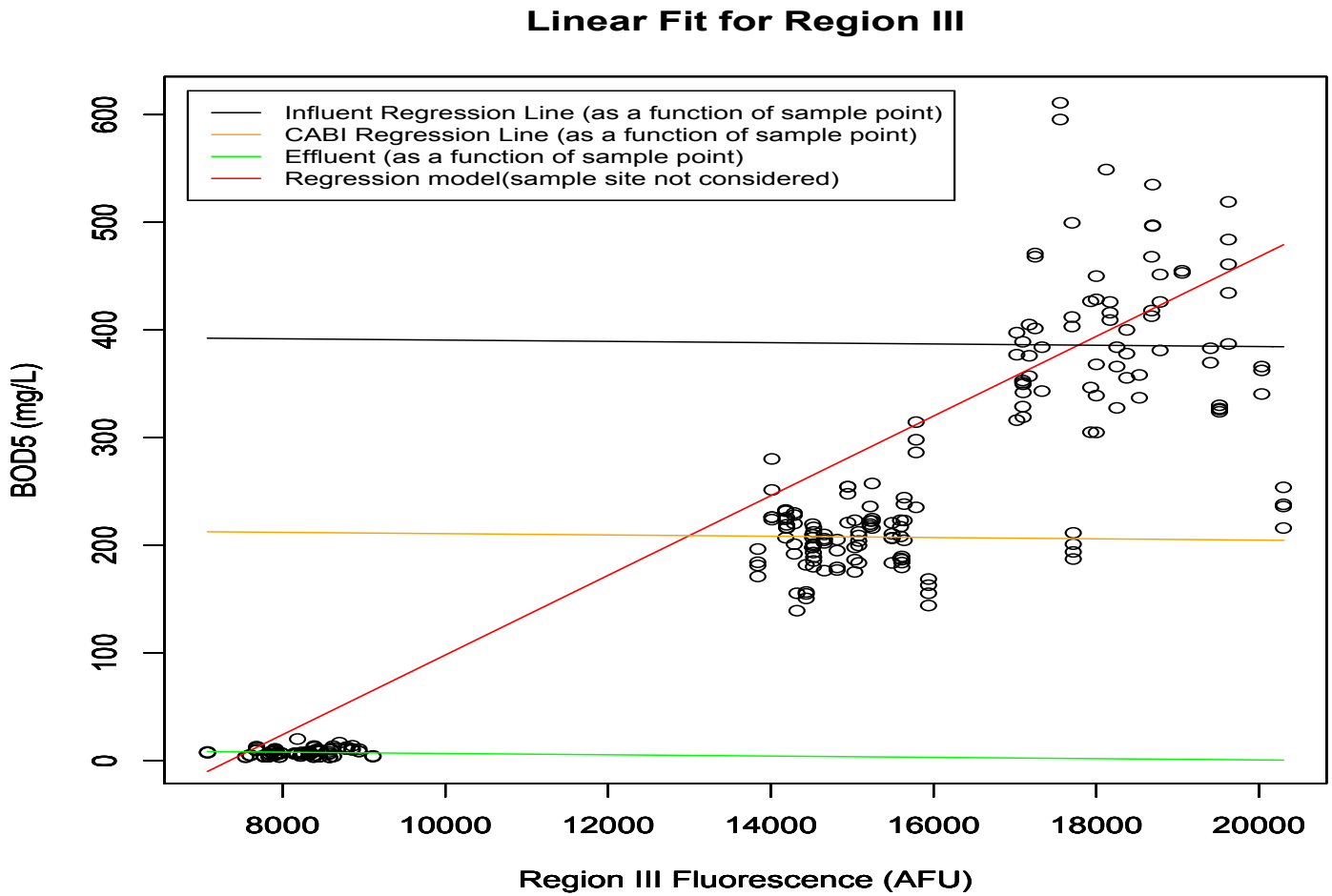

Figure 4.19: Linear regression fits between BOD_5 and region III fluorescence for influent, CABI, and effluent samples. The influent, CABI, and effluent regression lines (Table 4.7 Eqn. II) that were developed with sample site are not good indicators for CABI or influent. The regression model that was developed with data from each sample site (Table 4.7 Eqn. I), but did not consider sample site as an independent variable, appears to estimate influent, but it overestimates both effluent and CABI.

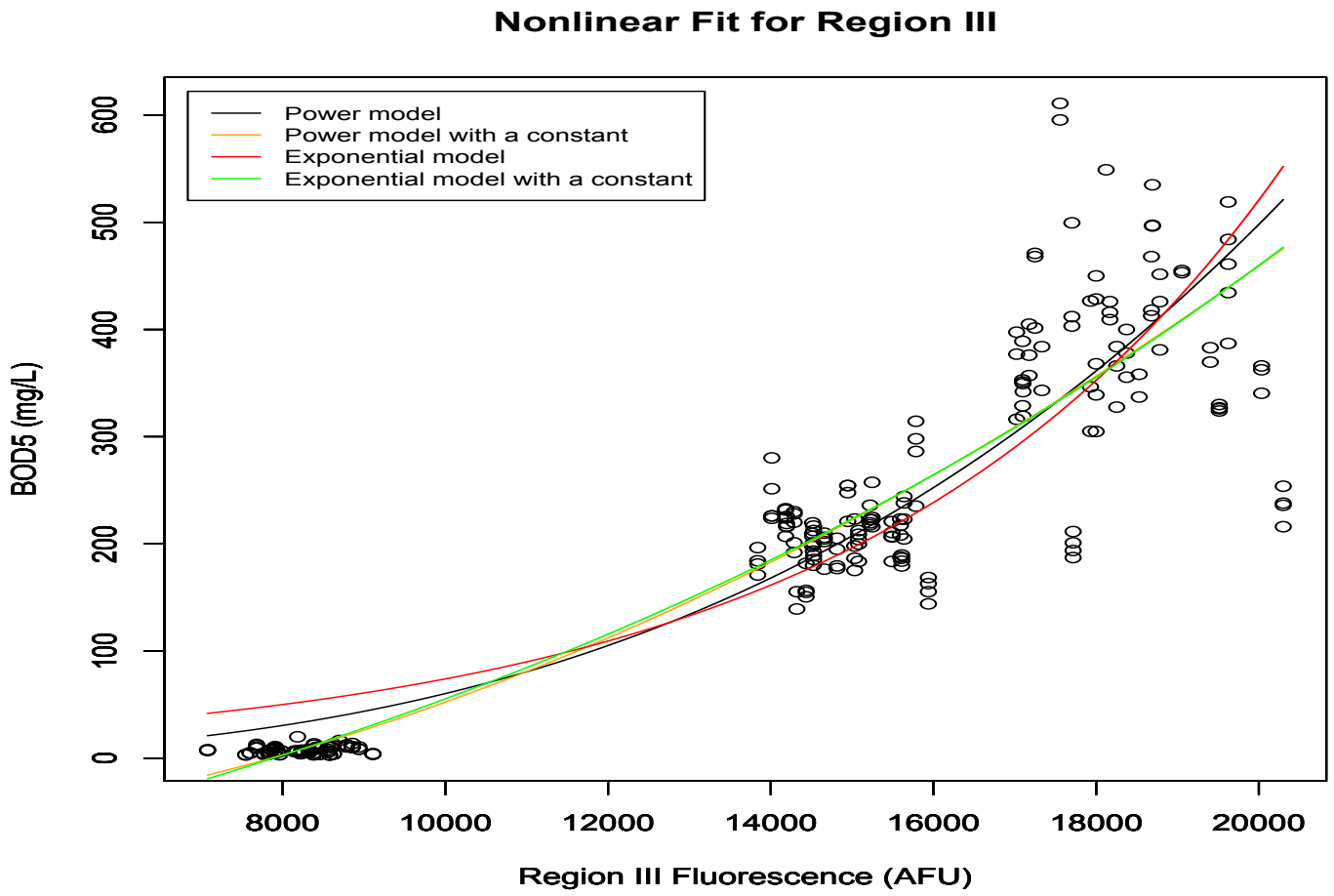

Figure 4.20: Nonlinear regression fits between BOD_5 and region III fluorescence for influent, CABI, and effluent samples. All four of the models appear to be poor estimators for effluent BOD_5, but they each appear to be reasonable estimators for CABI and influent BOD_5.

Table 4.7: Linear and Nonlinear Regression Fits for Region III Fluorescence

Region III Regression Models				
Reference Equation	**Generic Model**	**Regression Model**	**r^2**	**Residual Standard Error**
Eqn. I	Eqn. 4.5 – Linear model	BOD_5= 0.0337*Region III-271.46	0.86	30.89
Eqn. II	Eqn. 4.6 – Linear model (sample site independent variable)	BOD_5= -0.0006*Region III + 179.82* Influent - 204.18*Effluent + 216.76	0.92	14.21
Eqn. IIa	Influent	BOD_5= -0.0006*Region III + 396.58	-	-
Eqn. IIb	CABI	BOD_5= -0.0006*Region III + 216.76	-	-
Eqn. IIc	Effluent	BOD_5= -0.0006*Region III + 12.58	-	-
Eqn. III	Eqn. 4.7 – Power model	$BOD_5 = (3.99x10^{-7})$ *Region $III^{3.045}$	0.83	40.63
Eqn. IV	Eqn. 4.8 – Power model with a constant	$BOD_5 = (1.50x10^{-6})$ *Region $III^{1.99}$-84.7	0.86	24.53
Eqn. V	Eqn. 4.9 – Exponential model	$BOD_5 = 10.50*e^{(\text{Region III})*0.000195}$	0.79	65.86
Eqn. VI	Eqn. 4.10 – Exponential model with constant	$BOD_5 = 207.2*e^{(\text{Region III})*0.00006854} - 355.6$	0.86	25.00
No final recommendations for a best fit regression model influent, CABI, or effluent				

4.5.6 BOD_5 and Peak T Correlations

Several studies (Reynolds and Ahmad, 1997; Reynolds 2002; Baker and Invararity, 2004; Hudson et al., 2008; Hur et al., 2010; Lai et al., 2011; Yang et al., 2014) concluded that BOD_5 correlated well with protein-like peaks near an excitation wavelength of 280 nm and an emission wavelength of 340 nm. The peak chosen for this research was at an excitation wavelength of 275 nm and an emission wavelength of 340 nm, which is similar to the literature.

Similar to the previous fluorescence approaches, linear, power, and exponential models were developed to describe the relationship between BOD_5 and peak T fluorescence (Figure 4.21, Figure 4.22, and Table 4.8). The linear regression model that was developed independent of the sample sites is not a good fit for effluent and it underestimates influent BOD_5. The linear model that considers the sample site as an independent variable in the regression model does not appear to be a good fit either. The slope of the equation is negative, which indicates that the fluorescence decreases as the BOD_5 increases.

The nonlinear exponential model is the worst fit followed by the exponential model with a constant. Figure 4.22 and the reported correlation coefficients indicate that the power function and the exponential function with a constant are nearly identical. The power model with a constant has a slightly lower residual standard error so it may be preferred.

The results of the peak T correlation indicate the linear model is not a best fit, which is inconsistent with the literature (Reynolds and Ahmad, 1997; Reynolds 2002; Baker and Invararity, 2004; Hudson et al., 2008; Hur et al., 2010; Lai et al., 2011;Yang et al., 2014). The power function with a constant appears to be a good surrogate to estimate BOD_5 for effluent and CABI samples, although it will likely underestimate the BOD_5 of influent.

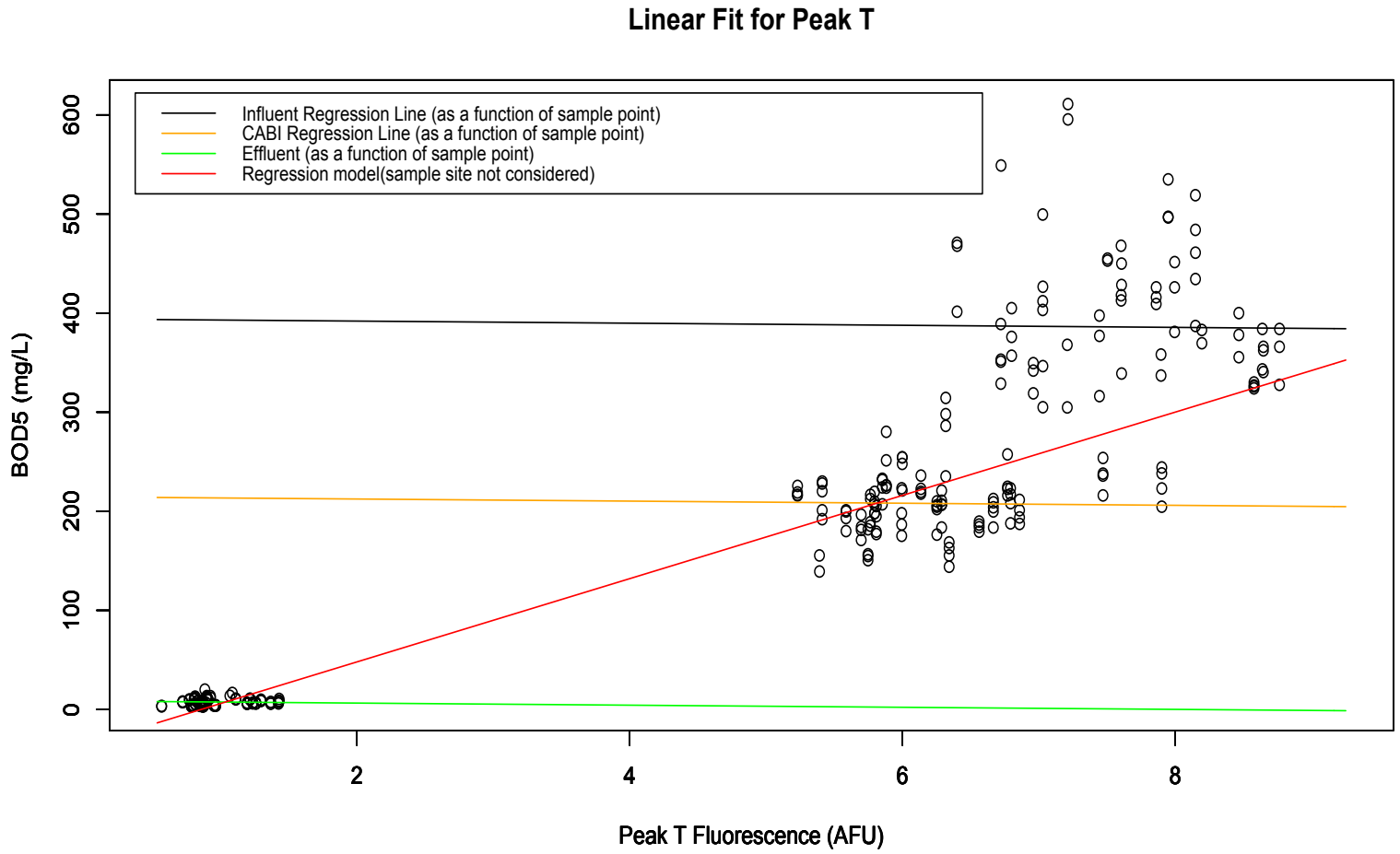

Figure 4.21: Linear regression fits between BOD_5 and peak T fluorescence for influent, CABI, and effluent samples. The regression model that was developed with sample site as an independent variable (Table 4.8 Eqn. II) is a poor fit for the data. The linear regression model that did not consider sample site as an independent variable (Table 4.8 Eqn. I) is a poor estimator for influent and effluent BOD_5.

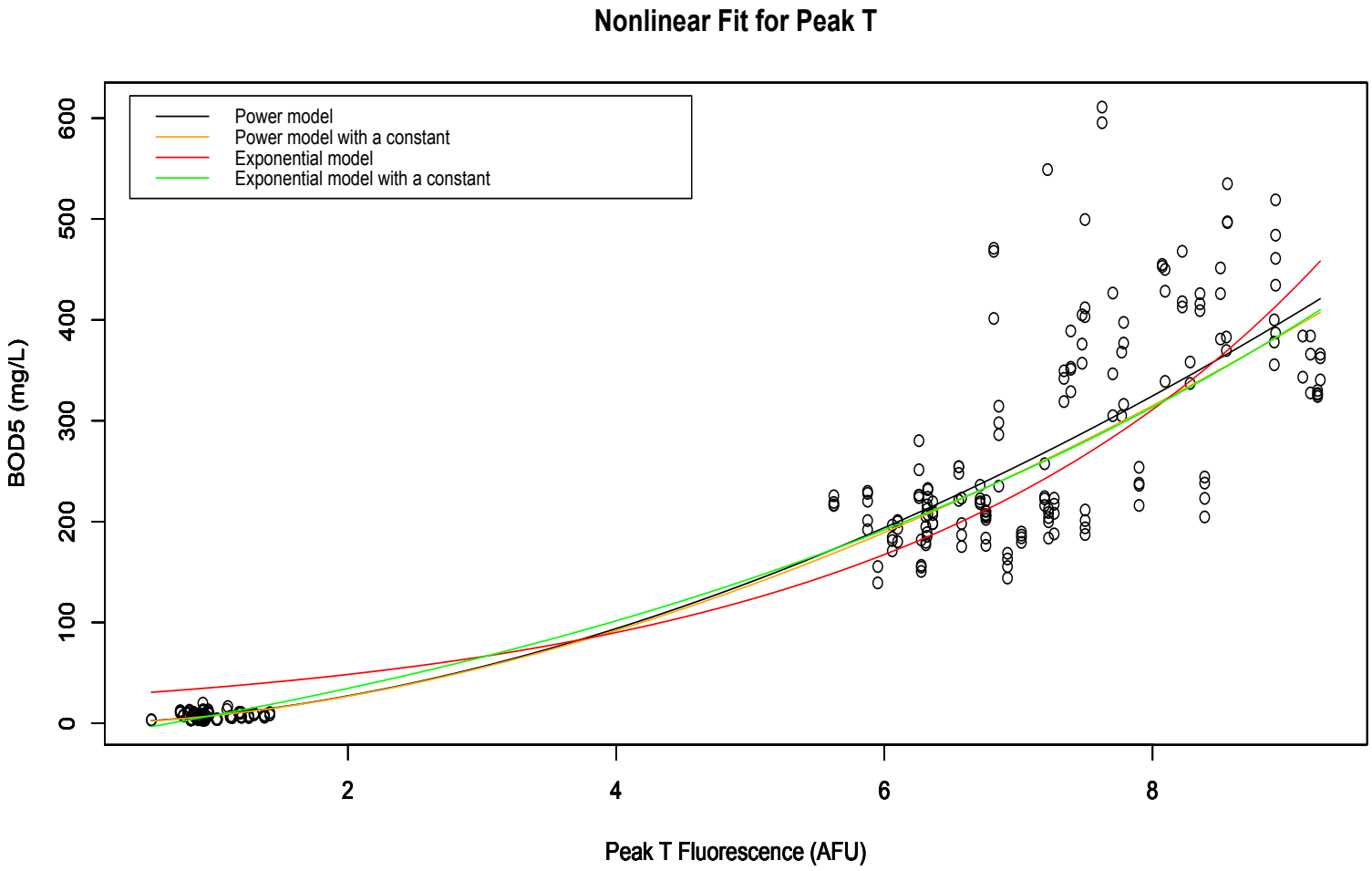

Figure 4.22: Nonlinear regression fits between BOD_5 and peak T fluorescence for influent, CABI, and effluent samples. The exponential model (Table 4.8 Eqn. V) is a poor estimator for effluent BOD_5. Each of the other models (Table 4.8 Eqn. III, Eqn. IV, and Eqn. VI) appears to be a reasonable fit for effluent and CABI, but they each underestimate influent BOD_5.

Table 4.8: Linear and Nonlinear Regression Fits for Peak T Fluorescence

Peak T Regression Models				
Reference Equation	**Generic Model**	**Regression Model**	**r^2**	**Residual Standard Error**
Eqn. I	Eqn. 4.5 – Linear model	BOD_5= 42.02*T-36.09	0.82	29.42
Eqn. II	Eqn. 4.6 – Linear model (sample site independent variable)	BOD_5= -1.06*T + 179.59*Influent - 205.97*Effluent + 214.58	0.92	14.24
Eqn. IIa	Influent	BOD_5= -1.06*T + 394.07	-	-
Eqn. IIb	CABI	BOD_5= -1.06*T + 214.48	-	-
Eqn. IIc	Effluent	BOD_5= -1.06*T + 8.51	-	-
Eqn. III	Eqn. 4.7 – Power model	$BOD_5 = (7.85) *T^{1.78}$	0.84	23.19
Eqn. IV	Eqn. 4.8 – Power model with a constant	[1]$BOD_5 = (7.95) *T^{1.77} - 0.36$	0.84	23.06
Eqn. V	Eqn. 4.9 – Exponential model	$BOD_5 = 26.05*e^{(T)*0.31}$	0.80	45.66
Eqn. VI	Eqn. 4.10 – Exponential model with constant	$BOD_5 =143.25*e^{(T)*0.149} - 158.39$	0.84	24.46
[1]Final recommendation for effluent and CABI No final recommendations are given for influent				

4.5.7 BOD_5 and Region I Correlations

Region I was chosen as a fluorescence correlation parameter because it contains the protein-like fluorescence peaks (i.e., peak T) most associated with BOD_5 as reported in the literature. Figure 4.23, Figure 4.24, and Table 4.9 show the results of the linear and nonlinear robust correlations that were developed. Similar to the findings of the peak T correlations, linear correlations do not appear to adequately describe the data (Figure 4.23).

The nonlinear exponential model is the worst fit; it overestimates effluent and underestimates influent BOD_5. Each of the other three nonlinear models has a similar trend line. They each appear to be reasonable estimators for effluent and CABI BOD_5, but they underestimate influent BOD_5. These conclusions are similar to the finding of the peak T regression models. A comparison of the statistical parameters (Table 4.9) indicates that the correlation coefficients of the three models are similar. The power model has the lowest residual standard error; therefore, it can be concluded that this is the better of the three models. The power model can be used to estimate effluent and CABI BOD_5. None of the models adequately estimated influent BOD_5, so peak T fluorescence is not recommended as an estimator for influent.

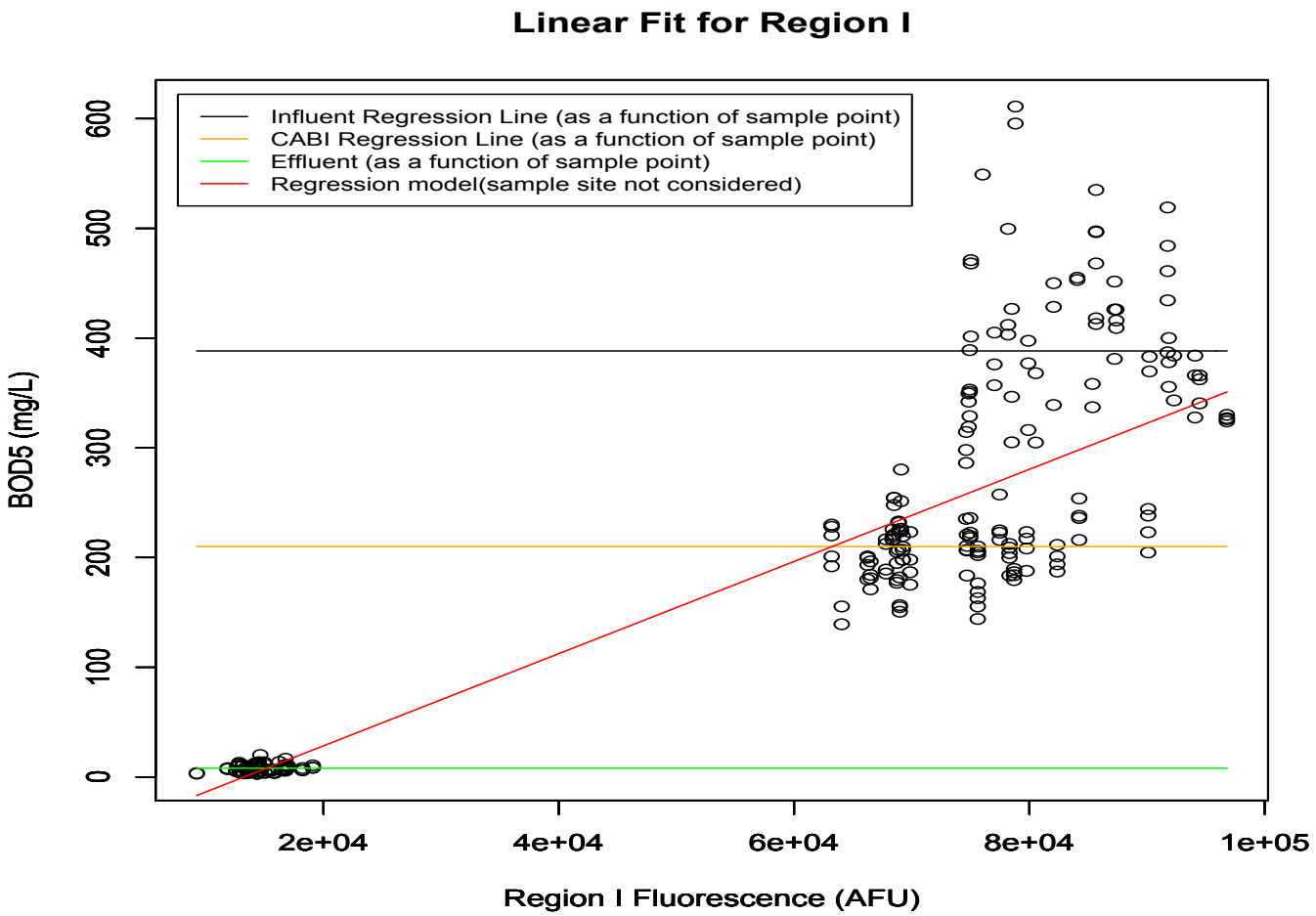

Figure 4.23: Linear regression fits between BOD_5 and peak T fluorescence for influent, CABI, and effluent samples. The regression model that was developed with sample site as an independent variable (Table 4.9 Eqn. II) is a poor fit for the data. The linear regression model that did not consider sample site as an independent variable (Table 4.9 Eqn. I) is a poor fit for effluent, it overestimates CABI, and it underestimates influent BOD_5.

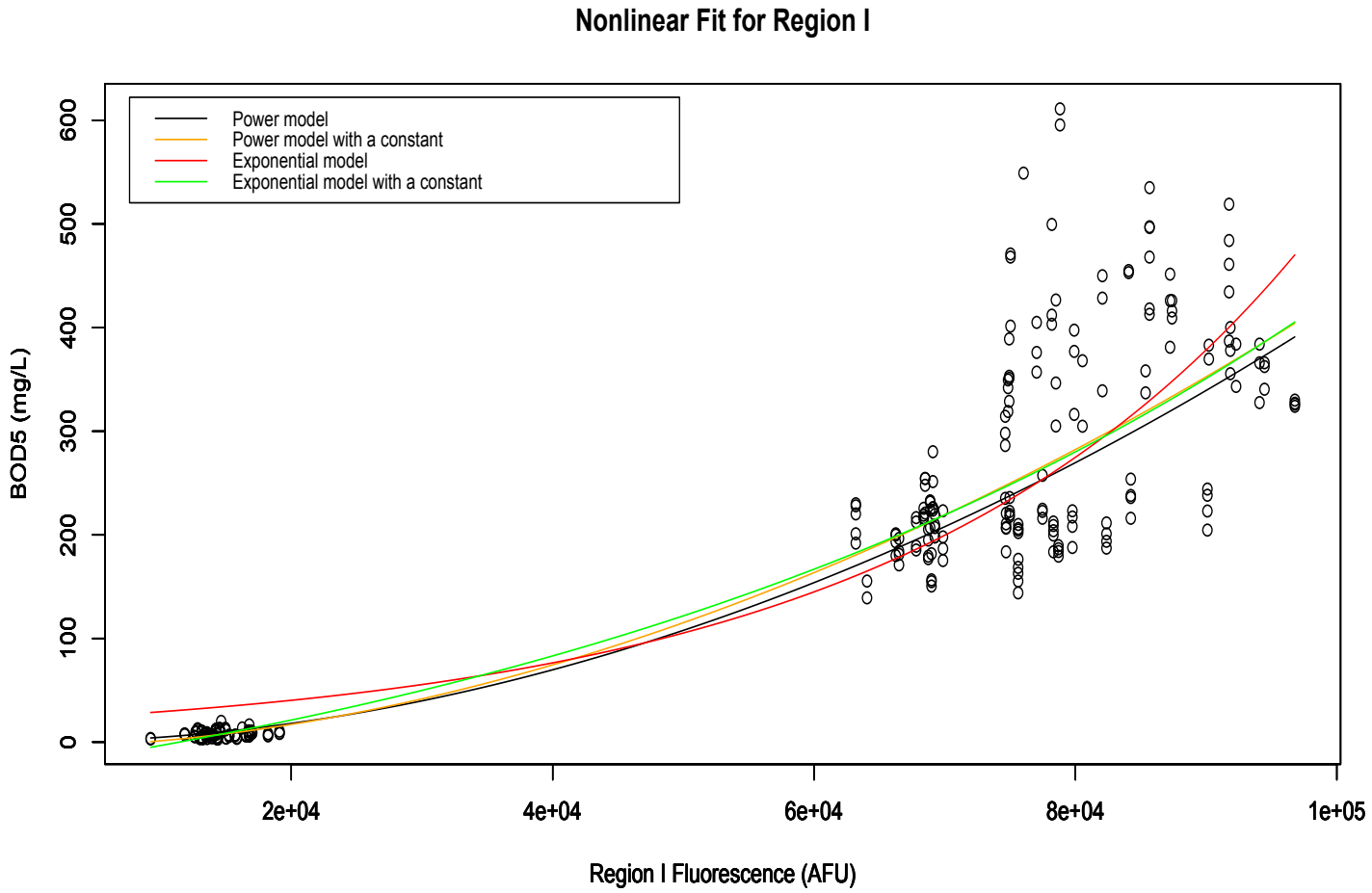

Figure 4.24: Nonlinear regression fits between BOD_5 and peak T fluorescence for influent, CABI, and effluent samples. The exponential model (Table 4.9 Eqn. V) is a poor estimator for effluent BOD_5. Each of the other models (Table 4.9 Eqn. III, Eqn. IV, and Eqn. VI) appears to be a reasonable fit for effluent and CABI, but they each underestimate influent BOD_5.

Table 4.9: Linear and Nonlinear Regression Fits for Region I Fluorescence

Region I Regression Models				
Reference Equation	**Generic Model**	**Regression Model**	**r^2**	**Residual Standard Error**
Eqn. I	Eqn. 4.5 – Linear model	BOD_5= 0.0042*Region I-55.64	0.80	33.79
Eqn. II	Eqn. 4.6 – Linear model (sample site independent variable)	BOD_5= 0*Region I + 178.25*Influent - 202.03*Effluent + 210.00	0.93	14.16
Eqn. IIa	Influent	BOD_5= 0*Region I + 388.25	-	-
Eqn. IIb	CABI	BOD_5= 0*Region I + 210.00	-	-
Eqn. IIc	Effluent	BOD_5= 0*Region I + 7.97	-	-
Eqn. III	Eqn. 4.7 – Power model	[1]$BOD_5 = (7.41x10^{-8})$ *Region $I^{1.95}$	0.81	22.22
Eqn. IV	Eqn. 4.8 – Power model with a constant	$BOD_5 = (2.28x10^{-7})$ *Region $I^{1.856}$ – 4.79	0.81	25.42
Eqn. V	Eqn. 4.9 – Exponential model	$BOD_5 = 21.28*e^{(\text{Region I})*0.0003198}$	0.77	44.56
Eqn. VI	Eqn. 4.10 – Exponential model with constant	$BOD_5 = 129.6*e^{(\text{Region I})*0.00001511} - 154$	0.80	26.11
[1]Final recommendation for effluent and CABI No final recommendations are given for influent				

4.5.8 Fluorescence Correlation Discussion

Four parameters were considered for the fluorescence correlation: the humic peak, region III, peak T, and region I. It can be concluded that the humic peak and region III are not the best surrogates for BOD_5 because they do not quantify biodegradable organic matter (Chen et al., 2003). Peaks near an excitation wavelength of 280 nm and an emission wavelength 340 nm are better surrogates for BOD_5 because they are indicators of labile organic matter that is removed during the treatment process (Hudson et al., 2007).

The best fit models for region I and peak T (ex = 275 nm and em = 340 nm) were the power function and the power function with a constant, respectively. Both of the models had similar correlation coefficients (peak T = 0.84 and region I = 0.81) and residual square errors (peak T = 23.06 and region I = 22.22). Similar statistical parameters were expected because the region represents the enclosed peaks. If the regions were defined appropriately to only include the protein like fluorescence, then it is reasonable that the region and the enclosed peaks should have similar statistical results. The results of peak T and region I fluorescence support this hypothesis. The use of a peak T fluorescence may be preferred to region I fluorescence because it takes more time to obtain the results of region fluorescence. Specifically, region fluorescence is the integrated area of several individual peaks. Therefore, the analyst must determine the fluorescence of multiple peaks to determine the region fluorescence; this is opposed to peak fluorescence, which only requires the analyst to determine the fluorescence at a specific point on the EEM graph.

The power function developed in this research is contradictory to previous studies that developed linear correlations, but the nonlinear trend can most likely be attributed to the observed IFE that was occurring in CABI and influent samples. Although it is not possible

to conclude with certainty, the literature suggests the model would have exhibited linear characteristics if the samples were diluted prior to fluorescence analysis.

An interesting trend emerged when the sample sites were considered as an independent variable in the linear model functions. The slope of region I (0) and region III (-0.0006) were nearly 0, whereas the slope of peak T (-1.06) was negative. It appears that the effluent sample sites are significantly contributing to the slope of the model, which is similar to the conclusions made in the TOC and COD correlations when sample site was not considered as an independent variable in the regression equation. The results were that the model was not a good predictor for either CABI or influent. As previously discussed, the ratios change as treatment progresses, indicating that independent correlations for sample sites should be developed or nonlinear regression models should be explored. Therefore, it can be concluded that the regression models that consider sample site are not an adequate representation of the physical relationship between BOD_5 and the alternate methods.

4.6 BOD_5 and Absorbance Correlations

The chosen wavelengths to correlate for this research were at 254 nm and 340 nm. Figure 4.25 and Figure 4.26 summarize the data used for the correlation for 254 nm absorbance and 340 nm absorbance, respectively. The first was chosen because it is the most common wavelength that absorbance is reported at (Tchobanoglous et al., 2003), and the latter was chosen because it falls in middle of the fluorescence spectrum. Several wavelengths have been successfully correlated based on linear regression (Comber et al., 1996; Wu et al., 2006; Chevakidagarn, 2006; Natarja et al., 2006; Michail and Idelvoitch, 1981). Nonlinear trends were observed in the absorbance data (Figure 4.25 and Figure 4.26), similar to the trends of the fluorescence data, which is contradictory to the literature.

4.6.1 BOD_5 and Absorbance at 254 nm Correlation

The linear trends are shown in Figure 4.27. From the figure, it is clear that the basic linear regression model is a poor estimator for each sample site and the linear model that considers each sample site as an independent variable is not a better indicator either. In fact, the regression line (Table 4.10) indicates that the slope is negative. Neither of the linear regression models are good predictors.

From Figure 4.28, the power and exponential functions without constants are poor fits for the effluent data. Their counterparts with constants appear to be the better fits, but the power function with a constant is slightly better due to the smaller residual standard error. Both of these models appear to overestimate CABI and underestimate influent BOD_5. It can be concluded that absorbance at 254 nm is not a good estimator for BOD_5.

4.6.2 BOD_5 and Absorbance at 340 nm Correlation

The results for the 340 nm absorbance correlations are summarized in Figure 4.29, 4.30, and Table 4.11. Both of the linear models are poor estimators for each of the sample sites. Figure 4.30 indicates that each of the nonlinear models are poor fits for the data as well. The power model passes through effluent, but is underestimates CABI and influent. The exponential model overestimates effluent and underestimates influent, but it appears to estimate CABI fairly well. The power and exponential models with constants are poor estimators for effluent, they overestimate CABI, and they underestimate influent. It can be concluded that the absorbance at 340 nm is a poor estimator for BOD_5.

4.6.3 Absorbance Correlation Discussion

A feasible correlation between absorbance and BOD_5 could not be found in this research, which is contradictory to other studies that have found strong relationships (Comber et al., 1996; Wu et al., 2006; Chevakidagarn, 2006; Natarja et al., 2006; Michail and Idelvoitch, 1981). Comber et al. (1996) concluded that the relationship between BOD_5 and absorbance weakened when samples were filtered, which may explain the weak correlation. Furthermore, absorbance is a measure of how much light passes through a sample, which in an indirect measurement of the organic matter. It can thus be concluded that absorbance is a poor surrogate for BOD_5 because of the method itself and because strong correlations could not be established.

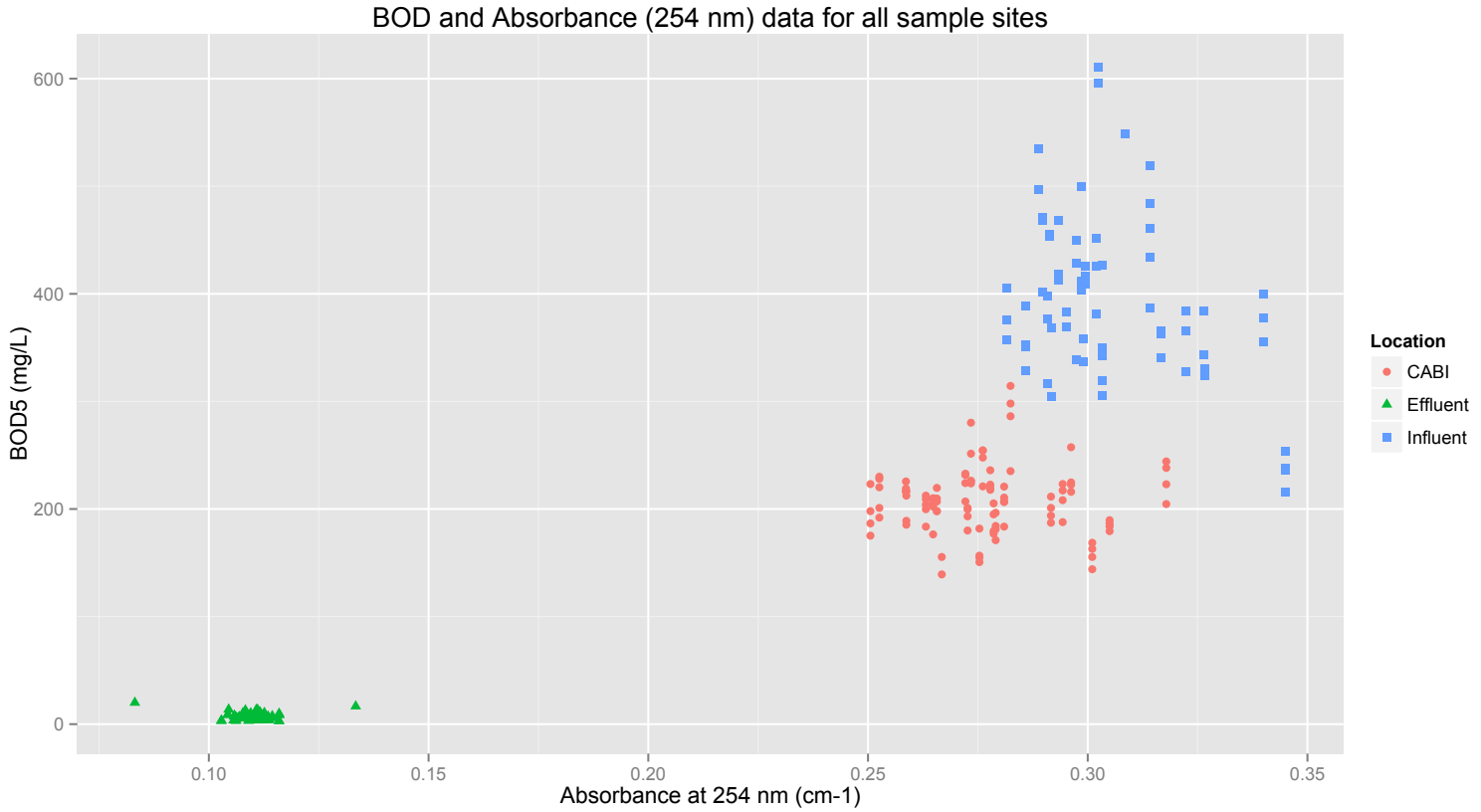

Figure 4.25: BOD_5 and absorbance data for absorbance at 254 nm.

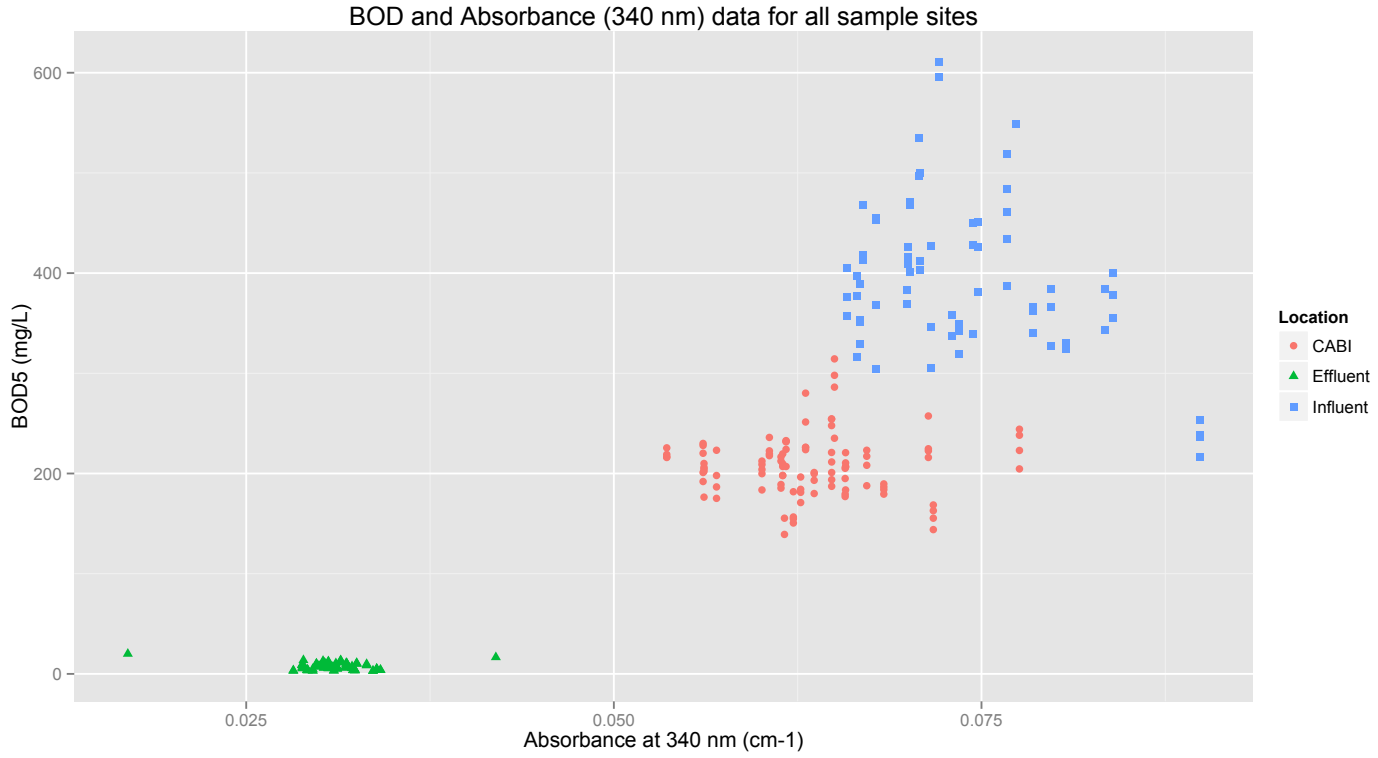

Figure 4.26: BOD_5 and absorbance data for absorbance at 340 nm.

