Biological Treatment of a Synthetic Dye Water and an Industrial Textile Wastewater Containing Azo Dye Compounds

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ABSTRACT

In this research, the ability of anaerobic and aerobic biological sludges to reduce and stabilize azo dye compounds was studied. Synthetic dye solutions and an industrial textile wastewater were both treated using anaerobic and aerobic biomass, separately and in sequential step-treatment processes. The primary objective was to reduce the wastewater color to an intensity that complies with the Virginia Pollutant Discharge Elimination System (VPDES) permit level. This level is set at 300 American Dye Manufactures Institute (ADMI) units. Further objectives were to achieve reductions in the total kjehdal nitrogen (TKN) and total organic carbon (TOC) in the wastewater. Anaerobic and aerobic treatment systems were both effective in reducing the wastewater color; however, anaerobic treatment generally produced the greatest color removal. Anaerobic/aerobic (ANA/AER) sequential step-treatment provided the best reductions in ADMI color, TKN and TOC. Anaerobic/aerobic/anaerobic/aerobic (ANA/AER/ANA/AER) sequential step-treatment did not yield greater reductions in ADMI color, TKN, or TOC as compared to ANA/AER sequential step-treatment.

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CHAPTER 1: INTRODUCTION

Azo dyes are used by a wide number of industries. While textile mills predominantly use them, azo dyes can also be found in the food, pharmaceutical, paper and printing, leather, and cosmetics industries. It is not surprising that these compounds have become a major environmental concern. Many of these dyes find their way into the environment via wastewater facilities. Because these compounds retain their color and structural integrity under exposure to sunlight, soil, bacteria and sweat, they also exhibit a high resistance to microbial degradation in wastewater treatment systems.

There is a continual demand to develop longer lasting, more applicable dyes. Azo dyes are second only to polymers in terms of the number of new compounds submitted for registration in the U.S. under the Toxic Substance Control Act (TSCA) (Brown and DeVito, 1993). The development of synthetic fabrics such as nylon, lycra, rayon, and polyester has required the production of new dyes that can effectively bond to these materials. The U.S. Department of Commerce has predicted a 3.5 fold increase in textile manufacturing between 1975 and 2020 (Ganesh, 1992; Walsh *et al.*, 1980). Azo dyes must be continually updated to produce colors that reflect the trends dictated by changing social ideas and styles. Brighter, longer lasting colors are often necessary to satisfy this demand.

Effective and economic treatment of a diversity of effluents containing azo has become a problem. No single treatment system is adequate for degrading the various dye structures. Currently, much research has been focused on chemically and physically degrading azo dyes in wastewaters. These methods include chemical oxidation, which uses strong oxidizers such as hydrogen peroxide or chlorine dioxide. Chemical oxidation is sometimes coupled with UV light exposure to increase the color removal. Other techniques involve electrochemical or wet oxidation, activated carbon adsorption, reverse osmosis, or coagulation/flocculation (Edwards, 2000). Many of these technologies are cost prohibitive, however, and therefore are not viable options for treating large waste streams.

Because of their recalcitrant nature, azo dyes often pass through activated sludge facilities with little or no reduction in color (Cariell *et al.* 1995; 1996; Pagga and Brown, 1986). Although some researchers have observed slight color reductions, their findings

are largely outweighed by those who have not (Loyd, 1992; Zissi *et al.* 1997). Reductions in the carbon content and oxygen demand of azo dye wastewaters following aerobic treatment are well cited (McCurdy, 1991 *as cited by* Horning 1977; Loyd, 1992; Pagga and Brown, 1986).

The anaerobic reduction of azo dyes to simpler compounds has been well researched (Chinwetkitvanich *et al.*, 2000; Razo-Flores *et al.* 1997; Brown and Laboureur, 1983; Chung *et al.*, 1978). These, and other studies, have all demonstrated the ability of anaerobic microbes and sludges to effectively reduce azo dyes to their intermediate structures, thus destroying the apparent color. Many of these intermediates are aromatic amines with constituent side groups. By reducing the dye compounds to their intermediates, the problem of aesthetic pollution is solved, but a larger and more deleterious problem may be created. Most azo dyes are non-toxic, but a higher percentage of their intermediates have been identified as carcinogens (Brown and DeVito, 1993). Because of the toxic potential of many aromatic amines, further degradation of the dye compound is necessary if toxicity is to be eliminated or reduced (Brown and DeVito, 1993; Levine, 1991).

The color concentration of a wastewater is often measured in American Dye Manufactures Institute (ADMI) units. In the Commonwealth of Virginia, the permit level for effluent discharges is 300 ADMI units. This level is set by the Virginia Pollutant Discharge Elimination System (VPDES). Not all wastewater treatment plants (WWTP) are able to continually comply with this requirement and are subject to fines.

One such facility is located near Martinsville, Virginia bordering the Lower Smith River in Henry County. This publicly owned treatment works (POTW) facility receives a large portion of its waste, nearly 80 percent, from a nearby textile mill. (The Lower Smith River treatment facility will be referred to as the POTW in following chapters.) The remaining 20 percent is composed of municipal wastewater. The average daily inflow is approximately 5.0 MGD and the ADMI color values range from 800-2000 units. Depending on the influent characteristics, chemical polymers may be added to the waste stream before primary clarification. These polymers bind with the untreated dye compounds and facilitate their removal by coagulation and settling. The treatment plant is composed of two open-air activated sludge basins that are mixed with large,

mechanical, surface aerators. Solids are removed in a clarifying chamber, before which polymer may or may not be added to the waste stream. Finally, the wastewater is passed through a chlorine contact tank and discharged into the Lower Smith River.

The primary objective of this study was to determine the best way to increase the color-reducing efficiency of the POTW without substantially affecting the long-term and short-term operating costs. Edwards (2000) conducted a study using chemical oxidation to reduce the color of the POTW effluent and other synthetic solutions containing several of the dyes found in POTW influent. Based on her findings and the preliminary results from this research, it was determined that the commercial dye Cypress Green had an ADMI color removal rate similar to the POTW influent. The study described herein investigated the following:

- degradation of Indigo Blue, Sultan Red, and Cypress Green azo dyes using biomass from the Henry County POTW
- degradation of Cypress Green dye in an anaerobic and an aerobic treatment system.
- degradation of Cypress Green dye in an anaerobic/aerobic (ANA/AER) sequential step-treatment system.
- degradation of Cypress Green dye in an anaerobic/aerobic/anaerobic/aerobic (ANA/AER/ANA/AER) sequential step-treatment system.
- degradation of the POTW influent by the method that yielded the greatest color reductions during the four evaluations listed above.

CHAPTER 2: LITERATURE REVIEW

Introduction

The treatment of textile effluents is of interest due to their toxic and esthetic impacts on receiving waters. While much research has been preformed to develop effective treatment technologies for wastewaters containing azo dyes, no single solution has been satisfactory for remediating the broad diversity of textile wastes. Human and ecological health concerns have prompted the government to require textile effluent discharges to have increasingly lower color and nitrogen levels. Despite being aware of the problem, many textile manufactures have failed to adequately remove azo dye compounds from their wastewaters. Until dye and textile manufactures are able to develop efficient technologies, allowing for increased dye-fiber bonding and lower dyehouse losses (Lewis, 1999), the problem of treating these types of wastes will fall to the wastewater treatment facilities.

This chapter will focus on the current problem created by textile effluents and azo dyes. More specifically, fiber-reactive azo dyes will be explored and effective techniques for degrading these types of compounds will be described. Because of their chemical properties, the health concerns related to azo dyes will be briefly discussed. Finally, several factors that may affect azo dye degradation in biological systems will be reviewed.

Understanding the basic composition of azo dyes and specifically fiber-reactive azo dyes is necessary to envision how these molecules can be destroyed. By understanding the general chemical structure of these compounds, consideration can be given to the toxic potential that they pose to the environment. To ensure the safety of effluents, proper technologies need to be used by treatment facilities when degrading fiber-reactive azo dyes. Previous research efforts have focused on various biological, chemical, and physical techniques for treating azo dye wastes. There is evidence that all three areas have potential for remediating dyehouse wastes. However, chemical treatment is often cost and application limited, while physical removal can lead to extra solid wastes and increased overhead. Biological treatment has been effective in reducing dyehouse effluents, and when used properly has a lower operating cost than other

remediation processes. Combinations of chemical and biological or physical and biological treatment have also proven to be effective. Because this research explores the use of microbial sludges for the treatment of a textile effluent, the focus of this literature review will be on biological techniques used for degrading wastewaters containing azo dyes.