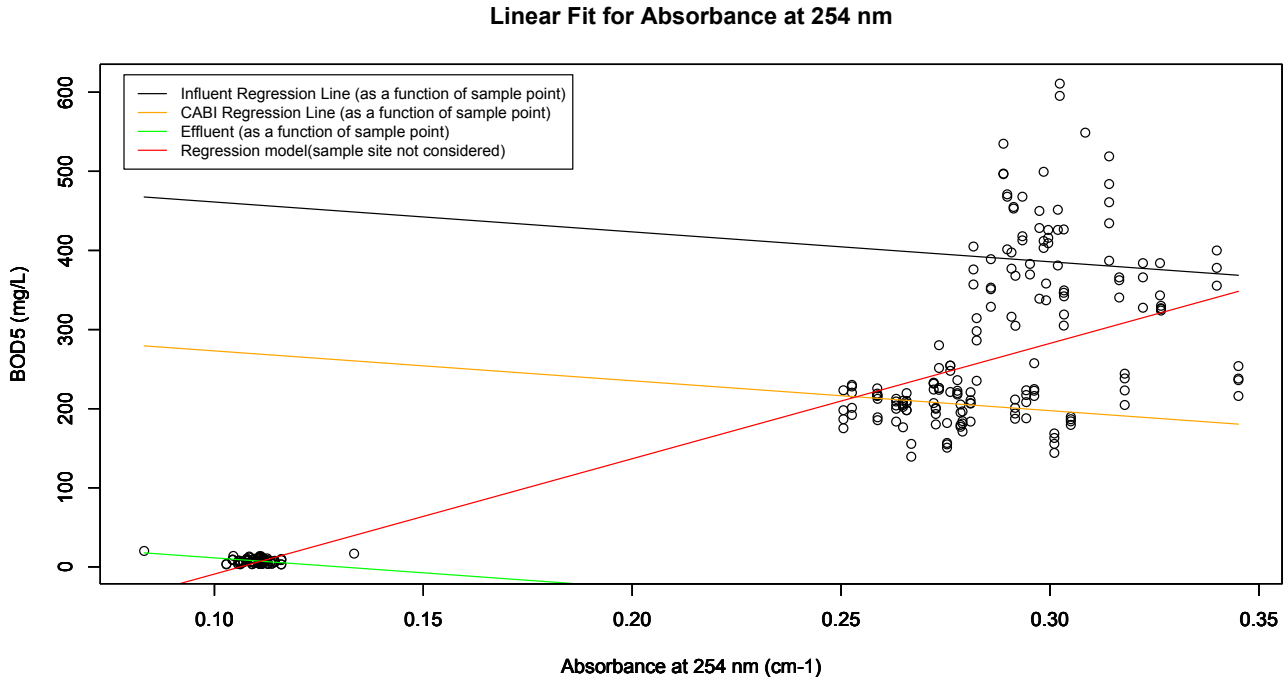

Figure 4.27: Linear regression fits for absorbance at 254 nm. The regression model that considers sample site as an independent variable (Table 4.10 Eqn. II) is a poor estimator for each of the samples sites. The linear model that does not consider sample site as an independent variable (Table 4.10 Eqn. I) is a poor fit for effluent, it overestimates CABI, and it underestimates influent BOD_5.

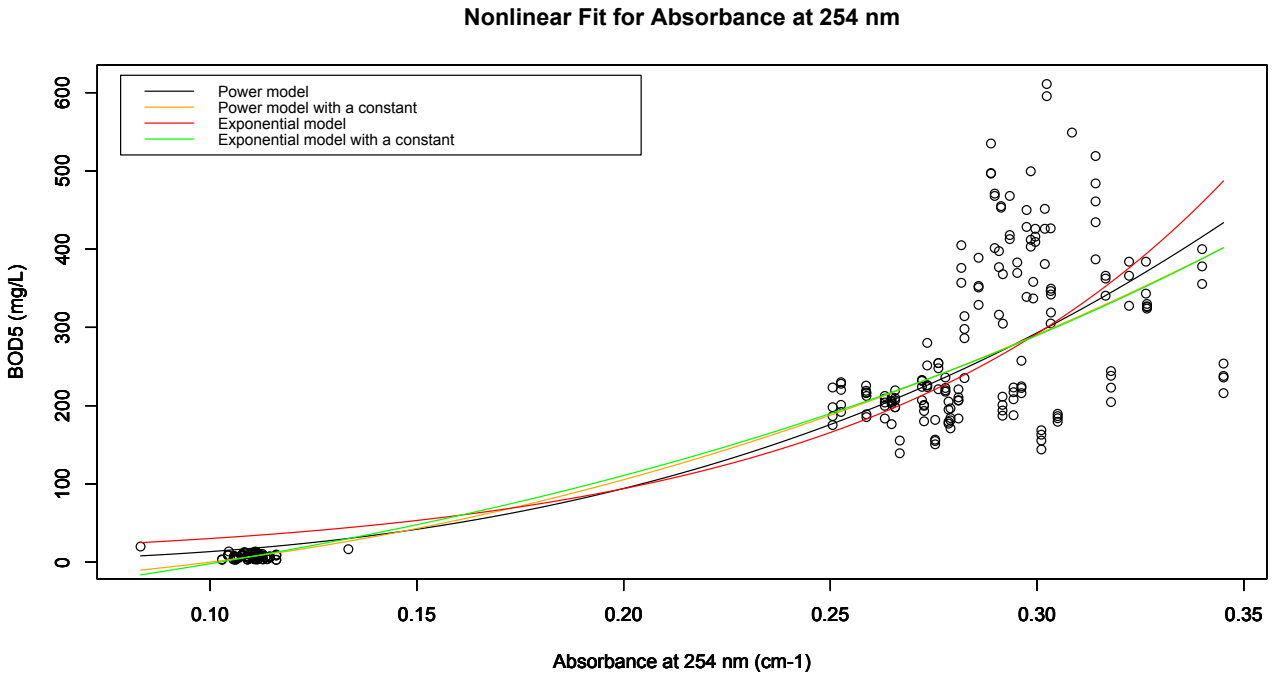

Figure 4.28: Nonlinear regression fits for absorbance at 254 nm. The power and exponential models (Table 4.10 Eqn. III and Eqn. V) overestimate effluent and CABI BOD_5, and they underestimate influent BOD_5. The models with constants (Table 4.10 Eqn. IV and Eqn. VI) overestimate CABI and they underestimate influent BOD_5.

Table 4.10: Absorbance at 254 nm Linear and Nonlinear Regression Models

Absorbance at 254 nm Regression Models				
Reference Equation	**Generic Model**	**Regression Model**	**r^2**	**Residual Standard Error**
Eqn. I	Eqn. 4.5 – Linear model	BOD_5= 1457.67*Abs - 155.07	0.77	31.93
Eqn. II	Eqn. 4.6 – Linear model (sample site independent variable)	BOD_5= -377.88*Abs + 188.28*Influent - 261.763*Effluent + 310.79	0.93	13.18
Eqn. IIa	Influent	BOD_5= -377.88*Abs + 499.07	-	-
Eqn. IIb	CABI	BOD_5= -377.88*Abs + 310.79	-	-
Eqn. IIc	Effluent	BOD_5= -377.88*Abs - 49.03	-	-
Eqn. III	Eqn. 4.7 – Power model	$BOD_5 = (8533) *Abs^{2.80}$	0.76	26.14
Eqn. IV	Eqn. 4.8 – Power model with a constant	$BOD_5 = (4090) *Abs^{2.11} - 31.64$	0.77	22.07
Eqn. V	Eqn. 4.9 – Exponential model	$BOD_5 = 9.73*e^{(Abs)*11.34}$	0.73	44.02
Eqn. VI	Eqn. 4.10 – Exponential model with constant	$BOD_5 =120.41*e^{(Abs)*4.63} - 193.20$	0.77	22.33
No final recommendations are given for influent, CABI, or effluent				

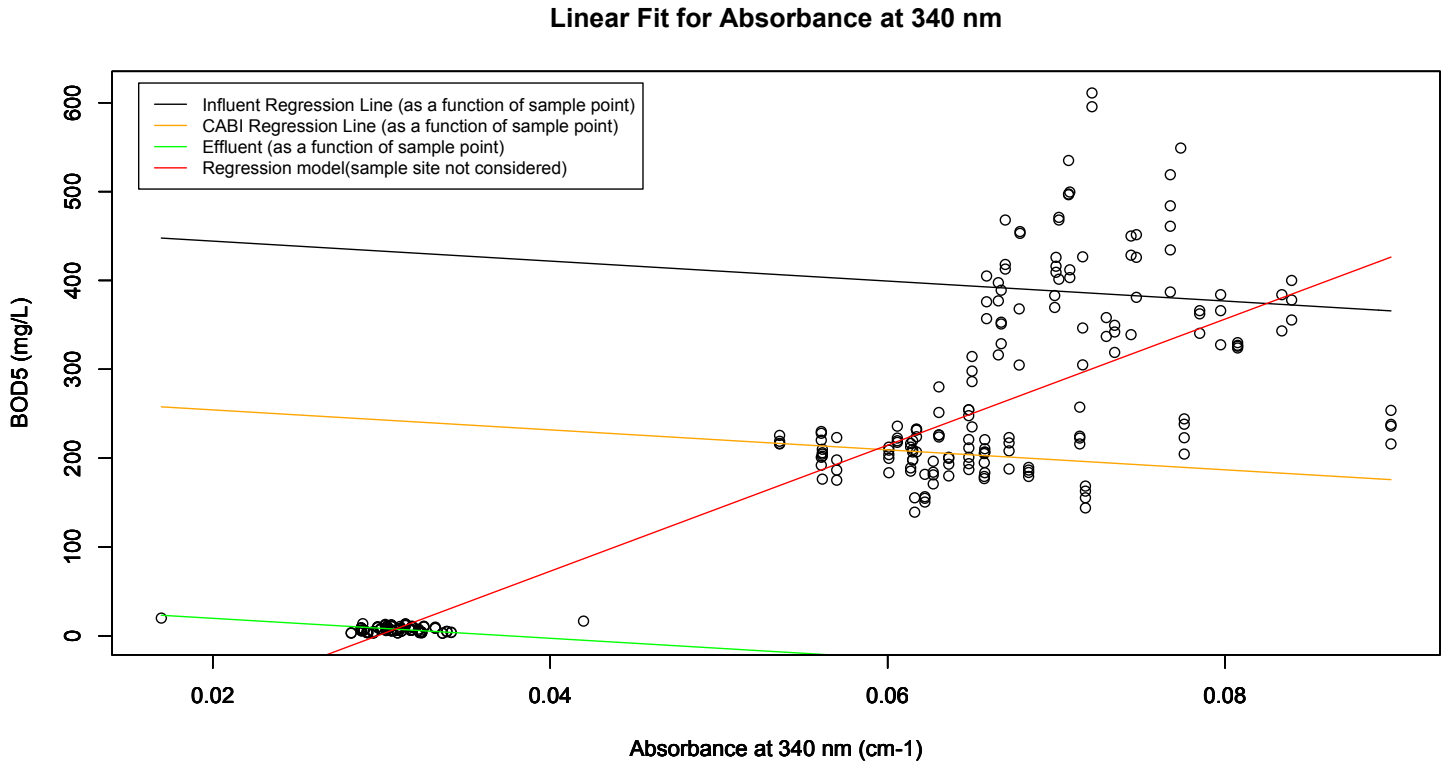

Figure 4.29: Linear regression fits for absorbance at 340 nm. The regression model that considers sample site as an independent variable (Table 4.11 Eqn. II) is a poor estimator for each of the sample sites. The linear model that does not consider sample site as an independent variable (Table 4.11 Eqn. I) is a poor fit for effluent, it overestimates CABI, and it underestimates influent BOD_5.

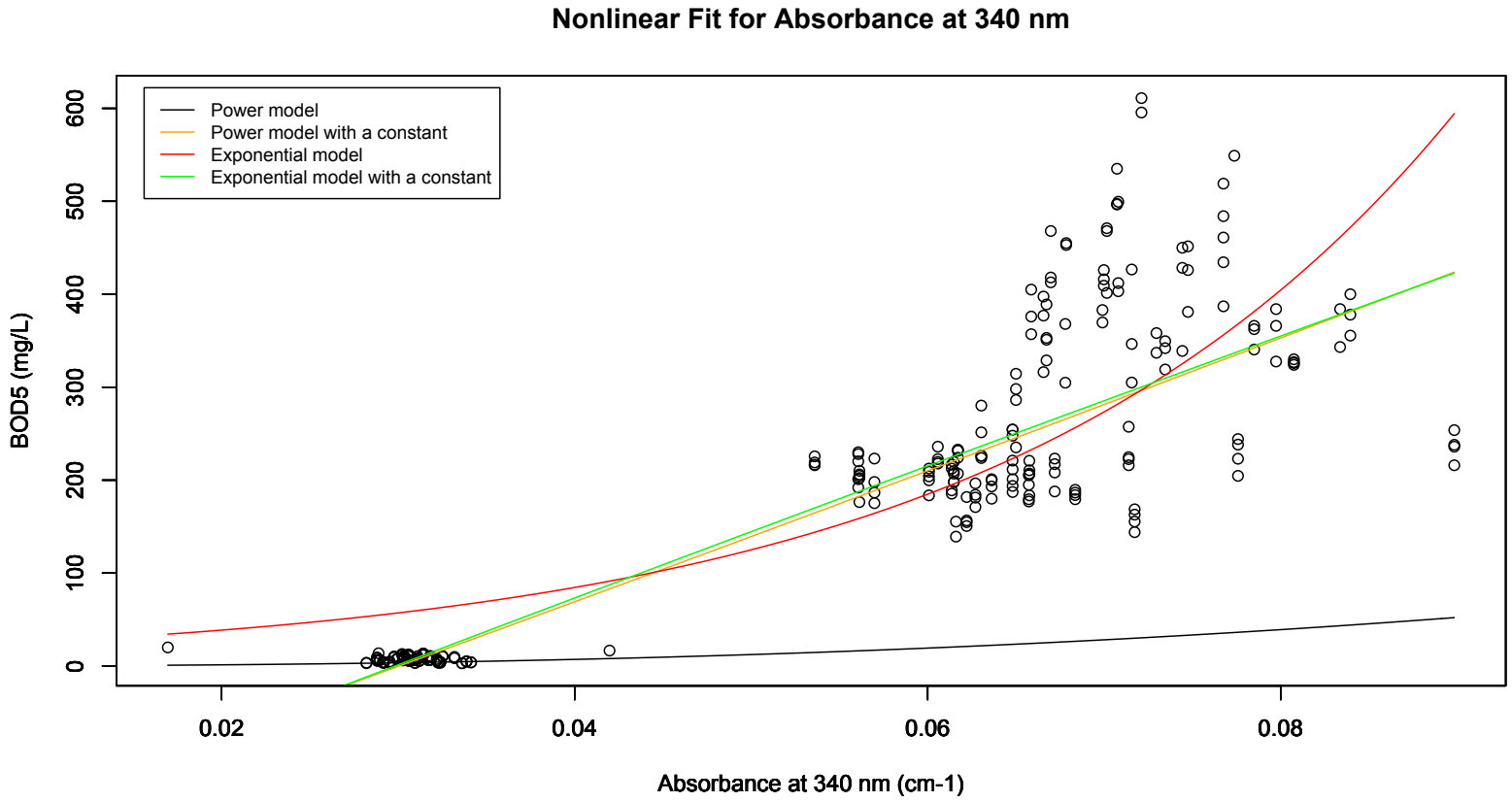

Figure 4.30: Nonlinear regression fits for absorbance at 340 nm. The power model (Table 4.11 Eqn. III) grossly underestimates the CABI and influent BOD_5. The exponential model (Table 4.11 Eqn. V) overestimates effluent and underestimates influent BOD_5. The exponential and power models with a constant (Table 4.11 Eqn. IV and VI) are poor fits for effluent, they overestimate CABI, and the underestimate influent BOD_5.

Table 4.11: Absorbance at 340 nm Linear and Nonlinear Regression Models

Absorbance at 340 nm Regression Models				
Reference Equation	**Generic Model**	**Regression Model**	**r^2**	**Residual Standard Error**
Eqn. I	Eqn. 4.5 – Linear model	BOD_5= 7094*Abs - 211	0.78	31.72
Eqn. II	Eqn. 4.6 – Linear model (sample site independent variable)	BOD_5= -1124*Abs + 189.94*Influent - 234.56*Effluent + 276.80	0.92	14.35
Eqn. IIa	Influent	BOD_5= -1124*Abs + 466.74	-	-
Eqn. IIb	CABI	BOD_5= -1124*Abs + 276.80	-	-
Eqn. IIc	Effluent	BOD_5= -1124*Abs + 42.24	-	-
Eqn. III	Eqn. 4.7 – Power model	$BOD_5 = (19550) *Abs^{2.46}$	0.73	50.23
Eqn. IV	Eqn. 4.8 – Power model with a constant	$BOD_5 = (7641) *Abs^{1.04} - 199.61$	0.78	31.23
Eqn. V	Eqn. 4.9 – Exponential model	$BOD_5 = 17.66*e^{(Abs)*39.14}$	0.66	76.89
Eqn. VI	Eqn. 4.10 – Exponential model with constant	$BOD_5 =-8871*e^{(Abs)*0.835} - 8652$	0.78	32.25
No final recommendations are given for influent, CABI, or effluent				

4.7 Final Recommendations for Alternate Test Procedures to Estimate BOD_5

Several surrogates were considered as alternatives to BOD_5, including COD, TOC, fluorescence, and absorbance. COD correlated well for all sample sites, TOC correlated well for effluent, and protein-like fluorescence correlated well for effluent and CABI, but not for influent. Absorbance and fluorescence that is not related to protein-like fluorescence, specifically humic-like and region III, did not correlate well and they are not recommended as a surrogates for BOD_5.

COD can be used to estimate BOD_5 for each sample site. Eqn. 4.11 (r^2=0.77) and Eqn. 4.12 (r^2=0.73) summarize the final models to estimate effluent and to estimate CABI or influent BOD_5. Eqn. 4.13 is valid for both influent and CABI, because the sample sites had similar ratios.

$$\text{Effluent } BOD_5 = 0.49 * COD - 7.78 \qquad \text{Eqn. 4.12}$$

$$\text{CABI or Influent } BOD_5 = 0.52 * COD + 5.09 \qquad \text{Eqn. 4.13}$$

TOC can be used to estimate BOD_5 of effluent, but it was not a good surrogate for influent or CABI. Eqn. 4.14 (r^2=0.87) summarizes the final model that can be used to estimate the BOD_5 of effluent.

$$\text{Effluent } BOD_5 = 2.25 * TOC - 12.07 \qquad \text{Eqn. 4.14}$$

Peak T and region I fluorescence can be used as a surrogate for BOD_5 for effluent and CABI, but use of these models would underestimate influent BOD_5. Eqn. 4.15 (r^2=0.84) and 4.16 (r^2=0.81) summarize the models. Both of these models are similar in terms of statistics, but peak T fluorescence can be obtained more quickly, so it is recommended.

$$\text{CABI or Effluent } BOD_5 = 7.95 * (\text{peak T fl.})^{1.77} - 0.36 \qquad \text{Eqn. 4.15}$$

$$\text{CABI or Effluent } BOD_5 = (7.41\text{x}10^{-8}) * (\text{region I fl.})^{1.95} \qquad \text{Eqn. 4.16}$$

A sensitivity analysis was conducted on each of the five recommended models to determine if the sample size was sufficient. Specifically, the sample size of each dataset was reduced to determine if the regression model and/or the correlation coefficients changed. A new dataset was created by randomly selecting 90% of data points from the original dataset and a new regression model was generated from new dataset. This process was repeated 1,000 times and the mean coefficients of the model and the mean correlation coefficients were determined, which were then compared with the models reported in the results. It was concluded that reducing the number of data points by 10% had minor impacts (i.e., they did not change by more than 5%) on the regression models and the correlation coefficients. Therefore, it can be concluded that a sufficient number of data points were used to generate each of the final recommended regression models.

4.8 Implications for WWTPs and their NPDES Permits

Wastewater treatment plants should consider available resources (e.g., TOC equipment, COD equipment, etc.) and their ultimate goals (e.g., NPDES alternate test procedure, in situ monitoring, process control) to determine which of these methods is the most practical for their plant. For instance, COD and BOD_5 had a good correlation for effluent, CABI, and influent. Many plants have Hach spectrophotometers at their facility, which can be used to complete the analysis, and the test is straightforward and easy to perform/interpret. This option may be beneficial for both process control and permit compliance. The TOC method was a good surrogate for effluent BOD_5; therefore, it may be a good option for permit compliance, but not necessarily process control. Fluorescence is not an approved Standard Method (2005); therefore, it is unlikely that it can currently be used as an alternate test procedure for NPDES permit compliance. Despite this fact, several studies

have shown that fluorescence can be used as a surrogate to BOD_5 and as a means to characterize the wastewater. It may be possible in the near future for the EPA to recognize fluorescence as a method for wastewater organic quantification, and thus treatment plants can use fluorescence as an alternate test method. In the meantime, treatment plants can use fluorescence to characterize their wastewater and potentially collect data for anticipated EPA changes that could allow them to use the method in lieu of BOD_5.

The models developed in this research are specific to the treatment facility where the samples were collected, but some of the generalized findings and conclusions can be used for other WWTPs. This research, along with the findings of previous studies, have concluded that correlation models are site specific due to the changing ratio as treatment progresses. This change in ratio is due to the selective removal of organics in the treatment process, and the fact that each alternate test procedure quantifies different types of organic matter. Furthermore, the correlation models are WWTP specific because each treatment facility operates at different efficiencies. The wastewater characteristics and the ratios can vary depending on the treatment facility efficiency, as well as the quality of wastewater influent (e.g., domestic versus industrial wastewater). Based on this logic, it can be further concluded that changes or additions to unit processes within the WWTP can potentially impact the correlation. Specifically, if a unit process selectively removes organic matter, it will change the BOD_5 ratio both at that point in the treatment process and downstream.

This research used samples collected from a single WWTP to develop correlations, which is highly recommended to other facilities. Clustering of data was observed at each sample site; the clusters of influent data were more spread and became tightly clustered as treatment progressed. This was expected, because influent wastewater is uncontrolled, but

the quality of wastewater is controlled as treatment progresses because the WWTP is engineered to remove organic matter. This type of data clustering is not ideal for developing site-specific correlations, but it is indicative of a treatment facility that is operating consistently. Combining samples collected from multiple treatment facilities is not preferred due to the difference in influent quality and difference in BOD_5 ratios among treatment plants, but this approach may lead to site-specific correlations. Data collected from multiple facilities will not be clustered due to the different influent wastewater characteristics and the different organic removal efficiencies, thus making it possible to develop site-specific correlations. Despite the potential positive results of this approach, combining samples collected from multiple sites is not recommended because the physical relationship and organic removal among multiple facilities is not identical.

WWTPs looking into alternate test procedures should consider the impacts of the testing methodology as well. In this research, samples were not diluted for the fluorescence testing to mimic in situ monitoring. This choice led to IFE, which quenched the fluorescence results, and may have caused the nonlinear relationships. In the TOC analysis, samples were acidified and sparged, which may have removed some of the organic matter and biased the results low. Although these are systematic errors, it is important to understand the impacts of the test procedure on the organic quantification. Slight changes to test procedure can impact the test results, which can lead to changes in the correlation model.

Part B: Removal Efficiency

4.9 Removal Efficiency of BOD_5, COD, TOC, and Absorbance

The removal efficiency of BOD_5, TOC, COD, and absorbance in the primary clarifier, biological treatment (i.e., the aeration basin), and the entire treatment plant was determined. The removal equations are summarized below. The removal for each sample day was calculated; only samples that were analyzed the same day as collection were included in the removal efficiency calculations. R statistical software was used to bootstrap 1,000 samples from the original dataset. The average and standard deviation of the bootstrap samples were used to construct a 95% confidence interval of the removal efficiency.

$$\text{Removal of primary clarifier} = \frac{\text{Influent} - \text{CABI}}{\text{Influent}} \text{x}100\% \qquad \text{Eqn. 4.17}$$

$$\text{Removal of biological treatment} = \frac{\text{CABI} - \text{Effluent}}{\text{CABI}} \text{x}100\% \qquad \text{Eqn. 4.18}$$

$$\text{Removal of treatment plant} = \frac{\text{Influent} - \text{Effluent}}{\text{Influent}} \text{x}100\% \qquad \text{Eqn. 4.19}$$

The removal efficiencies of each method are summarized in Table 4.12 and Figures 4.31 to 4.36 summarize the boxplots for each removal. The n value given in Table 4.12 represents the original number of data points, but as mentioned 1,000 bootstrap samples were created from the original dataset. The boxplots were plotted based on the original dataset.

Table 4.12: Removal Efficiency of BOD_5, COD, TOC, and Absorbance

		Primary		Biological Treatment		Entire Plant	
Method	**n**	**Mean**	**95% Confidence interval (±)**	**Mean**	**95% Confidence interval (±)**	**Mean**	**95% Confidence interval (±)**
BOD_5	21	48.15	4.75	96.26	0.84	98.13	0.36
COD	36	44.93	1.99	91.76	0.68	95.52	0.34
TOC	50	9.15	3.28	85.37	0.79	86.82	0.70
Absorbance 254 nm	21	8.27	2.42	61.08	1.05	64.29	1.12
Absorbance 340 nm	21	14.99	4.59	52.60	2.08	59.60	2.93

Figure 4.34 is a boxplot of the TOC removal, based on historical TOC data. The following discussion will address how it is related to the current data and its relevance. Unfortunately, historical data were not available for the other methods to make similar comparisons.

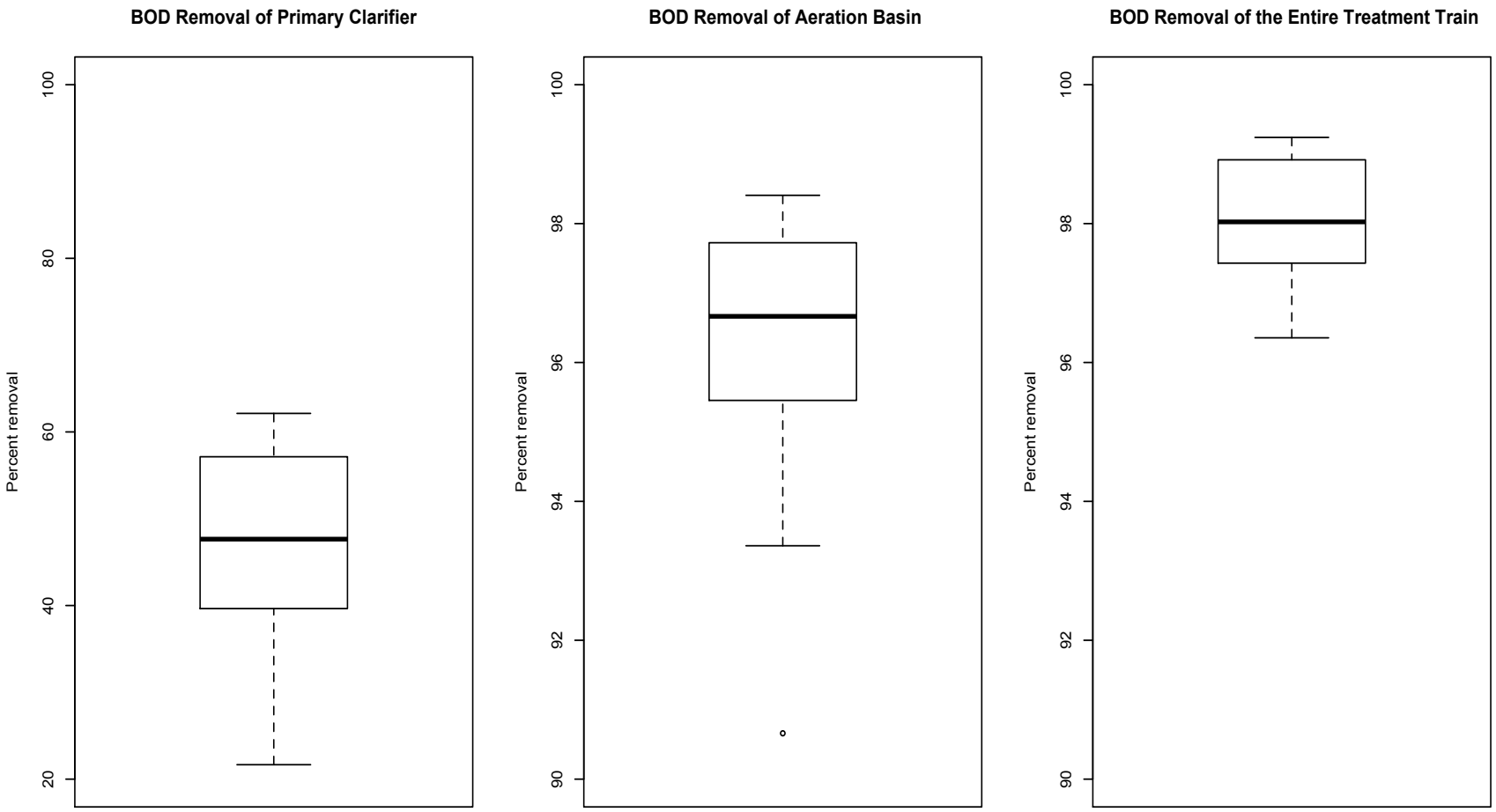

Figure 4.31: Boxplot of the BOD_5 removals of the primary clarifier, biological treatment, and the entire treatment process.

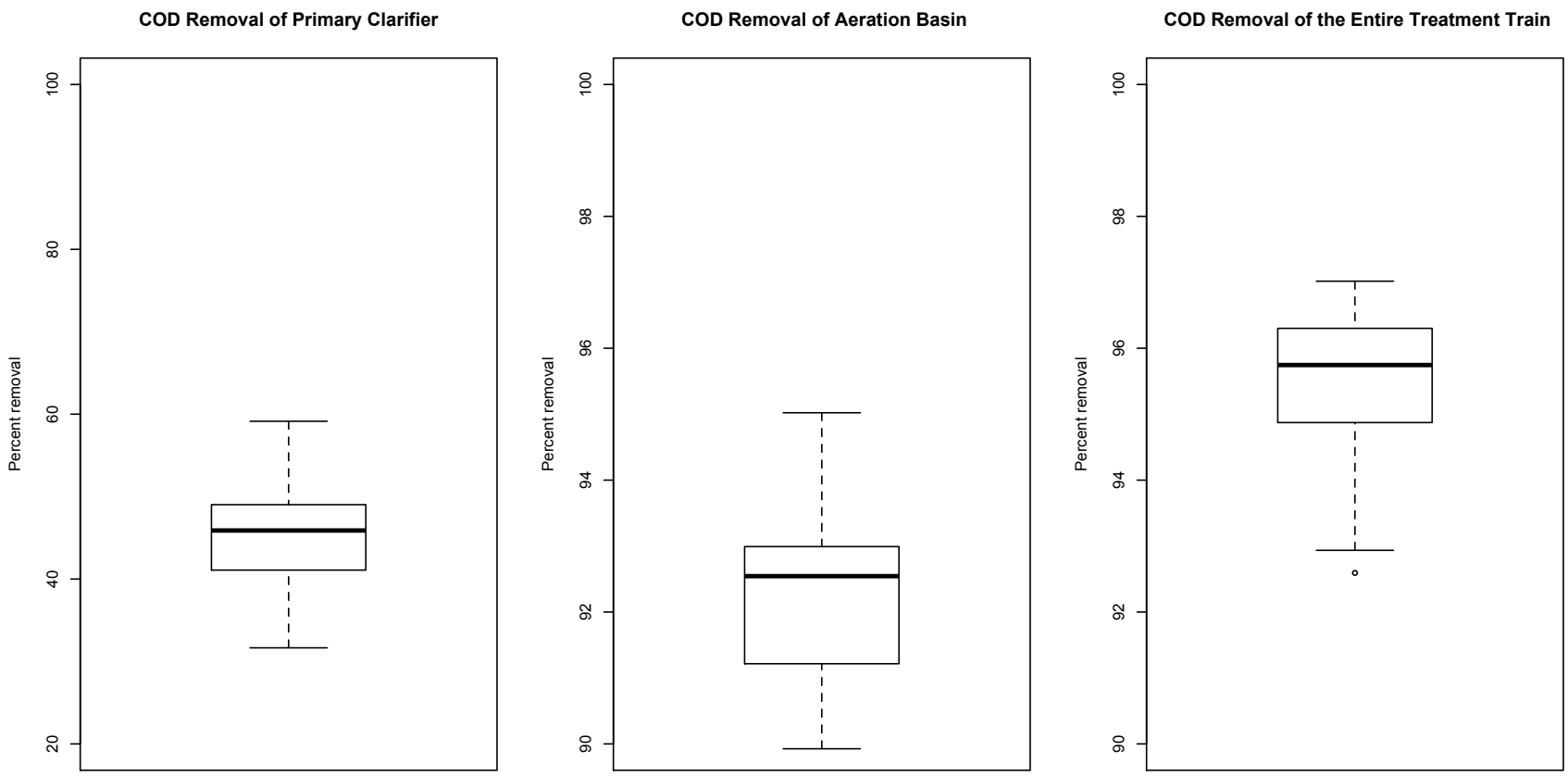

Figure 4.32: Boxplot of the COD removals of the primary clarifier, biological treatment, and the entire treatment process.

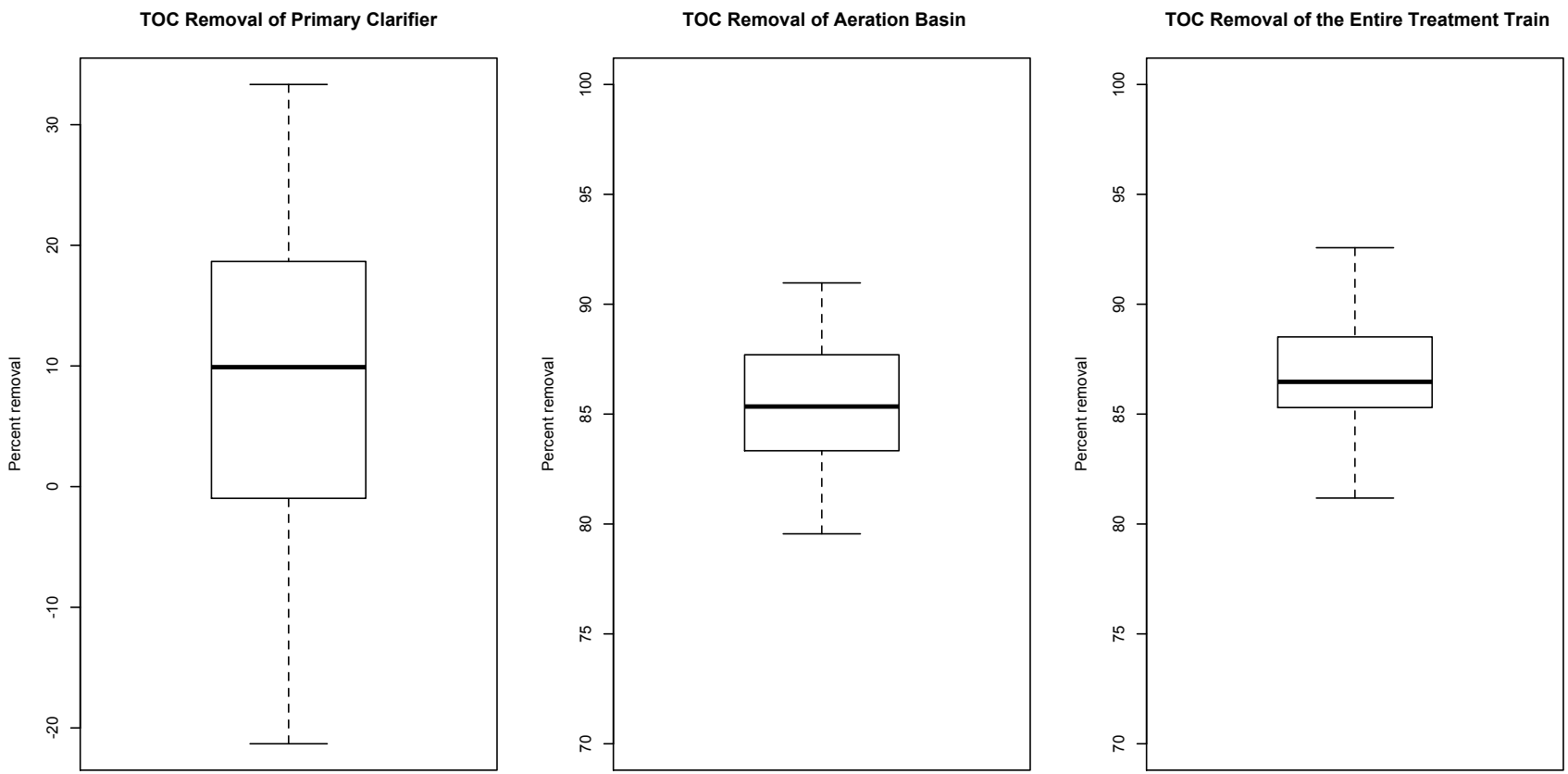

Figure 4.33: Boxplot of the TOC removals of the primary clarifier, biological treatment, and the entire treatment process.

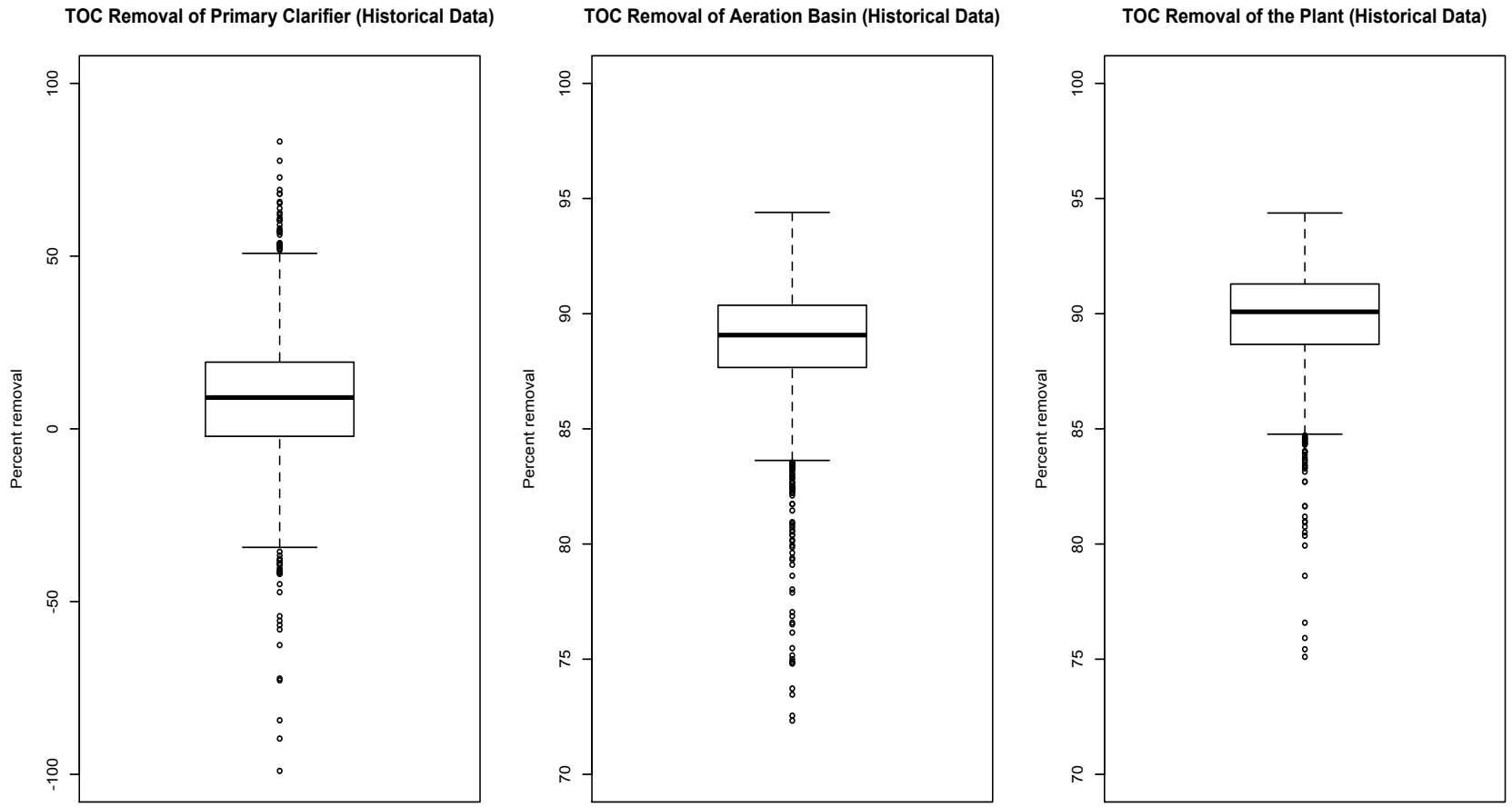

Figure 4.34: Boxplot of the historical TOC removals of the primary clarifier, biological treatment, and the entire treatment process.

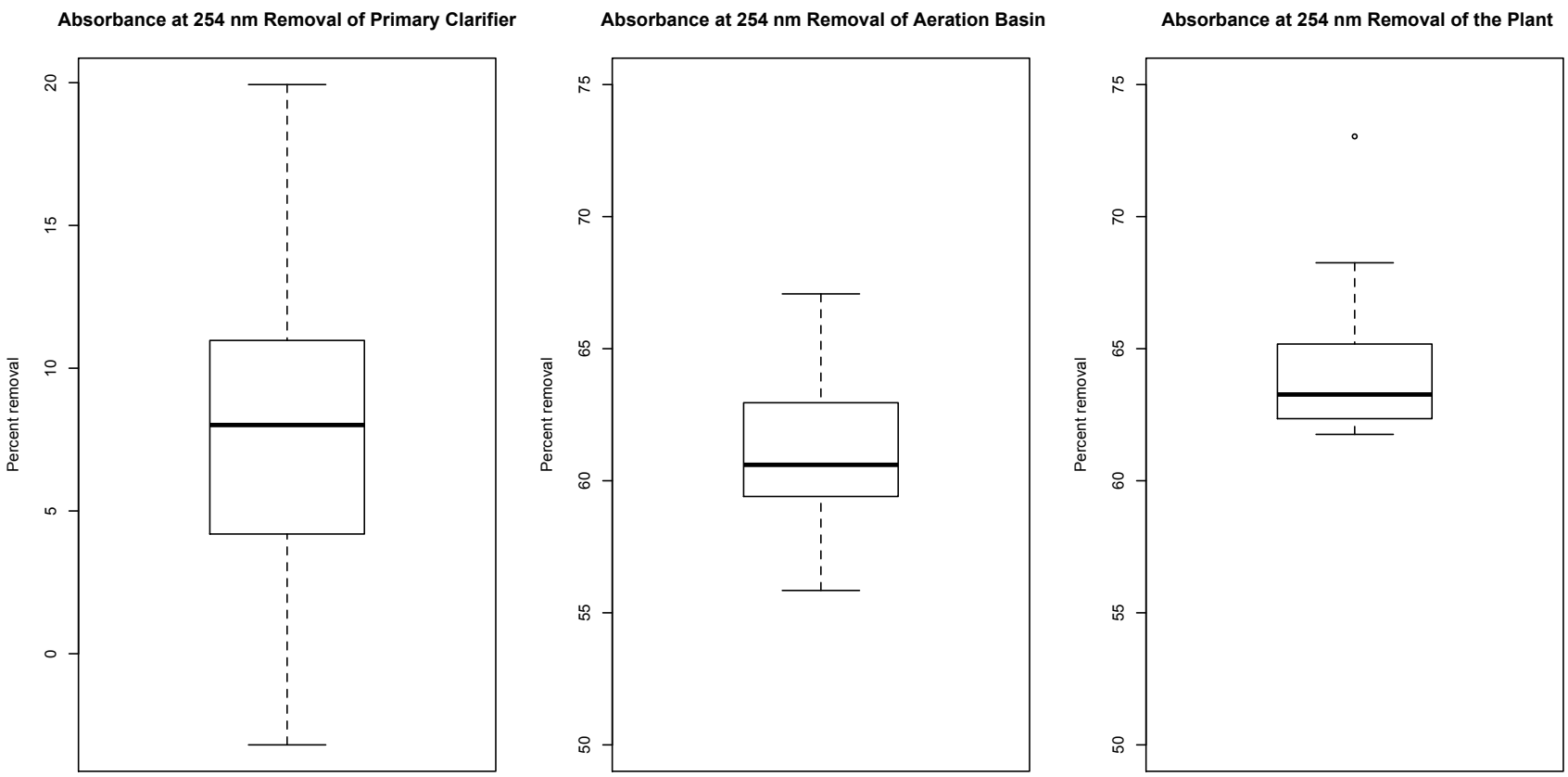

Figure 4.35: Boxplot of the absorbance at 254 nm removals of the primary clarifier, biological treatment, and the entire treatment process. .