Azo Dyes and Intermediates

Azo dyes contain a least one nitrogen-nitrogen (N=N) double bond, however many different structures are possible. (Zollinger, 1991). Monoazo dyes have only one N=N double bond, while diazo and triazo dyes contain two and three N=N double bonds, respectively. The azo groups are generally connected to benzene and naphthalene rings, but can also be attached to aromatic heterocycles or enolizable aliphatic groups (Zollinger, 1991). These side groups are necessary for imparting the color of the dye, with many different shades and intensities being possible (McCurdy, 1992). A common example of an azo dye can be seen in Figure 1. When describing a dye molecule, nucleophiles are referred to as *auxochromes*, while the aromatic groups are called *chromophores*. Together, the dye molecule is often described as a *chromogen*. The absorption and reflection of visible and UV irradiation is ultimately responsible for the observed color of the dye (Zollinger, 1991).

Synthesis of most azo dyes involves diazotization of a primary aromatic amine, followed by coupling with one or more nucleophiles. Amino- and hydroxy- groups are commonly used coupling components (Zollinger, 1991). Because of the diversity of dye components available for synthesis, a large number of structurally different azo dyes exist and are used in industry (McCurdy, 1991). World wide production of organic dyes is currently estimated at nearly 450,000 tons, with 50,000 tons being lost in effluents during application and manufacture (Lewis, 1999).

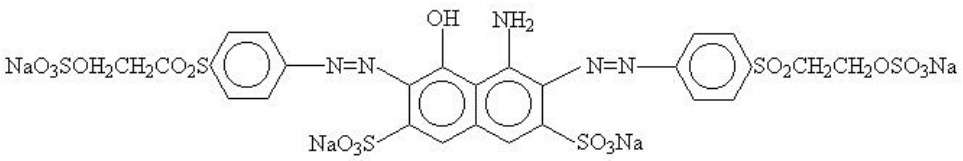
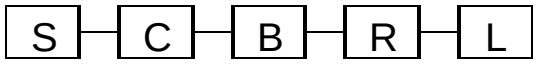


Figure 1: Example of an azo dye structure (Remazol Black 5).

There are a number of different classes of azo dyes, but this study will focus only on fiber-reactive azo dyes. Eighty to ninety-five percent of all reactive dyes are based on the azo chromogen (Zollinger, 1991; Edwards, 2000 *as cited by* Shore, 1990). Reactive dyes are colored compounds that contain one or two functional groups capable of forming covalent bonds with the active sites in fibers. A carbon or phosphorous atom of the dye molecule will bond to hydroxyl groups in cellulose, amino, thiol, and hydroxyl groups in wool, or amino groups in polyamides (Zollinger, 1991; Kirk-Othmer, 1979). Most fiber-reactive azo dyes are used for dyeing cellulosic materials, such as cotton, and are a major source of dye waste in textile effluents. Between 20-50 percent of the reactive dye used by the textile industry is lost in exhaust and wash water (Lewis, 1999).

Fiber-reactive azo dyes exhibit a high wet-fastness, due to their ability to covalently bond to substrates. However, dyes that hydrolyze in solution prior to bonding to a substrate are often lost in the washing processes (Loyd, 1992). Schematically, fiber-reactive azo dyes can be viewed as follows:



where:

S = Water solubilizing group
C = Chromogen
B = Bringing group (can be part of chromogen)
R = Reactive group
L = Fiber reactive or leaving group

The bridging group serves to combine the chromogen with the reactive group of the dye molecule. The bridging group must be stable, soluble in water, and exhibit a certain degree of flexibility. Amino and alkylamino groups are generally used for this purpose. The reactive group serves to bond the dye molecule to a substrate via nucleophilic substitution or addtion (Kirk-Othmer, 1979). Mono-, di-, and trichlorotriazinyl are all examples of reactive functional groups. Approximately 200 different reactive groups are listed in the patent literature (Zollinger, 1991). A common example of a fiber-reactive azo dye can be seen in Figure 2. Twenty-two new reactive azo dye structures were submitted for patent in early 1998, which was nearly five times as many from other

classes of azo dyes (Freeman and Sokolowska, 1999). With such a disproportionate production rate, it is not surprising that a large percentage of dye pollution problems are related to fiber-reactive azo dyes.

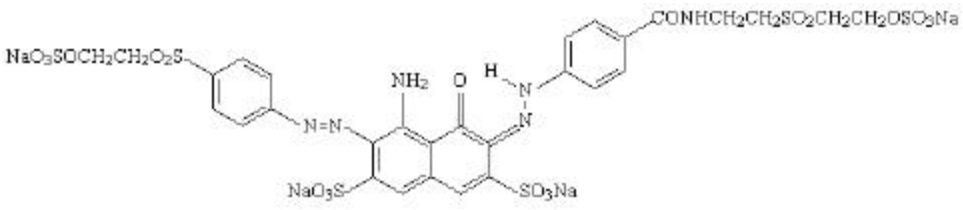


Figure 2: Example of a fiber reactive azo dye (C.I. Reactive Blue 238).

Toxicity Considerations

While this study does not directly address the problem of toxicity created by the release and degradation of azo dyes, consideration of this problem is still warranted. The potential for toxic effects to the environment and humans, resulting form the exposure to dyes and dye metabolites, is not a new concern. As early as 1895 increased rates in bladder cancer were observed in workers involved in dye manufacturing (Rehn, 1895). Since that time, many studies have been conducted showing the toxic potential of azo dyes. A complete review of these studies is beyond the scope of this paper; however, a broader understanding of the problem can be found in the works of Brown and DeVito (1993) as well as Levine (1991). Both papers indicate that the problem associated with azo dyes is created by the dye metabolites.

As mentioned previously, azo dyes are primarily composed of aromatic amines. Substituted benzene and naphthalene rings are common constituents of azo dyes, and have been identified as potentially carcinogenic agents (IARC, 1982). While most azo dyes themselves are non-toxic a significantly larger portion of their metabolites are (Ganesh, 1992). An investigation of several hundred commercial textile samples revealed that nearly 10 percent were mutagenic in the Ames test (McCarthy, 1997). Another study conducted on 45 combined effluents from textile finishing plants showed that 27 percent of the wastewater samples were mutagenic in the Ames test (McCarthy, 1997 *as cited in* Jager, 1996).

Most dyes that have been shown to be carcinogenic are no longer used; however, a complete investigation of all dyestuffs is impossible (Brown and DeVito, 1993). Other concerns are the impurities within commercial dye products and the additives used during the dyeing process. Many textile effluents contain heavy metals that are complexed in the azo dyes. High concentrations of salt are often used to force fiber-reactive dyes out of solution and onto substrates (Zollinger, 1991). These compounds can cause high electrolyte and conductivity concentrations in the dye wastewater, leading to acute and chronic toxicity problems.

Understanding the dye structures and how they are degraded is crucial to understanding how toxic by-products are created. Brown and DeVito (1993) have compiled a three-part list of the biological mechanisms thought to be responsible for carcinogenic activation of azo dye compounds. This list is based on an extensive review of the literature regarding azo dye toxicity, and places each mechanism in order of their frequency of citation. Brown and DeVito (1993) postulate that:

- Azo dyes may be toxic only after reduction and cleavage of the azo linkage, producing aromatic amines.
- Azo dyes with structures containing free aromatic amine groups that can be metabolically oxidized without azo reduction may cause toxicity.
- Azo dye toxic activation may occur following direct oxidation of the azo linkage producing highly reactive electrophilic diazonium salts.

An example of a reductive pathway that is mediated by intestinal anaerobic bacteria and leads to the formation of benzidines is shown in Figure 3 (Brown and DeVito, 1993). Similar reactions and by-products would be possible in an anaerobic treatment system. Further examples of toxic aromatic amines, which could be created from the degradation of azo dye compounds, can be seen in Table 1.