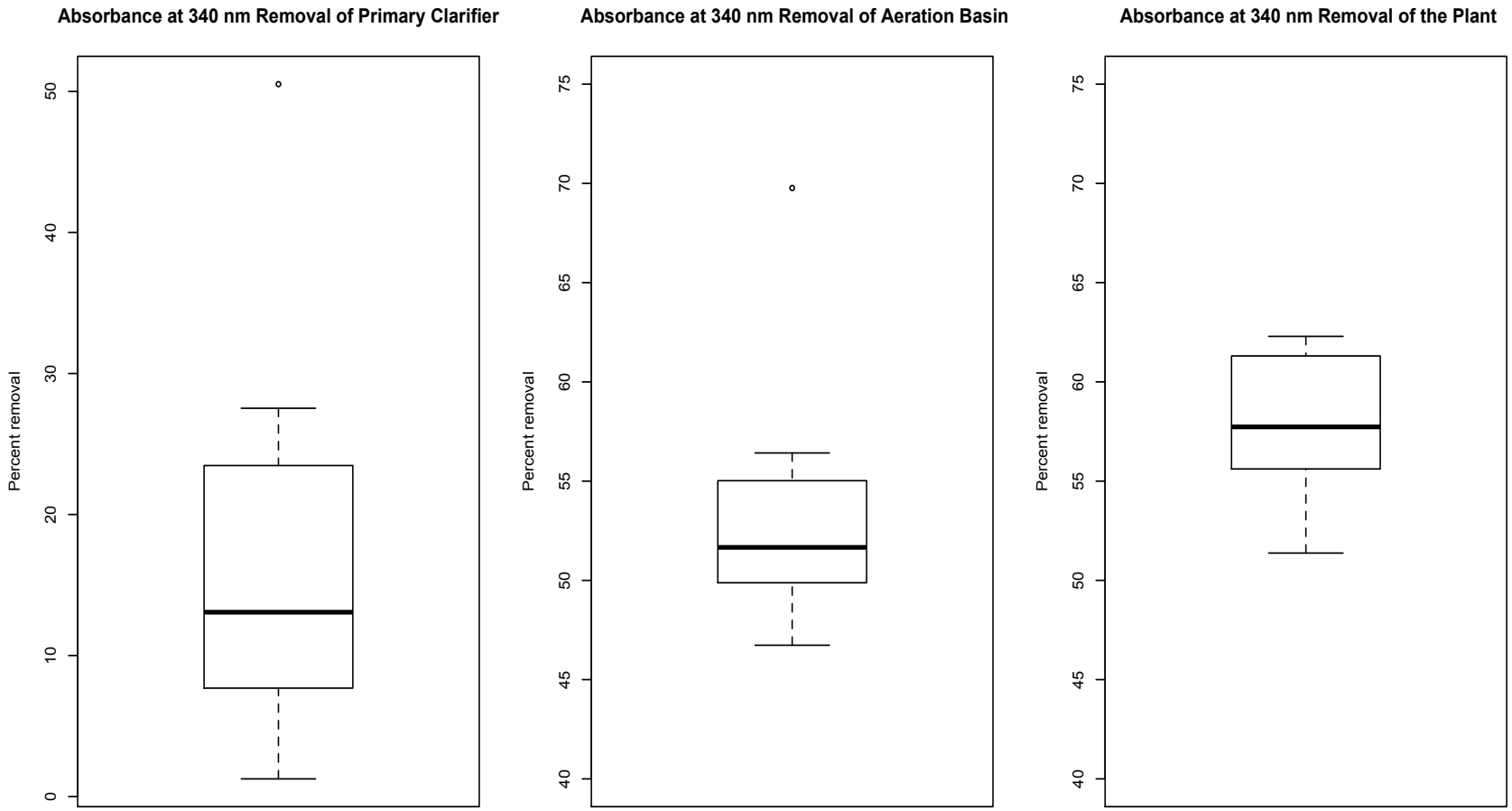

Figure 4.36: Boxplot of the absorbance at 340 nm removals of the primary clarifier, biological treatment, and the entire treatment process.

The results indicate that approximately 48% of BOD_5 is removed in the primary clarifiers, 96% of BOD_5 entering the biological treatment is removed, and 98% of BOD_5 is removed during the entire process. Tchobanoglous et al. (2003) reported that between 50 and 80% of colloidal biodegradable organic matter are removed in the primary clarifier when chemical precipitates are used, which is consistent with the findings of this research albeit on the lower end of this range. As expected, biological treatment, using activated sludge, is extremely effective at removing biodegradable organic matter. The removal of the treatment plant indicates that the facility is removing nearly all of the biodegradable organic matter that enters the plant. The plant's NPDES permit specifies that the 30-day average removal of TSS and BOD_5 must be a minimum of 85%. Figure 4.31 shows that the wastewater treatment removed over 96% of the BOD_5 during data collection for this research.

The results of COD removal (Table 4.12 and Figure 4.32) are similar to the removals observed for BOD_5, except that the COD removals are slightly less than the BOD_5 values. COD quantifies the organic matter based on the organics that are oxidized using potassium dichromate, which can also include some inorganic matter and non-biodegradable organic matter. Inorganics are not readily biodegradable; therefore, they are not readily removed during biological treatment. The BOD_5 test does not quantify these inorganics or non-biodegradable organics, so the method will not reflect their lack of removal, whereas the COD test will. Furthermore, the chemicals used in the COD test are capable of oxidizing more biodegradable organic matter than the microorganisms used in the BOD_5 test. The effect is that observed COD removals will be slightly less than the observed BOD_5 removals.

The average TOC removal in the primary clarifier was quite low, and Figure 4.33 indicates that there were actually increases in TOC in the primary clarifier. The mean removals for the biological treatment and the entire treatment plant are similar.

Historical TOC data that were collected from 2010 to 2014 were statistically analyzed to determine the TOC removal efficiencies. The removal efficiency was calculated for each data point (n=1,642) and 1,000 bootstrap samples were used to calculate the mean, standard deviation, and confidence intervals. The boxplot of the historical data are shown in Figure 4.34. The average TOC removal of the primary clarifier was determined to be 8.03±0.94%, the average removal of biological treatment was 88.58±0.15%, and the removal of the entire plant was 89.78±0.11%. The historical means are similar to the means calculated for this research (9.15±2.38%, 85.37±0.79%, and 86.82±0.70%). A comparison between the current and historical boxplots (Figure 4.33 and Figure 4.34) indicates that the current data did not capture the large variations that can occur in primary removal efficiencies. Specifically, the historical data indicate that the removal varied from -100% to +90% (Figure 4.34), but the removals observed in this research ranged from -20% to +30%. This is further support that longer sampling periods are necessary to develop adequate correlations.

A mass balance of the primary clarifier is necessary to understand the organic loading and why TOC sometimes increased. Organics are input into the primary clarifier through influent wastewater and through return streams, which include the centrate return (Figure 3.1), as well as thickened sludge overflow and supernatant from the anaerobic digestion of the primary and secondary sludge. Organics either leave the system through primary sludge or they are sent to the aeration basin for biological treatment. Tchobanoglous et al. (2003)

provides an excellent mass balance schematic and example of how to calculate the BOD_5 and TSS in the system for a secondary treatment facility.

Primary clarifiers are designed to remove particulate, suspended, and colloidal organic matter, which leave the primary clarifier as sludge. Particulate and suspended organic matter is removed by sedimentation and colloidal organic matter can be removed with coagulants. Colloidal organic matter repels one another at a molecular level; coagulants overcome the molecular repulsion so that the colloidal matter can be brought together into large flocs that can be removed through sedimentation, which leave the primary clarifier in the form of sludge (Tchobanoglous et al., 2003). In addition, colloidal organic matter is dissolved into the water during primary treatment, and thus becomes soluble organic matter, which is then sent to the aeration basin. The soluble organic matter is quantified at CABI with the TOC test, but it may not be quantified at influent; the result is an apparent increase in TOC between the two sample sites. This is because the organics are particulate at influent and may not be quantified in the influent TOC analysis due to needle occlusion and/or coarse filtration involved in the analysis of the sample.

The more critical reason for the increase in organic loading can be related to the return flows from sludge handling. The sludge from the secondary clarifier is thickened and blended with primary sludge. Some of the overflow from this process is sent to the primary clarifier, which has a typical BOD_5 value of 250 mg/L, but it can range from 6 to 400 mg/L. The sludge is then sent through an anaerobic digester, which separates the sludge into supernatant and biosolids. The supernatant from this process is returned back to the primary clarifier and can have a typical BOD_5 value of 1,000 mg/L, but it can range from 500 to 5,000 mg/L. The biosolids are sent to solids dewatering, where the liquid centrate is

removed from the solids. The centrate is returned to the primary clarifier and can have BOD_5 values that range from 100 to 2,000 mg/L, with an average value of 1,000 mg/L (Tchobanoglous et al., 2003).

Clearly, the return streams have a significant impact on the BOD_5 loading in the primary clarifier, even if the return flow is a small fraction of the total flow. A formal mass balance that considered actual flow rates and the characteristics of the influent, sludge, and effluent would need to be conducted to fully understand the impact of the return flows. This type of data was not collected in this research and it is not within the objectives of this thesis. Regardless, return flows clearly impact the treatment process and should be researched. Specifically, the characterization of return flows (i.e., TOC, BOD_5, COD, and fluorescence) would be extremely beneficial to plant operators. They could better understand if the sludge returned is being adequately treated or if it is building up with the system. In other words, research should be conducted to determine if the sludge characterized by biodegradable or recalcitrant organic matter. This type of information could help WWTP designers determine if side stream treatment is more appropriate rather than sending the water back to the primary clarifier for biological treatment, particularly if the return flows are not predominantly characterized by biodegradable organic matter. Additionally, chemicals are often added to the WWTP flow to target the return flows, which are a small percentage of the total flow. It is possible that side stream treatment would be more cost effective, particularly in terms of chemical additives, and result in a better quality effluent because the side stream organics would be targeted.

A literature review did not produce any articles where researchers have characterized sludge by their TOC and fluorescence characteristics, and this type of analysis was not

conducted in this research. As mentioned, this type of information would be invaluable. In fact, the results of this research indicate that BOD_5 had an overall positive removal, but it was on the lower end of the range indicated by Tchobanoglous et al. (2003), which may indicate an influx in biodegradable organic matter. In other words, the true removal of biodegradable organic matter is possibly higher than the observed 48%, but the observed removal is dampened due to an increase in BOD_5 caused by the return flow. Both the historical removal and current removal of TOC had rather large variability and indicated that the TOC increased, presumably due to the return stream. It should be mentioned that the removal of BOD_5 was always positive is this research, which may indicate that the return stream is predominantly characterized by TOC and to a lesser extent biodegradable organic matter (i.e., COD and BOD_5). It also supports that fact that BOD_5 and TOC do not quantify the same type of organic matter.

The removal of organics related to absorbance at 254 nm was approximately 8% in the primary clarifier and just over 60% for biological treatment and the entire plant. The removal in the primary clarifier increased in some instances. These results are similar to the observations made for the TOC data. As mentioned, the return flows increase the organics in the primary clarifier. Likely, the absorbance at 254 nm is quantifying these organics, whereas BOD_5 and COD do not.

The mean removals of absorbance at 340 nm were slightly higher than the removal at 254 nm and negative removals were not observed in the primary clarifier. Despite this observation, negative removals may be observed during longer data collection periods. This is believed because the TOC variations were large for data collected from 2010 to 2014, which were not captured in this research.

4.10 Removal Efficiency of Fluorescence

The removals of the peaks and regions are shown in Table 4.13. The fluorescence EEM graphs for influent, CABI, and effluent are shown in Figure 4.37, Figure 4.38, and Figure 4.39, respectively. The fluorescence scale (0 to 10 AFU) for influent and CABI are the same, so that one can visually see how the sample changes during treatment in the primary clarifier. The fluorescence of effluent is much smaller, so the fluorescence scale (0 to 2 AFU) was changed accordingly.

Table 4.13: Removal Efficiency of Fluorescence of the Primary Clarifier, Biological Treatment, and the Entire Treatment Process for Each Region and Peak

		Primary		**Biological Treatment**		**Entire Plant**	
Fluorescence parameter (ex/em) – Region	**n**	**Mean**	**95% Confidence interval (±)**	**Mean**	**95% Confidence interval (±)**	**Mean**	**95% Confidence interval (±)**
Total	39	10.09	1.3	67.83	0.86	70.96	0.65
Region I	39	12.03	1.48	80.26	0.78	82.66	0.62
Region II	39	3.10	1.35	54.20	1.03	55.73	0.89
Region III	39	18.24	1.14	45.01	0.9	55.07	0.69
Protein peak (280/331) – Region I	39	17.19	1.54	84.74	0.98	87.41	0.73
Humic peak (342/436) – Region III	39	27.73	1.23	40.79	1.05	57.02	0.71
Peak A (260/450) – Region II	39	2.18	1.41	46.6	0.81	47.78	0.86
Peak C (330/450) – Region III	39	24.3	1.35	48.76	0.92	61.28	0.68
Peak T (275/340) – Region I	39	16.45	1.34	85.59	0.81	87.98	0.63

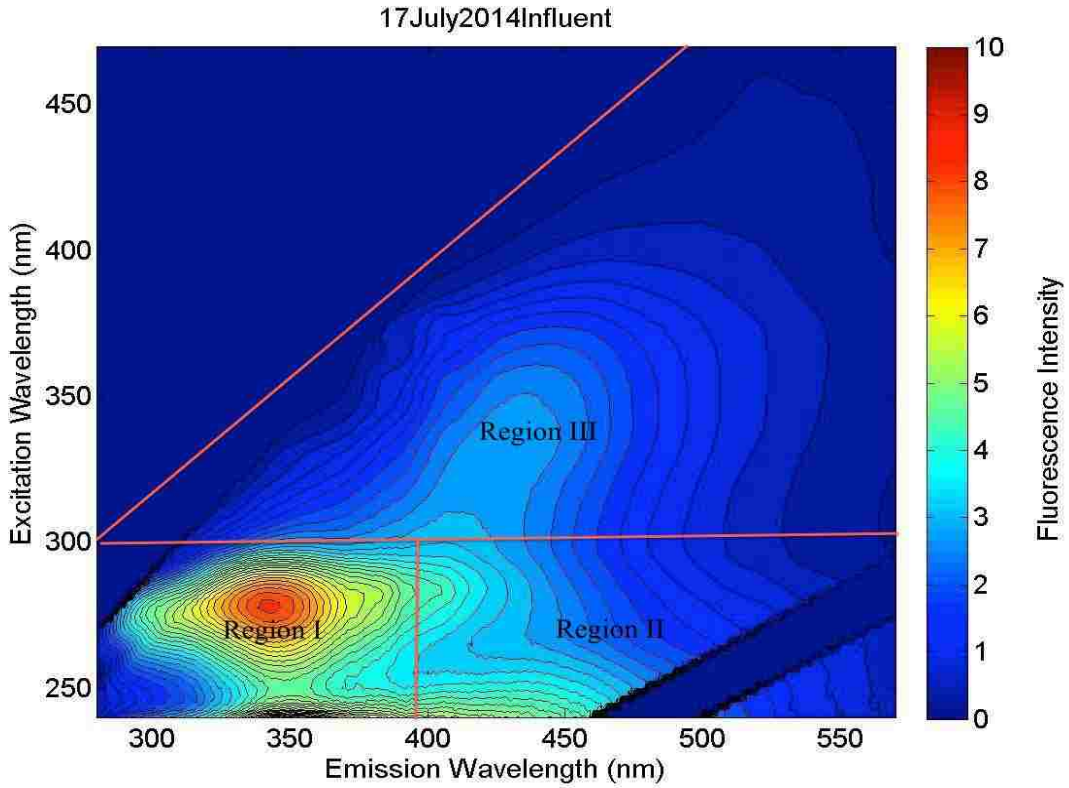

Figure 4.37: Fluorescence EEM graph of influent sample analyzed on 7/17/2014.

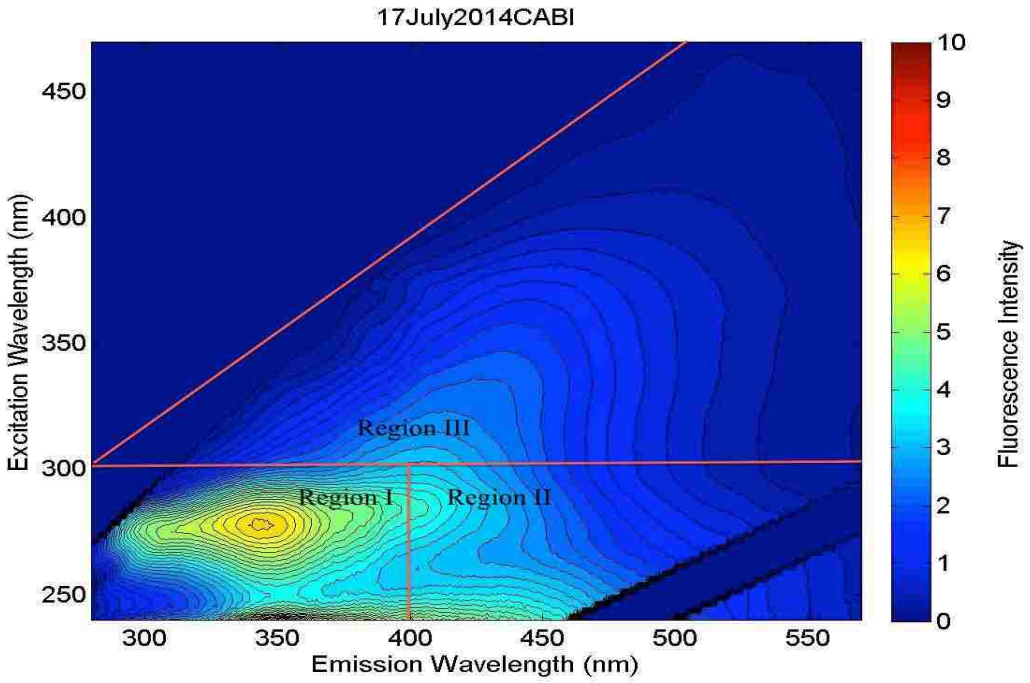

Figure 4.38: Fluorescence EEM graph of CABI sample analyzed on 7/17/2014.

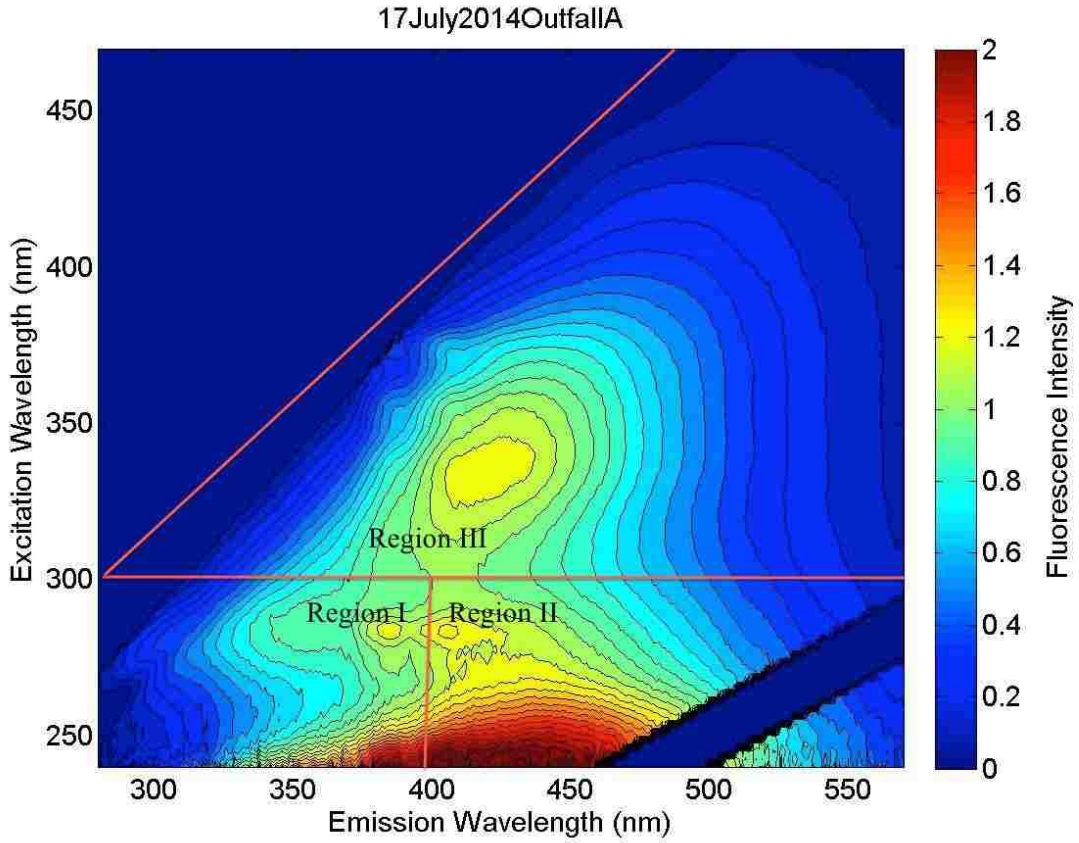

Figure 4.39: Fluorescence EEM graph of effluent sample analyzed on 7/17/2014.

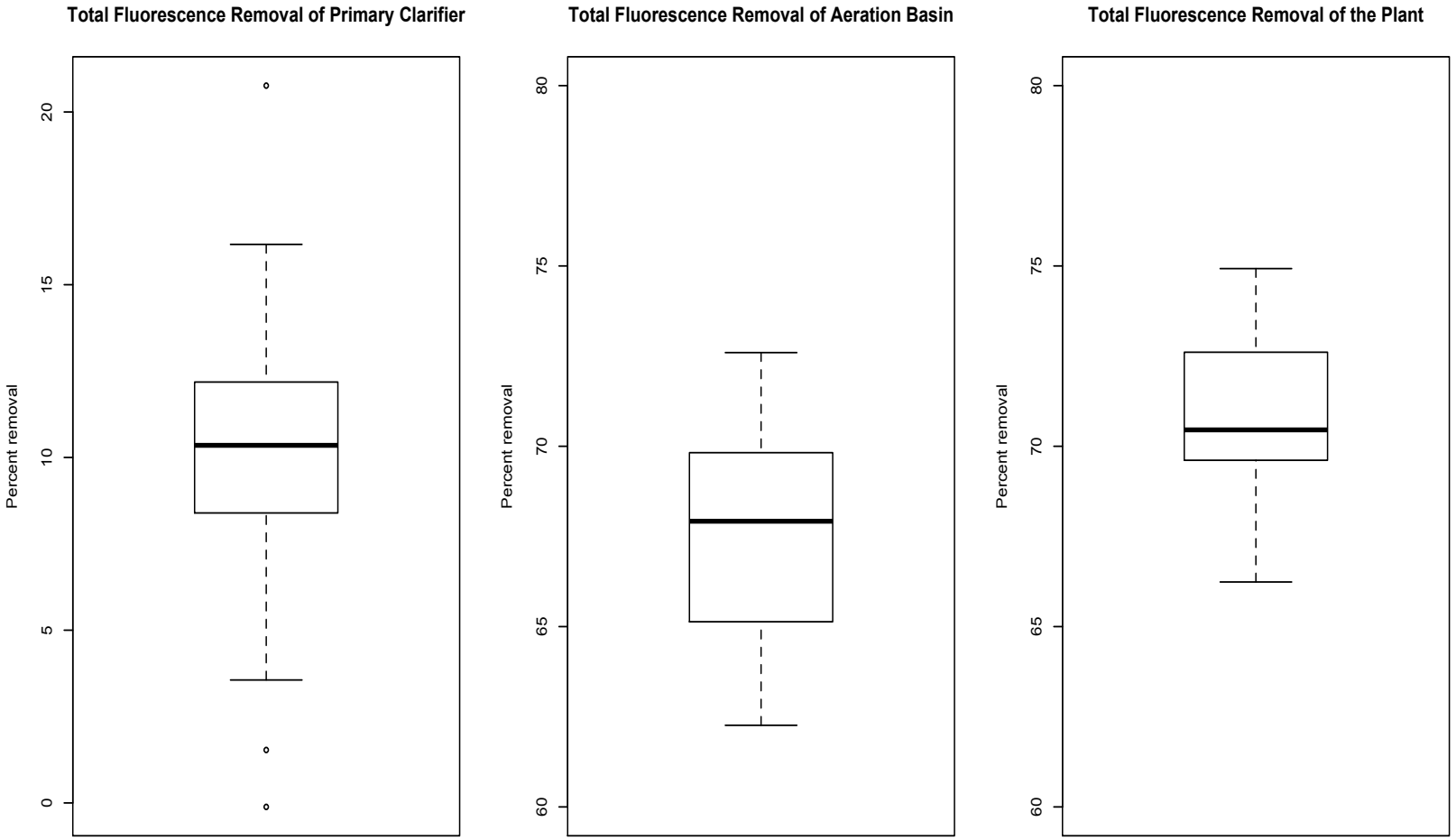

Figure 4.40: Boxplot of total fluorescence removals of the primary clarifier, biological treatment, and the entire treatment process.

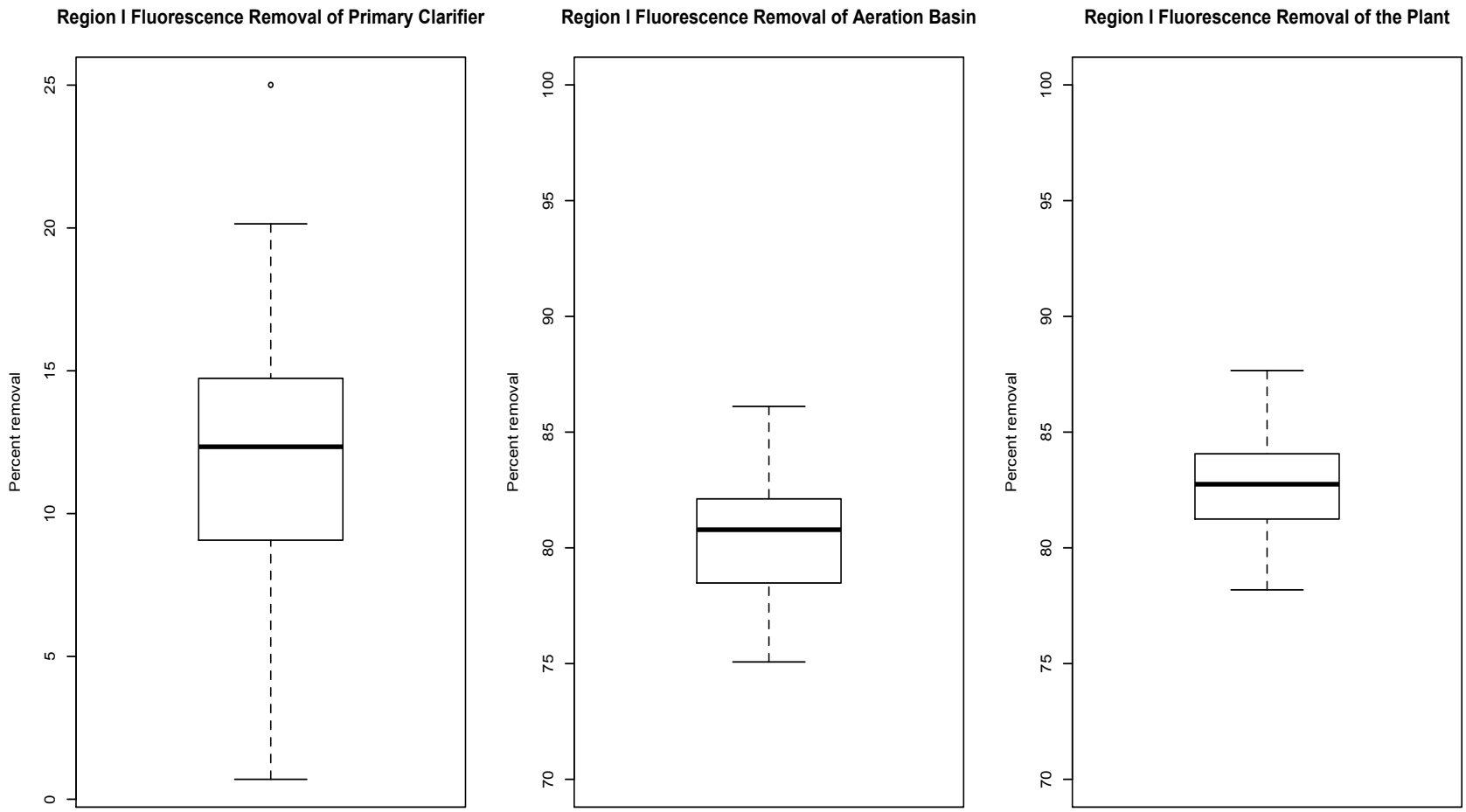

Figure 4.41: Boxplot of region I fluorescence removals of the primary clarifier, biological treatment, and the entire treatment process.

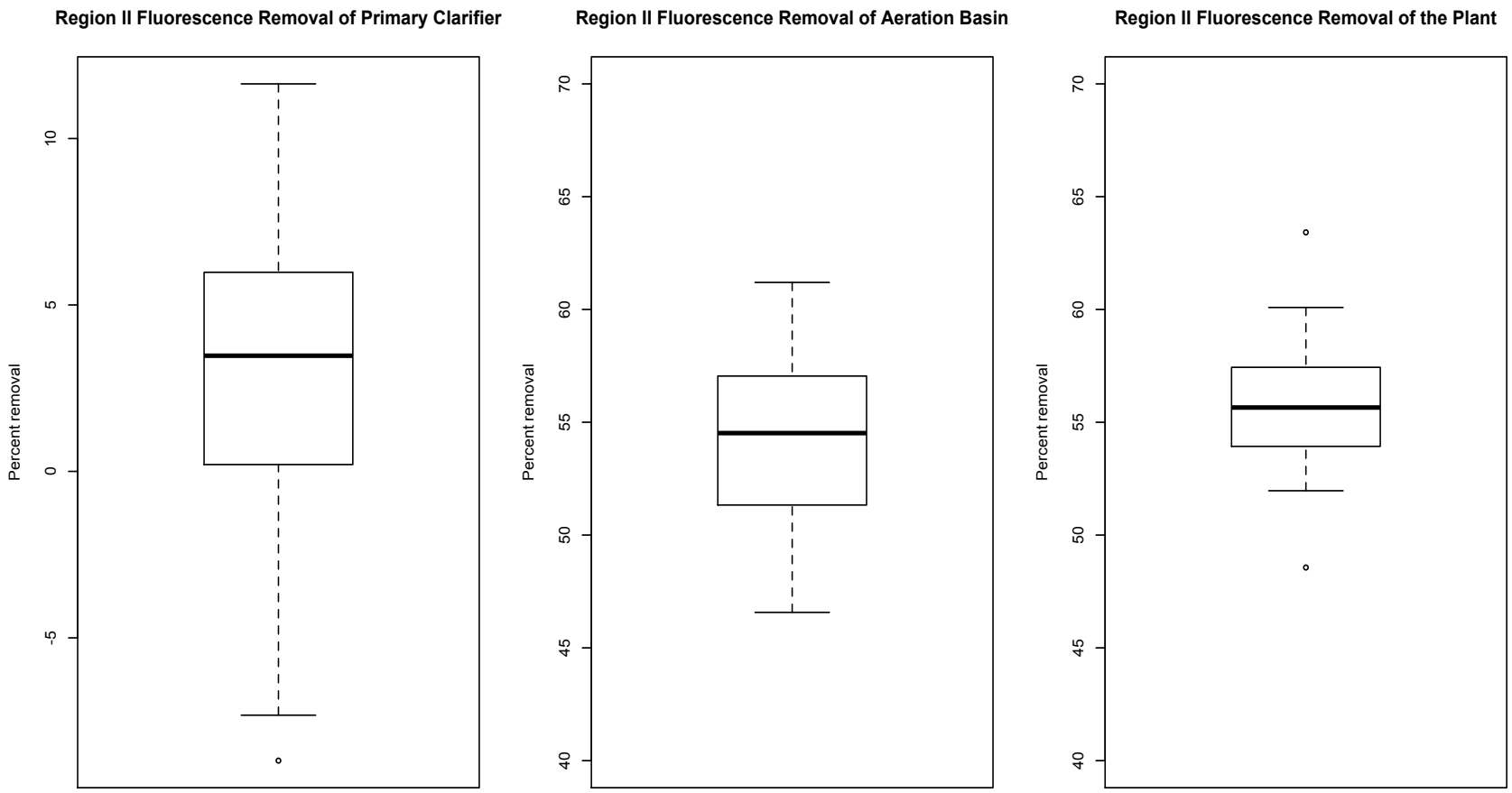

Figure 4.42: Boxplot of region II fluorescence removals of the primary clarifier, biological treatment, and the entire treatment process.

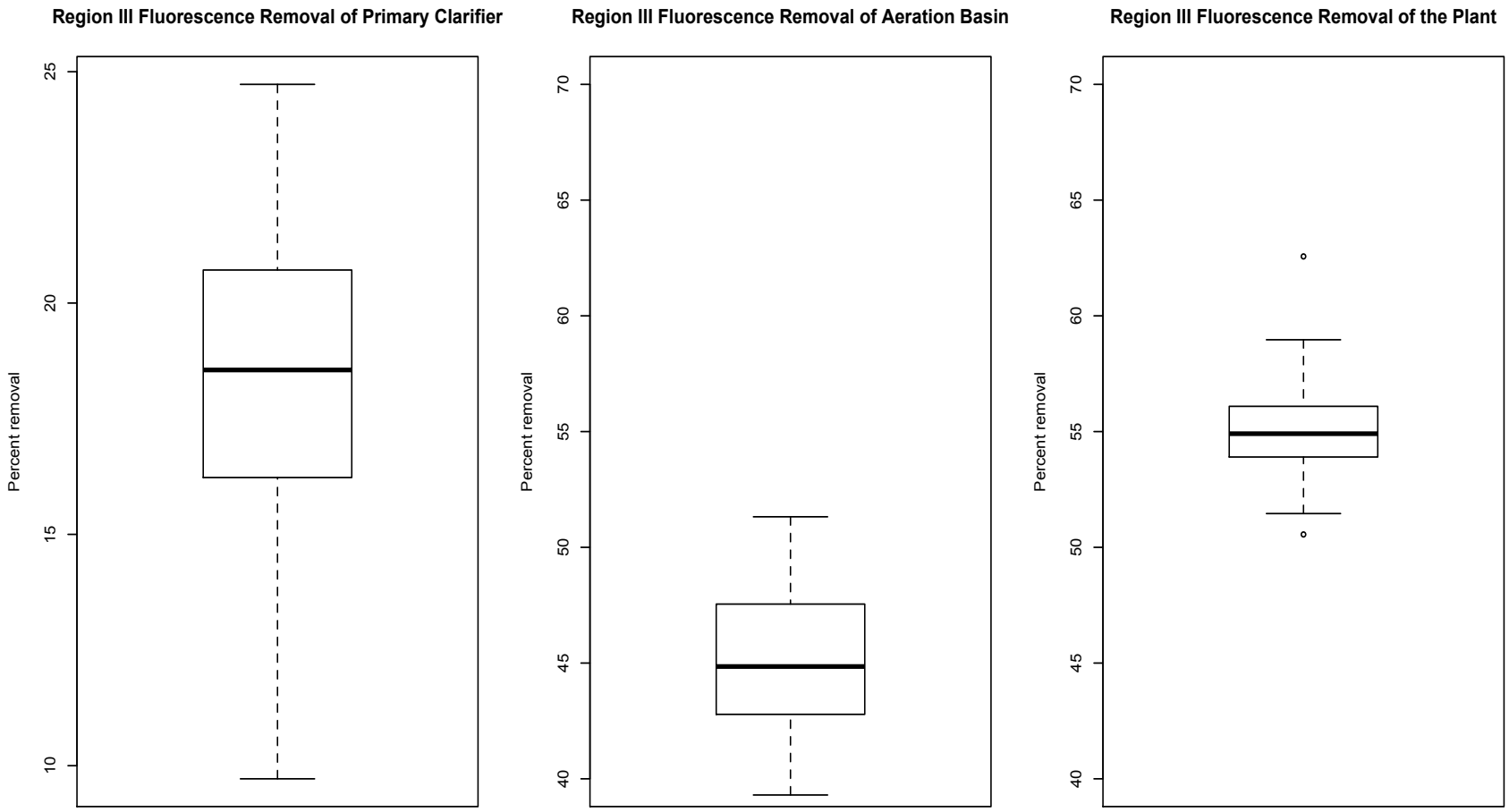

Figure 4.43: Boxplot of region III fluorescence removals of the primary clarifier, biological treatment, and the entire treatment process.

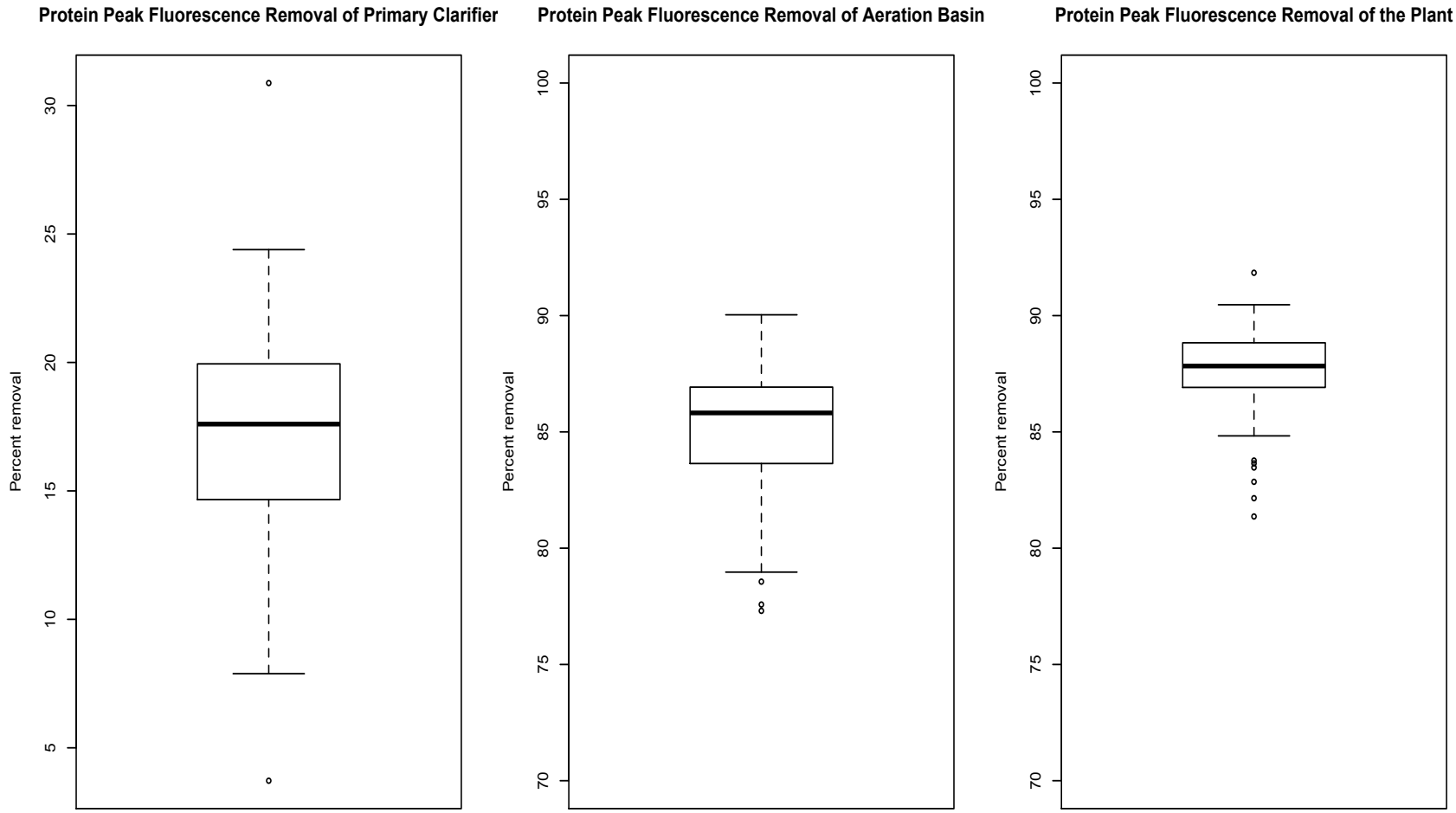

Figure 4.44: Boxplot of protein peak fluorescence removals of the primary clarifier, biological treatment, and the entire treatment process.

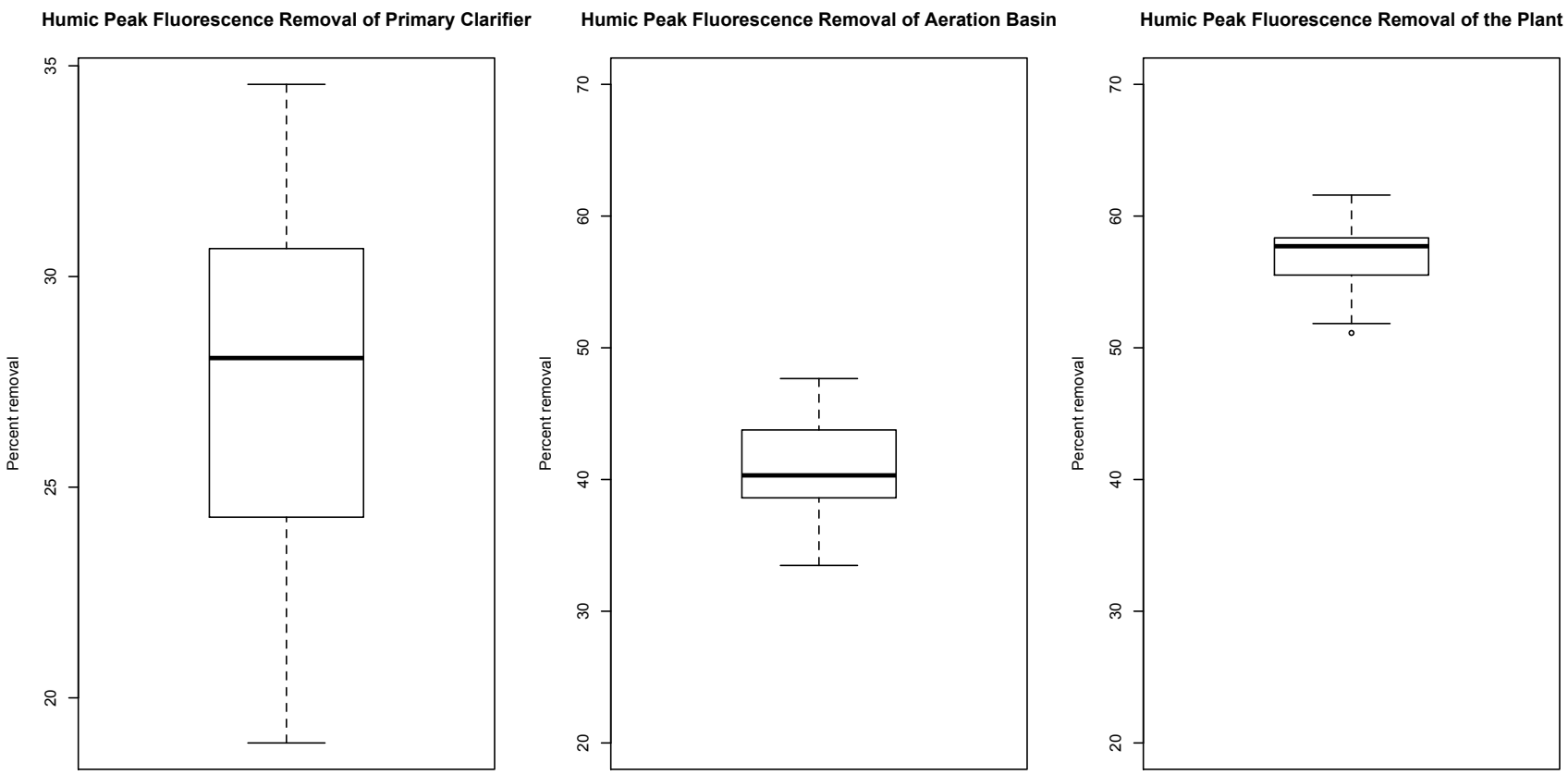

Figure 4.45: Boxplot of humic peak fluorescence removals of the primary clarifier, biological treatment, and the entire treatment process.