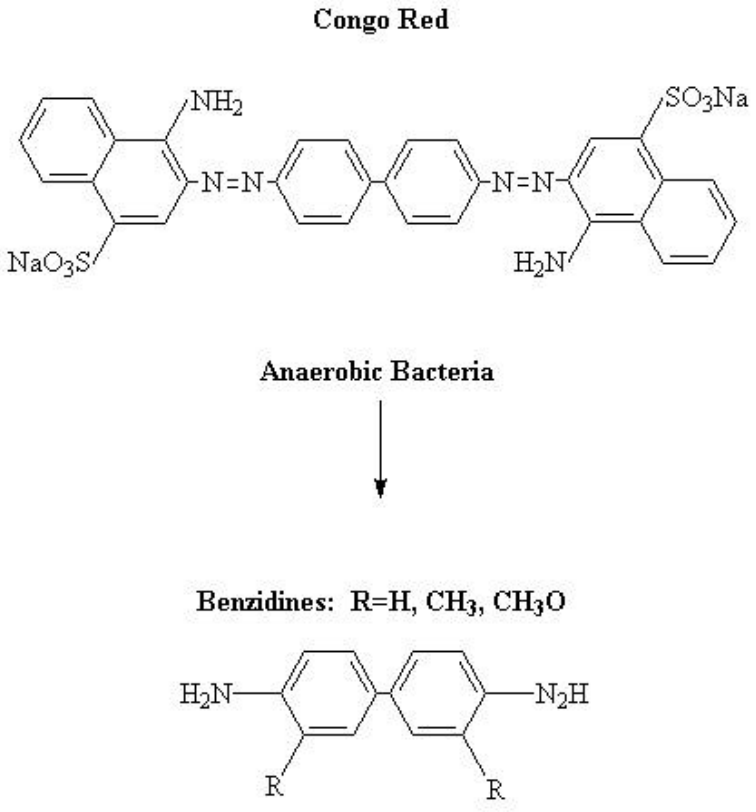


Figure 3: Example of azo dye reduction via anaerobic bacterium (Brown and Devito, 1993).

Aromatic Amine Group	Human Carcinogen Evidence
1-Napthylamine	Slight/Mixed
2-Napthylamine	Good
2,5-Diamiotoluene	Slight
3,3'-Dichlorobenzidine	Slight/Mixed
3,3'-Dimethoxybenzidine	Slight/Mixed
3,3'-Dimethylbenzidine	Slight
4-Biphenylamine	Good
4-Nitrobiphenyl	Slight/Mixed
4,4'-Methylenebis(2-chloroaniline)	Slight
Auramine	Slight
Benzidine	Good
Magenta	Slight
N-Phenyl-2-napthylamine	Some
N,N-Bis(2-chloroethyl)-napthylamine	Good

Table 1: Aromatic amines, and potential dye metabolites, that may be considered human carcinogens based on supporting evidence (Brown and Devito, 1993 *as cited by* Cartwright, 1983).

Anaerobic Treatment

Anaerobic reduction of azo dyes using microbial sludges can be an effective and economic treatment process for removing color from dyehouse effluents. Previous studies have demonstrated the ability of anaerobic bacteria to reductively cleave the azo linkages in reactive dyes (Chinwetkitvanich, 2000; Razo-Flores *et al.*, 1997; Loyd, 1992, Ganesh, 1992; Brown and Hamburger, 1987; Brown and Laboureur, 1983, Chung *et al.*, 1978). Although this effectively alters the chromogen and destroys the observed color of the dye, many aromatic groups are not susceptible to anaerobic reduction. However, there is evidence that some azo dye metabolites may be fully stabilized in anaerobic environments (Razo-Flores, 1997; Weber and Wolfe, 1987).

Chung *et al.* (1978) conducted a study measuring the degradability of seven azo dyes using intestinal and other major anaerobes. The studies were carried out using isolated strains of bacterium in suspended cell mediums containing the different azo dyes. Although the dyes studied were not fiber-reactive dyes, their findings showed that the reduction of azo compounds could be accomplished by intestinal and other major anaerobes. Furthermore, the presence of aromatic intermediates was also detected in measurable amounts for each dye. Toxicity tests were not conducted, but some of the intermediates had been previously determined to be mutagenic.

In a three-part research series, Brown *et al.* (1983, 1986, 1987) studied the degradability of various azo dyes in both anaerobic and aerobic systems. In the first study, Brown and Laboureur (1983) investigated the anaerobic degradability of 22 commercial dyes. Of the dyes studied, four monoazo and six diazo dyes showed substantial biodegradation, while two polyazo dyes showed moderate to variable reductions.

Later in 1987, Brown and Hamburger conducted a study on 14 azo dyes subjected to anaerobic sludge digestion followed by aerobic treatment. This project focused on both the reduction of the dye molecules as well as the production and subsequent degradation of dye metabolites. Brown and Hamburger's results confirmed the findings from earlier research, showing decolorization of the azo dyes. Confirming the cleavage of azo linkages, the production of metabolites was also observed, but at less than theoretical concentrations. Speculation was made as to why these concentrations were

low, but no conclusive evidence was provided. They do show that, based on the yield of metabolites, further dye reduction in anaerobic environments is in general, neither rapid nor appreciable.