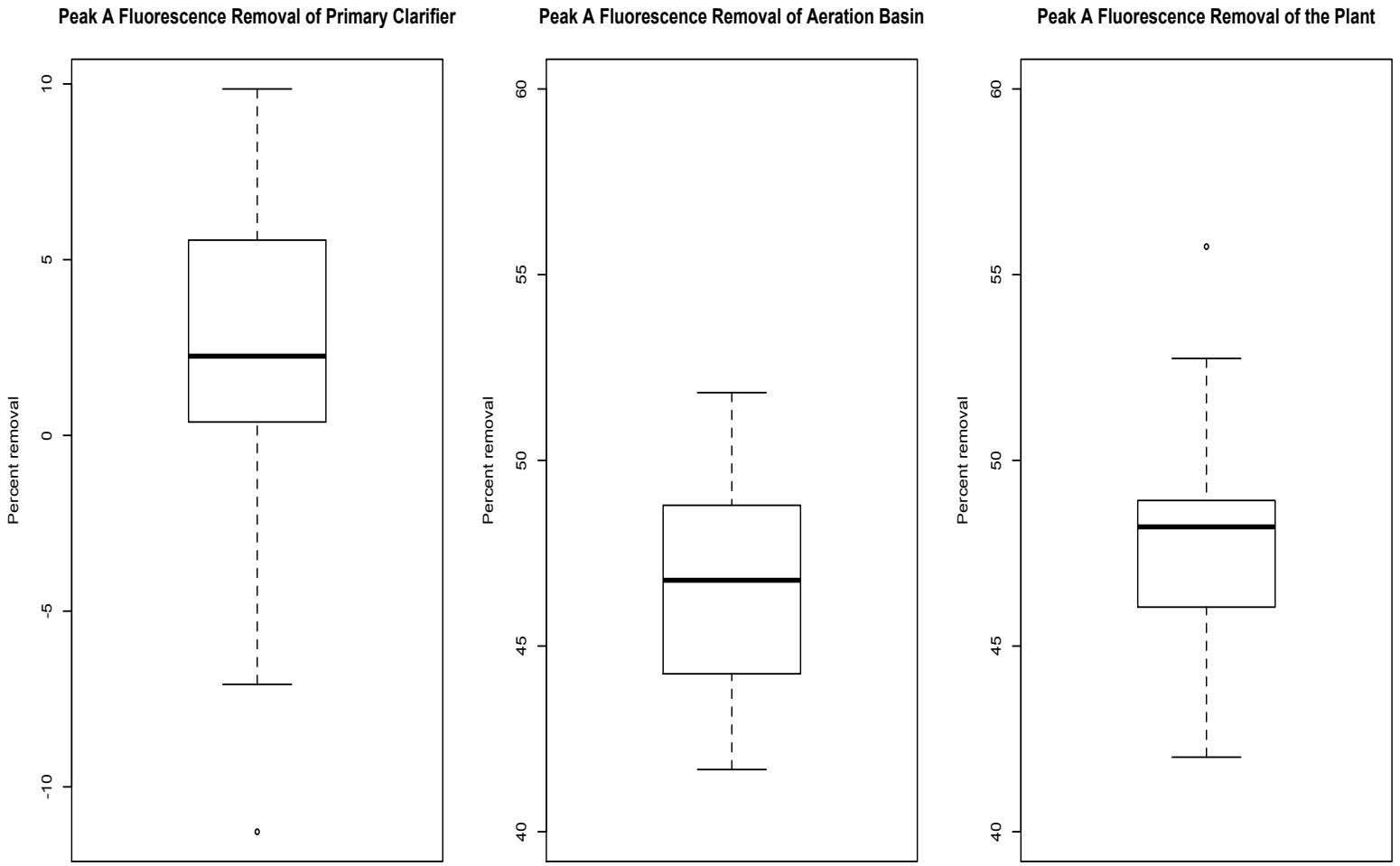

Figure 4.46: Boxplot of peak A fluorescence removals of the primary clarifier, biological treatment, and the entire treatment process.

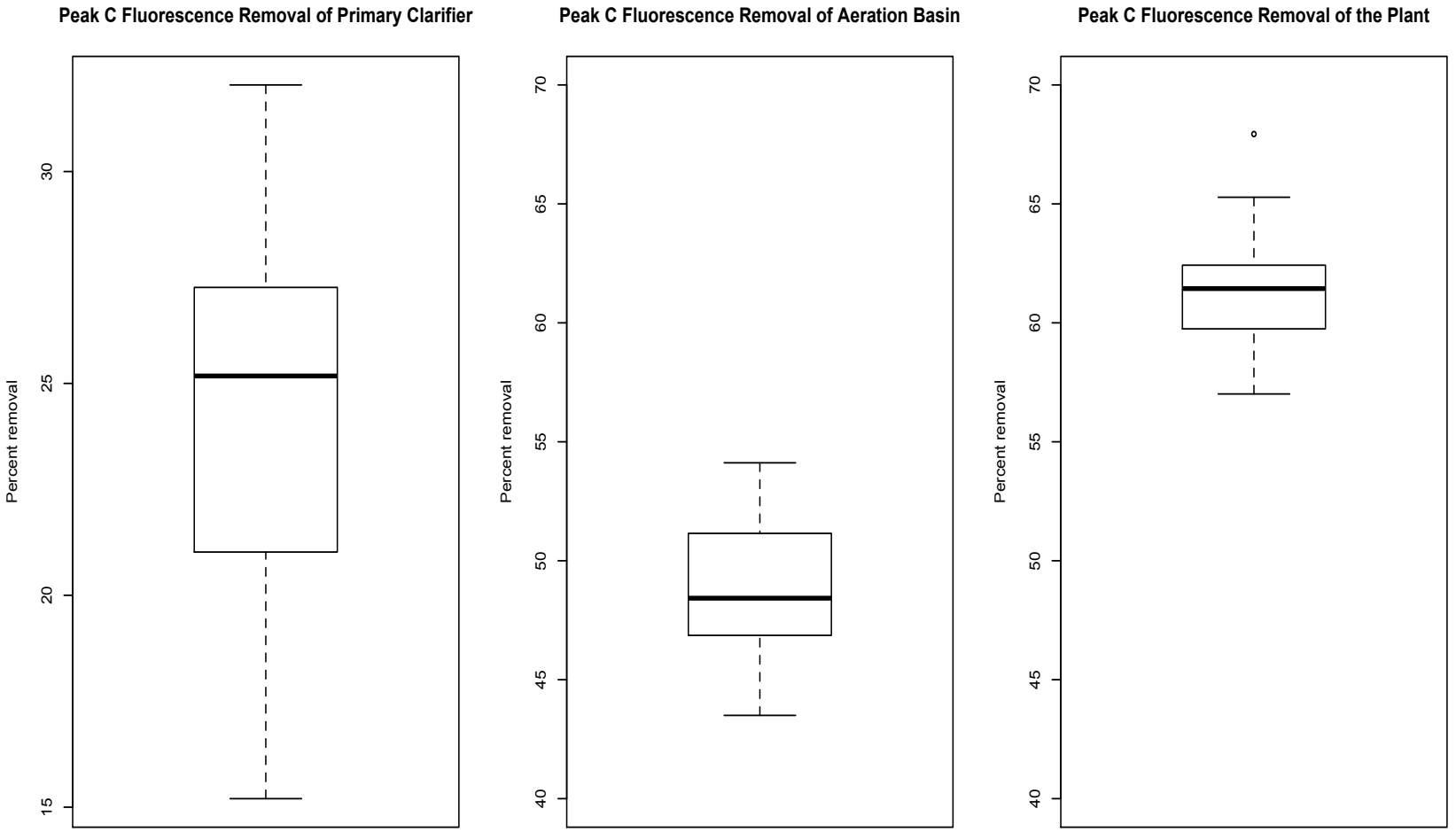

Figure 4.47: Boxplot of peak C fluorescence removals of the primary clarifier, biological treatment, and the entire treatment process.

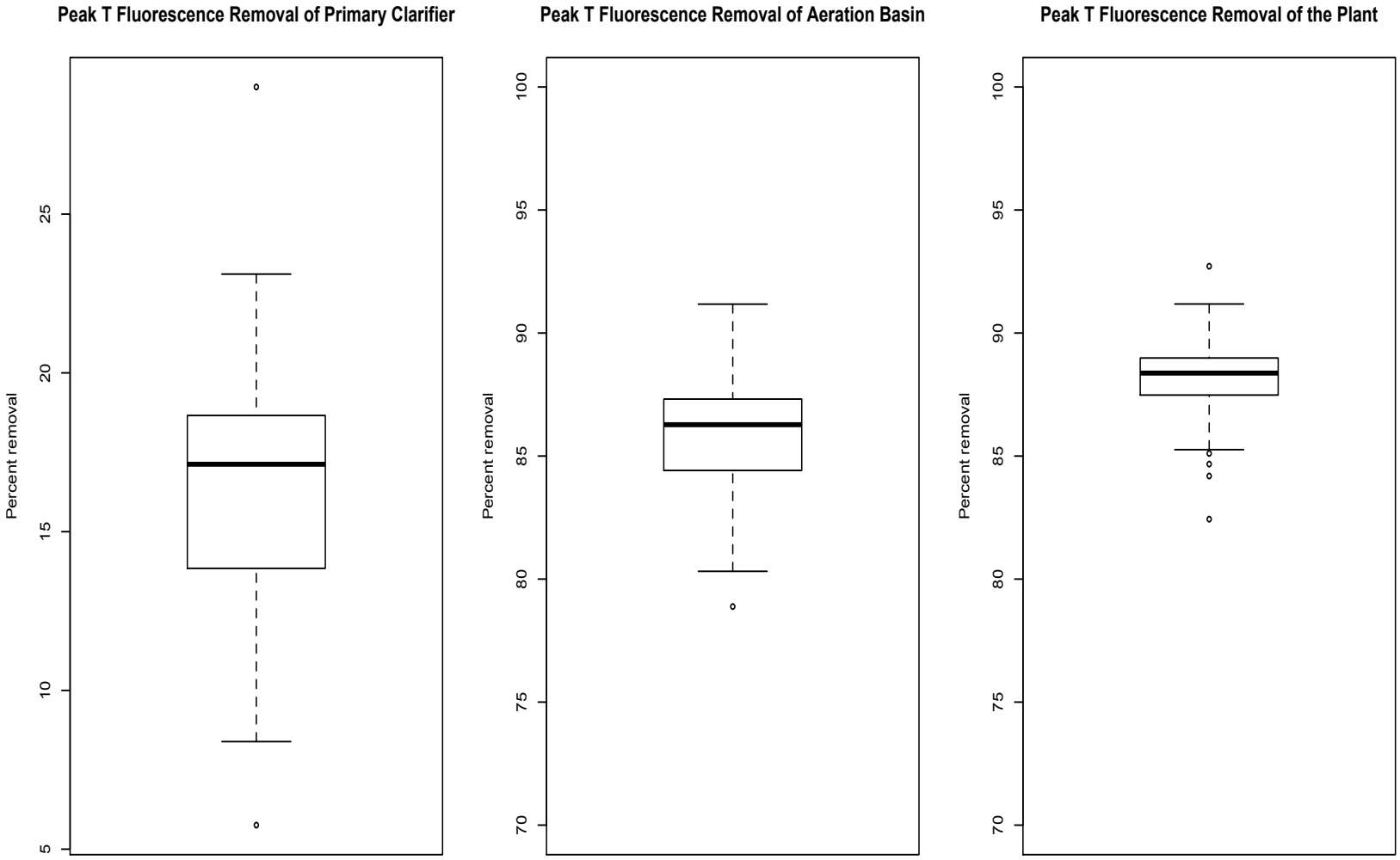

Figure 4.48: Boxplot of peak T fluorescence removals of the primary clarifier, biological treatment, and the entire treatment process.

Table 4.13 summarizes the means and confidence intervals for each of the fluorescence parameters. The region that the peak is associated with is noted in the table for clarity.

Some of the total fluorescence was removed during primary clarification (mean=10%), but the majority of the total fluorescence was removed during biological treatment in the aeration basin (mean basin = 68% and mean plant=71%). The boxplots (Figure 4.38) indicate that up to 75% of the total fluorescence was removed during treatment. A better understanding of the organics removed can be determined by assessing each region and the peaks contained within that region.

Region I is the region most related to BOD_5 and protein-like fluorescence; it contains peak T and the protein peak defined in this research. The mean removals for the primary clarifier, biological treatment, and the plant were similar for the protein peak (17%, 85%, and 87%), T peak (16%, 86%, and 88%), and region I (12%, 80%, and 83%, respectively). Figures 4.35 and 4.36 indicate that the fluorescence peak in region I was the most dominant for influent and CABI, but the fluorescence decreased after primary clarification, which was consistent with BOD_5 removal observations. This is expected because domestic wastewater contains 40-60% proteins, which are readily removed during the treatment process (Tchobanoglous et al., 2003). Region I and the associated peaks had the highest removal efficiency for both biological treatment and the entire plant compared with the other parameters. Figure 4.37 indicates that protein-like (region I) fluorescence is less dominant than fulvic-like (region II) or humic-like (region III) fluorescence. This further supports the literature, which has concluded that up to 90% of the fluorescence associated with region I and the peaks is removed during the wastewater treatment process and that these peaks are

most related to the wastewater treatment process (Hudson et al., 2007; Henderson et al., 2009).

Region II is associated with fulvic-like fluorescence and contains peak A. There does not appear to be a significant change in fulvic-like fluorescence during primary clarification (Figure 4.37 and 4.38), which is consistent with the mean removal values determined for peak A (2%) and region II (3%). Observation of the boxplots (Figure 4.40 and Figure 4.44) indicated that the fluorescence associated with this region increased (i.e., the removal is negative) during primary clarification. None of the other peaks or regions (with the exception of total region) had negative removals. This is an important observation because it may indicate a link between TOC and absorbance to fulvic-like organic compounds, both of which also had negative removals that were also observed in the primary clarifier. It may also be an indicator that fulvic-like compounds are likely characteristic of the return flows. Park et al. (2010) suggested that humic and fulvic-like materials may be byproducts of the biological process, which could be characteristic of the secondary clarifier sludge returned to the primary clarifier. Approximately 50% of the fulvic-like fluorescence is removed during the entire treatment process.

Region III is associated with humic-like fluorescence and it contains the humic-like peak and peak C. The observed fluorescence was less than region I, but greater than the fluorescence observed in region II. The primary clarifier was most efficient at removing humic peak fluorescence (28%), peak C fluorescence (24%), and region III fluorescence (18%), compared with the other peaks and regions. Approximately 45% of the remaining humic-like fluorescence was removed during biological treatment, resulting in total

fluorescence removals of 57%, 61%, and 55% for the humic peak, peak C, and region III, respectively.

It can be concluded that the primary clarifier removes humic-like fluorescence followed by protein-like fluorescence, but it does not remove fulvic-like fluorescence. In fact, fulvic-like fluorescence increased in the primary clarifier presumably due to the return flows associated with sludge treatment. Biological treatment is the most effective at removing protein-like fluorescence, and to a lesser degree it removes humic and fulvic-like fluorescence. The resulting effluent has very little protein-like fluorescence, but it is characterized by humic and fulvic-like fluorescence, which is consistent with Hudson et al. (2008), Henderson et al. (2009), and Yang et al. (2014). The decreases observed in the peaks and regions were consistent with Ahmad and Reynolds (1995) and Reynolds (2002), who concluded that peaks near excitation wavelengths of 280 nm (e.g., region I, peak T, and protein peak) were associated with biodegradable matter and peaks near excitation wavelength of 350 nm (e.g., region III and the humic peak) were associated with nonbiodegradable organic matter.

4.11 Relationship Between the Removal Efficiencies and Correlations

The strongest correlation was developed for BOD_5 and COD. A comparison of the removal efficiencies indicates that they had the most similar removal efficiencies for the primary clarifier, biological treatment, and the entire treatment plant. This further supports the conclusion that COD is a good surrogate for BOD_5 for the domestic wastewater used in this research.

TOC was related to effluent BOD_5, but it did not relate well to the influent or CABI samples. The TOC removal in the primary clarifier (9%) was far less than the BOD_5 removal

(49%) indicating that TOC and BOD_5 should not be related for this portion of treatment. TOC removal for biological treatment (85%), was slightly less than the removal observed for BOD_5 (96%). The similar removals observed during biological treatment may explain why effluent correlated better than the rest of the treatment process. A comparison between TOC removals and fulvic-like fluorescence removals indicate that they both increased in the primary clarifier, which may be an indicator that they are related to one another.

The absorbance correlations indicated that the relationship was nonlinear and weak for BOD_5 at both 254 nm and 340 nm. The removal efficiencies indicate that absorbance is not readily removed in the primary clarifier and 60% is removed during the entire treatment process. Recall that absorbance is a measure of how much light passes through a sample, based on the presence of chromophores. Therefore, it is not a direct indicator of biodegradable organic matter. It can be concluded that absorbance is not a good surrogate for BOD_5 or biodegradable organic matter. However, the removal efficiencies suggest that it may be a good indicator for total fluorescence, region II fluorescence, and/or region III fluorescence.

BOD_5 was compared to the humic-like peak and region III. It was concluded that these fluorescence parameters were not good surrogates for BOD_5 because they are not associated with labile organic matter. The removal efficiencies observed in this research show that less than 60% of humic-like fluorescence is removed during treatment, which supports the fact that it is not labile. This can be extended to fulvic-like peaks and region II as well.

It was concluded that region I and peak T were the best fluorescence parameters to correlate to BOD_5 because they are the most related to biodegradable organic matter. The removal efficiencies show that region I, peak T, and the protein peak had similar removal

efficiencies (<20%) in the primary clarifier, but they were much less than the observed BOD_5 removal (49%). On the other hand, the average removal efficiencies of biological treatment (80-86%) and the entire treatment train (83-88%) were more similar to the observed BOD_5 removal (96% and 98%, respectively). It is not known why there is a stark difference in removal efficiencies between fluorescence and BOD_5 in the primary clarifier, but it may be due to either IFE quenching and/or the centrate return. If IFE were not occurring in the sample, then the observed influent and CABI fluorescence would have been higher. The change in fluorescence would have been different, which could have potentially increased the removal efficiency making it more comparable to BOD_5. The other reason may be due to the return streams that increase the organic loading in the primary clarifier. It is possible that some of the organic matter is fluorescent but not necessarily biodegradable. The impact would be an increase in fluorescence in region I, peak T, and protein peak in the primary clarifier, which would make the apparent removal seem less than it truly is.

Part C: Method Development

4.12 Interpretation of the t-test Results

T-tests were used to compare datasets for the holding time tests and for the integration time comparison. The null hypothesis of the t-test was that the means were equal and the alternate hypothesis was that the means were not equal. When the p value of the t-test is greater the 0.05, then one accepts the null hypothesis as being plausible. When p is less than 0.05, then null hypothesis is rejected and one accepts that the means are different. It is important to clarify that the t-test does not definitively say the means are equal, only that it

is plausible that they are equal. The term "plausible" is used when the null hypothesis is accepted, rather that erroneously saying that the means are equal.

4.13 Grouped BOD_5 Data Holding Test

Samples were collected and analyzed for BOD_5 in two groups. One group was analyzed the same day it was collected and the other group of samples was stored overnight with headspace and analyzed the day after collection. The overnight samples were not tested the same day as collected and have no relation to the first group of samples; the two groups were sampled and analyzed independent of one another. The results are presented in Figures 4.49, 4.50, and 4.51. Notice that the x-axis is labeled index, this is because the data were randomly plotted and they are not paired with anything. The purpose of these plots was to illustrate the relative BOD_5 values with one another for data collected and analyzed over a period-of-time.

Figure 4.49 demonstrates that influent samples that were analyzed the same day had consistently higher BOD_5 results compared with samples that were analyzed the following day and the two-sample t-test ($p < 2.2e^{-16}$) shows that the mean values between the groups are different. Figure 4.50 indicates that CABI samples also had higher BOD_5 when they were analyzed the same day, although the discrepancy is not as pronounced compared with the influent. Furthermore, the two-sample t-test indicated the sample groups were statistically different ($p=3.53e^{-7}$). Figure 4.51 shows that the effluent data were clustered on top of one another and most likely holding time did not impact the results. A two sample t-test indicated that the $p=0.699$, so it is plausible that the sample groups were the same.

The results indicate that holding the samples with a headspace overnight impacted BOD_5 analysis of influent and CABI samples, but did not have a significant effect on effluent samples.

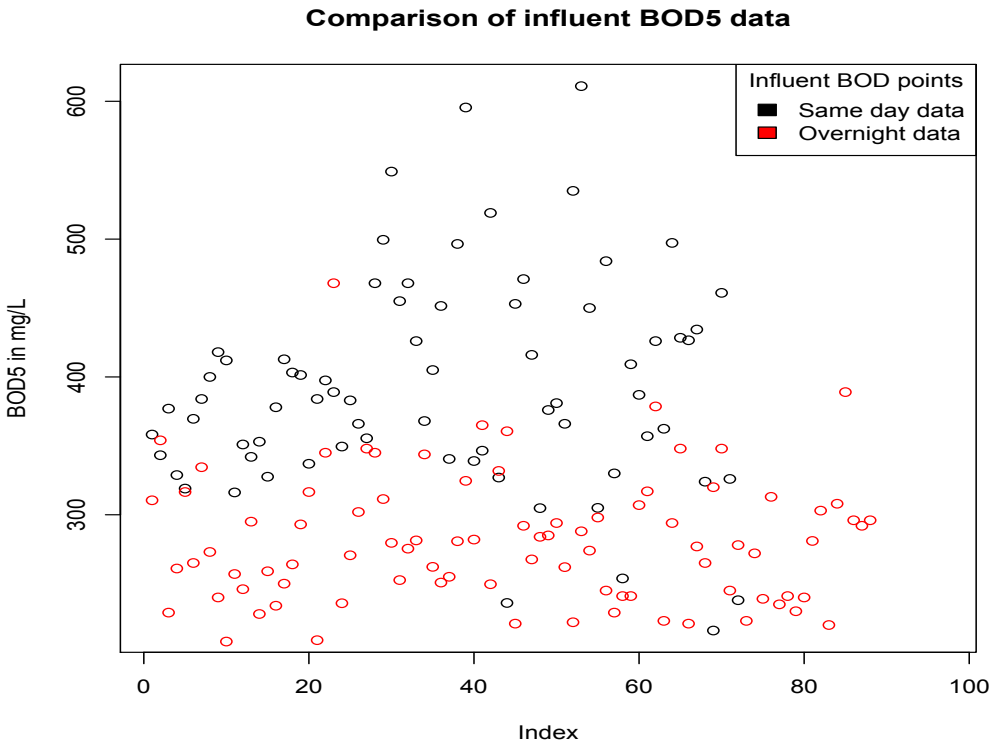

Figure 4.49: Holding tests results for influent grouped BOD_5 data.

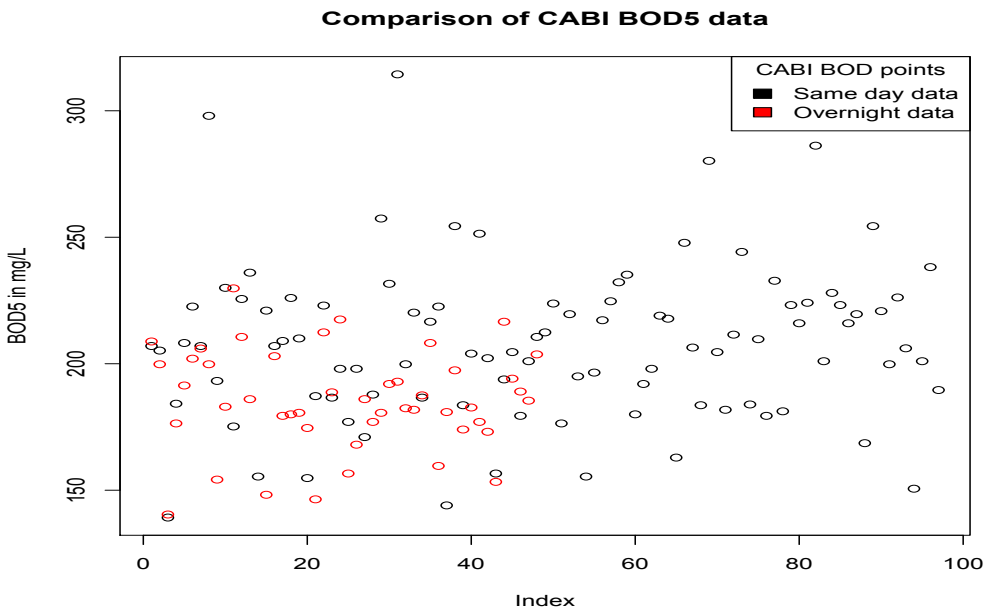

Figure 4.50: Holding tests results for CABI grouped BOD_5 data.

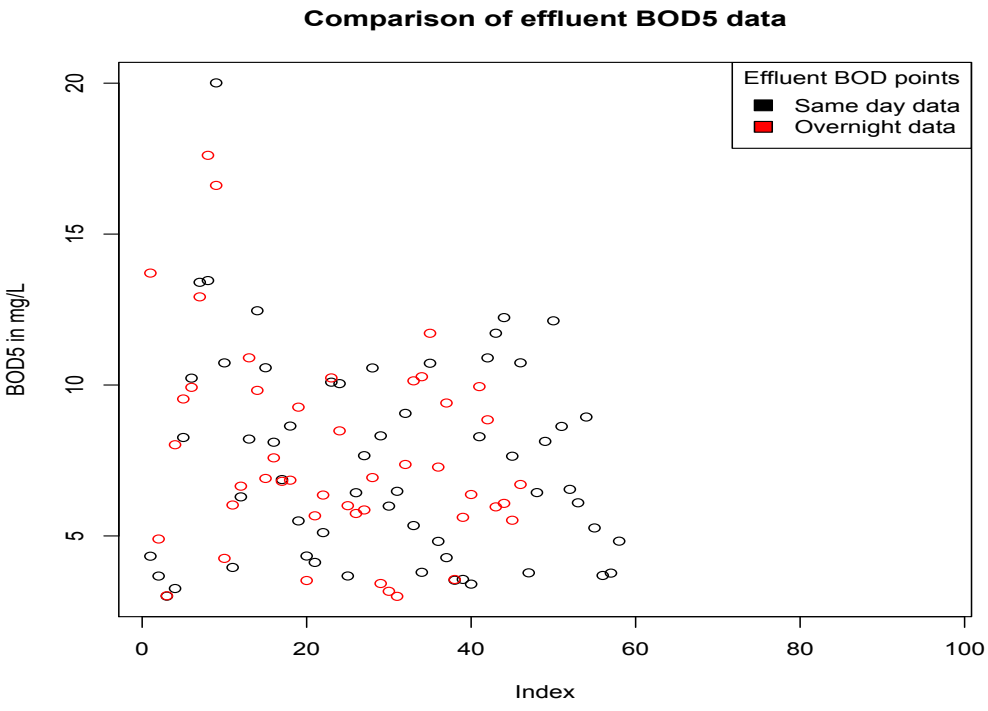

Figure 4.51: Holding tests results for effluent grouped BOD_5 data.

4.14 Holding Tests

4.14.1 BOD_5 Holding Test

In the previous section, two groups of BOD_5 data were compared. One group of samples was analyzed the same day and the other group was analyzed the following day, but the samples were not related to one another. It was shown that influent and CABI systematically differ from one another, but effluent samples were plausibly similar. This section takes these results a step further to investigate how an individual sample result changes overnight when it is held overnight a) with headspace and b) without headspace.

Samples (influent, CABI, and effluent) were collected and analyzed the same day. The samples were then refrigerated overnight and reanalyzed the following day. The influent sample was refrigerated overnight in two amber bottles, one that had headspace and another that did not have headspace. The CABI sample was stored in an amber bottle with headspace. The effluent sample was refrigerated overnight in a polyethylene cubitainer with headspace. The results from the initial BOD_5 analysis were directly compared with the sample counterpart analyzed the following day using the two-sample t-test. Two trials were conducted to determine if there was consistency among the variations.

Table 4.14 summarizes the mean and the standard deviations of the datasets. It is clear that there was a downward trend of BOD_5 between the results from day 1 and the results from day 2, which was consistent with the observations made in the grouped BOD_5 data. The standard deviation and the t-test results are not reported for effluent in trial A because only two sample dilutions were valid.

It is unsurprising that the observed BOD_5 of samples held overnight is less than the BOD_5 samples analyzed the same day that they were collected. BOD is a first order reaction,

modeled by $BOD_t=BOD_U(1-e^{-kt})$, where k values typically range from 0.12 to 0.23 day^{-1}. The k value is the reaction rate of BOD and it increases as the fraction of soluble organic matter increases, but it is also temperature dependent (Tchobanoglous et al., 2003). If a k value of 0.23 d^{-1} at 20°C is considered, then it can be approximated that 21% of the ultimate BOD is consumed during the first day, 68% is consumed by the fifth day, and 75% by the sixth day during incubation. The reaction rate will be less under refrigerated conditions (4°C), because the k value will be lower due to the colder temperature. In other words, when the sample is refrigerated overnight the k value is small and reaction rate is slower, but the k value will increase under incubation temperatures and increase the reaction rate. In addition, some of the colloidal organic matter may be dissolved into the sample and become soluble, which will increase the k value and bias the BOD_5 results high. This is because bacteria are able to oxidize soluble organic matter easier than particulate or colloidal organic matter.

The results in Table 4.15 indicate that influent and CABI samples that were held with headspace overnight significantly degraded. The influent sample that was held without headspace in trial B was statistically different than the initial sample, but the results from trial A indicate that the means were plausibly the same.

Influent and CABI samples have high microbe populations and concentrations of organic matter. Microbes are consuming organics, despite refrigeration, during the holding time because there is oxygen present (i.e., there is a headspace). This process is the same mechanism upon which the BOD_5 test is based. The headspace in the sample increases the amount of oxygen available (as opposed to samples stored without headspace), which further encourages microbial activity. Effluent water has fewer microbes present and most of the

biodegradable BOD has already been used; therefore, the presence of oxygen via the headspace does not impact the measurement much.

Table 4.14: Summary Statistics of the BOD_5 Holding Test Trials for Influent, CABI, and Effluent

		Trial A			Trial B		
Sample	**Sample day**	**Mean (mg/L)**	**SD**	**n**	**Mean (mg/L)**	**SD**	**n**
Influent	Day 1	327	3	4	236	16	4
	Day 2 without headspace	307	13	4	277	21	4
	Day 2 with headspace	226	12	4	188	7	4
CABI	Day 1	228	18	4	185	16	4
	Day 2	165	8	4	159	7	4
Effluent	Day 1	3.95	N/A	2	4.92	0.17	3
	Day 2	3.58	N/A	2	4.67	0.38	3

Table 4.15: Summary of the t-test Results of the BOD_5 Holding Test Trials for Influent, CABI, and Effluent

BOD t-test p value			
Sample	**Test**	**Trial A**	**Trial B**
Influent	Day 1 vs Day 2 full	0.0589	0.0239
	Day 1 vs Day 2 partial	0.0003	0.0043
CABI	Day 1 vs Day 2 full	0.0025	0.0008
Effluent	Day 1 vs Day 2 partial	N/A	0.3706

4.14.2 COD Holding Test

The COD holding test was conducted similar to the BOD_5 holding test. Samples (influent, CABI, and effluent) were collected and analyzed the same day. The samples were then refrigerated overnight. Influent and CABI samples were stored both with and without headspace in amber glassware. The effluent samples were stored overnight with headspace.

Table 4.16 summarizes the means and standard deviations from the two trials conducted. The results indicate the samples held overnight without headspace were similar to the samples analyzed the same day. Samples that were stored with headspace deteriorated more quickly, and thus had smaller COD values than the initial analysis.

The t-test (Table 4.17) indicates that it is plausible that the means of the first day were the same as samples held overnight without headspace for both influent and CABI. Influent and CABI samples held with headspace had similar means in trial A and different means in trial B. Similar to the BOD_5 test, the effluent sample means were not statistically different.

These results indicate that sample degradation is accelerated when samples are held overnight with headspace. The degree of degradation can vary based on the sample and likely the amount of headspace (e.g., more headspace means more degradation), thus creating variability in whether or not the degradation significantly changes the quality of the sample.

Table 4.16: Summary Statistics of the COD Holding Test Trials for Influent, CABI, and Effluent Samples

		Trial A			Trial B		
Sample	**Sample day**	**Mean (mg/L)**	**SD**	**n**	**Mean (mg/L)**	**SD**	**n**
Influent	Day 1	691	22	3	683	14	3
	Day 2 with headspace	669	10	3	668	26	3
	Day 2 without headspace	617	5	3	663	32	3
CABI	Day 1	396	8	3	467	1	3
	Day 2 with headspace	389	5	3	494	22	3
	Day 2 without headspace	373	3	3	473	26	3
Effluent	Day 1	32	2	3	30	2	3
	Day 2	31	1	3	32	1	3

Table 4.17: Results of the t-test for the COD Holding Test for Influent, CABI, and Effluent Samples

COD t-test p value			
Sample	**Test**	**Trial A**	**Trial B**
Influent	Day 1 vs Day 2 full bottle	0.2253	0.4531
	Day 1 vs Day 2 partially full	0.0243	0.4041
	Day 2 full vs Day 2 partially full	0.0041	0.8531
CABI	Day 1 vs Day 2 full bottle	0.2632	0.1633
	Day 1 vs Day 2 partially full	0.0258	0.7301
	Day 2 full bottle vs Day 2 partially full	0.0098	0.3423
Effluent	Day 1 vs Day 2 partially full bottle	0.2381	0.1346

4.14.3 Absorbance Holding Test

Samples (influent, CABI, and effluent) were analyzed and compared similar to the BOD_5 and COD holding tests. Samples were analyzed the same day that they were collected and then reanalyzed the following day. The results from the two analyses were compared using the two-sample t-test.

The wavelengths analyzed for the absorbance test ranged from 240 to 470 nm. The absorbance is summarized in Figures 4.52 (Trial A) and 4.53 (Trial B) for each sample that was analyzed in the trial. As expected, the absorbance decreases as the treatment progresses, with the most considerable change in absorbance from CABI to effluent. The t-test was conducted for each wavelength spectrum (λ = 240 to 470 nm in 1 nm increments) measured in the experiment, as summarized in Table 4.18. Overall, the samples were either statistically different or plausibly the same across the entire wavelength spectrum. The only exception to this was the CABI sample, which was plausibly the same for part of the spectrum and different for the other part of the spectrum.

It appears that the influent sample did not degrade significantly when it was held overnight with and without headspace. Variable results were observed in the CABI and effluent samples. The absorbance tends not to change overnight for more polluted samples and becomes more variable as the sample progresses through treatment. This is contradictory to the previous holding experiments. Table 4.18 also reports the overall trend of the sample.

The sample is undergoing different processes during the holding time, which can impact the sample in a variety of ways and thus create inconsistent and unpredictable results, as observed in these trials. Biological activity is occurring that is oxidizing the organic substrate, which will bias the results low. Also, some of the soluble colloidal matter is

becoming dissolved into the sample, which will bias the results high. The rate that these mechanisms change the sample will vary depending on the sample, thus creating a situation where it is difficult to predict if and how the absorbance parameters will change.

Table 4.18: Results of the Absorbance (λ = 240 to 470 nm) Holding Test Trials for Influent, CABI, and Effluent Samples

Absorbance t-test results (comparison of mean)			
Sample	**Comparison**	**Trial A**	**Trial B**
Influent	Day 1 vs Day 2 full	Plausible for all λ	Plausible for all λ
	Day 1 vs Day 2 partial	Plausible for all λ	Plausible for all λ
	Day 2 full vs Day 2 partial	Plausible for all λ	Plausible for all λ
	Trend in absorbance	Increase all λ	Decrease all λ
CABI	Day 1 vs Day 2 full	Different for all λ	Plausible for all λ
	Day 1 vs Day 2 partial	Different for all λ	Plausible for λ 470-313 nm Different for λ 314-240 nm
	Day 2 full vs Day 2 partial	Plausible for all λ	Plausible for λ 470-323 nm Different for λ 324-240 nm
	Trend in absorbance	Increase all λ	General decrease
Effluent	Day 1 vs Day 2 full	Different for all λ	Plausible for all λ
	Trend in absorbance	Increase all λ	Variable increase/decrease

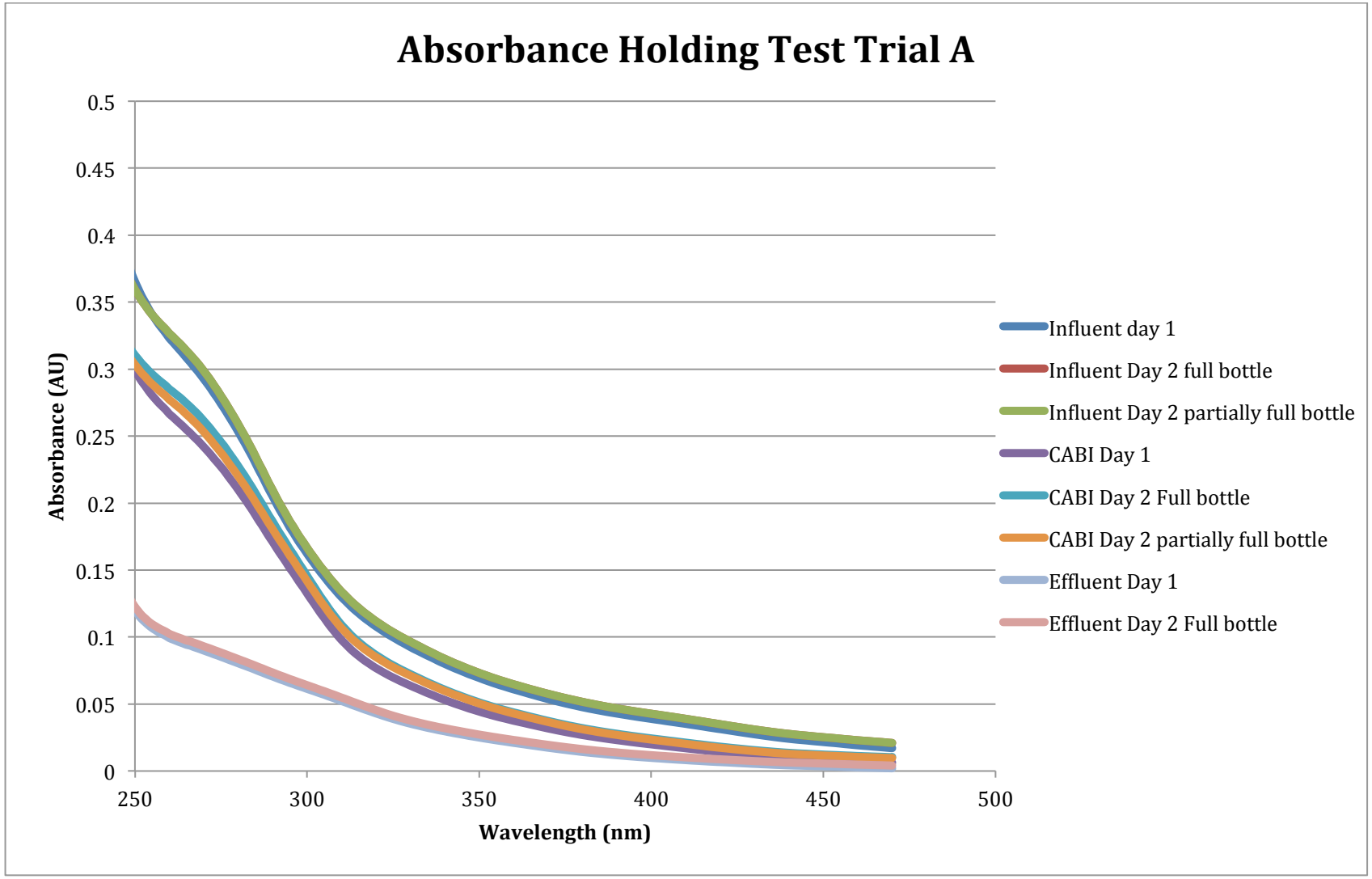

Figure 4.52: Absorbance (λ = 240 to 470 nm) results for holding test trial A.

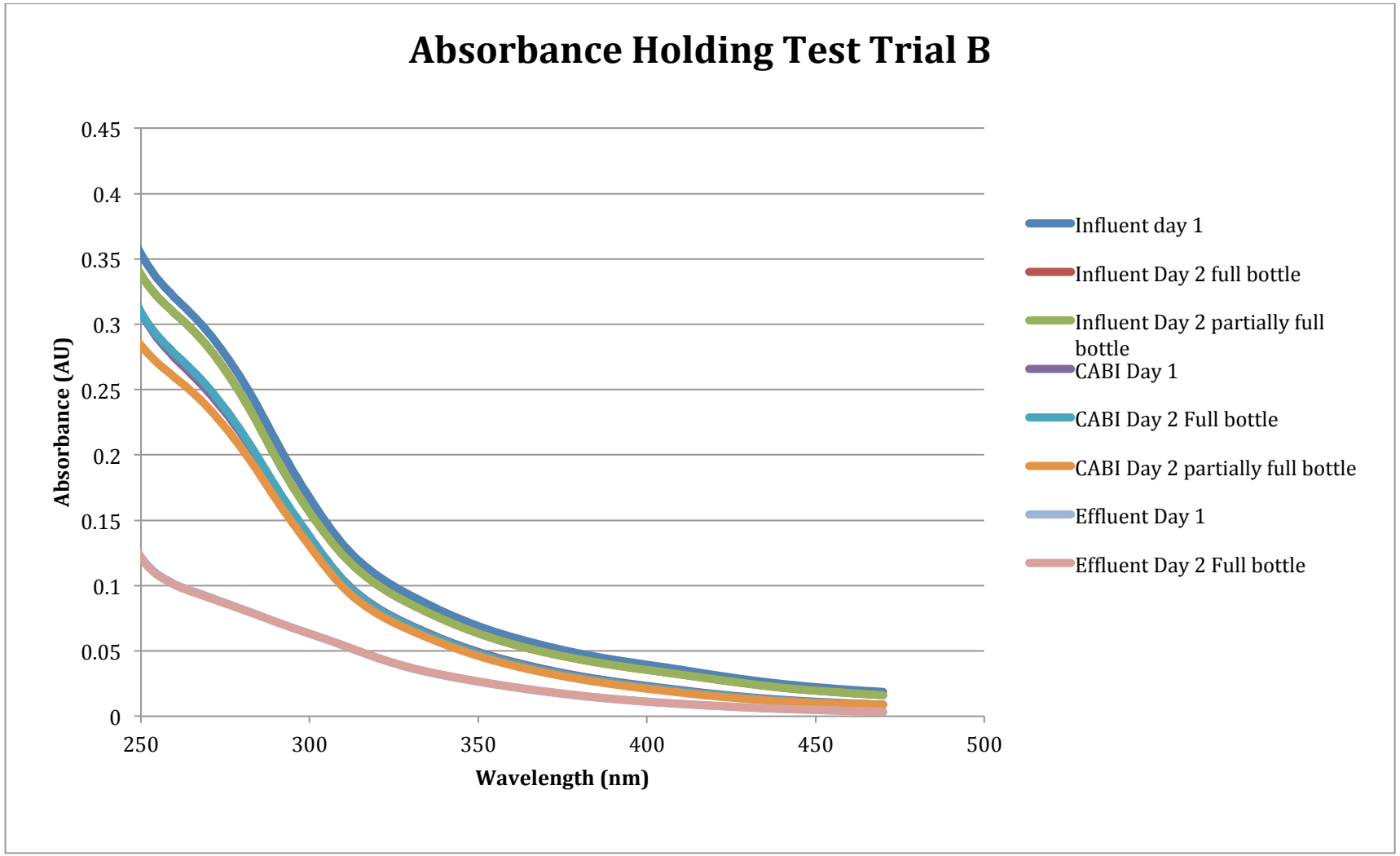

Figure 4.53: Absorbance (λ = 240 to 470 nm) results for holding test trial B.

4.14.4 Fluorescence Holding Test

Samples (influent, CABI, and effluent) were analyzed and compared similar to the BOD_5 and COD holding tests. Samples were analyzed the same day that they were collected and then reanalyzed the following day. The results from the two analyses were compared using the two-sample t-test. It should be mentioned that samples were not diluted for this experiment; therefore, fluorescence quenching likely occurred with CABI and influent samples. It is impossible to know how quenching impacted the results of day 1 and day 2, and if the degree of quenching was the same. For simplicity, the following discussion assumes that quenching was the same for both days. In actuality, quenching likely had more impact on samples analyzed the same day that they were collected, because the sample had more organic matter than when it was held overnight (i.e., biological degradation) and analyzed the following day.

Tables 4.19 to 4.23 summarize the fluorescence results and the t-test p values. Interestingly, the influent trial A sample did not significantly degrade overnight when it was held both with and without headspace. The results from the overnight samples were also compared and it can be concluded that it is plausible that the overnight sample stored with headspace is the same as the overnight sample stored without headspace. This was not true in the other trial conducted. There was degradation observed in the second influent trial, specifically for the parameters of region II, the humic-like peak, and peak A. Recall that peak A is located in region II and the humic-like peak is located in region III; therefore, there are some inconsistencies with the results in terms of degradation over fluorescence areas.

The CABI sample degraded significantly when it was held overnight without headspace, and even more with headspace. When the sample was held with headspace nearly

all of the fluorescence regions and peaks were lower than the previous day. For samples held without headspace, degradation was inhibited by the lack of oxygen, but it affected region III in trial A (including the humic-like peak and peak C). In trial B, sample degradation was observed in region I, region II, region III, the total of the regions, and peak A. The t-test also indicates that the overnight sample stored with headspace is not the same as the overnight sample without headspace in either of the trials.

The effluent samples were stored overnight without headspace for the fluorescence holding time tests. The results of the two trials indicate that the sample analyzed the first day was plausibly similar to the sample analyzed the following day. In both of the trials, region III was statistically different, indicating that it may be the most prone to degradation in effluent samples stored overnight.

The findings of the fluorescence holding tests were consistent with the previous COD and BOD_5 holding tests. This is an indication that all of the organic quantification methods are sensitive to the holding time and headspace. The changes in fluorescence were inconsistent among the samples; therefore, it can be concluded that samples degrade overnight when stored, but it is not possible to make inferences about the type (e.g., specific peaks or regions) of degradation.

Table 4.19: Fluorescence Holding Test for Influent Trial A

Influent - Trial A Hold Test (Fluorescence units in AFU)									
	Total	Region I	Region II	Region III	Proteins (280/331)	Humic (342/436)	A (260/450)	C (330/450)	T (275/340)
Day 1 test run A	168,567	96,162	50,740	21,665	8.653	3.237	3.222	3.428	9.070
Day 1 test run B	167,580	95,822	50,135	21,624	8.672	3.217	3.183	3.408	9.067
Day 1 test run C	173,948	100,332	51,410	22,206	9.264	3.277	3.282	3.473	9.524
Day 2 full bottle test run A	168,570	98,146	49,786	20,638	8.966	3.200	3.159	3.355	9.318
Day 2 full bottle test run B	173,163	100,804	50,916	21,443	9.324	3.253	3.237	3.449	9.634
Day 2 full bottle test run C	165,695	95,080	49,503	21,112	8.642	3.171	3.162	3.361	9.029
Day 2 partially full test A	163,622	93,292	49,273	21,057	8.545	3.174	3.125	3.362	8.870
Day 2 partially full test B	170,395	97,987	50,675	21,734	9.133	3.224	3.244	3.420	9.389
Day 2 partially full test C	165,306	94,078	49,726	21,503	8.620	3.182	3.184	3.374	8.974
T-test results for Day 1 vs Day 2 full bottle	*0.778	*0.808	*0.290	*0.065	*0.704	*0.301	*0.324	*0.269	*0.669
T-test results for Day 1 vs Day 2 partially full bottle	*0.275	*0.321	*0.192	*0.217	*0.739	*0.101	*0.377	*0.124	*0.552
T-test results Day 2 full bottle vs Day 2 partially full bottle	*0.416	*0.260	*0.781	*0.297	*0.477	*0.636	*0.973	*0.933	*0.351
* When p>0.05 accept the null hypothesis that the means are different.									

Table 4.20: Fluorescence Holding Test for Influent Trial B

Influent - Trial B Hold Test (Fluorescence units in AFU)									
	Total	Region I	Region II	Region III	Proteins (280/331)	Humic (342/436)	A (260/450)	C (330/450)	T (275/340)
Day 1 test run A	158,088	89,819	49,026	19,243	7.913	3.035	3.184	3.005	8.441
Day 1 test run B	158,575	90,149	49,031	19,394	7.932	3.021	3.171	3.031	8.465
Day 1 test run C	165,943	95,374	50,472	20,096	8.648	3.079	3.263	3.146	9.061
Day 2 full bottle test run A	152,702	87,652	46,399	18,651	7.941	2.944	2.974	2.895	8.375
Day 2 full bottle test run B	152,198	87,118	46,365	18,715	7.886	2.945	3.004	2.912	8.340
Day 2 full bottle test run C	155,362	89,445	46,877	19,040	8.204	2.970	3.036	2.946	8.585
Day 2 partially full test A	149,550	84,754	45,991	18,805	7.651	2.957	3.035	2.955	8.142
Day 2 partially full test B	147,902	83,597	45,548	18,757	7.553	2.943	3.050	2.941	8.026
Day 2 partially full test C	150,452	85,252	46,082	19,118	7.777	2.963	3.085	3.023	8.214
T-test results for Day 1 vs Day 2 full bottle	*0.085	*0.165	0.017	*0.081	*0.603	0.019	0.007	*0.069	*0.392
T-test results Day 1 vs Day 2 partially full bottle	0.036	0.048	0.011	*0.106	*0.166	0.025	0.020	*0.175	*0.112
T-test results Day 2 full bottle vs Day 2 partially full bottle	0.032	0.018	0.045	*0.609	0.049	*0.938	*0.089	*0.148	0.036
*When p>0.05 accept the null hypothesis that the means are different.									

Table 4.21: Fluorescence Holding Test for CABI Trial A

CABI - Trial A Hold Test (Fluorescence units in AFU)									
	Total	Region I	Region II	Region III	Proteins (280/331)	Humic (342/436)	A (260/450)	C (330/450)	T (275/340)
Day 1 test run A	149,095	85,585	48,096	15,414	7.100	2.125	3.034	2.206	7.756
Day 1 test run B	148,486	84,794	48,098	15,595	7.009	2.186	2.991	2.257	7.625
Day 1 test run C	148,920	84,901	48,361	15,658	7.038	2.190	3.054	2.271	7.634
Day 2 full bottle test run A	146,668	83,131	47,820	15,717	6.924	2.271	3.039	2.326	7.509
Day 2 full bottle test run B	148,906	84,832	48,151	15,923	7.122	2.314	3.067	2.367	7.716
Day 2 full bottle test run C	147,000	83,469	47,620	15,911	7.026	2.328	3.074	2.363	7.590
Day 2 partially full test A	146,166	81,078	48,443	16,646	6.652	2.495	3.157	2.481	7.355
Day 2 partially full test B	147,073	81,732	48,648	16,693	6.747	2.515	3.173	2.490	7.427
Day 2 partially full test C	147,858	82,568	48,596	16,695	6.856	2.500	3.156	2.483	7.602
T-test results for full bottle	*0.196	*0.117	*0.164	0.041	*0.719	0.008	*0.207	0.015	*0.425
T-test results for partially full bottle	*0.053	0.006	0.030	0.003	0.023	0.002	0.013	0.006	*0.084
T-test results Day 2 full bottle vs Day 2 partially full bottle	*0.598	0.042	0.032	0.005	0.030	0.004	0.004	0.001	*0.208
* When p>0.05 accept the null hypothesis that the means are different.									

Table 4.22: Fluorescence Holding Test for CABI Trial B

CABI - Trial B Hold Test (Fluorescence units in AFU)									
	Total	Region I	Region II	Region III	Proteins (280/331)	Humic (342/436)	A (260/450)	C (330/450)	T (275/340)
Day 1 test run A	136,955	77,873	44,389	14,693	6.412	1.990	2.828	2.086	6.930
Day 1 test run B	138,621	78,800	44,834	14,987	6.564	2.074	2.868	2.145	7.079
Day 1 test run C	139,268	78,584	45,378	15,306	6.498	2.151	2.949	2.202	7.007
Day 2 full bottle test run A	132,758	76,216	42,572	13,970	6.376	1.882	2.685	1.995	6.829
Day 2 full bottle test run B	133,428	76,412	42,752	14,265	6.450	1.955	2.723	2.050	6.838
Day 2 full bottle test run C	133,387	76,152	42,922	14,312	6.419	1.989	2.708	2.072	6.838
Day 2 partially full test A	131,819	73,208	43,460	15,151	6.032	2.180	2.828	2.206	6.593
Day 2 partially full test B	132,748	73,548	43,813	15,387	6.078	2.250	2.835	2.253	6.637
Day 2 partially full test C	131,771	72,485	43,837	15,449	5.964	2.265	2.868	2.270	6.500
T-test results for full bottle	0.012	0.012	0.011	0.025	*0.221	*0.091	0.030	*0.069	*0.058
T-test results for partially full bottle	0.005	0.000	0.040	*0.193	0.002	*0.054	*0.403	*0.079	0.002
T-test results Day 2 full bottle vs Day 2 partially full bottle	*0.056	0.007	0.004	0.001	0.001	0.002	0.001	0.003	0.023
* When p>0.05 accept the null hypothesis that the means are different.									

Table 4.23: Fluorescence Holding Test for Effluent Trial A and Trial B

Effluent - Trial A Hold Test (Fluorescence units in AFU)									
	Total	Region I	Region II	Region III	Proteins (280/331)	Humic (342/436)	A (260/450)	C (330/450)	T (275/340)
Day 1 test run A	43,605	14,683	20,217	8,705	0.976	1.391	1.607	1.194	1.042
Day 1 test run B	43,102	14,524	19,928	8,650	0.971	1.363	1.578	1.168	1.012
Day 1 test run C	42,597	14,241	19,765	8,590	0.921	1.335	1.555	1.145	0.962
Day 2 full bottle test A	42,686	14,456	19,721	8,509	0.930	1.360	1.561	1.162	0.974
Day 2 full bottle test B	42,168	14,439	19,340	8,389	0.947	1.317	1.538	1.130	0.982
Day 2 full bottle test C	41,907	14,279	19,268	8,360	0.923	1.311	1.516	1.114	0.949
T-test results	*0.088	*0.568	*0.052	0.018	*0.332	*0.208	*0.106	*0.170	*0.257
*Statistically significant at 95%									

Effluent- Trial B Hold Test									
	Total	Region I	Region II	Region III	Proteins (280/331)	Humic (342/436)	A (260/450)	C (330/450)	T (275/340)
Day 1 test run A	39,481	12,899	18,444	8,138	0.822	1.273	1.452	1.082	0.864
Day 1 test run B	39,181	12,757	18,333	8,091	0.809	1.256	1.439	1.066	0.851
Day 1 test run C	39,784	13,141	18,513	8,130	0.850	1.260	1.481	1.071	0.898
Day 2 full bottle test A	38,180	12,435	17,873	7,873	0.801	1.234	1.429	1.042	0.831
Day 2 full bottle test B	36,335	11,814	16,923	7,598	0.758	1.177	1.329	0.996	0.789
Day 2 full bottle test C	38,304	12,752	17,729	7,824	0.872	1.205	1.420	1.021	0.890
T-test results	*0.089	*0.149	*0.084	0.049	*0.676	*0.060	*0.170	0.046	*0.376
* When p>0.05 accept the null hypothesis that the means are different.									

4.14.5 Implications of the Holding Tests

The results of the holding tests show a systematic pattern. Influent samples tend to be the most prone to degradation, but this is accelerated when the sample is stored with headspace. CABI samples are also prone to degradation, but to a lesser degree than influent. The effluent tends to be the most consistent when held overnight. Influent and CABI samples contain microorganisms and organic matter; therefore, they degrade more quickly than effluent, which has minimal microorganisms (due to the WWTP filtration process) and biodegradable organic matter (due to the WWTP biological treatment).

As shown in the previous results sections, headspace impacts the quality of the results for samples held overnight. There is more oxygen available to microbes to consume the substrate, thus decreasing the organics quantified during the experiments. Despite the logic behind this, samples may be held with headspace for two reasons. First, lab personal often ask that samples to be brought into the laboratory with headspace. This headspace gives the analyst adequate room in the bottle to shake the sample and properly homogenize it to conduct their experiments. Despite this request, it would be recommended that the samples be collected and transported to the lab without headspace. Prior to analysis, the analyst should transfer the sample to a larger container. The larger container can be used to homogenize the sample and partition it for different testing procedures.

The second reason that an analyst may leave headspace in their sample container is because they do not fully understand the method and the sensitivity to experimental conditions. Many people refer to various versions of the Standard Methods to obtain information to conduct their respective tests; the 21st edition (2005) was referenced for this research. The organic quantification methods are in part 5000, and broken into the respective

subsections (i.e., COD, BOD, and TOC). A small part of the introduction of part 5000 refers the reader to part 1000, which addresses quality control and assurance measures. A single paragraph in sample storage and preservation subsection of part 1000 addresses headspace, which the methods say is "important in preservation of samples with volatile organic compounds and radon" (Standard Methods, 2005). An analyst that is performing a method that quantifies the aggregate organic matter can easily overlook this obscure reference to headspace. Furthermore, the Standard Methods specifies volatile organic matter, which is not quantified in the BOD_5 test. These experiments have clearly demonstrated that headspace will accelerate the rate of biodegradable decomposition in the sample, which will bias the test results low. Therefore, it would be highly recommended that the Standard Methods more directly address the issue of headspace in the context of each method. The analyst will be far less likely to overlook the importance of the issue and it will provide for better quality control and assurance.

Sample headspace brings up another issue that is related to the traditional composite process. Samples are typically collected over a 24-hour period using a refrigerated compositor with a pump assembly that draws the water sample and then transfers it to a cubitainer via tubing. The cubitainer is open to the atmosphere and it is full of air while the sample is being collected, slowly filling during each composite event. Early event composites are prone to the most degradation, whereas composites that are collected later in the composite period are less prone to degradation due to time and air. The degree of degradation is influenced by many factors including time, available oxygen, and temperature each of which are changing during the composite period (Tchobanoglous et al., 2003). It can be concluded that the sample collection method is not ideal and a more proper method would

include collection containers that minimize air space, minimize exposure to the atmosphere, and expand as the sample fills the container.

4.15 Fluorescence Integration Times

The integration time (i.e., amount of time that the detector stays open to capture light) of the fluorescence test will impact how long it takes to analyze the sample and also the quality of the results. It was found that the time to analyze a sample was approximately 15 minutes, 10 minutes, and 5 minutes for integration settings of 3 seconds, 2 seconds, and 1 second. Longer integration times will reduce the signal-noise ratio, but there is a point of diminishing return after 3 seconds, as discussed in the literature review. Based on this information from the literature review, integration times of 1s, 2s, and 3s were compared.

Two criteria were identified to compare the test results: the two sample t-test and the normalized standard deviation. For the two-sample t-test, the samples were conservatively assumed to have unequal variance.

Table 4.24 (influent), 4.25 (CABI), and 4.26 (effluent) present the raw data and the results of the t-test. The influent has fewer parameters that are statistically significant compared with CABI and influent. This sample is the most polluted with organic carbon, so it tends to be the most variable due to the sample matrix. It is interesting to note that the parameters (total region, region I, A, and M) that were statistically significant for CABI and effluent were similar in the 3s and 2s integration comparison. It is possible that some of the organic matter that caused the influent regions to be insignificant were removed or stabilized in the primary clarifier.

Overall, the t-test shows that the results increase with higher integration times, which is expected because the detector is exposed to the sample longer. Incrementally increasing

the integration times may have some use when trying to identify pollutants in the water, for instance there may be a minimum integration setting to identify particular pollutants. For this research, all three integration settings quantified the peaks. As such, the t-test was determined to be an inferior method to justify the integration setting for this research.

The alternate method was to normalize the standard deviation to the mean of the samples. The literature suggested that protein-like peaks and tryptophan peaks were best to correlate to BOD_5. These two peaks and region I (related to protein-like peaks) were determined to be the primary baseline for comparison. The normalized standard deviation (NSD) sharply decreased from when the integration time was increased to from 1 to 2 seconds, but it was similar when the integration time was increase from 2 to 3 seconds. The CABI data also indicated a general decrease from 1 to 2 seconds. But there was an increase in the NSD from 2 to 3 seconds. Observation of the data indicate that this may be due to lower value reported for test run A. The integration times for 1s and 3s were compared for CABI indicate the NSD are similar, verifying the aforementioned observation. A skewed data point was also reported in the effluent sample for the 2s integration. Comparison of the 1s and 3s effluent integration times indicated a general decrease or similarity for the NSD.

Excluding the skewed data, there seems to be a downward trend in NSD associated with increased integration times. Therefore, it can be concluded that a 3 second integration time is preferred over 1 or 2 seconds for these datasets. As mentioned, longer integration times increase the analysis time and they will not significantly reduce the signal to noise interference associated with the equipment, so it is unreasonable to consider longer integration times. Based on this analysis, a 3 second integration time was implemented for the three sample sites in this research.

Table 4.24: Fluorescence Integration Results for Influent

Influent (Fluorescence units in AFU)											
Setting	**Run**	**Total**	**Region I**	**Region II**	**Region III**	**Proteins**	**Humic**	**A**	**C**	**M**	**T**
3s integration	Test run A	196,527	118,451	57,203	20,872	10.35	2.72	3.60	2.85	9.43	10.85
	Test run B	202,150	121,399	59,150	21,602	10.69	2.77	3.74	2.91	9.72	11.20
	Test run C	202,957	122,820	58,707	21,430	10.81	2.77	3.73	2.92	9.86	11.35
2s integration	Test run A	188,521	115,714	53,010	19,797	10.61	3.02	3.39	2.92	10.04	11.10
	Test run B	192,758	118,107	54,006	20,644	11.03	3.25	3.46	3.07	10.36	11.49
	Test run C	187,460	114,152	52,936	20,372	10.62	3.20	3.39	3.06	9.96	10.99
1s integration	Test run A	167,048	100,194	47,546	19,308	9.40	3.14	3.09	2.92	8.78	9.68
	Test run B	157,261	92,837	45,696	18,729	8.44	3.06	3.07	2.85	8.04	8.89
	Test run C	155,073	91,029	45,169	18,874	8.35	3.07	3.10	2.85	7.94	8.81
2 sample t-test	3s versus 2s	0.015	0.048	0.004	0.037	*0.500	0.022	0.010	*0.121	*0.062	*0.790
	3s versus 1s	0.002	0.004	0.000	0.001	0.019	0.001	0.004	*0.553	0.019	0.008
	2s versus 1s	0.007	0.009	0.003	0.016	0.015	*0.461	0.003	*0.086	0.009	0.008
* When p>0.05 accept the null hypothesis that the means are different.											
Normalized SD	3s	0.017	0.018	0.017	0.018	0.023	0.011	0.020	0.012	0.023	0.023
	2s	0.015	0.017	0.011	0.021	0.022	0.037	0.012	0.029	0.021	0.023
	1s	0.040	0.051	0.027	0.016	0.067	0.014	0.004	0.015	0.056	0.053

Table 4.25: Fluorescence Integration Results for CABI

CABI (Fluorescence units in AFU)											
Setting	**Run**	**Total**	**Region I**	**Region II**	**Region III**	**Proteins**	**Humic**	**A**	**C**	**M**	**T**
3s integration	Test run A	143,364	81,892	46,033	15,440	7.07	2.23	3.08	2.21	6.80	7.48
	Test run B	140,304	80,140	44,889	15,275	6.95	2.24	2.99	2.21	6.67	7.30
	Test run C	146,883	85,048	46,134	15,700	7.56	2.31	3.05	2.27	7.16	7.86
2s integration	Test run A	137,737	79,908	43,450	14,379	7.04	1.99	2.83	2.02	6.78	7.47
	Test run B	141,805	82,953	44,112	14,739	7.44	2.04	2.93	2.06	7.12	7.80
	Test run C	142,540	83,002	44,448	15,090	7.52	2.13	2.96	2.12	7.14	7.81
1s integration	Test run A	127,969	74,564	39,412	13,993	6.52	1.99	2.59	1.95	6.43	7.00
	Test run B	124,663	71,180	39,498	13,984	6.43	2.07	2.61	2.02	6.15	6.72
	Test run C	121,407	68,569	39,161	13,677	6.13	1.98	2.64	1.96	5.85	6.48
2 sample t-test	3s versus 2s	*0.311	*0.830	0.031	0.048	*0.590	0.017	*0.050	0.011	*0.500	*0.4914
	3s versus 1s	0.002	0.009	0.002	0.001	0.026	0.003	0.001	0.001	0.030	0.0219
	2s versus 1s	0.003	0.011	0.002	0.035	0.008	*0.434	0.009	*0.065	0.016	0.0084
* When p>0.05 accept the null hypothesis that the means are different.											
Normalized SD	3s	0.023	0.030	0.015	0.014	0.045	0.021	0.016	0.016	0.037	0.038
	2s	0.018	0.022	0.012	0.024	0.035	0.033	0.023	0.023	0.029	0.025
	1s	0.026	0.042	0.004	0.013	0.033	0.024	0.011	0.019	0.047	0.039

Table 4.26: Fluorescence Integration Results for Effluent

Effluent (Fluorescence units in AFU)											
Setting	**Run**	**Total**	**Region I**	**Region II**	**Region III**	**Proteins**	**Humic**	**A**	**C**	**M**	**T**
3s integration	Test run A	42,787	14,052	20,149	8,585	0.86	1.33	1.61	1.13	0.91	0.91
	Test run B	43,886	14,630	20,601	8,655	0.93	1.33	1.67	1.13	0.97	0.96
	Test run C	42,349	13,910	19,843	8,596	0.86	1.33	1.58	1.13	0.91	0.90
2s integration	Test run A	38,114	12,172	18,081	7,860	0.74	1.20	1.48	1.01	0.79	0.79
	Test run B	38,220	12,314	18,035	7,871	0.76	1.19	1.46	1.02	0.81	0.81
	Test run C	44,665	17,503	19,016	8,147	1.27	1.22	1.53	1.04	1.29	1.33
1s integration	Test run A	34,883	10,365	16,955	7,563	0.59	1.13	1.33	0.97	0.64	0.65
	Test run B	34,770	10,285	16,984	7,502	0.58	1.12	1.37	0.96	0.64	0.63
	Test run C	34,476	10,015	16,916	7,545	0.54	1.14	1.37	0.97	0.61	0.59
2 sample t-test	3s versus 2s	*0.342	*0.920	0.012	0.016	*0.837	0.003	0.017	0.009	*0.856	*0.809
	3s versus 1s	0.002	0.001	0.004	0.000	0.001	0.001	0.002	0.000	0.001	0.000
	2s versus 1s	*0.121	*0.164	0.046	0.041	*0.179	0.002	0.009	0.030	*0.177	*0.184
* When p>0.05 accept the null hypothesis that the means are different.											
Normalized SD	3s	0.018	0.027	0.019	0.004	0.048	0.001	0.027	0.002	0.038	0.037
	2s	0.093	0.217	0.030	0.020	0.328	0.011	0.024	0.018	0.294	0.314
	1s	0.006	0.018	0.002	0.004	0.048	0.008	0.019	0.002	0.027	0.050

CHAPTER 5

CONCLUSIONS

NPDES permits are used to regulate the organic pollution, as determined by the BOD_5 test, discharged into the environment by wastewater treatment plants. The BOD_5 test takes five days to obtain the results, which makes it desirable to seek alternate test procedure methods. In fact, treatment plants can seek approval through their NPDES permit-governing agency to use alternate test methods in lieu of the BOD_5 method. A review of the literature indicated that COD, TOC, fluorescence, and absorbance could be used as a surrogate for BOD_5. The primary benefit to these alternate test methods is that the results can be obtained quickly, typically within minutes or a few hours as opposed to the five days required for the BOD_5 method. In fact, on-line instrumentation is available for some of the alternate test procedures, which would allow treatment plants to estimate their BOD_5 concentrations in real-time.

This research explored the use of the alternate methods as a surrogate for BOD_5 using influent, primary clarifier effluent, and finished effluent samples that were collected from an operational wastewater treatment facility located in Nevada. The uninhibited BOD_5, COD, and TOC results were determined in accordance with the Standard Methods (2005). Samples for the BOD_5 and COD test were not filtered. Samples that were used for the TOC test were

filtered through a 1.2-micron filter, acidified and sparged to remove the inorganics. Fluorescence was determined for an excitation range from 240 to 470 nm using 1-nm increments and a 3-second integration time. The samples were filtered through a 0.7-micron filter, and they were not diluted. Equipment corrections were used to correct for IFE and Rayleigh interference. The fluorescence was also normalized to the area of the Raman peak at a 350 nm excitation wavelength.

Each of the methods quantifies organic matter differently. BOD_5 quantifies the biodegradable organic matter. COD quantifies more of the biodegradable organic matter than BOD_5 as well as some inorganics, which is attributed to the harsh and effective oxidation of potassium dichromate. TOC quantifies the organic carbon after mineralization to CO_2. Fluorescence is arguably the most versatile method, because it can identify organic compounds based on their peaks (e.g., humic-like, fulvic-like, protein-like, etc.). Absorbance is a measure of how much light is absorbed by a sample, which might be related to chromophores present in organic matter but can also be related to some inorganics as well.

Samples were analyzed the same day that they were collected for correlations between BOD_5 and the alternate tests. The correlations developed in this research have shown that COD can be correlated to BOD_5 and used to predict the BOD_5 of influent, primary clarifier effluent (i.e., CABI) and finished effluent. The relationship between TOC and BOD_5 was strong for the finished effluent, but weak for influent and CABI samples; therefore, TOC should not be used as a surrogate for those sample sites. Fluorescence was related to BOD_5 based on data for region I and peak T using a power function. Presumably, IFE interference quenched some of the fluorescence, particularly for the more concentrated samples (i.e., influent and CABI) and created a nonlinear relationship that caused this

research to differ from the literature. It was concluded that humic-like and region III fluorescence are poor surrogates for BOD_5 because they are not largely linked to biodegradable organic matter.

Interestingly, when the results of the correlation and the removal efficiencies are compared, it is shown that BOD_5 correlates the best to alternate test procedures that have similar removal efficiencies. This was true for COD, which had the most similar removal throughout the treatment process, and for TOC, which had the most similar removal during biological treatment. Region I and protein/tryptophan-like peaks had similar removals during the biological process as well; the literature has suggested that these are the most related to the wastewater treatment process. Humic- and fulvic-like fluorescence are most related to nonbiodegradable organic matter, and they are not easily removed during treatment, which was also observed in this research.

It was shown that labile and humic fluorescence were removed in the primary clarifier. Protein-like fluorescence was effectively removed during biological treatment. Nearly half of the humic- and fulvic-like fluorescence was removed during the treatment processes, mostly during biological treatment. It was observed that TOC, fulvic-like fluorescence, and absorbance increased in the primary clarifier, which was most likely due to the return flows associated with sludge treatment that are sent to the primary clarifiers. Although impossible to say with certainty, it is likely that these parameters are related because they exhibited similar increases in the primary clarifier and similar removal efficiency trends.

Experiments were conducted to see if holding a sample overnight would impact the quality of the results. It was determined that influent samples were the most prone to

degradation, which was followed by primary clarifier effluent samples. The effluent sample was not as susceptible to degradation as the other samples. It was also concluded that samples stored overnight with headspace degraded more than their counterpart that was not stored with headspace. It can be concluded that the water matrix and the available air in the storage container have an impact on the sample when it is held overnight.

In particular, influent and CABI samples have large concentrations of biodegradable organic matter and microorganisms. When these samples are analyzed following a day of storage, the results are biased low because the native microorganisms consume the organic matter and utilize ambient dissolved oxygen. These findings are not novel, but they are extremely important. It is not uncommon for lab analysts to ask sample handlers to bring in samples with headspace so that they can properly homogenize the sample. It is recommended for the sample to be stored without headspace, and the lab analyst should transfer the sample to a larger container to be homogenized. Additionally, samples are typically collected during a 24-hour composite period with a cubitainer. Samples are deposited into the cubitainer through a lid opening, which is exposed to the environment. The cubitainer is initially full of air, and fills with each composite event. A better composite method would be one that has a sample container that is not exposed to the environment and that is capable of expanding with each sample event. This type of container would limit the excess oxygen in the sample and also prevent contamination from the environment.

The second part of the method development explored the use of 1-, 2-, and 3-second fluorescence integration times. The signal to noise ratio decreases as the integration time increases, but there is a point of diminishing return after 3 seconds. Furthermore, the time that it takes to analyze a sample increases with increased integration times, so this must also

be considered. Comparison of the integration times using the two-sample t-test was not practical, because the observed fluorescence will always increase with increased integration times. Instead, the methods were compared using their normalized standard deviations. It was concluded that the 3-second integration was the most practical for this research because it had the lowest normalized standard deviation for peaks related to BOD_5.

Future research should look into the changing ratios between BOD_5 and the alternate test methods. Previous researchers have identified BOD_5/TOC and BOD_5/COD ratios and discussed that this is a reason not to combine sample sites, but little has been done to embrace the change in ratio and consider it as part of a dynamic relationship as treatment progresses. The use of fluorescence to characterize the wastewater may be a useful tool when investigating the relationships between BOD_5, TOC, and COD. For instance, this research showed that TOC was more related to fulvic-like fluorescence than biodegradable fluorescence, which made it a bad surrogate for influent and primary clarifier effluent BOD_5 which is related to biodegradable fluorescence. TOC and BOD_5 had similar removals during biological treatment, which may be an explanation as to why finished effluent correlated well in this research.

The negative removals observed in this research were attributed to return flows associated with sludge treatment. It is known that these flows are highly concentrated with BOD and TSS, although little is known about the organic characterization of the centrate. Future research should determine if centrate is characterized by biodegradable organic matter (e.g., COD or BOD_5) or organic carbons (e.g., TOC). The return flows can also be characterized as protein-, fulvic-, or humic-like with fluorescence spectroscopy. A better understanding of the return flows can help plant operators understand if the flows are being

adequately treated when they are returned to the primary clarifier and if there is a buildup of organic matter during the process. Furthermore, plant designers can determine if side stream treatment is a more adequate way to treat these return flows, particularly if the flows are not predominantly biodegradable organic matter. This may also reduce the need for coagulants and precipitants in the primary clarifier, which would have an economical benefit.

Standardized fluorescence methods do not exist, but fluorescence is gaining considerable attention for a wide range of uses. Therefore, it is essential that standardized fluorescence settings be developed that address integration times, integration increments, how to address interferences, and acceptance of a standard (e.g., quinine sulfate). This research showed that increases in integration time will increase the fluorescence results. Raman interference is overcome by normalizing the sample to a Raman peak or a Raman area. IFE interference is overcome by software corrections, dilutions, or normalizing the data to a specific absorbance wavelength. These arbitrary choices make it difficult to reproduce the results of other researchers, to decide which approaches to make during analysis, and to directly compare the results of different research projects. For instance, dilution was not done in this research, which resulted in a nonlinear model that could not be compared with other research due to the IFE quenching interference.

This research has shown that alternate methods, particularly COD and protein-like fluorescence, can be used as surrogate for BOD_5. WWTPs can develop correlations that are specific to their treatment plant and submit the information to their NPDES issuer to obtain permission to use an alternate test procedure in lieu of BOD_5. When WWTPs are developing their correlations, they should keep in mind the change in ratios, the removal efficiency of each unit process, and possible designs that can increase the organic loading (e.g., return

flows). If a WWTP can successfully develop correlations, then BOD_5 can be estimated rapidly, allowing the plant to make changes to the treatment process quickly and efficiently, which will save time and money and ultimately protect the environment from organic pollution.

APPENDIX A

BOD_5 DATA COLLECTION AND RESULTS

Table A.1: Benchmark Data Collection Sheet for BOD_5 Test

BOD bottles prepared by: _____ First sample in: _____

Read back completed by: _____ First sample read back: _____

Date sample tested: _____ Verify chlorine: _____

Sample name (dup=duplicate)	Volume Sample mL	Volume Seed mL	Temp °C	Initial DO mg/L	Final DO mg/L	Initial-Final DO mg/L	Calculated BOD mg/L
Blank dup. A	0	0					
Blank dup. B	0	0					
GGA A	6	2					
GGA B	6	2					
Seed Blank 1	3	0					
Seed Blank 2	5	0					
Seed Blank 3	7	0					
Seed Blank 4	10	0					

Date sample collected: _____ pH Influent _____

Data Set #: _____ pH CABI _____

pH Effluent _____

Influent 1	2	0					
Influent 2	3	0					
Influent 3	5	0					
Influent dup A	3	0					
Influent dup B	3	0					
CABI 1	3	0					
CABI 2	5	0					
CABI 3	10	0					
Effluent dup 1	100	2					
Effluent dup 2	150	2					
Effluent dup 3	200	2					

Table A.2: Sample Calculations for BOD_5 Test

BOD bottles prepared by:	Evelyn	First sample in:	1030 AM
Read back completed by:	Evelyn	First sample read back:	940 AM
Date sample tested:	7/16/14	Verify chlorine:	0

Sample name	Volume Sample (mL)	Volume Seed (mL)	Temp (°C)	Initial DO (mg/L)	Final DO (mg/L)	Initial-Final DO (mg/L)	Calculated BOD (mg/L)
Blank Dup A	0	0	18.2	8.78	8.78	0.00	OK water
Blank Dup B	0	0	18.2	8.82	8.81	0.01	OK water
*If Initial-final DO>0.2 mg/L, then the dilution water is inadequate for BOD test							
GGA A	6	2	18.3	8.77	3.75	5.02	210
GGA B	6	2	18.3	8.78	3.66	5.12	215
						Average GGA BOD	212
						Is the GGA within reasonable limits?	Yes
BOD is based on the average for samples that had a minimum uptake of 2 mg/L but did not have a final DO<1.0 mg/L. GGA standard is 198ppm ± 30.5							
Seed Blank 1	3	0	18.3	8.81	7.53	1.28	Unqualified
Seed Blank 2	5	0	18.3	8.82	6.79	2.03	0.41
Seed Blank 3	7	0	18.3	8.82	5.92	2.90	0.41
Seed Blank 4	10	0	18.4	8.81	4.62	4.19	0.42
						Average seed strength	0.41
Average seed strength is based on the average for samples that had a minimum uptake of 2 mg/L but did not have a final DO<1.0 mg/L							

Date sample collected 7/15/2014	pH Influent	7.39
	pH CABI	7.24
	pH Effluent	7.90

All of the averages are based on samples that had a minimum uptake of 2 mg/L but did not have a final DO<1.0 mg/L.							
Influent 1	2	0	18.4	8.81	6.97	1.84	Unqualified
Influent 2	3	0	18.4	8.82	6.17	2.65	265
Influent 3	5	0	18.4	8.80	3.00	5.80	348
Influent Dup A	3	0	18.4	8.82	5.98	2.84	284
Influent Dup B	3	0	18.4	8.81	5.61	3.20	320
						Average Influent BOD	304
						Standard deviation Influent BOD	37
CABI 1	3	0	18.4	8.83	6.81	2.02	202
CABI 2	5	0	18.5	8.81	5.86	2.95	177
CABI 3	10	0	18.5	8.78	0.87	7.91	Unqualified
						Average CABI BOD	190
						Standard Deviation CABI BOD	N/A
Effluent dup 1	100	2	18.4	8.90	5.40	3.50	8.02
Effluent dup 2	150	2	18.5	8.85	4.34	4.51	7.37
Effluent dup 3	200	2	18.6	8.84	3.16	5.68	7.28
						Average Effluent BOD	7.56
						Standard Deviation Effluent BOD	0.40

Table A.3: Quality Control Parameter Check for BOD_5 Samples

Quality Control Parameter Check	Pass	Fail
Dilution water blank does not deplete >0.2 mg/L	✔	×
GGA within 198±30.5 mg/L	✔	×
Replicates do not differ by >30% for high and low values	✔	×
Seed controls meet dilution criteria	✔	×
Final DO must be >1 mg/L. If all samples have a final DO<1mg/L, then report value as "greater than." Data points marked with "×" are greater than values. These values were not included in final correlation.	✔	×

Table A.4: Influent BOD_5 Results with Quality Control Parameters (see Table A.3)

BOD Results for Influent									
Holding Time (NR=Not reported due to quality control fail)	Date (2014) Month/Day	BOD (mg/L)	Standard Deviation (SD)	Normalized SD	Dilution Water	GGA	Sample Replicates	Seed Replicates	Final DO>1 mg/L
NR	6/5	412	N/A	N/A	×	×	×	×	✓
NR	6/10	349	18	0.05	×	✓	✓	✓	✓
NR	6/11	348	19	0.05	×	✓	✓	✓	✓
NR	6/18	326	36	0.11	×	×	✓	✓	✓
NR	6/19	331	27	0.08	×	×	✓	✓	✓
NR	6/19	346	25	0.07	×	×	✓	✓	✓
NR	6/20	475	N/A	N/A	×	×	N/A	✓	✓
Overnight	6/24	318	20	0.06	✓	✓	✓	✓	✓
Same Day	6/25	348	N/A	N/A	✓	✓	✓	✓	✓
Overnight	6/26	384	50	0.13	✓	✓	✓	✓	✓
Same Day	6/27	364	N/A	N/A	✓	✓	✓	✓	✓
Overnight	7/1	227	7	0.03	✓	✓	✓	✓	✓
Same Day	7/2	364	42	0.12	✓	✓	✓	✓	✓
Same Day	7/3	355	25	0.07	✓	✓	✓	✓	✓
Overnight	7/8	275	13	0.05	✓	✓	✓	✓	✓
Same Day	7/9	337	16	0.05	✓	✓	✓	✓	✓
Overnight	7/10	289	22	0.08	✓	✓	✓	✓	✓
Same Day	7/11	376	N/A	N/A	✓	✓	✓	✓	✓
Overnight	7/15	304	37	0.12	✓	✓	✓	✓	✓
Same Day	7/16	359	29	0.08	✓	✓	✓	✓	✓
Overnight	7/17	321	30	0.09	✓	✓	✓	✓	✓
Same Day	7/18	378	22	0.06	✓	✓	✓	✓	✓
Overnight	7/22	281	29	0.10	✓	✓	✓	✓	✓
Same Day	7/23	433	30	0.07	✓	✓	✓	✓	✓
Overnight	7/24	265	18	0.07	✓	✓	✓	✓	✓
Same Day	7/25	438	53	0.12	✓	✓	✓	✓	✓
Overnight	7/29	226	19	0.08	✓	✓	✓	✓	✓
Same Day	7/30	549	N/A	N/A	✓	✓	N/A	✓	✓
Overnight	7/31	273	13	0.05	✓	✓	✓	✓	✓
Same Day	8/1	454	N/A	N/A	✓	✓	✓	✓	✓
Overnight	8/5	260	21	0.08	✓	×	✓	✓	✓
Same Day	8/6	447	39	0.09	✓	×	✓	✓	✓
Overnight	8/7	312	22	0.07	✓	✓	✓	✓	✓

Same Day	8/6	447	39	0.09	✓	×	✓	✓	✓
Overnight	8/7	312	22	0.07	✓	✓	✓	✓	✓
Same Day	8/8	417	8	0.02	✓	✓	✓	✓	✓
Overnight	8/12	245	17	0.07	✓	✓	✓	✓	✓
Same Day	8/13	336	N/A	N/A	✓	✓	✓	✓	✓
Overnight	8/14	243	14	0.06	✓	✓	✓	✓	✓
Same Day	8/15	379	24	0.06	✓	✓	✓	✓	✓
Overnight	8/19	243	9	0.04	✓	✓	✓	✓	✓
Same Day	8/20	420	36	0.09	✓	✓	✓	✓	✓
Overnight	8/21	250	22	0.09	✓	✓	✓	✓	✓
Same Day	8/22	356	14	0.04	✓	✓	✓	✓	✓
Overnight	8/26	284	39	0.14	✓	✓	✓	✓	✓
Same Day	8/27	510	22	0.04	✓	✓	✓	✓	✓
Overnight	8/28	293	17	0.06	✓	✓	✓	✓	✓
Same Day	8/29	603	N/A	N/A	✓	✓	✓	✓	✓
Overnight	9/2	332	38	0.11	✓	✓	✓	✓	✓
Same Day	9/3	406	59	0.15	✓	✓	✓	✓	✓
Overnight	9/4	225	17	0.08	✓	✓	✓	✓	✓
Same Day	9/5	359	62	0.17	✓	✓	✓	✓	✓
Results from the holding test									
Trial (not reported because of GGA failure)									
Day 1	9/9	457	50	0.11	✓	✓	✓	✓	✓
Day 2 with headspace	9/10	260	20	0.08	✓	×	✓	✓	✓
*Day 2 influent was collected from a different aliquot & the GGA failed, so this was not included in the final analysis.									
Trial A									
Day 1	9/11	327	3	0.01	✓	✓	✓	✓	✓
Day 2 with headspace	9/12	226	12	0.05	✓	✓	✓	✓	✓
Day 2 w/out headspace	9/12	307	13	0.04	✓	✓	✓	✓	✓
Trial B									
Day 1 (46)	9/16	236	16	0.07	✓	✓	✓	✓	✓
Day 2 with headspace	9/17	188	7	0.04	✓	✓	✓	✓	✓
Day 2 w/out headspace	9/17	277	21	0.08	✓	✓	✓	✓	✓
Results from the solubility test (not reported)									
Trial A									
Day 1 unfiltered	9/17	277	N/A	N/A	✓	✓	✓	✓	✓
Day 1 filtered	9/17	63	N/A	N/A	✓	✓	✓	✓	✓
Day 2 unfiltered	9/18	231	19	0.08	✓	✓	✓	✓	✓
Day 2 filtered	9/18	65	N/A	N/A	✓	✓	✓	✓	✓

Table A.5: CABI BOD_5 Results with Quality Control Parameters (see Table A.3)

BOD Results for CABI									
Holding Time (NR=Not reported due to quality control fail)	Date (2014) Month/Day	BOD (mg/L)	Standard Deviation (SD)	Normalized SD	Dilution Water	GGA	Sample Replicates	Seed Replicates	Final DO >1 mg/L
NR	6/5	N/A	N/A	N/A	×	×	×	×	×
NR	6/10	242	N/A	N/A	×	✓	N/A	✓	✓
NR	6/11	233	N/A	N/A	×	✓	N/A	✓	✓
NR	6/17	217	N/A	N/A	×	×	✓	✓	✓
NR	6/18	224	14	0.06	×	×	✓	✓	✓
NR	6/19	235	N/A	N/A	×	×	✓	✓	✓
NR	6/20	221	19	0.09	×	×	✓	✓	✓
Overnight	6/24	199	N/A	N/A	✓	✓	✓	✓	✓
Same Day	6/25	201	16	0.08	✓	✓	✓	✓	✓
Overnight	6/26	209	N/A	N/A	✓	✓	✓	✓	✓
Same Day	6/27	206	9	0.04	✓	✓	✓	✓	✓
Overnight	7/1	149	N/A	N/A	✓	✓	✓	✓	✓
Same Day	7/2	189	13	0.07	✓	✓	✓	✓	✓
Same Day	7/3	147	N/A	N/A	✓	✓	✓	✓	✓
Overnight	7/8	172	N/A	N/A	✓	✓	✓	✓	✓
Same Day	7/9	183	10	0.05	✓	✓	✓	✓	✓
Overnight	7/10	189	N/A	N/A	✓	✓	✓	✓	✓
Same Day	7/11	209	15	0.07	✓	✓	✓	✓	✓
Overnight	7/15	190	N/A	N/A	✓	✓	✓	✓	✓
Same Day	7/16	230	19	0.08	✓	✓	✓	✓	✓
Overnight	7/17	194	13	0.07	✓	✓	✓	✓	✓
Same Day	7/18	226	11	0.05	✓	✓	✓	✓	✓
Overnight	7/22	196	N/A	N/A	✓	✓	✓	✓	✓
Same Day	7/23	283	34	0.12	✓	✓	✓	✓	✓
Overnight	7/24	174	N/A	N/A	✓	✓	✓	✓	✓
Same Day	7/25	194	10	0.05	✓	✓	✓	✓	✓
Overnight	7/29	183	N/A	N/A	✓	✓	✓	✓	✓
Same Day	7/30	214	17	0.08	✓	✓	✓	✓	✓
Overnight	7/31	206	N/A	N/A	✓	✓	✓	✓	✓
Same Day	8/1	196	21	0.11	✓	✓	✓	✓	✓
Overnight	8/5	199	N/A	N/A	✓	×	✓	✓	✓
Same Day	8/6	219	4	0.02	✓	×	✓	✓	✓

Overnight	8/7	197	N/A	N/A	✓	✓	✓	✓	✓
Same Day	8/8	224	8	0.04	✓	✓	✓	✓	✓
Overnight	8/12	143	N/A	N/A	✓	✓	✓	✓	✓
Same Day	8/13	158	11	0.07	✓	✓	✓	✓	✓
Overnight	8/14	165	N/A	N/A	✓	✓	✓	✓	✓
Same Day	8/15	244	16	0.07	✓	✓	✓	✓	✓
Overnight	8/19	201	0	0.00	✓	✓	✓	✓	✓
Same Day	8/20	206	14	0.07	✓	✓	✓	✓	✓
Overnight	8/21	177	N/A	N/A	✓	✓	✓	✓	✓
Same Day	8/22	202	11	0.05	✓	✓	✓	✓	✓
Overnight	8/26	181	N/A	N/A	✓	✓	✓	✓	✓
NR	8/27	255	48	0.19	✓	✓	×	✓	✓
Overnight	8/28	179	N/A	N/A	✓	✓	✓	✓	✓
Same Day	8/29	242	24	0.10	✓	✓	✓	✓	✓
Overnight	9/2	174	N/A	N/A	✓	✓	✓	✓	✓
Same Day	9/3	200	13	0.07	✓	✓	✓	✓	✓
Overnight	9/4	150	N/A	N/A	✓	✓	✓	✓	✓
Same Day	9/5	161	4	0.02	✓	✓	✓	✓	✓
Results from the holding test									
Trial (not reported because of GGA failure on day 2)									
Day 1	9/9	198	10	0.05	✓	✓	✓	✓	✓
Day 2 with headspace	9/10	143	5	0.03	✓	×	✓	✓	✓
*Day 2 the GGA failed, so this was not included in the final analysis.									
Trial A									
Day 1	9/11	228	18	0.08	✓	✓	✓	✓	✓
Day 2 with headspace	9/12	165	8	0.05	✓	✓	✓	✓	✓
Trial B									
Day 1	9/16	185	4	0.02	✓	✓	✓	✓	✓
Day 2 with headspace	9/17	159	1	0.01	✓	✓	✓	✓	✓

Table A.6: Effluent BOD_5 Results with Quality Control Parameters (see Table A.3)

BOD Results for Effluent										
Holding Time (NR=Not reported due to quality control fail)	Date (2014) Month/Day	Report as greater than (>)	BOD (mg/L)	Standard Deviation (SD)	Normalized SD	Dilution Water	GGA	Sample Replicates	Seed Replicates	Final DO>1 mg/L
NR	6/5/	-	N/A	N/A	N/A	×	×	×	×	×
NR	6/10	-	3.73	N/A	N/A	×	✓	✓	✓	✓
NR	6/11	-	3.41	N/A	N/A	×	✓	✓	✓	✓
NR	6/17	-	6.17	N/A	N/A	×	×	✓	✓	✓
NR	6/18	-	7.18	N/A	N/A	×	×	✓	✓	✓
NR	6/19	>	8.16	N/A	N/A	×	×	✓	✓	×
NR	6/20	>	8.11	N/A	N/A	×	×	✓	✓	×
NR	6/24	>	7.49	N/A	N/A	✓	✓	✓	✓	×
NR	6/25	>	7.66	N/A	N/A	✓	✓	✓	✓	×
NR	6/26	>	8.07	N/A	N/A	✓	✓	✓	✓	×
NR	6/27	>	8.08	N/A	N/A	✓	✓	✓	✓	×
Overnight	7/1	-	13.71	N/A	N/A	✓	✓	✓	✓	✓
Same Day	7/2	-	4.30	N/A	N/A	✓	✓	✓	✓	✓
Same Day	7/3	-	3.60	N/A	N/A	✓	✓	✓	✓	✓
Overnight	7/8	-	3.83	0.93	0.24	✓	✓	×	✓	✓
Same Day	7/9	-	3.28	N/A	N/A	✓	✓	✓	✓	✓
Overnight	7/10	-	3.01	N/A	N/A	✓	✓	✓	✓	✓
Same Day	7/11	-	3.33	N/A	N/A	✓	✓	✓	✓	✓
Overnight	7/15	-	7.56	0.40	0.05	✓	✓	✓	✓	✓
Same Day	7/16	-	8.06	0.37	0.05	✓	✓	✓	✓	✓
Overnight	7/17	-	9.69	0.39	0.04	✓	✓	✓	✓	✓
Same Day	7/18	-	10.41	0.43	0.04	✓	✓	✓	✓	✓
Overnight	7/22	-	9.82	0.51	0.05	✓	✓	✓	✓	✓
Same Day	7/23	-	12.56	N/A	N/A	✓	✓	✓	✓	✓
Overnight	7/24	-	12.32	N/A	N/A	✓	✓	✓	✓	✓
Same Day	7/25	-	12.85	N/A	N/A	✓	✓	✓	✓	✓
Overnight	7/29	-	17.61	N/A	N/A	✓	✓	N/A	✓	✓
Same Day	7/30	-	20.01	N/A	N/A	✓	✓	N/A	✓	✓
Overnight	7/31	-	16.61	N/A	N/A	✓	✓	N/A	✓	✓
Same Day	8/1	-	10.50	0.40	0.04	✓	✓	✓	✓	✓
NR	8/5	>	23	N/A	N/A	✓	×	N/A	✓	×
NR	8/6	-	5.79	0.28	0.05	✓	×	✓	✓	✓
Overnight	8/7	-	3.78	0.41	0.11	✓	✓	✓	✓	✓

Same Day	8/8	-	3.80	0.14	0.04	✓	✓	✓	✓	✓
Overnight	8/12	-	5.77	0.22	0.04	✓	✓	✓	✓	✓
Same Day	8/13	-	6.39	0.08	0.01	✓	✓	✓	✓	✓
Overnight	8/14	-	6.46	0.16	0.02	✓	✓	✓	✓	✓
Same Day	8/15	-	8.00	0.30	0.04	✓	✓	✓	✓	✓
Overnight	8/19	-	10.36	0.49	0.05	✓	✓	✓	✓	✓
Same Day	8/20	-	11.72	1.01	0.09	✓	✓	✓	✓	✓
Overnight	8/21	-	9.05	0.69	0.08	✓	✓	✓	✓	✓
Same Day	8/22	-	9.17	1.22	0.13	✓	✓	✓	✓	✓
Overnight	8/26	-	6.29	0.53	0.08	✓	✓	✓	✓	✓
Same Day	8/27	-	6.88	1.10	0.16	✓	✓	✓	✓	✓
Overnight	8/28	-	6.47	0.98	0.15	✓	✓	✓	✓	✓
Same Day	8/29	-	6.48	0.38	0.06	✓	✓	✓	✓	✓
Overnight	9/2	-	6.06	0.67	0.11	✓	✓	✓	✓	✓
Same Day	9/3	-	8.88	0.22	0.02	✓	✓	✓	✓	✓
Overnight	9/4	-	6.83	0.12	0.02	✓	✓	✓	✓	✓
Same Day	9/5	-	5.37	0.12	0.02	✓	✓	✓	✓	✓
Results from the holding test										
Trial (not reported because of GGA failure day 2)										
Day 1	9/9	-	3.95	0.35	0.09	✓	✓	✓	✓	✓
Day 2 with headspace	9/10	-	3.55	0.06	0.02	✓	×	✓	✓	✓
*Day 2 the GGA failed, so this was not included in the final analysis.										
Trial A										
Day 1	9/11	-	3.95	N/A	N/A	✓	✓	✓	✓	✓
Day 2 with headspace	9/12	-	3.58	N/A	N/A	✓	✓	✓	✓	✓
Trial B										
Day 1	9/16	-	4.92	0.17	0.03	✓	✓	✓	✓	✓
Day 2 with headspace	9/17	-	4.67	0.38	0.08	✓	✓	✓	✓	✓

APPENDIX B

COD DATA COLLECTION AND RESULTS

Table B.1: Benchmark Data Collection Sheet for COD Test

COD test prepared by: _____

Read back completed by: _____

Date sample collected: _____

Date sample tested: _____

Data Set # _____

	Test run A	Test run B	Test run C
High range blank		-	-
Influent			
CABI			
Control (500 mg/L)		-	-
Low range blank		-	-
Effluent			
Control 50 mg/L		-	-

Table B.2: Sample Calculations for COD Test

COD test prepared by:	Evelyn
Read back completed by:	Evelyn
Date sample collected:	8/19/14
Date sample tested:	8/19/14

	Test run A	Test run B	Test run C	Average (mg/L)	Standard Deviation
High range blank	0	-	-	0	N/A
Influent	793	714	671	726	62
CABI	503	431	424	453	44
Control (500 mg/L)	496	-	-	496	Control within reason
Low range blank	0	-	-	0	N/A
Effluent	38	-	-	38	N/A
Control 50 mg/L	48	-	-	48	Control within reason

Table B.3: Influent COD Results

Compiled COD results for Influent					
Analysis Day NR=Not reported (see notes)	Date	COD (mg/L)	Standard Deviation (SD)	Normalized SD	Notes
NR	6/17/14	974	157	0.16	BOD results invalid
NR	6/18/14	720	122	0.17	BOD results invalid
NR	6/19/14	694	92	0.13	BOD results invalid
NR	6/20/14	798	52	0.07	BOD results invalid
Same Day	6/24/14	744	7	0.01	
Same Day	6/25/14	748	27	0.04	
Same Day	6/26/14	829	71	0.09	
Same Day	6/27/14	668	17	0.03	
Same Day	7/1/14	654	39	0.06	
Same Day	7/2/14	674	6	0.01	
Same Day	7/3/14	710	16	0.02	
Same Day	7/8/14	692	22	0.03	
Same Day	7/9/14	727	29	0.04	
Same Day	7/10/14	728	33	0.05	
Same Day	7/11/14	645	17	0.03	
Same Day	7/15/14	646	15	0.02	
Same Day	7/16/14	794	113	0.14	
Same Day	7/17/14	755	46	0.06	
Same Day	7/18/14	790	78	0.10	
Same Day	7/22/14	714	40	0.06	
Same Day	7/23/14	764	16	0.02	
Same Day	7/24/14	737	136	0.18	
Same Day	7/25/14	772	N/A	N/A	
Same Day	7/29/14	637	17	0.03	
Same Day	7/30/14	886	95	0.11	
Same Day	7/31/14	621	14	0.02	
Same Day	8/1/14	725	32	0.04	
Same Day	8/5/14	639	7	0.01	
Same Day	8/6/14	627	12	0.02	
Same Day	8/7/14	727	5	0.01	
Same Day	8/8/14	723	24	0.03	
Same Day	8/12/14	657	13	0.02	
Same Day	8/13/14	752	59	0.08	
Same Day	8/14/14	704	19	0.03	
Same Day	8/15/14	766	41	0.05	

Same Day	8/19/14	726	62	0.09	
Same Day	8/20/14	779	82	0.11	
Same Day	8/21/14	772	37	0.05	
Same Day	8/22/14	706	39	0.06	
Same Day	8/26/14	722	49	0.07	
Same Day	8/27/14	706	N/A	N/A	
Same Day	8/28/14	788	N/A	N/A	
Same Day	8/29/14	747	6	0.01	
Same Day	9/2/14	718	21	0.03	
Same Day	9/3/14	772	7	0.01	
Same Day	9/4/14	706	17	0.02	
Same Day	9/5/14	771	79	0.10	
Same Day	9/9/14	581	19	0.03	
Same Day	9/11/14	676	29	0.04	
Same Day	9/16/14	612	16	0.03	
Holding Test results					
Trial A					
Day 1	9/30/14	691	22	0.03	
Day 2 without headspace	10/1/14	669	10	0.01	
Day 2 with headspace	10/1/14	617	5	0.01	
Trial B					
Day 1	10/1/14	683	14	0.02	
Day 2 without headspace	10/2/14	668	26	0.04	
Day 2 with headspace	10/2/14	663	32	0.05	

Table B.4: CABI COD Results

Compiled COD results for CABI					
Analysis Day NR=Not reported (see notes)	Date	COD (mg/L)	Standard Deviation (SD)	Normalized SD	Notes
NR	6/17/14	410	10	0.02	BOD results invalid
NR	6/18/14	413	22	0.05	BOD results invalid
NR	6/19/14	364	N/A	N/A	BOD results invalid
NR	6/20/14	431	37	0.09	BOD results invalid
Same Day	6/24/14	438	N/A	N/A	
Same Day	6/25/14	411	22	0.05	
Same Day	6/26/14	450	N/A	N/A	
Same Day	6/27/14	410	13	0.03	
Same Day	7/1/14	382	N/A	N/A	
Same Day	7/2/14	404	N/A	N/A	
Same Day	7/3/14	343	N/A	N/A	
Same Day	7/8/14	371	5	0.01	
Same Day	7/9/14	401	N/A	N/A	
Same Day	7/10/14	361	3	0.01	
Same Day	7/11/14	387	N/A	N/A	
Same Day	7/15/14	412	12	0.03	
Same Day	7/16/14	424	N/A	N/A	
Same Day	7/17/14	470	61	0.13	
Same Day	7/18/14	399	N/A	N/A	
Same Day	7/22/14	410	15	0.04	
Same Day	7/23/14	419	N/A	N/A	
Same Day	7/24/14	398	34	0.09	
Same Day	7/25/14	374	N/A	N/A	
Same Day	7/29/14	335	9	0.03	
Same Day	7/30/14	362	N/A	N/A	
Same Day	7/31/14	369	10	0.03	
Same Day	8/1/14	339	N/A	N/A	
Same Day	8/5/14	321	6	0.02	
Same Day	8/6/14	355	N/A	N/A	
Same Day	8/7/14	374	8	0.02	
Same Day	8/8/14	386	N/A	N/A	
Same Day	8/12/14	378	N/A	N/A	
Same Day	8/13/14	398	23	0.06	
Same Day	8/14/14	411	N/A	N/A	

Same Day	8/15/14	461	17	0.04	
Same Day	8/19/14	453	44	0.10	
Same Day	8/20/14	407	N/A	N/A	
Same Day	8/21/14	473	117	0.25	
Same Day	8/22/14	389	N/A	N/A	
Same Day	8/26/14	431	34	0.08	
Same Day	8/28/14	412	25	0.06	
Same Day	8/29/14	405	N/A	N/A	
Same Day	9/2/14	353	6	0.02	
Same Day	9/3/14	370	N/A	N/A	
Same Day	9/4/14	401	30	0.07	
Same Day	9/5/14	377	N/A	N/A	
Same Day	9/9/14	333	10	0.03	
Same Day	9/11/14	462	22	0.05	
Same Day	9/16/14	411	13	0.03	
Holding Test results					
Trial A					
Day 1	9/30/14	396	8	0.02	
Day 2 without headspace	10/1/14	389	5	0.01	
Day 2 with headspace	10/1/14	373	3	0.01	
Trial B					
Day 1	10/1/14	467	1	0.00	
Day 2 without headspace	10/2/14	494	22	0.04	
Day 2 with headspace	10/2/14	473	26	0.05	

Table B.5: Effluent COD Results

Compiled COD results for Effluent					
Analysis Day NR=Not reported (see notes)	Date	COD (mg/L)	Standard Deviation (SD)	Normalized SD	Notes
NR	6/17/14	25	2	0.08	BOD results invalid
NR	6/18/14	30	N/A	N/A	BOD results invalid
NR	6/19/14	31	3	0.10	BOD results invalid
NR	6/20/14	36	N/A	N/A	BOD results invalid
NR	6/24/14	43	4	0.09	BOD results invalid
NR	6/25/14	42	N/A	N/A	BOD results invalid
NR	6/26/14	38	2	0.05	BOD results invalid
NR	6/27/14	31	N/A	N/A	BOD results invalid
Same Day	7/1/14	45	12	0.27	
Same Day	7/2/14	30	N/A	N/A	
Same Day	7/3/14	26	3	0.12	
Same Day	7/8/14	25	N/A	N/A	
Same Day	7/9/14	23	1	0.04	
Same Day	7/10/14	26	N/A	N/A	
Same Day	7/11/14	22	0	0.00	
Same Day	7/15/14	36	N/A	N/A	
Same Day	7/16/14	30	2	0.07	
Same Day	7/17/14	33	N/A	N/A	
Same Day	7/18/14	32	1	0.03	
Same Day	7/22/14	32	N/A	N/A	
Same Day	7/23/14	37	1	0.03	
Same Day	7/24/14	37	N/A	N/A	
Same Day	7/25/14	44	31	0.70	
Same Day	7/29/14	45	N/A	N/A	
Same Day	7/30/14	47	6	0.13	
Same Day	7/31/14	46	N/A	N/A	
Same Day	8/1/14	39	3	0.08	
NR	8/5/14	79	N/A	N/A	BOD results invalid
NR	8/6/14	28	1	0.04	BOD results invalid
Same Day	8/7/14	28	N/A	N/A	
Same Day	8/8/14	27	8	0.30	
Same Day	8/12/14	31	3	0.10	
Same Day	8/13/14	33	N/A	N/A	
Same Day	8/14/14	30	1	0.03	
Same Day	8/15/14	34	N/A	N/A	
Same Day	8/19/14	38	N/A	N/A	

Same Day	8/20/14	41	6	0.15	
NR	8/21/14	N/A	N/A	N/A	COD control fail
NR	8/22/14	N/A	N/A	N/A	COD control fail
NR	8/26/14	N/A	N/A	N/A	COD control fail
NR	8/27/14	N/A	N/A	N/A	COD control fail
Same Day	8/28/14	33	N/A	N/A	
Same Day	8/29/14	29	3	0.10	
Same Day	9/2/14	26	N/A	N/A	
Same Day	9/3/14	27	2	0.07	
Same Day	9/4/14	28	N/A	N/A	
Same Day	9/5/14	23	1	0.04	
Same Day	9/9/14	23	4	0.17	
Same Day	9/11/14	23	2	0.09	
Same Day	9/16/14	26	2	0.08	
Holding Test results					
Trial A					
HT Day 1	9/30/14	32	2	0.06	
HT Day 2	10/1/14	31	1	0.03	
Trial B					
HT Day 1	10/1/14	30	2	0.07	
HT Day 2	10/2/14	32	1	0.03	

APPENDIX C

FLUORESCENCE DATA RESULTS

Table C.1: Influent Fluorescence Results for EEM Regions

Influent Fluorescence for EEM Regions												
Date	**Total**	**SD Total**	**Normalized SD**	**Region I**	**SD Region I**	**Normalized SD**	**Region II**	**SD Region II**	**Normalized SD**	**Region III**	**SD Region III**	**Normalized SD**
6/17/14	154,014	-	-	89,317	-	-	45,866	-	-	18,831	-	-
6/18/14	158,201	-	-	93,455	-	-	46,628	-	-	18,117	-	-
6/19/14	155,886	1,006	0.01	90,011	734	0.01	47,036	348	0.01	18,839	100	0.01
6/20/14	151,805	-	-	88,771	-	-	45,734	-	-	17,300	-	-
6/24/14	160,050	-	-	93,514	-	-	48,543	-	-	17,993	-	-
6/25/14	148,609	2,854	0.02	85,366	1,930	0.02	44,713	579	0.01	18,529	348	0.02
6/26/14	148,383	-	-	84,495	-	-	45,388	-	-	18,500	-	-
6/27/14	154,449	-	-	92,292	-	-	44,824	-	-	17,333	-	-
7/1/14	162,397	6,906	0.04	95,302	5,370	0.06	47,644	1,250	0.03	19,451	405	0.02
7/2/14	138,287	-	-	79,906	-	-	41,359	-	-	17,023	-	-
7/3/14	133,219	-	-	74,931	-	-	41,192	-	-	17,096	-	-
7/8/14	154,768	-	-	87,899	-	-	47,758	-	-	19,111	-	-
7/9/14	132,789	3,609	0.03	74,844	2,712	0.04	40,844	744	0.02	17,101	216	0.01
7/10/14	155,388	-	-	89,045	-	-	47,597	-	-	18,746	-	-

7/11/14	157,035	-	-	90,206	-	-	47,425	-	-	19,403	-	-
7/15/14	132,394	389	0.00	74,909	186	0.00	40,773	395	0.01	16,713	129	0.01
7/16/14	160,251	-	-	94,085	-	-	47,913	-	-	18,253	-	-
7/17/14	143,573	-	-	81,684	-	-	44,250	-	-	17,639	-	-
7/18/14	157,090	7,904	0.05	91,851	6,243	0.07	46,864	1,358	0.03	18,374	334	0.02
7/22/14	168,667	-	-	96,319	-	-	52,136	-	-	20,212	-	-
7/23/14	151,285	-	-	85,666	-	-	46,939	-	-	18,680	-	-
7/24/14	129,777	-	-	72,647	-	-	40,452	-	-	16,678	-	-
7/25/14	138,653	5,023	0.04	78,190	3,739	0.05	42,760	950	0.02	17,702	349	0.02
7/29/14	163,611	-	-	91,922	-	-	50,981	-	-	20,708	-	-
7/30/14	136,668	-	-	76,028	-	-	42,517	-	-	18,122	-	-
7/31/14	142,652	232	0.00	80,021	640	0.01	44,091	280	0.01	18,539	304	0.02
8/1/14	150,029	-	-	84,071	-	-	46,904	-	-	19,055	-	-
8/5/14	147,493	-	-	81,288	-	-	48,572	-	-	17,633	-	-
8/6/14	136,987	2,078	0.02	75,029	1,556	0.02	44,709	510	0.01	17,248	98	0.01
8/7/14	137,214	-	-	77,077	-	-	42,743	-	-	17,394	-	-
8/8/14	152,329	-	-	87,389	-	-	46,771	-	-	18,169	-	-
8/12/14	138,227	-	-	78,315	-	-	42,064	-	-	17,847	-	-
8/13/14	142,658	2,124	0.01	80,538	1,439	0.02	44,120	544	0.01	18,000	146	0.01
8/14/14	141,729	-	-	79,583	-	-	44,122	-	-	18,025	-	-
8/15/14	135,211	-	-	77,032	-	-	41,003	-	-	17,176	-	-

8/19/14	156,279	5,078	0.03	88,182	3,548	0.04	48,221	1,208	0.03	19,876	331	0.02
8/20/14	152,895	-	-	87,240	-	-	46,871	-	-	18,784	-	-
8/21/14	139,229	-	-	77,936	-	-	43,800	-	-	17,492	-	-
8/22/14	163,934	4,492	0.03	94,464	3,347	0.04	49,436	995	0.02	20,034	194	0.01
8/26/14	150,008	-	-	85,531	-	-	46,269	-	-	18,208	-	-
8/27/14	149,008	-	-	85,671	-	-	44,644	-	-	18,692	-	-
8/28/14	141,271	3,590	0.03	81,157	2,363	0.03	43,152	849	0.02	16,962	383	0.02
8/29/14	139,583	-	-	78,818	-	-	43,208	-	-	17,557	-	-
9/2/14	141,100	-	-	79,505	-	-	42,926	-	-	18,669	-	-
9/3/14	142,990	4,674	0.03	82,060	3,417	0.04	42,930	864	0.02	18,000	468	0.03
9/4/14	140,746	-	-	79,563	-	-	43,334	-	-	17,850	-	-
9/5/14	140,398	-	-	78,503	-	-	43,963	-	-	17,931	-	-
9/9/14	162,796	6,427	0.04	91,754	4,390	0.05	51,418	1,322	0.03	19,624	825	0.04
9/11/14	168,182	-	-	96,787	-	-	51,881	-	-	19,514	-	-
9/16/14	152,522	-	-	84,233	-	-	47,990	-	-	20,300	-	-

Table C.2: Influent Fluorescence Results for EEM Peaks

Influent Fluorescence for EEM Peaks															
Date	**Proteins (280/331)**	**SD Proteins**	**Normalized SD**	**Humic (342/436)**	**SD Humic**	**Normalized SD**	**A (260/450)**	**SD A**	**Normalized SD**	**C (330/450)**	**SD C**	**Normalized SD**	**T (275/340)**	**SD T**	**Normalized SD**
6/17/14	8.026	-	-	2.792	-	-	3.143	-	-	2.858	-	-	8.534	-	-
6/18/14	8.697	-	-	2.748	-	-	3.048	-	-	2.714	-	-	9.129	-	-
6/19/14	8.266	0.065	0.01	2.849	0.011	0.00	3.173	0.019	0.01	2.850	0.037	0.01	8.795	0.041	0.00
6/20/14	8.252	-	-	2.722	-	-	2.982	-	-	2.591	-	-	8.669	-	-
6/24/14	8.604	-	-	2.785	-	-	3.190	-	-	2.692	-	-	9.003	-	-
6/25/14	7.895	0.227	0.03	2.787	0.021	0.01	2.952	0.051	0.02	2.894	0.041	0.01	8.280	0.211	0.03
6/26/14	7.892	-	-	2.857	-	-	3.173	-	-	2.744	-	-	8.377	-	-
6/27/14	8.638	-	-	2.667	-	-	2.913	-	-	2.530	-	-	9.120	-	-
7/1/14	8.635	0.680	0.08	2.984	0.058	0.02	3.154	0.053	0.02	2.917	0.076	0.03	9.197	0.600	0.07
7/2/14	7.444	-	-	2.648	-	-	2.698	-	-	2.578	-	-	7.784	-	-
7/3/14	6.721	-	-	2.672	-	-	2.852	-	-	2.658	-	-	7.390	-	-
7/8/14	7.786	-	-	3.002	-	-	3.128	-	-	2.927	-	-	8.329	-	-
7/9/14	6.959	0.318	0.05	2.702	0.034	0.01	2.744	0.051	0.02	2.687	0.042	0.02	7.339	0.293	0.04

7/10/14	7.867	-	-	3.008	-	-	3.270	-	-	2.752	-	-	8.542	-	-
7/11/14	8.195	-	-	3.088	-	-	3.059	-	-	3.009	-	-	8.550	-	-
7/15/14	6.660	0.036	0.01	2.684	0.001	0.00	2.713	0.039	0.01	2.589	0.019	0.01	7.046	0.044	0.01
7/16/14	8.764	-	-	2.890	-	-	3.160	-	-	2.730	-	-	9.177	-	-
7/17/14	7.537	-	-	2.809	-	-	3.082	-	-	2.598	-	-	8.064	-	-
7/18/14	8.466	0.798	0.09	2.898	0.081	0.03	2.995	0.095	0.03	2.777	0.055	0.02	8.907	0.703	0.08
7/22/14	8.524	-	-	3.181	-	-	3.409	-	-	3.182	-	-	9.137	-	-
7/23/14	7.603	-	-	2.939	-	-	3.071	-	-	2.877	-	-	8.221	-	-
7/24/14	6.339	-	-	2.645	-	-	2.665	-	-	2.625	-	-	6.857	-	-
7/25/14	7.028	0.468	0.07	2.835	0.055	0.02	2.763	0.057	0.02	2.690	0.058	0.02	7.496	0.405	0.05
7/29/14	7.991	-	-	3.323	-	-	3.366	-	-	3.260	-	-	8.749	-	-
7/30/14	6.723	-	-	2.974	-	-	2.787	-	-	2.810	-	-	7.217	-	-
7/31/14	7.161	0.109	0.02	3.082	0.025	0.01	2.963	0.030	0.01	2.914	0.063	0.02	7.755	0.055	0.01
8/1/14	7.503	-	-	3.143	-	-	3.114	-	-	2.906	-	-	8.072	-	-
8/5/14	6.753	-	-	2.802	-	-	3.301	-	-	2.633	-	-	7.264	-	-
8/6/14	6.400	0.202	0.03	2.853	0.029	0.01	3.064	0.049	0.02	2.666	0.019	0.01	6.816	0.161	0.02
8/7/14	6.706	-	-	2.935	-	-	2.916	-	-	2.677	-	-	7.259	-	-
8/8/14	7.860	-	-	2.917	-	-	3.056	-	-	2.737	-	-	8.353	-	-
8/12/14	7.255	-	-	2.993	-	-	2.952	-	-	2.705	-	-	7.816	-	-

8/13/14	7.208	0.165	0.02	3.017	0.040	0.01	2.963	0.064	0.02	2.789	0.031	0.01	7.769	0.163	0.02
8/14/14	6.898	-	-	3.057	-	-	2.920	-	-	2.790	-	-	7.573	-	-
8/15/14	6.800	-	-	2.863	-	-	2.689	-	-	2.643	-	-	7.474	-	-
8/19/14	8.072	0.478	0.06	3.209	0.052	0.02	3.247	0.086	0.03	3.130	0.063	0.02	8.543	0.448	0.05
8/20/14	7.995	-	-	3.124	-	-	3.171	-	-	2.890	-	-	8.506	-	-
8/21/14	6.955	-	-	2.884	-	-	3.019	-	-	2.657	-	-	7.404	-	-
8/22/14	8.646	0.387	0.04	3.282	0.038	0.01	3.340	0.067	0.02	3.101	0.029	0.01	9.250	0.375	0.04
8/26/14	7.756	-	-	2.987	-	-	3.147	-	-	2.725	-	-	8.252	-	-
8/27/14	7.948	-	-	3.155	-	-	3.059	-	-	2.865	-	-	8.558	-	-
8/28/14	7.663	0.319	0.04	2.722	0.094	0.03	2.899	0.053	0.02	2.497	0.078	0.03	7.977	0.253	0.03
8/29/14	7.213	-	-	2.845	-	-	2.902	-	-	2.652	-	-	7.623	-	-
9/2/14	7.304	-	-	2.935	-	-	2.884	-	-	2.993	-	-	7.765	-	-
9/3/14	7.606	0.440	0.06	2.979	0.095	0.03	2.872	0.083	0.03	2.716	0.092	0.03	8.093	0.387	0.05
9/4/14	7.002	-	-	2.883	-	-	2.813	-	-	2.763	-	-	7.570	-	-
9/5/14	7.029	-	-	2.905	-	-	2.893	-	-	2.757	-	-	7.703	-	-
9/9/14	8.148	0.599	0.07	3.162	0.186	0.06	3.522	0.069	0.02	2.969	0.149	0.05	8.917	0.513	0.06
9/11/14	8.577	-	-	3.219	-	-	3.366	-	-	3.025	-	-	9.230	-	-
9/16/14	7.470	-	-	3.114	-	-	3.139	-	-	3.145	-	-	7.899	-	-

Table C.3: CABI Fluorescence Results for EEM Regions

CABI Fluorescence for EEM Region												
Date	**Total**	**SD Total**	**Normalized SD**	**Region I**	**SD Region I**	**Normalized SD**	**Region II**	**SD Region II**	**Normalized SD**	**Region III**	**SD Region III**	**Normalized SD**
6/17/14	137,527	-	-	76,779	-	-	45,027	-	-	15,721	-	-
6/18/14	130,453	891	0.01	71,914	761	0.01	43,431	162	0.00	15,108	106	0.01
6/19/14	134,736	-	-	75,298	-	-	44,601	-	-	14,837	-	-
6/20/14	139,907	-	-	77,718	-	-	46,708	-	-	15,481	-	-
6/24/14	130,773	1,946	0.01	72,811	1,535	0.02	43,110	393	0.01	14,852	54	0.00
6/25/14	124,425	-	-	67,812	-	-	42,086	-	-	14,527	-	-
6/26/14	125,034	-	-	68,784	-	-	41,475	-	-	14,775	-	-
6/27/14	124,477	4,784	0.04	69,242	4,210	0.06	40,721	459	0.01	14,514	170	0.01
7/1/14	136,143	-	-	76,105	-	-	44,882	-	-	15,156	-	-
7/2/14	126,038	-	-	68,737	-	-	42,485	-	-	14,815	-	-
7/3/14	118,053	-	-	64,052	-	-	39,680	-	-	14,321	-	-
7/8/14	143,299	4,580	0.03	79,854	3,436	0.04	47,472	898	0.02	15,973	323	0.02
7/9/14	121,258	-	-	66,498	-	-	40,920	-	-	13,840	-	-
7/10/14	142,101	978	0.01	78,592	806	0.01	47,138	196	0.00	16,371	143	0.01

7/11/14	141,137	-	-	79,766	-	-	45,777	-	-	15,594	-	-
7/15/14	121,297	-	-	68,179	-	-	39,384	-	-	13,733	-	-
7/16/14	137,438	2,317	0.02	77,475	2,010	0.03	44,718	279	0.01	15,246	172	0.01
7/17/14	127,718	-	-	71,154	-	-	41,918	-	-	14,646	-	-
7/18/14	124,474	-	-	68,881	-	-	41,409	-	-	14,183	-	-
7/22/14	144,556	4,026	0.03	81,563	3,143	0.04	47,162	809	0.02	15,830	195	0.01
7/23/14	135,883	-	-	74,627	-	-	45,472	-	-	15,785	-	-
7/24/14	112,766	1,682	0.01	61,091	1,419	0.02	38,059	261	0.01	13,616	129	0.01
7/25/14	121,237	-	-	66,250	-	-	40,464	-	-	14,523	-	-
7/29/14	141,369	-	-	78,178	-	-	47,142	-	-	16,050	-	-
7/30/14	117,008	2,602	0.02	63,194	1,969	0.03	39,526	494	0.01	14,288	168	0.01
7/31/14	127,629	-	-	70,389	-	-	42,451	-	-	14,789	-	-
8/1/14	127,832	-	-	69,863	-	-	42,937	-	-	15,032	-	-
8/5/14	133,961	3,242	0.02	72,532	3,192	0.04	46,052	223	0.00	15,377	192	0.01
8/6/14	127,896	-	-	68,386	-	-	45,315	-	-	14,195	-	-
8/7/14	137,372	-	-	76,540	-	-	45,873	-	-	14,960	-	-
8/8/14	135,511	1,313	0.01	74,966	1,017	0.01	45,324	236	0.01	15,221	96	0.01
8/12/14	136,109	-	-	74,857	-	-	45,719	-	-	15,533	-	-
8/13/14	137,582	-	-	75,614	-	-	46,029	-	-	15,938	-	-
8/14/14	136,119	1,319	0.01	76,824	1,355	0.02	44,492	211	0.00	14,804	233	0.02
8/15/14	124,762	-	-	68,498	-	-	41,317	-	-	14,946		0.00

8/19/14	143,132	-	-	82,066	-	-	46,062	-	-	15,005	-	-
8/20/14	135,746	1,533	0.01	74,691	1,343	0.02	45,565	194	0.00	15,490	150	0.01
8/21/14	122,801	-	-	68,202	-	-	40,661	-	-	13,938	-	-
8/22/14	138,748	-	-	78,313	-	-	45,355	-	-	15,080	-	-
8/26/14	132,301	1,454	0.01	73,905	1,266	0.02	43,566	249	0.01	14,830	116	0.01
8/27/14	137,808	-	-	77,625	-	-	44,986	-	-	15,196	-	-
8/28/14	127,180	-	-	70,789	-	-	42,051	-	-	14,340	-	-
8/29/14	123,701	1,401	0.01	69,092	814	0.01	40,595	417	0.01	14,013	241	0.02
9/2/14	132,810	-	-	74,633	-	-	43,552	-	-	14,625	-	-
9/3/14	134,248	-	-	75,624	-	-	43,967	-	-	14,658	-	-
9/4/14	126,105	-	-	69,819	-	-	42,055	-	-	14,231	-	-
9/5/14	125,865	542	0.00	68,985	350	0.01	42,446	170	0.00	14,435	96	0.01
9/9/14	151,819	-	-	82,375	-	-	51,727	-	-	17,717	-	-
9/11/14	154,048	-	-	90,098	-	-	48,311	-	-	15,639	-	-
9/16/14	139,556	-	-	78,700	-	-	45,246	-	-	15,610	-	-

Table C.4: CABI Fluorescence Results for EEM Peaks

CABI Fluorescence for EEM Peaks															
Date	**Proteins (280/331)**	**SD Proteins**	**Normalized SD**	**Humic (342/436)**	**SD Humic**	**Normalized SD**	**A (260/450)**	**SD A**	**Normalized SD**	**C (330/450)**	**SD C**	**Normalized SD**	**T (275/340)**	**SD T**	**Normalized SD**
6/17/14	6.560	-	-	2.252	-	-	3.195	-	-	2.197	-	-	7.000	-	-
6/18/14	6.151	0.086	0.01	2.192	0.028	0.01	3.081	0.026	0.01	2.141	0.029	0.01	6.638	0.080	0.01
6/19/14	6.415	-	-	2.085	-	-	3.087	-	-	2.084	-	-	6.901	-	-
6/20/14	6.717	-	-	2.236	-	-	3.257	-	-	2.228	-	-	7.235	-	-
6/24/14	6.324	0.138	0.02	2.140	0.013	0.01	2.999	0.024	0.01	2.145	0.034	0.02	6.741	0.150	0.02
6/25/14	5.764	-	-	2.043	-	-	3.020	-	-	2.014	-	-	6.319	-	-
6/26/14	5.866	-	-	2.116	-	-	2.972	-	-	2.051	-	-	6.383	-	-
6/27/14	5.795	0.334	0.06	2.020	0.043	0.02	2.852	0.042	0.01	1.981	0.029	0.01	6.361	0.364	0.06
7/1/14	6.529	-	-	2.191	-	-	3.083	-	-	2.141	-	-	7.072	-	-
7/2/14	5.808	-	-	2.116	-	-	2.889	-	-	2.089	-	-	6.309	-	-
7/3/14	5.391	-	-	2.141	-	-	2.761	-	-	2.070	-	-	5.951	-	-
7/8/14	6.674	0.371	0.06	2.296	0.051	0.02	3.162	0.058	0.02	2.271	0.039	0.02	7.220	0.341	0.05
7/9/14	5.697	-	-	1.964	-	-	2.714	-	-	1.961	-	-	6.059	-	-

7/10/14	6.562	0.092	0.01	2.300	0.052	0.02	3.213	0.010	0.00	2.237	0.034	0.02	7.115	0.077	0.01
7/11/14	6.792	-	-	2.189	-	-	3.045	-	-	2.188	-	-	7.266	-	-
7/15/14	5.829	-	-	1.931	-	-	2.641	-	-	1.895	-	-	6.290	-	-
7/16/14	6.770	0.281	0.04	2.168	0.049	0.02	2.984	0.011	0.00	2.140	0.035	0.02	7.195	0.257	0.04
7/17/14	6.197	-	-	2.154	-	-	2.842	-	-	2.062	-	-	6.591	-	-
7/18/14	5.852	-	-	2.003	-	-	2.775	-	-	1.980	-	-	6.324	-	-
7/22/14	7.076	0.437	0.06	2.263	0.046	0.02	3.185	0.057	0.02	2.217	0.034	0.02	7.683	4.451	0.58
7/23/14	6.317	-	-	2.383	-	-	3.108	-	-	2.261	-	-	6.853	-	-
7/24/14	5.224	0.206	0.04	2.006	0.043	0.02	2.657	0.017	0.01	1.921	0.035	0.02	5.683	0.157	0.03
7/25/14	5.586	-	-	2.132	-	-	2.732	-	-	2.082	-	-	6.099	-	-
7/29/14	6.622	-	-	2.336	-	-	3.230	-	-	2.277	-	-	7.217	-	-
7/30/14	5.411	0.249	0.05	2.056	0.047	0.02	2.716	0.036	0.01	1.991	0.039	0.02	5.875	0.215	0.04
7/31/14	6.083	-	-	2.150	-	-	2.940	-	-	2.070	-	-	6.641	-	-
8/1/14	5.994	-	-	2.198	-	-	3.020	-	-	2.130	-	-	6.576	-	-
8/5/14	5.989	0.409	0.07	2.204	0.076	0.03	3.222	0.004	0.00	2.159	0.054	0.03	6.265	0.501	0.08
8/6/14	5.230	-	-	2.019	-	-	2.997	-	-	2.052	-	-	5.623	-	-
8/7/14	6.128	-	-	2.133	-	-	2.963	-	-	2.138	-	-	6.650	-	-
8/8/14	6.135	0.119	0.02	2.196	0.023	0.01	3.006	0.008	0.00	2.191	0.018	0.01	6.714	0.115	0.02
8/12/14	6.227	-	-	2.233	-	-	3.049	-	-	2.223	-	-	6.929	-	-
8/13/14	6.342	-	-	2.405	-	-	3.103	-	-	2.355	-	-	6.918	-	-

8/14/14	6.641	0.182	0.03	2.098	0.071	0.03	3.047	0.035	0.01	2.070	0.054	0.03	7.137	0.161	0.02
8/15/14	5.998	-	-	2.218	-	-	2.992	-	-	2.113	-	-	6.555	-	-
8/19/14	7.028	-	-	2.109	-	-	2.998	-	-	2.127	-	-	7.544	-	-
8/20/14	6.287	0.150	0.02	2.271	0.036	0.02	3.100	0.017	0.01	2.248	0.026	0.01	6.756	0.174	0.03
8/21/14	5.781	-	-	1.997	-	-	2.721	-	-	2.012	-	-	6.159	-	-
8/22/14	6.666	-	-	2.148	-	-	3.053	-	-	2.148	-	-	7.224	-	-
8/26/14	6.292	0.175	0.03	2.146	0.034	0.02	2.940	0.017	0.01	2.133	0.027	0.01	6.817	0.200	0.03
8/27/14	6.669	-	-	2.191	-	-	3.053	-	-	2.172	-	-	7.246	-	-
8/28/14	6.027	-	-	2.057	-	-	2.883	-	-	2.049	-	-	6.408	-	-
8/29/14	5.881	0.092	0.02	1.976	0.067	0.03	2.741	0.041	0.01	1.973	0.051	0.03	6.258	0.094	0.01
9/2/14	6.172	-	-	2.098	-	-	2.769	-	-	2.126	-	-	6.637	-	-
9/3/14	6.252	-	-	2.044	-	-	2.857	-	-	2.076	-	-	6.757	-	-
9/4/14	5.671	-	-	1.962	-	-	2.710	-	-	2.010	-	-	6.156	-	-
9/5/14	5.747	0.061	0.01	2.040	0.032	0.02	2.789	0.047	0.02	2.063	0.025	0.01	6.275	0.071	0.01
9/9/14	6.858	-	-	2.539	-	-	3.586	-	-	2.517	-	-	7.497	-	-
9/11/14	7.901	-	-	2.149	-	-	3.108	-	-	2.225	-	-	8.388	-	-
9/16/14	6.562	-	-	2.147	-	-	2.883	-	-	2.180	-	-	7.022	-	-

Table C.5: Effluent Fluorescence Results for EEM Regions

Effluent Fluorescence for EEM Regions												
Date	**Total**	**SD Total**	**Normalized SD**	**Region I**	**SD Region I**	**Normalized SD**	**Region II**	**SD Region II**	**Normalized SD**	**Region III**	**SD Region III**	**Normalized SD**
6/17/14	55,353	3,622	0.07	24,500	3,293	0.13	21,457	226	0.01	9,397	204	0.02
6/18/14	42,369	-	-	13,970	-	-	19,963	-	-	8,436	-	-
6/19/14	44,655	-	-	15,275	-	-	20,594	-	-	8,786	-	-
6/20/14	41,647	1,049	0.03	13,556	623	0.05	19,638	316	0.02	8,453	116	0.01
6/24/14	44,670	-	-	14,481	-	-	20,794	-	-	9,395	-	-
6/25/14	44,012	-	-	15,274	-	-	20,370	-	-	8,368	-	-
6/26/14	44,893	312	0.01	15,336	257	0.02	20,879	245	0.01	8,677	76	0.01
6/27/14	43,309	-	-	15,042	-	-	19,856	-	-	8,410	-	-
7/1/14	47,276	-	-	16,243	-	-	22,175	-	-	8,858	-	-
7/2/14	41,689	-	-	13,938	-	-	19,530	-	-	8,222	-	-
7/3/14	41,647	1,049	0.03	13,556	623	0.05	19,638	316	0.02	8,453	116	0.01
7/8/14	43,067	-	-	14,312	-	-	20,373	-	-	8,382	-	-
7/9/14	34,236	-	-	9,235	-	-	17,455	-	-	7,545	-	-

7/10/14	43,170	-	-	14,377	-	-	20,215	-	-	8,578	-	-
7/11/14	40,734	157	0.00	13,233	55	0.00	19,539	103	0.01	7,962	30	0.00
7/15/14	35,750	-	-	11,842	-	-	16,830	-	-	7,077	-	-
7/16/14	42,951	-	-	14,290	-	-	20,338	-	-	8,322	-	-
7/17/14	38,713	174	0.00	12,690	77	0.01	18,345	72	0.00	7,678	26	0.00
7/18/14	39,929	-	-	13,143	-	-	18,878	-	-	7,909	-	-
7/22/14	43,802	-	-	14,575	-	-	20,808	-	-	8,419	-	-
7/23/14	45,137	83	0.00	14,956	75	0.01	21,560	166	0.01	8,620	37	0.00
7/24/14	39,126	-	-	12,833	-	-	18,613	-	-	7,680	-	-
7/25/14	43,375	-	-	14,499	-	-	20,489	-	-	8,387	-	-
7/29/14	49,998	790	0.02	16,960	174	0.01	23,937	434	0.02	9,102	193	0.02
7/30/14	43,239	-	-	14,632	-	-	20,423	-	-	8,184	-	-
7/31/14	48,163	-	-	16,784	-	-	22,680	-	-	8,699	-	-
8/1/14	46,361	1,114	0.02	16,918	899	0.05	20,851	207	0.01	8,592	18	0.00
8/5/14	51,888	-	-	15,448	-	-	25,597	-	-	10,843	-	-
8/6/14	35,767	-	-	7,672	-	-	20,106	-	-	7,990	-	-
8/7/14	39,750	415	0.01	13,021	161	0.01	18,955	222	0.01	7,775	62	0.01
8/8/14	39,853	-	-	13,008	-	-	19,014	-	-	7,831	-	-
8/12/14	40,383	125	0.00	13,495	67	0.01	19,045	77	0.00	7,843	22	0.00
8/13/14	42,279	-	-	14,018	-	-	20,005	-	-	8,256	-	-
8/14/14	41,875	-	-	13,717	-	-	19,982	-	-	8,176	-	-

8/15/14	40,244	326	0.01	13,399	335	0.03	18,984	20	0.00	7,860	11	0.00
8/19/14	46,869	-	-	16,727	-	-	21,288	-	-	8,854	-	-
8/20/14	45,235	-	-	14,223	-	-	22,225	-	-	8,787	-	-
8/21/14	45,665	978	0.02	17,002	885	0.05	20,203	97	0.00	8,460	15	0.00
8/22/14	50,373	-	-	19,097	-	-	22,338	-	-	8,939	-	-
8/26/14	45,327	-	-	16,528	-	-	20,464	-	-	8,335	-	-
8/27/14	48,215	569	0.01	18,220	445	0.02	21,426	78	0.00	8,569	49	0.01
8/28/14	44,676	-	-	16,774	-	-	19,669	-	-	8,234	-	-
8/29/14	43,303	-	-	15,705	-	-	19,448	-	-	8,151	-	-
9/2/14	41,902	747	0.02	15,244	510	0.03	18,722	174	0.01	7,936	75	0.01
9/3/14	40,151	-	-	13,535	-	-	18,719	-	-	7,897	-	-
9/4/14	40,664	387	0.01	13,821	377	0.03	18,861	61	0.00	7,982	59	0.01
9/5/14	39,263	-	-	12,611	-	-	18,754	-	-	7,899	-	-
9/9/14	47,410	-	-	15,830	-	-	22,470	-	-	9,110	-	-
9/11/14	45,064	-	-	15,009	-	-	21,431	-	-	8,625	-	-
9/16/14	38,242	-	-	13,087	-	-	17,556	-	-	7,599	-	-

Table C.6: Effluent Fluorescence Results for EEM Peaks

Effluent Fluorescence for EEM Peaks															
Date	**Proteins (280/331)**	**SD Proteins**	**Normalized SD**	**Humic (342/436)**	**SD Humic**	**Normalized SD**	**A (260/450)**	**SD A**	**Normalized SD**	**C (330/450)**	**SD C**	**Normalized SD**	**T (275/340)**	**SD T**	**Normalized SD**
6/17/14	1.672	0.254	0.15	1.326	0.012	0.01	1.681	0.025	0.01	1.133	0.013	0.01	1.789	0.283	0.16
6/18/14	0.889	-	-	1.297	-	-	1.603	-	-	1.104	-	-	0.925	-	-
6/19/14	1.001	-	-	1.359	-	-	1.627	-	-	1.174	-	-	1.064	-	-
6/20/14	0.850	0.055	0.06	1.306	0.027	0.02	1.564	0.035	0.02	1.111	0.021	0.02	0.885	0.052	0.06
6/24/14	0.981	-	-	1.541	-	-	1.565	-	-	1.707	-	-	0.978	-	-
6/25/14	1.007	-	-	1.298	-	-	1.633	-	-	1.115	-	-	1.036	-	-
6/26/14	1.008	0.019	0.02	1.330	0.011	0.01	1.653	0.034	0.02	1.136	0.008	0.01	1.039	0.025	0.02
6/27/14	0.970	-	-	1.298	-	-	1.551	-	-	1.107	-	-	1.011	-	-
7/1/14	1.069	-	-	1.398	-	-	1.744	-	-	1.205	-	-	1.098	-	-
7/2/14	0.854	-	-	1.275	-	-	1.565	-	-	1.080	-	-	0.897	-	-
7/3/14	0.850	0.055	0.06	1.306	0.027	0.02	1.564	0.035	0.02	1.111	0.021	0.02	0.885	0.052	0.06
7/8/14	0.875	-	-	1.308	-	-	1.621	-	-	1.118	-	-	0.935	-	-
7/9/14	0.568	-	-	1.200	-	-	1.427	-	-	1.014	-	-	0.535	-	-

7/10/14	0.869	-	-	1.306	-	-	1.624	-	-	1.117	-	-	0.919	-	-
7/11/14	0.785	0.004	0.01	1.227	0.006	0.00	1.565	0.023	0.01	1.054	0.010	0.01	0.829	0.003	0.00
7/15/14	0.722	-	-	1.078	-	-	1.333	-	-	0.920	-	-	0.773	-	-
7/16/14	0.863	-	-	1.294	-	-	1.632	-	-	1.103	-	-	0.922	-	-
7/17/14	0.772	0.004	0.00	1.178	0.008	0.01	1.457	0.005	0.00	1.007	0.002	0.00	0.807	0.004	0.01
7/18/14	0.807	-	-	1.222	-	-	1.474	-	-	1.036	-	-	0.850	-	-
7/22/14	0.909	-	-	1.325	-	-	1.630	-	-	1.143	-	-	0.964	-	-
7/23/14	0.926	0.006	0.01	1.326	0.002	0.00	1.695	0.016	0.01	1.142	0.006	0.01	0.958	0.005	0.01
7/24/14	0.814	-	-	1.179	-	-	1.414	-	-	1.009	-	-	0.819	-	-
7/25/14	0.899	-	-	1.275	-	-	1.563	-	-	1.099	-	-	0.920	-	-
7/29/14	1.040	0.007	0.01	1.405	0.029	0.02	1.821	0.044	0.02	1.235	0.024	0.02	1.070	0.011	0.01
7/30/14	0.885	-	-	1.250	-	-	1.552	-	-	1.090	-	-	0.919	-	-
7/31/14	1.086	-	-	1.333	-	-	1.715	-	-	1.170	-	-	1.105	-	-
8/1/14	1.112	0.130	0.12	1.289	0.008	0.01	1.655	0.014	0.01	1.104	0.012	0.01	1.202	0.157	0.13
8/5/14	0.760	-	-	1.895	-	-	2.053	-	-	1.583	-	-	0.588	-	-
8/6/14	0.040	-	-	1.275	-	-	1.656	-	-	1.103	-	-	0.000	-	-
8/7/14	0.787	0.014	0.02	1.204	0.005	0.00	1.500	0.027	0.02	1.032	0.007	0.01	0.830	0.015	0.02
8/8/14	0.800	-	-	1.205	-	-	1.526	-	-	1.027	-	-	0.836	-	-
8/12/14	0.859	0.012	0.01	1.210	0.009	0.01	1.527	0.017	0.01	1.036	0.010	0.01	0.909	0.010	0.01
8/13/14	0.894	-	-	1.258	-	-	1.587	-	-	1.081	-	-	0.950	-	-
8/14/14	0.845	-	-	1.252	-	-	1.571	-	-	1.074	-	-	0.890	-	-

8/15/14	0.827	0.051	0.06	1.203	0.002	0.00	1.486	0.013	0.01	1.029	0.003	0.00	0.852	0.063	0.07
8/19/14	1.216	-	-	1.377	-	-	1.640	-	-	1.173	-	-	1.189	-	-
8/20/14	0.900	-	-	1.402	-	-	1.745	-	-	1.202	-	-	0.750	-	-
8/21/14	1.296	0.108	0.08	1.329	0.007	0.01	1.563	0.009	0.01	1.131	0.007	0.01	1.301	0.178	0.14
8/22/14	1.429	-	-	1.392	-	-	1.714	-	-	1.200	-	-	1.418	-	-
8/26/14	1.259	-	-	1.304	-	-	1.566	-	-	1.100	-	-	1.206	-	-
8/27/14	1.425	0.073	0.05	1.329	0.011	0.01	1.672	0.003	0.00	1.139	0.007	0.01	1.378	0.067	0.05
8/28/14	1.368	-	-	1.282	-	-	1.523	-	-	1.073	-	-	1.261	-	-
8/29/14	1.237	-	-	1.252	-	-	1.539	-	-	1.064	-	-	1.124	-	-
9/2/14	1.195	0.071	0.06	1.240	0.015	0.01	1.472	0.021	0.01	1.039	0.010	0.01	1.137	0.070	0.06
9/3/14	0.864	-	-	1.204	-	-	1.477	-	-	1.022	-	-	0.918	-	-
9/4/14	0.872	0.023	0.03	1.216	0.014	0.01	1.481	0.010	0.01	1.030	0.012	0.01	0.932	0.033	0.04
9/5/14	0.815	-	-	1.210	-	-	1.489	-	-	1.032	-	-	0.868	-	-
9/9/14	0.965	-	-	1.365	-	-	1.805	-	-	1.181	-	-	1.027	-	-
9/11/14	0.952	-	-	1.341	-	-	1.721	-	-	1.153	-	-	1.020	-	-
9/16/14	0.867	-	-	1.196	-	-	1.389	-	-	1.008	-	-	0.903	-	-

APPENDIX D

TOC RESULTS

Table D.1: TOC Results for Influent, CABI, and Effluent

TOC Data			
Date	Influent	CABI	Effluent
6/17/14	68.7	59.4	8.2
6/18/14	61	74	8.8
6/19/14	64.5	56.5	9.1
6/20/14	76.8	62	10
6/24/14	77.6	58.8	11.2
6/25/14	72.8	57.1	11.2
6/26/14	67.5	54.5	10.8
6/27/14	64.6	57.5	9.5
7/1/14	63.9	62.2	10.4
7/2/14	62	62.6	7.7
7/3/14	88.7	62.8	7
7/8/14	90.5	64.6	6.9
7/9/14	90.2	65.6	6.7
7/10/14	65.7	59.6	6.8
7/11/14	78.3	74.2	6.7
7/15/14	69.2	60.9	8.9
7/16/14	73.1	62.6	9.2
7/17/14	64	63.2	10
7/18/14	75.3	59.7	10
7/22/14	86.3	70.5	9.3
7/23/14	78.2	66.3	10.1
7/24/14	72.4	61	10.6
7/25/14	74.9	57.5	10.3
7/29/14	67.5	56.5	10.6
7/30/14	73.4	59.7	11.4
7/31/14	65	58.2	11.9
8/1/14	66.9	53.5	9.7
8/5/14	57.1	53.9	7.6
8/6/14	60.6	62	7.4
8/7/14	62.6	68.5	6.8
8/8/14	59.6	65.4	6.8
8/12/14	56	59	8
8/13/14	60	57.4	8.6
8/14/14	61	58.1	8.7
8/15/14	58	63.8	9.2
8/19/14	58.6	62.4	10.4
8/20/14	54.2	56.5	10.2
8/21/14	65.3	58.3	9.6

8/22/14	75.8	58.7	9.4
8/26/14	69.4	64.9	8.3
8/27/14	62.8	63.7	8.7
8/28/14	59.8	59	8.8
8/29/14	64.6	58.6	8.9
9/2/14	76.9	66	8.4
9/3/14	66.3	67.1	9.2
9/4/14	68.5	62.1	8.6
9/5/14	81.9	54.6	7.7
9/9/14	59.2	59.9	6.8
9/11/14	64.6	64.1	6.8
9/16/14	67.7	69.9	7.3

APPENDIX E

ABSORBANCE RESULTS

Table E.1: Influent Absorbance at 254 nm and 340 nm Results

Absorbance Results for Influent		
Date	254 nm	340 nm
6/25/14	0.299	0.073
6/27/14	0.326	0.083
7/2/14	0.291	0.067
7/3/14	0.286	0.067
7/9/14	0.303	0.073
7/11/14	0.295	0.070
7/16/14	0.322	0.080
7/18/14	0.340	0.084
7/23/14	0.293	0.067
7/25/14	0.298	0.071
7/30/14	0.308	0.077
8/1/14	0.291	0.068
8/6/14	0.290	0.070
8/8/14	0.300	0.070
8/13/14	0.292	0.145
8/15/14	0.282	0.066
8/20/14	0.302	0.075
8/22/14	0.317	0.078
8/27/14	0.289	0.071
8/29/14	0.302	0.072
9/3/14	0.297	0.074
9/5/14	0.303	0.072
9/9/14	0.314	0.077
9/11/14	0.327	0.081
9/16/14	0.345	0.090

Table E.2: CABI Absorbance at 254 nm and 340 nm Results

Absorbance Results for CABI		
Date	254 nm	340 nm
6/25/14	0.259	0.061
6/27/14	0.266	0.061
7/2/14	0.279	0.066
7/3/14	0.267	0.062
7/9/14	0.279	0.063
7/11/14	0.294	0.067
7/16/14	0.296	0.071
7/18/14	0.272	0.062
7/23/14	0.282	0.065
7/25/14	0.273	0.064
7/30/14	0.253	0.056
8/1/14	0.251	0.057
8/6/14	0.259	0.054
8/8/14	0.278	0.061
8/13/14	0.301	0.072
8/15/14	0.276	0.065
8/20/14	0.281	0.066
8/22/14	0.263	0.060
8/29/14	0.273	0.063
9/3/14	0.265	0.056
9/5/14	0.275	0.062
9/9/14	0.292	0.065
9/11/14	0.318	0.078
9/16/14	0.305	0.068

Table E.3: Effluent Absorbance at 254 nm and 340 nm Results

Absorbance Results for Effluent		
Date	254 nm	340 nm
7/1/14	0.105	0.029
7/2/14	0.111	0.032
7/3/14	0.106	0.029
7/8/14	0.106	0.030
7/9/14	0.103	0.028
7/10/14	0.116	0.034
7/11/14	0.109	0.031
7/15/14	0.106	0.030
7/16/14	0.111	0.031
7/17/14	0.110	0.032
7/18/14	0.112	0.033
7/22/14	0.108	0.030
7/23/14	0.111	0.031
7/24/14	0.108	0.030
7/25/14	0.111	0.031
7/30/14	0.083	0.017
7/31/14	0.133	0.042
8/1/14	0.111	0.030
8/7/14	0.111	0.032
8/8/14	0.113	0.032
8/12/14	0.106	0.031
8/13/14	0.111	0.032
8/14/14	0.109	0.029
8/15/14	0.108	0.030
8/19/14	0.112	0.032
8/20/14	0.111	0.031
8/21/14	0.116	0.033
8/22/14	0.113	0.031
8/26/14	0.109	0.031
8/27/14	0.111	0.030
8/28/14	0.114	0.032
8/29/14	0.112	0.031
9/2/14	0.107	0.029
9/3/14	0.104	0.029
9/4/14	0.113	0.032
9/5/14	0.111	0.031
9/9/14	0.111	0.029
9/11/14	0.114	0.034
9/16/14	0.110	0.034

APPENDIX F

COMBINED BOD_5, COD, TOC, and FLUORESCENCE RESULTS

Table F.1: Influent Results for BOD_5, COD, TOC, and Fluorescence

Influent Results for Each Method														
Date	BOD Hold time	Sample use[1&2]	BOD	COD	TOC	Total	Region I	Region II	Region III	Protein	Humic	A	C	T
[1]Comparative (Comp)- Overnight BOD samples were compared with the same day samples used for correlations. The results from the other methods were used to calculate removal efficiency. None of these samples were used for correlation models.														
[2]Correlation (Corr)- Samples were analyzed the same day that they were collected. These were used for the final correlations and also included in the removal efficiency analysis.														
6/24/14	Overnight	Comp	318	744	77.6	160,050	93,514	48,543	17,993	8.60	2.78	3.19	2.69	9.00
6/25/14	Same Day	Corr	348	748	72.8	148,609	85,366	44,713	18,529	7.90	2.79	2.95	2.89	8.28
6/26/14	Overnight	Comp	384	829	67.5	148,383	84,495	45,388	18,500	7.89	2.86	3.17	2.74	8.38
6/27/14	Same Day	Corr	364	668	64.6	154,449	92,292	44,824	17,333	8.64	2.67	2.91	2.53	9.12
7/1/14	Overnight	Comp	227	654	63.9	162,397	95,302	47,644	19,451	8.63	2.98	3.15	2.92	9.20
7/2/14	Same Day	Corr	364	674	62	138,287	79,906	41,359	17,023	7.44	2.65	2.70	2.58	7.78
7/3/14	Same Day	Corr	355	710	88.7	133,219	74,931	41,192	17,096	6.72	2.67	2.85	2.66	7.39
7/8/14	Overnight	Comp	275	692	90.5	154,768	87,899	47,758	19,111	7.79	3.00	3.13	2.93	8.33
7/9/14	Same Day	Corr	337	727	90.2	132,789	74,844	40,844	17,101	6.96	2.70	2.74	2.69	7.34
7/10/14	Overnight	Comp	289	728	65.7	155,388	89,045	47,597	18,746	7.87	3.01	3.27	2.75	8.54
7/11/14	Same Day	Corr	376	645	78.3	157,035	90,206	47,425	19,403	8.19	3.09	3.06	3.01	8.55
7/15/14	Overnight	Comp	304	646	69.2	132,394	74,909	40,773	16,713	6.66	2.68	2.71	2.59	7.05
7/16/14	Same Day	Corr	359	794	73.1	160,251	94,085	47,913	18,253	8.76	2.89	3.16	2.73	9.18
7/17/14	Overnight	Comp	321	755	64	143,573	81,684	44,250	17,639	7.54	2.81	3.08	2.60	8.06
7/18/14	Same Day	Corr	378	790	75.3	157,090	91,851	46,864	18,374	8.47	2.90	3.00	2.78	8.91
7/22/14	Overnight	Comp	281	714	86.3	168,667	96,319	52,136	20,212	8.52	3.18	3.41	3.18	9.14
7/23/14	Same Day	Corr	433	764	78.2	151,285	85,666	46,939	18,680	7.60	2.94	3.07	2.88	8.22
7/24/14	Overnight	Comp	265	737	72.4	129,777	72,647	40,452	16,678	6.34	2.64	2.66	2.62	6.86
7/25/14	Same Day	Corr	438	772	74.9	138,653	78,190	42,760	17,702	7.03	2.83	2.76	2.69	7.50

7/29/14	Overnight	Comp	226	637	67.5	163,611	91,922	50,981	20,708	7.99	3.32	3.37	3.26	8.75
7/30/14	Same Day	Corr	549	886	73.4	136,668	76,028	42,517	18,122	6.72	2.97	2.79	2.81	7.22
7/31/14	Overnight	Comp	273	621	65	142,652	80,021	44,091	18,539	7.16	3.08	2.96	2.91	7.76
8/1/14	Same Day	Corr	454	725	66.9	150,029	84,071	46,904	19,055	7.50	3.14	3.11	2.91	8.07
8/5/14	Overnight	Comp	260	639	57.1	147,493	81,288	48,572	17,633	6.75	2.80	3.30	2.63	7.26
8/6/14	Same Day	Corr	447	627	60.6	136,987	75,029	44,709	17,248	6.40	2.85	3.06	2.67	6.82
8/7/14	Overnight	Comp	312	727	62.6	137,214	77,077	42,743	17,394	6.71	2.93	2.92	2.68	7.26
8/8/14	Same Day	Corr	417	723	59.6	152,329	87,389	46,771	18,169	7.86	2.92	3.06	2.74	8.35
8/12/14	Overnight	Comp	245	657	56	138,227	78,315	42,064	17,847	7.26	2.99	2.95	2.70	7.82
8/13/14	Same Day	Corr	336	752	60	142,658	80,538	44,120	18,000	7.21	3.02	2.96	2.79	7.77
8/14/14	Overnight	Comp	243	704	61	141,729	79,583	44,122	18,025	6.90	3.06	2.92	2.79	7.57
8/15/14	Same Day	Corr	379	766	58	135,211	77,032	41,003	17,176	6.80	2.86	2.69	2.64	7.47
8/19/14	Overnight	Comp	243	726	58.6	156,279	88,182	48,221	19,876	8.07	3.21	3.25	3.13	8.54
8/20/14	Same Day	Corr	420	779	54.2	152,895	87,240	46,871	18,784	8.00	3.12	3.17	2.89	8.51
8/21/14	Overnight	Comp	250	772	65.3	139,229	77,936	43,800	17,492	6.95	2.88	3.02	2.66	7.40
8/22/14	Same Day	Corr	356	706	75.8	163,934	94,464	49,436	20,034	8.65	3.28	3.34	3.10	9.25
8/26/14	Overnight	Comp	284	722	69.4	150,008	85,531	46,269	18,208	7.76	2.99	3.15	2.72	8.25
8/27/14	Same Day	Corr	510	706	62.8	149,008	85,671	44,644	18,692	7.95	3.15	3.06	2.87	8.56
8/28/14	Overnight	Comp	293	788	59.8	141,271	81,157	43,152	16,962	7.66	2.72	2.90	2.50	7.98
8/29/14	Same Day	Corr	603	747	64.6	139,583	78,818	43,208	17,557	7.21	2.85	2.90	2.65	7.62
9/2/14	Overnight	Comp	332	718	76.9	141,100	79,505	42,926	18,669	7.30	2.94	2.88	2.99	7.76
9/3/14	Same Day	Corr	406	772	66.3	142,990	82,060	42,930	18,000	7.61	2.98	2.87	2.72	8.09
9/4/14	Overnight	Comp	225	706	68.5	140,746	79,563	43,334	17,850	7.00	2.88	2.81	2.76	7.57
9/5/14	Same Day	Corr	359	771	81.9	140,398	78,503	43,963	17,931	7.03	2.90	2.89	2.76	7.70
9/9/14	Same Day	Corr	457	581	59.2	162,796	91,754	51,418	19,624	8.15	3.16	3.52	2.97	8.92
9/11/14	Same Day	Corr	327	676	64.6	168,182	96,787	51,881	19,514	8.58	3.22	3.37	3.02	9.23
9/16/14	Same Day	Corr	236	612	67.7	152,522	84,233	47,990	20,300	7.47	3.11	3.14	3.15	7.90

Table F.2: CABI Results for BOD_5, COD, TOC, and Fluorescence

CABI Results for Each Method														
Date	BOD Hold time	Sample use[1&2]	BOD	COD	TOC	Total	Reg. I	Reg. II	Reg. III	Protein	Humic	A	C	T
[1]Comparative- Overnight BOD samples were compared with the same day samples used for correlations. The results from the other methods were used to calculate removal efficiency. None of these samples were used for correlation models.														
[2]Correlation - Samples were analyzed the same day that they were collected. These were used for the final correlations and also included in the removal efficiency analysis.														
6/24/14	Overnight	Comp	199	438	58.8	130,773	72,811	43,110	14,852	6.32	2.14	3.00	2.14	6.74
6/25/14	Same Day	Corr	201	411	57.1	124,425	67,812	42,086	14,527	5.76	2.04	3.02	2.01	6.32
6/26/14	Overnight	Comp	209	450	54.5	125,034	68,784	41,475	14,775	5.87	2.12	2.97	2.05	6.38
6/27/14	Same Day	Corr	206	410	57.5	124,477	69,242	40,721	14,514	5.80	2.02	2.85	1.98	6.36
7/1/14	Overnight	Comp	149	382	62.2	136,143	76,105	44,882	15,156	6.53	2.19	3.08	2.14	7.07
7/2/14	Same Day	Corr	189	404	62.6	126,038	68,737	42,485	14,815	5.81	2.12	2.89	2.09	6.32
7/3/14	Same Day	Corr	147	343	62.8	118,053	64,052	39,680	14,321	5.39	2.14	2.76	2.07	5.95
7/8/14	Overnight	Comp	172	371	64.6	143,299	79,854	47,472	15,973	6.67	2.30	3.16	2.27	7.22
7/9/14	Same Day	Corr	183	401	65.6	121,258	66,498	40,920	13,840	5.70	1.96	2.71	1.96	6.06
7/10/14	Overnight	Comp	189	361	59.6	142,101	78,592	47,138	16,371	6.56	2.30	3.21	2.24	7.12
7/11/14	Same Day	Corr	209	387	74.2	141,137	79,766	45,777	15,594	6.79	2.19	3.04	2.19	7.27
7/15/14	Overnight	Comp	190	412	60.9	121,297	68,179	39,384	13,733	5.83	1.93	2.64	1.89	6.29
7/16/14	Same Day	Corr	230	424	62.6	137,438	77,475	44,718	15,246	6.77	2.17	2.98	2.14	7.20
7/17/14	Overnight	Comp	194	470	63.2	127,718	71,154	41,918	14,646	6.20	2.15	2.84	2.06	6.59
7/18/14	Same Day	Corr	226	399	59.7	124,474	68,881	41,409	14,183	5.85	2.00	2.78	1.98	6.32
7/22/14	Overnight	Comp	196	410	70.5	144,556	81,563	47,162	15,830	7.08	2.26	3.19	2.22	7.68
7/23/14	Same Day	Corr	283	419	66.3	135,883	74,627	45,472	15,785	6.32	2.38	3.11	2.26	6.85
7/24/14	Overnight	Comp	174	398	61	112,766	61,091	38,059	13,616	5.22	2.01	2.66	1.92	5.68
7/25/14	Same Day	Corr	194	374	57.5	121,237	66,250	40,464	14,523	5.59	2.13	2.73	2.08	6.10

7/29/14	Overnight	Comp	183	335	56.5	141,369	78,178	47,142	16,050	6.62	2.34	3.23	2.28	7.22
7/30/14	Same Day	Corr	214	362	59.7	117,008	63,194	39,526	14,288	5.41	2.06	2.72	1.99	5.88
7/31/14	Overnight	Comp	206	369	58.2	127,629	70,389	42,451	14,789	6.08	2.15	2.94	2.07	6.64
8/1/14	Same Day	Corr	196	339	53.5	127,832	69,863	42,937	15,032	5.99	2.20	3.02	2.13	6.58
8/5/14	Overnight	Comp	199	321	53.9	133,961	72,532	46,052	15,377	5.99	2.20	3.22	2.16	6.27
8/6/14	Same Day	Corr	219	355	62	127,896	68,386	45,315	14,195	5.23	2.02	3.00	2.05	5.62
8/7/14	Overnight	Comp	197	374	68.5	137,372	76,540	45,873	14,960	6.13	2.13	2.96	2.14	6.65
8/8/14	Same Day	Corr	224	386	65.4	135,511	74,966	45,324	15,221	6.14	2.20	3.01	2.19	6.71
8/12/14	Overnight	Comp	143	378	59	136,109	74,857	45,719	15,533	6.23	2.23	3.05	2.22	6.93
8/13/14	Same Day	Corr	158	398	57.4	137,582	75,614	46,029	15,938	6.34	2.40	3.10	2.36	6.92
8/14/14	Overnight	Comp	165	411	58.1	136,119	76,824	44,492	14,804	6.64	2.10	3.05	2.07	7.14
8/15/14	Same Day	Corr	244	461	63.8	124,762	68,498	41,317	14,946	6.00	2.22	2.99	2.11	6.56
8/19/14	Overnight	Comp	201	453	62.4	143,132	82,066	46,062	15,005	7.03	2.11	3.00	2.13	7.54
8/20/14	Same Day	Corr	206	407	56.5	135,746	74,691	45,565	15,490	6.29	2.27	3.10	2.25	6.76
8/21/14	Overnight	Comp	177	473	58.3	122,801	68,202	40,661	13,938	5.78	2.00	2.72	2.01	6.16
8/22/14	Same Day	Corr	202	389	58.7	138,748	78,313	45,355	15,080	6.67	2.15	3.05	2.15	7.22
8/26/14	Overnight	Comp	181	431	64.9	132,301	73,905	43,566	14,830	6.29	2.15	2.94	2.13	6.82
8/28/14	Overnight	Comp	179	412	59	127,180	70,789	42,051	14,340	6.03	2.06	2.88	2.05	6.41
8/29/14	Same Day	Corr	242	405	58.6	123,701	69,092	40,595	14,013	5.88	1.98	2.74	1.97	6.26
9/2/14	Overnight	Comp	174	353	66	132,810	74,633	43,552	14,625	6.17	2.10	2.77	2.13	6.64
9/3/14	Same Day	Corr	200	370	67.1	134,248	75,624	43,967	14,658	6.25	2.04	2.86	2.08	6.76
9/4/14	Overnight	Comp	150	401	62.1	126,105	69,819	42,055	14,231	5.67	1.96	2.71	2.01	6.16
9/5/14	Same Day	Corr	161	377	54.6	125,865	68,985	42,446	14,435	5.75	2.04	2.79	2.06	6.28
9/9/14	Same Day	Corr	198	333	59.9	151,819	82,375	51,727	17,717	6.86	2.54	3.59	2.52	7.50
9/11/14	Same Day	Corr	223	462	64.1	154,048	90,098	48,311	15,639	7.90	2.15	3.11	2.23	8.39
9/16/14	Same Day	Corr	185	411	69.9	139,556	78,700	45,246	15,610	6.56	2.15	2.88	2.18	7.02

Table F.3: Effluent Results for BOD_5, COD, TOC, and Fluorescence

Effluent Results for Each Method														
Date	BOD Hold time	Sample use[1&2]	BOD	COD	TOC	Total	Reg. I	Reg. II	Reg. III	Protein	Humic	A	C	T
[1]Comparative- Overnight BOD samples were compared with the same day samples used for correlations. The results from the other methods were used to calculate removal efficiency. None of these samples were used for correlation models.														
[2]Correlation - Samples were analyzed the same day that they were collected. These were used for the final correlations and also included in the removal efficiency analysis.														
7/1/14	Overnight	Comp	13.71	45	10.4	47,276	16,243	22,175	8,858	1.07	1.40	1.74	1.20	1.10
7/2/14	Same Day	Corr	4.30	30	7.7	41,689	13,938	19,530	8,222	0.85	1.28	1.56	1.08	0.90
7/3/14	Same Day	Corr	3.60	26	7	41,647	13,556	19,638	8,453	0.85	1.31	1.56	1.11	0.89
7/8/14	Overnight	Comp	3.83	25	6.9	43,067	14,312	20,373	8,382	0.88	1.31	1.62	1.12	0.94
7/9/14	Same Day	Corr	3.28	23	6.7	34,236	9,235	17,455	7,545	0.57	1.20	1.43	1.01	0.53
7/10/14	Overnight	Comp	3.01	26	6.8	43,170	14,377	20,215	8,578	0.87	1.31	1.62	1.12	0.92
7/11/14	Same Day	Corr	3.33	22	6.7	40,734	13,233	19,539	7,962	0.78	1.23	1.56	1.05	0.83
7/15/14	Overnight	Comp	7.56	36	8.9	35,750	11,842	16,830	7,077	0.72	1.08	1.33	0.92	0.77
7/16/14	Same Day	Corr	8.06	30	9.2	42,951	14,290	20,338	8,322	0.86	1.29	1.63	1.10	0.92
7/17/14	Overnight	Comp	9.69	33	10	38,713	12,690	18,345	7,678	0.77	1.18	1.46	1.01	0.81
7/18/14	Same Day	Corr	10.41	32	10	39,929	13,143	18,878	7,909	0.81	1.22	1.47	1.04	0.85
7/22/14	Overnight	Comp	9.82	32	9.3	43,802	14,575	20,808	8,419	0.91	1.33	1.63	1.14	0.96
7/23/14	Same Day	Corr	12.56	37	10.1	45,137	14,956	21,560	8,620	0.93	1.33	1.69	1.14	0.96
7/24/14	Overnight	Comp	12.32	37	10.6	39,126	12,833	18,613	7,680	0.81	1.18	1.41	1.01	0.82
7/25/14	Same Day	Corr	12.85	44	10.3	43,375	14,499	20,489	8,387	0.90	1.27	1.56	1.10	0.92
7/29/14	Overnight	Comp	17.61	45	10.6	49,998	16,960	23,937	9,102	1.04	1.41	1.82	1.24	1.07
7/30/14	Same Day	Corr	20.01	47	11.4	43,239	14,632	20,423	8,184	0.88	1.25	1.55	1.09	0.92
7/31/14	Overnight	Comp	16.61	46	11.9	48,163	16,784	22,680	8,699	1.09	1.33	1.71	1.17	1.11
8/1/14	Same Day	Corr	10.50	39	9.7	46,361	16,918	20,851	8,592	1.11	1.29	1.65	1.10	1.20

8/7/14	Overnight	Comp	3.78	28	6.8	39,750	13,021	18,955	7,775	0.79	1.20	1.50	1.03	0.83
8/8/14	Same Day	Corr	3.80	27	6.8	39,853	13,008	19,014	7,831	0.80	1.20	1.53	1.03	0.84
8/12/14	Overnight	Comp	5.77	31	8	40,383	13,495	19,045	7,843	0.86	1.21	1.53	1.04	0.91
8/13/14	Same Day	Corr	6.39	33	8.6	42,279	14,018	20,005	8,256	0.89	1.26	1.59	1.08	0.95
8/14/14	Overnight	Comp	6.46	30	8.7	41,875	13,717	19,982	8,176	0.85	1.25	1.57	1.07	0.89
8/15/14	Same Day	Corr	8.00	34	9.2	40,244	13,399	18,984	7,860	0.83	1.20	1.49	1.03	0.85
8/19/14	Overnight	Comp	10.36	38	10.4	46,869	16,727	21,288	8,854	1.22	1.38	1.64	1.17	1.19
8/20/14	Same Day	Corr	11.72	41	10.2	45,235	14,223	22,225	8,787	0.90	1.40	1.75	1.20	0.75
8/21/14	Overnight	Comp	9.05	N/A	9.6	45,665	17,002	20,203	8,460	1.30	1.33	1.56	1.13	1.30
8/22/14	Same Day	Corr	9.17	N/A	9.4	50,373	19,097	22,338	8,939	1.43	1.39	1.71	1.20	1.42
8/26/14	Overnight	Comp	6.29	N/A	8.3	45,327	16,528	20,464	8,335	1.26	1.30	1.57	1.10	1.21
8/27/14	Same Day	Corr	6.88	N/A	8.7	48,215	18,220	21,426	8,569	1.42	1.33	1.67	1.14	1.38
8/28/14	Overnight	Comp	6.47	33	8.8	44,676	16,774	19,669	8,234	1.37	1.28	1.52	1.07	1.26
8/29/14	Same Day	Corr	6.48	29	8.9	43,303	15,705	19,448	8,151	1.24	1.25	1.54	1.06	1.12
9/2/14	Overnight	Comp	6.06	26	8.4	41,902	15,244	18,722	7,936	1.19	1.24	1.47	1.04	1.14
9/3/14	Same Day	Corr	8.88	27	9.2	40,151	13,535	18,719	7,897	0.86	1.20	1.48	1.02	0.92
9/4/14	Overnight	Comp	6.83	28	8.6	40,664	13,821	18,861	7,982	0.87	1.22	1.48	1.03	0.93
9/5/14	Same Day	Corr	5.37	23	7.7	39,263	12,611	18,754	7,899	0.82	1.21	1.49	1.03	0.87
9/9/14	Same Day	Corr	3.95	23	6.8	47,410	15,830	22,470	9,110	0.96	1.36	1.80	1.18	1.03
9/11/14	Same Day	Corr	3.77	23	6.8	45,064	15,009	21,431	8,625	0.95	1.34	1.72	1.15	1.02
9/16/14	Same Day	Corr	4.92	26	7.3	38,242	13,087	17,556	7,599	0.87	1.20	1.39	1.01	0.90

APPENDIX G

R CODES

The corresponding Excel files are available upon request. All referenced data files were developed from data reported in the previous appendices.

G.1 R Code for BOD and COD Regression Models and Summary Statistics

```
#-------Import BOD and COD Data-----------------------------------------------------------------
library(XLConnect)
CODBOD=loadWorkbook("BODCOD.xlsx")
effluent=readWorksheet(CODBOD, sheet="effluent")
all=readWorksheet(CODBOD, sheet="All")

#-------Summary Statistics-----------------------------------------------------------

#Determine summary statistics
library(boot)
library(pastecs)
summary(influent)
stat.desc(influent)
summary(cabi)
stat.desc(cabi)
stat.desc(effluent)
summary(effluent)

#Determine ratios
y1<-influent$COD/influent$BOD
summary(y1)
y2<-cabi$COD/cabi$BOD
summary(y2)
y3<-effluent$COD/effluent$BOD
summary(y3)

#--------Linear Models-----------------------------------------------------------------------

#influent
lminfluent=lm(BOD~COD, influent)
summary(lminfluent)
#r2=0.02763 --> bad model

#CABI
lmcabi=lm(BOD~COD, cabi)
summary(lmcabi)
#r2=0.1453 --> bad model

#effluent
lmeffluent=lm(BOD~COD, effluent)
summary(lmeffluent)
#r2=0.7682
#Residual SE 1.713
```

```
qqnorm(lmeffluent$residuals)
shapiro.test(lmeffluent$residuals)
#Errors are non normal p=0.02408

#influent and cabi combined
lminfluentcabi=lm(BOD~COD, influentcabi)
summary(lminfluentcabi)
#r2=0.7274
#Residual SE 55.75
#Check residuals for error assumption
qqnorm(lminfluentcabi$residuals)
shapiro.test(lminfluentcabi$residuals)
#Errors are non normal p=1.012e-08

#all data combined
lmalldata=lm(BOD~COD, all)
summary(lmalldata)
#r2=0.9189
qqnorm(lmalldata$residuals)
shapiro.test(lmalldata$residuals)
#Errors are non normal p=2.734e-16

#Errors are not normally distributed; therefore, use robust regression.

#------Robust Linear Regression ---------------------------------

# Errors are non-normal, use Robust Regression
library(MASS)
library(sandwich)
library(lmtest)

#Try robust linear model for all data combined
rlmall <- rlm(BOD~COD,data=all)
summary(rlmall)
#BOD=0.5389*COD-9.7876
#Residual SE 20.16
r2all<-lm(all$BOD~ rlmall $fitted.values)
summary(r2all)
#Psuedo r2 = 0.9189

#Try robust linear model for all data combined by location
rlmall2 <- rlm(BOD~COD+factor(Location),data=all)
summary(rlmall2)
#BOD=0.1996*COD+110.8749*Influent-127.7315*Effluent+129.0188
#Residual SE 16.21
r2infcabi0<-lm(all$BOD~ rlmall2 $fitted.values)
```

```
summary(r2infcabi0)
#Psuedo r2 = 0.9252
#Check the coefficients
coeftest(rlmall2, df = Inf, vcov = vcovHC(rlmall2, type = "const"))
coeftest(rlmall2, df = Inf, vcov = vcovHC(rlmall2, type = "HC"))
coeftest(rlmall2, df = Inf, vcov = vcovHC(rlmall2, type = "HC0"))
coeftest(rlmall2, df = Inf, vcov = vcovHC(rlmall2, type = "HC3"))
coeftest(rlmall2, df = Inf, vcov = vcovHC(rlmall2, type = "HC4"))

#Robust linear model for influent/cabi combined
rlminfcab0 <- rlm(BOD~COD,data=influentcabi)
summary(rlminfcab0)
#BOD=0.5176*COD+5.0854
#Residual SE 41.17
r2infcabi0<-lm(influentcabi$BOD~ rlminfcab0 $fitted.values)
summary(r2infcabi0)
#Psuedo r2 = 0.7274

#Robust linear model for influent/cabi combined by location
rlminfcab <- rlm(BOD~COD+factor(Location),data=influentcabi)
summary(rlminfcab)
#BOD=0.1792*COD+119.8388*Influent+136.0053
#Residual SE 35.87
r2infcabi<-lm(influentcabi$BOD~rlminfcab $fitted.values)
summary(r2infcabi)
#Psuedo r2 = 0.7586
#Check the coefficients
coeftest(rlminfcab, df = Inf, vcov = vcovHC(rlminfcab, type = "const"))
coeftest(rlminfcab, df = Inf, vcov = vcovHC(rlminfcab, type = "HC"))
coeftest(rlminfcab, df = Inf, vcov = vcovHC(rlminfcab, type = "HC0"))
coeftest(rlminfcab, df = Inf, vcov = vcovHC(rlminfcab, type = "HC3"))
coeftest(rlminfcab, df = Inf, vcov = vcovHC(rlminfcab, type = "HC4"))

#Robust linear model for effluent
rlmeffluent <- rlm(BOD~COD,data=effluent)
summary(rlmeffluent)
#BOD=0.4876*COD-7.7833
#Residual SE 1.931
effr2<-lm(effluent$BOD~rlmeffluent$fitted.value)
summary(effr2)
#r2=0.7682
```

G.2 R Code for BOD and TOC Regression Models and Summary Statistics

```
#-------Import data-------------------------------------------------------------
library(XLConnect)
TOCBOD=loadWorkbook("BODTOC.xlsx")
influent=readWorksheet(TOCBOD, sheet="influent")
cabi=readWorksheet(TOCBOD, sheet="cabi")
influentcabi=readWorksheet(TOCBOD, sheet="influentcabi")
effluent=readWorksheet(TOCBOD, sheet="effluent")
all=readWorksheet(TOCBOD, sheet="all")
cabieff=readWorksheet(TOCBOD, sheet="cabieffluent")

#-------Summary Statistics------------------------------------------------------
#Determine summary statistics
library(boot)
library(pastecs)
summary(influent)
stat.desc(influent)
summary(cabi)
stat.desc(cabi)
stat.desc(effluent)
summary(effluent)

#Determine BOD/TOC ratios
y1<-influent$BOD/influent$TOC
y2<-cabi$BOD/cabi$TOC
y3<-effluent$BOD/effluent$TOC
summary(y1)
summary(y2)
summary(y3)

#--------Linear Models-----------------------------------------------------------

#build linear models
lminfluent=lm(BOD~TOC, influent)
summary(lminfluent)
#r2=0.06485 -> bad model

lmcabi=lm(BOD~TOC, cabi)
summary(lmcabi)
#r2=0.0361 -> bad model

#influent and cabi combined
lminfluentcabi=lm(BOD~TOC, influentcabi)
```

```
summary(lminfluentcabi)
#r2=0.111-> bad model

#effluent
lmeffluent=lm(BOD~TOC, effluent)
summary(lmeffluent)
#r2=0.8663 -> ok model
#Residual SE 1.237
qqnorm(lmeffluent$residuals)
shapiro.test(lmeffluent$residuals)
#Data are not normally distributed =5.9e-08

#all data combined
lmalldata=lm(BOD~TOC, all)
summary(lmalldata)
#r2=0.7493
#Residual SE 79.54
#combined data appear to be the best fit
qqnorm(lmalldata$residuals)
shapiro.test(lmalldata$residuals)
#Data are not normally distributed p=2.845e-15

#Errors are not normally distributed; therefore, use robust regression.

#------Robust Regression ------------------------------------------------

#Perform robust regression on effluent and all data point
library(MASS)
library(sandwich)
library(lmtest)

#Robust linear model for all sites combined
rlmall <- rlm(BOD~TOC,data=all)
summary(rlmall)
#Residual SE 27.92
#BOD=4.4126*TOC-31.7059
#Residual SE 20.16
r2all<-lm(all$BOD~ rlmall $fitted.values)
summary(r2all)
#Psuedo r2 = 0.7493

#Robust linear model for all sites combined by location
rlmall2 <- rlm(BOD~TOC +factor(Location),data=all)
summary(rlmall2)
#Residual SE 14.87
#BOD=-0.9427*TOC+184.4776*Inf-249.2292*Effluent+264.9775
```

```
r2all2<-lm(all$BOD~ rlmall2 $fitted.values)
summary(r2all2)
#Psuedo r2 = 0.9277
#Check coefficients
coeftest(rlmall2, df = Inf, vcov = vcovHC(rlmall2, type = "const"))
coeftest(rlmall2, df = Inf, vcov = vcovHC(rlmall2, type = "HC"))
coeftest(rlmall2, df = Inf, vcov = vcovHC(rlmall2, type = "HC0"))
coeftest(rlmall2, df = Inf, vcov = vcovHC(rlmall2, type = "HC3"))
coeftest(rlmall2, df = Inf, vcov = vcovHC(rlmall2, type = "HC4"))

#Robust linear model for influent/cabi combined
rlminfcab0 <- rlm(BOD~TOC,data=influentcabi)
summary(rlminfcab0)
#BOD=4.8462*TOC-44.1455
#Residual SE 80.83
r2infcabi0<-lm(influentcabi$BOD~ rlminfcab0 $fitted.values)
summary(r2infcabi0)
#Psuedo r2 = 0.111

#Robust linear model for influent/cabi combined by location
rlminfcab <- rlm(BOD~TOC+factor(Location.),data=influentcabi)
summary(rlminfcab)
#BOD=-1.0708*TOC+188.9198*Influent+272.4721
#Residual SE 33.82
r2infcabi<-lm(influentcabi$BOD~rlminfcab $fitted.values)
summary(r2infcabi)
#Psuedo r2 = 0.7445
#okay fit, but the slope is not representative of the data
#Check the coefficients
coeftest(rlminfcab, df = Inf, vcov = vcovHC(rlminfcab, type = "const"))
coeftest(rlminfcab, df = Inf, vcov = vcovHC(rlminfcab, type = "HC"))
coeftest(rlminfcab, df = Inf, vcov = vcovHC(rlminfcab, type = "HC0"))
coeftest(rlminfcab, df = Inf, vcov = vcovHC(rlminfcab, type = "HC3"))
coeftest(rlminfcab, df = Inf, vcov = vcovHC(rlminfcab, type = "HC4"))

#Robust linear model for effluent
rlmeffluent <- rlm(BOD~TOC,data=effluent)
summary(rlmeffluent)
#Residual SE 0.8721
#BOD=2.2456*TOC-12.0691
r2eff<-lm(effluent$BOD~rlmeffluent$fitted.value)
summary(r2eff)
#r2=0.8663
```

G.3 BOD and Fluorescence Models

```
library(XLConnect)
fl=loadWorkbook("Fluorescence.xlsx")
influent=readWorksheet(fl, sheet="influent")
cabi=readWorksheet(fl, sheet="cabi")
influentcabi=readWorksheet(fl, sheet="influentcabi")
effluent=readWorksheet(fl, sheet="effluent")
all=readWorksheet(fl, sheet="all")


#----Linear influent correlations-----------------------------------------------------------------
#build linear models for influent
lmtotalinf=lm(BOD~Total, influent)
summary(lmtotalinf)
#r2=0.0139

lmregionIinf=lm(BOD~RegionI, influent)
summary(lmregionIinf)
#r2=0.01319

lmregionIIinf=lm(BOD~RegionII, influent)
summary(lmregionIIinf)
#r2=0.007189

lmregionIIIinf=lm(BOD~RegionIII, influent)
summary(lmregionIIIinf)
#r2=0.0354

lmProteinsinf=lm(BOD~Proteins, influent)
summary(lmProteinsinf)
#r2=0.01184

lmHumicsinf=lm(BOD~Humics, influent)
summary(lmHumicsinf)
#r2=0.0014

lmAinf=lm(BOD~A, influent)
summary(lmAinf)
#r2=0.0002

lmCinf=lm(BOD~C, influent)
summary(lmCinf)
#r2=0.0642
```

```
lmMinf=lm(BOD~M, influent)
summary(lmMinf)
#r2=0.0056

lmTinf=lm(BOD~T, influent)
summary(lmTinf)
#r2=0.0068

#No significant correlation coefficients

#----Linear CABI correlations----------------------------------------------------------------------

lmtotalcabi=lm(BOD~Total, cabi)
summary(lmtotalcabi)
#r2=0.0069

lmregionIcabi=lm(BOD~RegionI, cabi)
summary(lmregionIcabi)
#r2=0.0113

lmregionIIcabi=lm(BOD~RegionII, cabi)
summary(lmregionIIcabi)
#r2=0.0016

lmregionIIIcabi=lm(BOD~RegionIII, cabi)
summary(lmregionIIIcabi)
#r2=0.0024

lmProteinscabi=lm(BOD~Proteins, cabi)
summary(lmProteinscabi)
#r2=0.0209

lmHumicscabi=lm(BOD~Humics, cabi)
summary(lmHumicscabi)
#r2=0.0015

lmAcabi=lm(BOD~A, cabi)
summary(lmAcabi)
#r2=0.0097

lmCcabi=lm(BOD~C, cabi)
summary(lmCcabi)
#r2=0.0015

lmMcabi=lm(BOD~M, cabi)
summary(lmMcabi)
```

```
#r2=0.0170

lmTcabi=lm(BOD~T, cabi)
summary(lmTcabi)
#r2=0.0144
#No significant correlation coefficients

#----Linear effluent correlations----------------------------------------------------------------

lmtotaleff=lm(BOD~Total, effluent)
summary(lmtotaleff)
#r2=0.1111

lmregionIeff=lm(BOD~RegionI, effluent)
summary(lmregionIeff)
#r2=0.0758

lmregionIIeff=lm(BOD~RegionII, effluent)
summary(lmregionIIeff)
#r2=0.1354

lmregionIIIeff=lm(BOD~RegionIII, effluent)
summary(lmregionIIIeff)
#r2=0.0680

lmProteinseff=lm(BOD~Proteins, effluent)
summary(lmProteinseff)
#r2=0.0262

lmHumicseff=lm(BOD~Humics, effluent)
summary(lmHumicseff)
#r2=0.0655

lmAeff=lm(BOD~A, effluent)
summary(lmAeff)
#r2=0.0492

lmCeff=lm(BOD~C, effluent)
summary(lmCeff)
#r2=0.0920

lmMeff=lm(BOD~M, effluent)
summary(lmMeff)
#r2=0.0287

lmTeff=lm(BOD~T, effluent)
```

```
summary(lmTeff)
#r2=0.0192
#No significant correlation coefficients

#----Linear Influent/CABI correlations-------------------------------------------------------
#build linear models for CABI and Influent
lmtotalc=lm(BOD~Total, influentcabi)
summary(lmtotalc)
#r2=0.2953

lmregionIc=lm(BOD~RegionI, influentcabi)
summary(lmregionIc)
#r2=0.3208

lmregionIIc=lm(BOD~RegionII, influentcabi)
summary(lmregionIIc)
#r2=0.0677

lmregionIIIc=lm(BOD~RegionIII, influentcabi)
summary(lmregionIIIc)
#r2=0.5331

lmProteinsc=lm(BOD~Proteins, influentcabi)
summary(lmProteinsc)
#r2=0.4314

lmHumicsc=lm(BOD~Humics,influentcabi)
summary(lmHumicsc)
#r2=0.6489
shapiro.test(lmHumicsc$residuals)
#nonnormal p=5.261e-6
rlmHumicsc=rlm(BOD~Humics,influentcabi)
summary(rlmHumicsc)
#model 205*Humic peak - 222.81
#Visually inspect model
 plot(influentcabi $BOD~ influentcabi $Humics, xlim=range(influentcabi $Humics),
 ylim=range(influentcabi$BOD) )
 par(new=T)
 curve(205*x-222, from=min(influentcabi $Humics), to=max(influentcabi$Humics),
 ylim=range(influentcabi $BOD),col="red")
 #Conclude that it is not a goof fit for the data

lmAc=lm(BOD~A, influentcabi)
summary(lmAc)
#r2=0.0355
```

```
lmCc=lm(BOD~C, influentcabi)
summary(lmCc)
#r2=0.5699

lmMc=lm(BOD~M, influentcabi)
summary(lmMc)
#r2=0.4407

lmTc=lm(BOD~T, influentcabi)
summary(lmTc)
#r2=0.4322


#----Linear Influent/CABI/effluent correlations----------------------------------------------

#Total of the regions
lmtotalall=lm(BOD~Total, all)
summary(lmtotalall)
#r2= 0.7915 and residual SE 72.55
shapiro.test(lmtotalall$residuals)
#non normal
rlmtotalall=rlm(BOD~Total+factor(Location), all)
summary(rlmtotalall)
#The Total fl. factor drops out, bad model
rlmtotalallb=rlm(BOD~Total, all)
summary(rlmtotalallb)
#y=0.0027*Total-111.0774
#Visually inspect model
 plot(all $BOD~ all $Humics, xlim=range(all $Humics), ylim=range(all$BOD) )
 par(new=T)
 curve(0.0027*x-111, from=min(all $Total), to=max(all$Total), ylim=range(all
 $BOD),col="red")
#Not a good model

lmregionI=lm(BOD~RegionI, all)
summary(lmregionI)
#r2= 0.7971
#residual SE 71.55
qqnorm(lmregionI$residuals)
shapiro.test(lmregionI$residuals)
#non normal

lmregionII=lm(BOD~RegionII, all)
summary(lmregionII)
#r2=0.7303
#residual SE 82.5
```

```
qqnorm(lmregionII$residuals)
shapiro.test(lmregionII$residuals)
#non normal

lmregionIII=lm(BOD~RegionIII, all)
summary(lmregionIII)
#r2=0.8618
#residual SE 59.07
qqnorm(lmregionIII$residuals)
shapiro.test(lmregionIII$residuals)
#non normal

lmProteins=lm(BOD~Proteins, all)
summary(lmProteins)
#r2=0.8261
#residual SE 66.26
qqnorm(lmregionIII$residuals)
shapiro.test(lmregionIII$residuals)
#non normal

lmHumics=lm(BOD~Humics,all)
summary(lmHumics)
#r2=0.8946
#residual SE 51.58
qqnorm(lmHumics$residuals)
shapiro.test(lmHumics$residuals)
#non normal

lmA=lm(BOD~A, all)
summary(lmA)
#r2=0.7128
#residual SE 85.13
qqnorm(lmA$residuals)
shapiro.test(lmA$residuals)
#non normal

lmC=lm(BOD~C, all)
summary(lmC)
#r2=0.8764
#residual SE 55.86
qqnorm(lmC$residuals)
shapiro.test(lmC$residuals)
#non normal

lmM=lm(BOD~M, all)
summary(lmM)
```

```
#r2= 0.8244
#residual SE 66.57
qqnorm(lmM$residuals)
shapiro.test(lmM$residuals)
#non normal

lmT=lm(BOD~T, all)
summary(lmT)
#r2= 0.8234
#residual SE 66.76
qqnorm(lmT$residuals)
shapiro.test(lmT$residuals)
#non normal

#----Robust regressions Humic peak-----------------------------------------------------------------

#Humic peak
rlmhumic <- rlm(BOD~Humics,data=all)
summary(rlmhumic)
#Residual 29.26
#Model a=227.6772 and b=-277.7612
r2humica<-lm(all$BOD~ rlmhumic $fitted.value)
summary(r2humica)
#r2=0.89463


rlmhumicb <- rlm(BOD~Humics +factor(Location),data=all)
summary(rlmhumicb)
#Model 14.65*Humic+166.75*Inf -187.3350*Effluent +176.2195
r2humicb<-lm(all$BOD~ rlmhumicb $fitted.value)
summary(r2humicb)
#R2=0.9295
coeftest(rlmhumicb, df = Inf, vcov = vcovHC(rlmhumicb, type = "const"))
coeftest(rlmhumicb, df = Inf, vcov = vcovHC(rlmhumicb, type = "HC"))
coeftest(rlmhumicb, df = Inf, vcov = vcovHC(rlmhumicb, type = "HC0"))
coeftest(rlmhumicb, df = Inf, vcov = vcovHC(rlmhumicb, type = "HC3"))
coeftest(rlmhumicb, df = Inf, vcov = vcovHC(rlmhumicb, type = "HC4"))

plot(all$BOD~all$Humics, xlim=range(all$Humics), ylim=range(all$BOD),  main="Linear
 Fit for Humic Peak", xlab="Humic Peak Fluorescence (AFU)", ylab="BOD5 (mg/L)"  )

legend('topleft', c("Influent Regression Line (as a function of sample point)", "CABI
 Regression Line (as a function of sample point)", "Effluent (as a function of sample point)",
 "Regression model(sample site not considered) "), lty=c(1,1,1,1), lwd=c(1, 1, 1,1),
 col=c('black', 'orange', 'green', 'red'), inset=0.02, cex=0.8)
```

```
#Sample sites not included
 par(new=T)
 curve(227.68*x-277.76, from=min(all$Humics),
                    to=max(all$Humics),
                    ylim=range(all$BOD),col="red" , xlab="Humic Peak Fluorescence (AFU)",
 ylab="BOD5 (mg/L)")

#Samples sites as part of linear function
#effluent
 par(new=T)
 curve(14.65*x-11.12, from=min(all$Humics),
                    to=max(all$Humics),
                    ylim=range(all$BOD),col="green" , xlab="Humic Peak Fluorescence
 (AFU)", ylab="BOD5 (mg/L)")

#CABI
 par(new=T)
 curve(14.65*x+176.22, from=min(all$Humics),
                    to=max(all$Humics),
                    ylim=range(all$BOD),col="orange" , xlab="Humic Peak Fluorescence
 (AFU)", ylab="BOD5 (mg/L)")


#influent
 par(new=T)
 curve(14.65*x+342.97, from=min(all$Humics),
                    to=max(all$Humics),
                    ylim=range(all$BOD),col="black" , xlab="Humic Peak Fluorescence
 (AFU)", ylab="BOD5 (mg/L)")


#Try a nonlinear power model ax^b
nlhumic<-nlrob((BOD~a*Humics^b), data=all, start=list(a=4e7, b=3))
summary(nlhumic)
#residual error 64.81
#model is a=29.6989 b=2.3878
r2humicc<-lm(all$BOD~fitted.values(nlhumic))
summary(r2humicc)
#r2=0.847

#Try a nonlinear power model ax^b+c
nlhumicb<-nlrob((BOD~a*Humics^b+c), data=all, start=list(a=1, b=1.494, c=-15))
summary(nlhumicb)
#residual error 31.97
#model is a=297.2757, b=0.8460, and c=-353.3478
r2humicd<-lm(all$BOD~fitted.values(nlhumicb))
```

```
summary(r2humicd)
#r2=0.90

#Exponential model without c
exphumic<-nlrob((BOD~a*exp(Humics*b)), data=all, start=list(a=1, b=1.494))
summary(exphumic)
#Residuals 90.8
#Model is a=19.96 and b=1.00
r2humice<-lm(all$BOD~fitted.values(exphumic))
summary(r2humice)

#Exponential model with c intercept
exphumicb<-nlrob((BOD~a*exp(Humics*b)+c), data=all, start=list(a=4e3, b=2e-2, c=-
 2.69e5), trace=T)
summary(exphumicb)
#Model a=-2.90e3, b=-9.443e-2, c=2.585e3
r2humicf<-lm(all$BOD~fitted.values(exphumicb))
summary(r2humicf)
#r2-0.90

 plot(all$BOD~all$Humics, xlim=range(all$Humics), ylim=range(all$BOD) ,
 main="Nonlinear Fit for Humic Peak", xlab="Humic Peak Fluorescence (AFU)",
 ylab="BOD5 (mg/L)" )

 legend('topleft', c("Power model", "Power model with a constant ", "Exponential model",
 "Exponential model with a constant"), lty=c(1,1,1,1), lwd=c(1, 1, 1,1), col=c('black',
 'orange', 'red', 'green'), inset=0.02, cex=0.8)

 par(new=T)
 curve(29.6989*x^2.3878, from=min(all$Humics),
                  to=max(all$Humics),
                  ylim=range(all$BOD),col="black", xlab="Humic Peak Fluorescence
 (AFU)", ylab="BOD5 (mg/L)" )

 par(new=T)
 curve(297.2757*x^0.8460-353.3478, from=min(all$Humics),
                  to=max(all$Humics),
                  ylim=range(all$BOD),col="orange", xlab="Humic Peak Fluorescence
 (AFU)", ylab="BOD5 (mg/L)" )

 par(new=T)
 curve(19.96*exp(x*1.00), from=min(all$Humics),
                  to=max(all$Humics),
                  ylim=range(all$BOD),col="red", xlab="Humic Peak Fluorescence (AFU)",
 ylab="BOD5 (mg/L)" )
```

```
par(new=T)
 curve(-2903*exp(x*-9.443e-2)+2.585e3, from=min(all$Humics),
                    to=max(all$Humics),
                    ylim=range(all$BOD),col="green", xlab="Humic Peak Fluorescence
 (AFU)", ylab="BOD5 (mg/L)" )


#----Robust regressions Region III-------------------------------------------------------------

#All of the data is non normal
#Perform a robust regression

#Region III
rlmregionIII <- rlm(BOD~RegionIII,data=all)
summary(rlmregionIII)
#Residual 30.89
#model a=0.0337 b=-271.96
r2region3a<-lm(all$BOD~rlmregionIII$fitted.value)
summary(r2region3a)
#r2=0.8613
coeftest(rlmregionIII, df = Inf, vcov = vcovHC(rlmregionIII, type = "const"))
coeftest(rlmregionIII, df = Inf, vcov = vcovHC(rlmregionIII, type = "HC"))
coeftest(rlmregionIII, df = Inf, vcov = vcovHC(rlmregionIII, type = "HC0"))
coeftest(rlmregionIII, df = Inf, vcov = vcovHC(rlmregionIII, type = "HC3"))
coeftest(rlmregionIII, df = Inf, vcov = vcovHC(rlmregionIII, type = "HC4"))

#Region III with sample sites as factor
rlmregionIIIb <- rlm(BOD~RegionIII +factor(Location),data=all)
summary(rlmregionIIIb)
#Residual 14.21
#model a=-0.0006, effluent=-204.1823, influent=179.8176, intercept=216.7640
r2reg3b<-lm(all$BOD~rlmregionIIIb$fitted.value)
summary(r2reg3b)
#r2=0.9292
coeftest(rlmregionIIIb, df = Inf, vcov = vcovHC(rlmregionIIIb, type = "const"))
coeftest(rlmregionIIIb, df = Inf, vcov = vcovHC(rlmregionIIIb, type = "HC"))
coeftest(rlmregionIIIb, df = Inf, vcov = vcovHC(rlmregionIIIb, type = "HC0"))
coeftest(rlmregionIIIb, df = Inf, vcov = vcovHC(rlmregionIIIb, type = "HC3"))
coeftest(rlmregionIIIb, df = Inf, vcov = vcovHC(rlmregionIIIb, type = "HC4"))


plot(all$BOD~all$RegionIII, xlim=range(all$RegionIII), ylim=range(all$BOD),
 main="Linear Fit for Region III", xlab="Region III Fluorescence (AFU)", ylab="BOD5
 (mg/L)"  )
```

```
legend('topleft', c("Influent Regression Line (as a function of sample point)", "CABI
 Regression Line (as a function of sample point)", "Effluent (as a function of sample point)",
 "Regression model(sample site not considered) "), lty=c(1,1,1,1), lwd=c(1, 1, 1,1),
 col=c('black', 'orange', 'green', 'red'), inset=0.02, cex=0.8)


par(new=T)
 curve(0.037*x-271.96, from=min(all$RegionIII),
                    to=max(all$RegionIII),
                    ylim=range(all$BOD),col="red" , xlab="Region III Fluorescence (AFU)",
 ylab="BOD5 (mg/L)")

par(new=T)
 curve(-0.0006*x+12.56, from=min(all$RegionIII),
                    to=max(all$RegionIII),
                    ylim=range(all$BOD),col="green" , xlab="Region III Fluorescence (AFU)",
 ylab="BOD5 (mg/L)")

par(new=T)
 curve(-0.0006*x+216.76, from=min(all$RegionIII),
                    to=max(all$RegionIII),
                    ylim=range(all$BOD),col="orange" , xlab="Region III Fluorescence
 (AFU)", ylab="BOD5 (mg/L)")


par(new=T)
 curve(-0.0006*x+396.58, from=min(all$RegionIII),
                    to=max(all$RegionIII),
                    ylim=range(all$BOD),col="black" , xlab="Region III Fluorescence (AFU)",
 ylab="BOD5 (mg/L)")

#Try a nonlinear power model ax^b
nlabsreg3<-nlrob((BOD~a*RegionIII^b), data=all, start=list(a=4e7, b=3))
summary(nlabsreg3)
#residual error 40.63
#model is a=3.987e-11 b=3.045
r2reg3<-lm(all$BOD~fitted.values(nlabsreg3))
summary(r2reg3)
#r2=0.833

#Try a nonlinear power model ax^b+c
nlabsreg3bb<-nlrob((BOD~a*RegionIII^b+c), data=all, start=list(a=9e-8, b=2.274, c=-9.54))
summary(nlabsreg3bb)
#Residuals 24.53
#Model a=1.502e-6, b=1.99, c=-8.470e1
r2reg3b<-lm(all$BOD~fitted.values(nlabsreg3bb))
```

```
summary(r2reg3b)
#r2=0.86

#Try an exponential model
expreg3a<-nlrob((BOD~a*exp(RegionIII*b)), data=all, start=list(a=1, b=4e-7))
summary(expreg3a)
#Residuals 65.86
#Model a=1.052e+1, b=1.951e-4
r2exp3<-lm(all$BOD~fitted.values(expreg3a))
summary(r2exp3)
#r2=0.79

#Try an exponential model with c
expreg3b<-nlrob((BOD~a*exp(RegionIII*b)+c), data=all, start=list(a=2.1e2, b=6.9e-5, c=-
 3.56e2))
summary(expreg3b)
#Residuals 25
#Model a=2.072e2, b=6.85e-5, c=-3.556+2
r2exp3b<-lm(all$BOD~fitted.values(expreg3b))
summary(r2exp3b)
#r2=0.86

 plot(all$BOD~all$RegionIII, xlim=range(all$RegionIII), ylim=range(all$BOD),
 main="Nonlinear Fit for Region III", xlab="Region III Fluorescence (AFU)", ylab="BOD5
 (mg/L)"   )

  legend('topleft', c("Power model", "Power model with a constant ", "Exponential model",
 "Exponential model with a constant"), lty=c(1,1,1,1), lwd=c(1, 1, 1,1), col=c('black',
 'orange', 'red', 'green'), inset=0.02, cex=0.8)

 par(new=T)
 curve(3.987e-11*x^3.045, from=min(all$RegionIII),
                    to=max(all$RegionIII),
                    ylim=range(all$BOD),col="black", xlab="Region III Fluorescence (AFU)",
 ylab="BOD5 (mg/L)")
 par(new=T)
 curve(1.502e-6*x^1.99-8.470e1, from=min(all$RegionIII),
                    to=max(all$RegionIII),
                    ylim=range(all$BOD),col="orange", xlab="Region III Fluorescence (AFU)",
 ylab="BOD5 (mg/L)")
 par(new=T)
 curve(1.052e1*exp(x*1.951e-4), from=min(all$RegionIII),
                    to=max(all$RegionIII),
                    ylim=range(all$BOD),col="red", xlab="Region III Fluorescence (AFU)",
 ylab="BOD5 (mg/L)")
par(new=T)
```

```
curve(2.072e2*exp(x*6.85e-5)-3.556e+2, from=min(all$RegionIII),
                    to=max(all$RegionIII),
                    ylim=range(all$BOD),col="green", xlab="Region III Fluorescence (AFU)",
ylab="BOD5 (mg/L)")



#----Robust linear regressions Peak T ---------------------------------------------------

rlmT<- rlm(BOD~T,data=all)
summary(rlmT)
#Residual 29.42
#Model a=42.0179 and b=-36.9042
r2Ta<-lm(all$BOD~ rlmT $fitted.value)
summary(r2Ta)
#r2=0.83
coeftest(rlmT, df = Inf, vcov = vcovHC(rlmT, type = "const"))
coeftest(rlmT, df = Inf, vcov = vcovHC(rlmT, type = "HC"))
coeftest(rlmT, df = Inf, vcov = vcovHC(rlmT, type = "HC0"))
coeftest(rlmT, df = Inf, vcov = vcovHC(rlmT, type = "HC3"))
coeftest(rlmT, df = Inf, vcov = vcovHC(rlmT, type = "HC4"))


rlmTb <- rlm(BOD~ T +factor(Location),data=all)
summary(rlmTb)
#Model -1.0621*T+179.5924*Influent -205.9731*Effluent +214.4779
r2Tb<-lm(all$BOD~ rlmTb $fitted.value)
summary(r2Tb)
#R2=0.9292 residual
coeftest(rlmTb, df = Inf, vcov = vcovHC(rlmTb, type = "const"))
coeftest(rlmTb, df = Inf, vcov = vcovHC(rlmTb, type = "HC"))
coeftest(rlmTb, df = Inf, vcov = vcovHC(rlmTb, type = "HC0"))
coeftest(rlmTb, df = Inf, vcov = vcovHC(rlmTb, type = "HC3"))
coeftest(rlmTb, df = Inf, vcov = vcovHC(rlmTb, type = "HC4"))

plot(all$BOD~all$Proteins, xlim=range(all$T), ylim=range(all$BOD),  main="Linear Fit for
 Peak T", xlab="Peak T Fluorescence (AFU)", ylab="BOD5 (mg/L)"  )

legend('topleft', c("Influent Regression Line (as a function of sample point)", "CABI
 Regression Line (as a function of sample point)", "Effluent (as a function of sample point)",
 "Regression model(sample site not considered) "), lty=c(1,1,1,1), lwd=c(1, 1, 1,1),
 col=c('black', 'orange', 'green', 'red'), inset=0.02, cex=0.8)

 par(new=T)
 curve(-1.06*x+394.07, from=min(all$T),
```

```
                to=max(all$T),
                ylim=range(all$BOD),col="black" , xlab="Peak T Fluorescence (AFU)",
ylab="BOD5 (mg/L)")
par(new=T)
curve(-1.06*x+ 214.48, from=min(all$T),
                to=max(all$T),
                ylim=range(all$BOD),col="orange" , xlab="Peak T Fluorescence (AFU)",
ylab="BOD5 (mg/L)")
par(new=T)
curve(-1.06*x+8.51, from=min(all$T),
                to=max(all$T),
                ylim=range(all$BOD),col="green" , xlab="Peak T Fluorescence (AFU)",
ylab="BOD5 (mg/L)")
par(new=T)
curve(42.02*x-36.09, from=min(all$T),
                to=max(all$T),
                ylim=range(all$BOD),col="red" , xlab="Peak T Fluorescence (AFU)",
ylab="BOD5 (mg/L)")


#Try a nonlinear power model ax^b
nlTpeak<-nlrob((BOD~a*T^b), data=all, start=list(a=4e7, b=3))
summary(nlTpeak)
#residual error 23.19
#model is a=7.85 b=1.78
r2T<-lm(all$BOD~fitted.values(nlTpeak))
summary(r2T)
#r2=0.8378


#Try a nonlinear power model ax^b+c
nlTpeakb<-nlrob((BOD~a*T^b+c), data=all, start=list(a=1, b=1.494, c=-15))
summary(nlTpeakb)
#residual error 23.06
#model is a=7.95, b=1.77, and c=-0.36266
r2Tb<-lm(all$BOD~fitted.values(nlTpeakb))
summary(r2Tb)
#r2=0.84


#Exponential model without c
expTa<-nlrob((BOD~a*exp(T*b)), data=all, start=list(a=1, b=1.494))
summary(expTa)
#Residuals 45.66
#Model is a=26.05 and b=0.31
r2Tc<-lm(all$BOD~fitted.values(expTa))
```

```
summary(r2Tc)
#r2=0.80

#Exponential model with c intercept
expTb <-nlrob((BOD~a*exp(T*b)+c), data=all, start=list(a=4e3, b=2e-2, c=-2.69e5))
summary(expTb)
#Residuals 24.46
#Model a=143.25, b=-0.149, c=-158.39
r2Td<-lm(all$BOD~fitted.values(expTb))
summary(r2Td)
#r2=0.84

 plot(all$BOD~all$T, xlim=range(all$T), ylim=range(all$BOD), main="Nonlinear Fit for
 Peak T", xlab="Peak T Fluorescence (AFU)", ylab="BOD5 (mg/L)"   )

   legend('topleft', c("Power model", "Power model with a constant ", "Exponential model",
 "Exponential model with a constant"), lty=c(1,1,1,1), lwd=c(1, 1, 1,1), col=c('black',
 'orange', 'red', 'green'), inset=0.02, cex=0.8)

 par(new=T)
 curve(7.85*x^1.79, from=min(all$T),
                    to=max(all$T),
                    ylim=range(all$BOD),col="black", xlab="Peak T Fluorescence (AFU)",
 ylab="BOD5 (mg/L)"  )
 par(new=T)
 curve(7.95*x^ 1.77-0.36266, from=min(all$T),
                    to=max(all$T),
                    ylim=range(all$BOD),col="orange", xlab="Peak T Fluorescence (AFU)",
 ylab="BOD5 (mg/L)"  )
 par(new=T)
 curve(26.05*exp(x*0.31), from=min(all$T),
                    to=max(all$T),
                    ylim=range(all$BOD),col="red", xlab="Peak T Fluorescence (AFU)",
 ylab="BOD5 (mg/L)"  )
 par(new=T)
 curve(143.25*exp(x*0.149)-158.39, from=min(all$T),
                    to=max(all$T),
                    ylim=range(all$BOD),col="green", xlab="Peak T Fluorescence (AFU)",
 ylab="BOD5 (mg/L)"  )


#----Robust regressions Region I-----------------------------------------------------

#Region I
rlmregionI <- rlm(BOD~RegionI,data=all)
summary(rlmregionI)
```

```
#Residual 33.79
#model a=0.0042 b=-55.6370
r2region1a<-lm(all$BOD~rlmregionI$fitted.value)
summary(r2region1a)
#r2=0.7964
coeftest(rlmregionI, df = Inf, vcov = vcovHC(rlmregionI, type = "const"))
coeftest(rlmregionI, df = Inf, vcov = vcovHC(rlmregionI, type = "HC"))
coeftest(rlmregionI, df = Inf, vcov = vcovHC(rlmregionI, type = "HC0"))
coeftest(rlmregionI, df = Inf, vcov = vcovHC(rlmregionI, type = "HC3"))
coeftest(rlmregionI, df = Inf, vcov = vcovHC(rlmregionI, type = "HC4"))

#Region III with sample sites as factor
rlmregionIb <- rlm(BOD~RegionI +factor(Location),data=all)
summary(rlmregionIb)
#Residual 14.16
#model a=0, effluent=-202.0265, influent=178.2512, intercept=210.0014
r2reg1b<-lm(all$BOD~rlmregionIb$fitted.value)
summary(r2reg1b)
#r2=0.9292
coeftest(rlmregionIb, df = Inf, vcov = vcovHC(rlmregionIb, type = "const"))
coeftest(rlmregionIb, df = Inf, vcov = vcovHC(rlmregionIb, type = "HC"))
coeftest(rlmregionIb, df = Inf, vcov = vcovHC(rlmregionIb, type = "HC0"))
coeftest(rlmregionIb, df = Inf, vcov = vcovHC(rlmregionIb, type = "HC3"))
coeftest(rlmregionIb, df = Inf, vcov = vcovHC(rlmregionIb, type = "HC4"))


plot(all$BOD~all$RegionI, xlim=range(all$RegionI), ylim=range(all$BOD),  main="Linear
 Fit for Region I", xlab="Region I Fluorescence (AFU)", ylab="BOD5 (mg/L)"  )

legend('topleft', c("Influent Regression Line (as a function of sample point)", "CABI
 Regression Line (as a function of sample point)", "Effluent (as a function of sample point)",
 "Regression model(sample site not considered) "), lty=c(1,1,1,1), lwd=c(1, 1, 1,1),
 col=c('black', 'orange', 'green', 'red'), inset=0.02, cex=0.8)


 par(new=T)
 curve(0*x+388.25, from=min(all$RegionI),
                   to=max(all$RegionI),
                   ylim=range(all$BOD),col="black" , xlab="Region I Fluorescence (AFU)",
 ylab="BOD5 (mg/L)")
 par(new=T)
 curve(0*x+210.0014, from=min(all$RegionI),
                   to=max(all$RegionI),
                   ylim=range(all$BOD),col="orange" , xlab="Region I Fluorescence (AFU)",
 ylab="BOD5 (mg/L)")
```

```
par(new=T)
curve(0*x+7.9749, from=min(all$RegionI),
                  to=max(all$RegionI),
                  ylim=range(all$BOD),col="green" , xlab="Region I Fluorescence (AFU)",
ylab="BOD5 (mg/L)")
par(new=T)
curve(0.0042*x-55.6370, from=min(all$RegionI),
                  to=max(all$RegionI),
                  ylim=range(all$BOD),col="red" , xlab="Region I Fluorescence (AFU)",
ylab="BOD5 (mg/L)")

library(robustbase)

#Try a nonlinear power model ax^b
nlreg2<-nlrob((BOD~a*RegionI^b), data=all, start=list(a=6.78e-09, b=2.2), trace=T)
summary(nlreg2)
#residual error 22.22
#model is a=7.41e-8 b=1.95
r2reg2<-lm(all$BOD~fitted.values(nlreg2))
summary(r2reg2)
#r2=0.81

#Try a nonlinear power model ax^b+c
nlregIb<-nlrob((BOD~a*RegionI^b+c), data=all, start=list(a=4.38e-5, b=1.38, c=-92),
 trace=T)
summary(nlregIb)
#Residuals 25.42
#Model a=2.28e-7, b=1.856, c=-4.79
r2reg2b<-lm(all$BOD~fitted.values(nlregIb))
summary(r2reg2b)
#r2=0.81

#Try an exponential model
expreg2a<-nlrob((BOD~a*exp(RegionI*b)), data=all, start=list(a=4, b=3e-6), trace=T)
summary(expreg2a)
#Residuals 44.56
#Model a=21.28, b=3.198e-5
r2reg2c<-lm(all$BOD~fitted.values(expreg2a))
summary(r2reg2c)

 #Try an exponential model with c
expreg2b<-nlrob((BOD~a*exp(RegionI*b)+c), data=all, start=list(a=2.3e3, b=4.4e-6, c=-
 2.5e3), trace=T)
summary(expreg2b)
#Residuals 43.53
#Model a=129.6, b=1.511e-5, c=-154.0
```

```
r2reg2d<-lm(all$BOD~fitted.values(expreg2b))
summary(r2reg2d)
#r2=0.80



plot(all$BOD~all$RegionI, xlim=range(all$RegionI), ylim=range(all$BOD) ,
 main="Nonlinear Fit for Region I", xlab="Region I Fluorescence (AFU)", ylab="BOD5
 (mg/L)"    )

   legend('topleft', c("Power model", "Power model with a constant ", "Exponential model",
 "Exponential model with a constant"), lty=c(1,1,1,1), lwd=c(1, 1, 1,1), col=c('black',
 'orange', 'red', 'green'), inset=0.02, cex=0.8)


  par(new=T)
 curve(7.41e-8*x^1.95, from=min(all$RegionI),
                     to=max(all$RegionI),
                     ylim=range(all$BOD),col="black", xlab="Region I Fluorescence (AFU)",
 ylab="BOD5 (mg/L)")
  par(new=T)
 curve(2.28e-7*x^1.856-4.79, from=min(all$RegionI),
                     to=max(all$RegionI),
                     ylim=range(all$BOD),col="orange", xlab="Region I Fluorescence (AFU)",
 ylab="BOD5 (mg/L)")
 par(new=T)
 curve(21.28*exp(x*3.198e-5), from=min(all$RegionI),
                     to=max(all$RegionI),
                     ylim=range(all$BOD),col="red", xlab="Region I Fluorescence (AFU)",
 ylab="BOD5 (mg/L)")
 par(new=T)
 curve(1.296e2*exp(x*1.511e-5)-1.540e2, from=min(all$RegionI),
                     to=max(all$RegionI),
                     ylim=range(all$BOD),col="green", xlab="Region I Fluorescence (AFU)",
 ylab="BOD5 (mg/L)")
```

G.4 BOD and Absorbance Models

```
#importdata
library(XLConnect)
abs=loadWorkbook("absorbancedata.xlsx")
influent=readWorksheet(abs, sheet="influent")
cabi=readWorksheet(abs, sheet="cabi")
influentcabi=readWorksheet(abs, sheet="influentcabi")
effluent=readWorksheet(abs, sheet="effluent")
all=readWorksheet(abs, sheet="all")


#Plot data
library(ggplot2)
qplot(Abs254,BOD, main= "BOD and Absorbance (254 nm) data for all sample sites",
 ylab="BOD5 (mg/L)", xlab="Absorbance at 254 nm (AU)", colour = Location, shape =
 Location, data = all)

qplot(Abs254,BOD, main= "BOD and Absorbance (254 nm) data for Effluent", colour =
 Location, shape = Location, data = effluent)


#-----BOD vs 254 nm linear--------------------------------------------------------------------
#First correlation is for BOD vs 254nm absorbance

lm254<-lm(BOD~Abs254, data=all)
shapiro.test(lm254$residuals)
#p=1.713e-11, data non normal use robust regression

library(robustbase)

rlm254a <- rlm(BOD~Abs254,data=all)
summary(rlm254a)
#SE=31.93
#Model a=1458.7 and b=-155.07
lm254r2a<-lm(all$BOD~ rlm254a $fitted.values)
summary(lm254r2a)
#R2=0.77

#Try a robust linear model
rlm254b <- rlm(BOD~Abs254 + factor(Location),data=all)
summary(rlm254b)
#Residual SE 13.18
#Model is BOD=-377.8830+188.2781*Inf-261.76*Eff+310.7882
lm254r2<-lm(all$BOD~rlm254b$fitted.values)
```

```
summary(lm254r2)
#r2=0.9292

#Plot the lines for each regression model
 plot(all$BOD~all$Abs254, xlim=range(all$Abs254), ylim=range(all$BOD),  main="Linear
 Fit for Absorbance at 254 nm", xlab="Absorbance at 254 nm (AU)", ylab="BOD5 (mg/L)" )
 legend('topleft', c("Influent Regression Line (as a function of sample point)", "CABI
 Regression Line (as a function of sample point)", "Effluent (as a function of sample point)",
 "Regression model(sample site not considered) "), lty=c(1,1,1,1), lwd=c(1, 1, 1,1),
 col=c('black', 'orange', 'green', 'red'), inset=0.02, cex=0.8)
  par(new=T)
 curve(-377.8830*x+499.07, from=min(all$Abs254), to=max(all$Abs254), ylim=
 range(all$BOD),col="black", xlab="Absorbance at 254 nm (AU)", ylab="BOD5 (mg/L)"  )
 par(new=T)
 curve(-377.8830*x+310.7882, from=min(all$Abs254), to=max(all$Abs254),ylim=
 range(all$BOD), col="orange", xlab="Absorbance at 254 nm (AU)", ylab="BOD5 (mg/L)"
 )

par(new=T)
curve(-377.8830*x+49.03, from=min(all$Abs254), to=max(all$Abs254), ylim=
 range(all$BOD), col="green", xlab="Absorbance at 254 nm (AU)", ylab="BOD5 (mg/L)"  )

  par(new=T)
 curve(1458.67*x-155.07, from=min(all$Abs254),  to=max(all$Abs254), ylim=
 range(all$BOD), col="red", xlab="Absorbance at 254 nm (AU)", ylab="BOD5 (mg/L)"  )

#Try a nonlinear power model ax^b
nlabs254<-nlrob((BOD~a*Abs254^b), data=all, start=list(a=4e7, b=3))
summary(nlabs254)
#residual error 26.14
#model is a=8533.16 b=2.80
r2nl254<-lm(all$BOD~fitted.values(nlabs254))
summary(r2nl254)
#r2=0.7624

#Try a nonlinear power model ax^b+c
nlabs254b<-nlrob((BOD~a*Abs254^b+c), data=all, start=list(a=4000, b=3, c=100))
summary(nlabs254b)
#residual error 22.07
#model is a=4090.4313 b=2.1087 c=-31.6428
r2nl254b<-lm(all$BOD~fitted.values(nlabs254b))
summary(r2nl254b)

#Try an exponential regression model ae^xb
nlabs254exp<-nlrob((BOD~a*exp(Abs254*b)), data=all, start=list(a=0.8, b=20))
summary(nlabs254exp)
```

```
#Residual error 44.02
#Model is a=11.34 and b=9.73
r2exp254<-lm(all$BOD~fitted.values(nlabs254exp))
summary(r2exp254)
#r2=0.7289

#Try an exponential regression model ae^xb+c
nlabs254expb<-nlrob((BOD~a*exp(Abs254*b)+c ), data=all, start=list(a=0.8, b=20, c=10))
summary(nlabs254expb)
#Residual error 22.33
#Model is a=120.41 and b=4.63 and c=-193.20
r2exp254b<-lm(all$BOD~fitted.values(nlabs254expb))
summary(r2exp254b)
#r2=0.771

#Plot the lines for each regression model
 plot(all$BOD~all$Abs254, xlim=range(all$Abs254), ylim=range(all$BOD) ,
  main="Nonlinear Fit for Absorbance at 254 nm", xlab="Absorbance at 254 nm (AU)",
  ylab="BOD5 (mg/L)" )
    legend('topleft', c("Power model", "Power model with a constant ", "Exponential model",
  "Exponential model with a constant"), lty=c(1,1,1,1), lwd=c(1, 1, 1,1), col=c('black',
  'orange', 'red', 'green'), inset=0.02, cex=0.8)
 par(new=T)
 curve(8533*x^2.80, from=min(all$Abs254), to=max(all$Abs254),
  ylim=range(all$BOD),col="black", xlab="Absorbance at 254 nm (AU)", ylab="BOD5
  (mg/L)")
 par(new=T)
 curve(4090*x^2.11-31.64, from=min(all$Abs254), to=max(all$Abs254),
  ylim=range(all$BOD), col="orange", xlab="Absorbance at 254 nm (AU)", ylab="BOD5
  (mg/L)")
par(new=T)
curve(9.73*exp(11.34*x), from=min(all$Abs254), to=max(all$Abs254),
  ylim=range(all$BOD), col="red", xlab="Absorbance at 254 nm (AU)", ylab="BOD5
  (mg/L)")
par(new=T)
curve(120.41*exp(4.63*x)-193.2, from=min(all$Abs254, to=max(all$Abs254),
  ylim=range(all$BOD), col="green", xlab="Absorbance at 254 nm (AU)", ylab="BOD5
  (mg/L
```

```
#-------BOD Abs340 correlations----------------------------------------------


lm340 <- rlm(BOD~Abs340 + factor(Location),data=all)
shapiro.test(lm340$residuals)
#nonnormal data, use robust regression p<2.2e-16


#Try a linear model
rlm340a <- rlm(BOD~Abs340,data=all)
summary(rlm340a)
#Residual SE 31.72
#Model is BOD=7094.19x-211.3323
lm340r2a<-lm(all$BOD~ rlm340a $fitted.values)
summary(lm340r2a)
#r2=0.78


#Try a robust linear model with factor
rlm340 <- rlm(BOD~Abs340 + factor(Location),data=all)
summary(rlm340)
#Residual SE 14.35
#Model is BOD=-1124.32*Abs+189.94*Inf-234.56*eff +276.80
lm340r2<-lm(all$BOD~rlm340$fitted.values)
summary(lm340r2)
#r2=0.9292


#Plot the lines for each line regression line
 plot(all$BOD~all$Abs340, xlim=range(all$Abs340), ylim=range(all$BOD),  main="Linear
 Fit for Absorbance at 340 nm", xlab="Absorbance at 340 nm (AU)", ylab="BOD5 (mg/L)"}
 legend('topleft', c("Influent Regression Line (as a function of sample point)", "CABI
 Regression Line (as a function of sample point)", "Effluent (as a function of sample point)",
 "Regression model(sample site not considered) "), lty=c(1,1,1,1), lwd=c(1, 1, 1,1),
 col=c('black', 'orange', 'green', 'red'), inset=0.02, cex=0.8)
  par(new=T)
 curve(-1124*x+466.74, from=min(all$Abs340), to=max(all$Abs340),
 ylim=range(all$BOD), col="black", xlab="Absorbance at 340 nm (AU)", ylab="BOD5
 (mg/L)" )
  par(new=T)
  curve(-1124*x+276.80, from=min(all$Abs340), to=max(all$Abs340),
 ylim=range(all$BOD), col="orange", xlab="Absorbance at 340 nm (AU)", ylab="BOD5
 (mg/L)" )

     par(new=T)
 curve(-1124*x+42.24, from=min(all$Abs340), to=max(all$Abs340), ylim=range(all$BOD),
```

```
 col="green", xlab="Absorbance at 340 nm (AU)", ylab="BOD5 (mg/L)" )
    par(new=T)
 curve(7094*x-211, from=min(all$Abs340), to=max(all$Abs340), ylim=range(all$BOD),
 col="red", xlab="Absorbance at 340 nm (AU)", ylab="BOD5 (mg/L)" )


#Try a nonlinear power model ax^b
nlabs340<-nlrob((BOD~a*Abs340^b), data=all, start=list(a=4e6, b=3))
summary(nlabs340)
#residual error 50.23
#model is a=19550 b=2.46
r2nl340<-lm(all$BOD~fitted.values(nlabs340))
summary(r2nl340)
#r2=0.7321

#Try a nonlinear power model ax^b+c
nlabs340b<-nlrob((BOD~a*Abs340^b+c), data=all, start=list(a=400000, b=3, c=1))
summary(nlabs340b)
#residual error 31.25
#model is a=7641.95 b=1.04 c=-199.61
r2nl340b<-lm(all$BOD~fitted.values(nlabs340b))
summary(r2nl340b)
#r2=0.7786

#Try an exponential regression model ae^xb
nlabs340exp<-nlrob((BOD~a*exp(Abs340*b)), data=all, start=list(a=0.8, b=20))
summary(nlabs340exp)
#Residual error 76.89
#Model is a=17.66 and b=39.14
r2exp340<-lm(all$BOD~fitted.values(nlabs340exp))
summary(r2exp340)
#r2=0.662

#Try an exponential regression model ae^xb+c
nlabs340exp<-nlrob((BOD~a*exp(Abs340*b)+c), data=all, start=list(a=-6442, b=-1.17,
 c=6220), trace=T)
summary(nlabs340exp)
#Residual 32.25
#Model a=-8870.5, b=-0.835, c=8652.3
r2exp340b<-lm(all$BOD~fitted.values(nlabs340exp))
summary(r2exp340b)

#Plot the lines for each regression model
 plot(all$BOD~all$Abs340, xlim=range(all$Abs340), ylim=range(all$BOD) ,
 main="Nonlinear Fit for Absorbance at 340 nm", xlab="Absorbance at 340 nm (AU)",
 ylab="BOD5 (mg/L)" )
```

```
   legend('topleft', c("Power model", "Power model with a constant ", "Exponential model",
 "Exponential model with a constant"), lty=c(1,1,1,1), lwd=c(1, 1, 1,1), col=c('black',
 'orange', 'red', 'green'), inset=0.02, cex=0.8)
 par(new=T)
 curve(19550*x^2.46, from=min(all$Abs340),  to=max(all$Abs340), ylim= range(all$BOD),
 col="black", xlab="Absorbance at 340 nm (AU)", ylab="BOD5 (mg/L)" )
 par(new=T)
 curve(7641*x^1.04-199.61, from=min(all$Abs340), to=max(all$Abs340), ylim=
 range(all$BOD), col="orange", xlab="Absorbance at 340 nm (AU)", ylab="BOD5 (mg/L)")
par(new=T)
curve(17.66*exp(39.14*x), from=min(all$Abs340),   to=max(all$Abs340), ylim=
 range(all$BOD), col="red", xlab="Absorbance at 340 nm (AU)", ylab="BOD5 (mg/L)")
par(new=T)
curve(-8870.5*exp(-0.835*x)+8652.3, from=min(all$Abs340), to=max(all$Abs340),
ylim=range(all$BOD), col="green", xlab="Absorbance at 340 nm (AU)", ylab="BOD5
 (mg/L)" )
```

G.5 Removal Efficiency (Example for absorbance at 340 nm)

```
#Removal efficiency was calculated in Excel worksheet and R was used to calculate
bootstrap samples and summary statstics
library(XLConnect)
removals=loadWorkbook("difference.xlsx")
abs340=readWorksheet(removals, sheet="removalAbs340")

primary.mean <- vector()
basin.mean <- vector()
plant.mean <- vector()
x1 <- vector()
x2 <- vector()
x3 <- vector()

B <- 1000  # number of bootstrap runs
for (i in 1:B)
{
x1 <- sample(abs340 $abs340primary,replace=TRUE)
x2 <- sample(abs340 $abs340basin,replace=TRUE)
x3 <- sample(abs340 $abs340plant,replace=TRUE)
primary.mean[i] <- mean(x1)
basin.mean[i] <- mean(x2)
plant.mean[i] <- mean(x3)
}
Primary.mean <- mean(primary.mean)
Basin.mean <- mean(basin.mean)
Plant.mean <- mean(plant.mean)

Primary.sd <- sd(primary.mean)
Basin.sd <- sd(basin.mean)
Plant.sd <- sd(plant.mean)

# Approximate 95% CIs
PrimaryL95 <- Primary.mean - 1.96*Primary.sd
PrimaryU95 <- Primary.mean + 1.96*Primary.sd

BasinL95 <- Basin.mean - 1.96*Basin.sd
BasinU95 <- Basin.mean + 1.96*Basin.sd

PlantL95 <- Plant.mean - 1.96*Plant.sd
PlantU95<- Plant.mean + 1.96*Plant.sd
```

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