More recently, Razo-Flores *et al*. (1997) investigated the fate of Mordant Orange 1 (MO1) and Azodisalicylate (ADS) under methanogenic conditions using continuous upflow-anaerobic-sludge-blanket (UASB) reactors. Their research focused on the reduction by-products, 5-aminosalicylic acid (5-ASA) and 1,4-phenylenediamine. Cosubstrates, VFA or glucose, were also fed to the reactors in order to supply the reducing equivalents needed for the reduction of the azo bonds. The results of this study demonstrated the ability of an anaerobic consortium to completely mineralize some azo dye compounds. The experiments were conducted using two reactor series. In the first reactor, only MO1 and VFA were fed for a 217-day period. Data from this period showed high decolorization, and high concentrations of 5-ASA and 1,4-phenylenediamine. In the second reactor, MO1 was fed for 217 days, which was followed by ADS, with and without glucose, for a 340-day period. Data from this reactor showed high decolorization throughout testing, but a lower concentration of 5-ASA. Razo-Flores *et al*. (1997) observed the complete mineralization of ADS with and without a cosubstrate, indicating the possibility for aromatic amine destruction in methanogenic environments. The compound, 1,4-phenylenediamine was not observed to degrade in either test reactor, indicating the specificity of aromatic amine utilization by anaerobes.

In studies conducted by Loyd (1992) and Ganesh (1992), the anaerobic reduction of textile mill effluents and the azo dyes Reactive Black 5 and Navy 106 were investigated, respectively. In both cases, laboratory scale anaerobic reactors were used for dye degradation. The results of Loyd and Ganesh were similar; both observed good decolorization with minimal nutrient removal. These findings concur with many studies found in the literature. While high decolorization of textile effluents is often achieved in anaerobic environments, poor TOC and nitrogen removals are usually observed.

Chinwetkitvanich *et al*. (2000) performed a study on various reactive dyebath effluents. The research examined the effect of co-substrate and initial color concentrations on fiber-reactive dye reduction efficiencies in UASB reactors. Five different experiments were conducted using a variation of red, blue, and black dye

synthetic wastewaters and also real dyehouse effluents composed of red, blue, and black dyes. Their results showed that by adding a cosubstrate, such as tapioca, increased reduction efficiencies could be achieved. However, at high levels of tapioca addition no enhancement was observed. Furthermore, Chinwetkitvanich *et al*. (2000) concluded that higher initial color concentrations might be deleterious to acid forming bacteria, resulting in a lower dye removal. Additionally, the authors suggest that sulfate-reducing bacteria might out-compete other anaerobic microorganisms for available organic carbon, but contribute minimally to decolorization. This could serve to limit the reduction equivalents necessary for dye degradation.

Aerobic Treatment

Conventional activated sludge treatment of wastes is often an effective and highly economic system for reducing organic pollutants in wastewater. A fair amount of research has been conducted assessing the viability of using activated sludge to treat textile effluents (Zissi *et al.*, 1997; Loyd, 1992; Shaul *et al*., 1991; Pagga and Brown, 1986). However, aerobic treatment of azo dye wastes has proven ineffective in most cases, but is often the typical method of treatment used today (Edwards, 2000 *citing* Yang *et al*., 1998). Because aerobic microbes cannot reduce azo linkages, their ability to destroy dye chromogens is less than anaerobic bacterium. However, aerobic sludges have been successfully used to stabilize dye metabolites (Brown and Laboureur, 1983).

Pagga and Brown (1983) conducted a study on 87 commercial dyesfuffs. Some of the dyestuffs were in a technically pure form, while others were in a sales form containing organic substances such as wetting agents. The exact class of each dye was not given. The tests were performed in a reactor designed to simulate the conditions of and adapted activated sludge wastewater treatment plant. The samples were tested for color and DOC removal following 42 days of treatment. Pagga and Brown concluded that “as expected from their structures and function, dyestuffs are most unlikely to [biodegrade] in short-term aerobic tests". They further indicate that the primary mechanism for removal of dyes in activated sludge systems may occur by adsorption onto the cell walls. Also, they concluded that DOC removal is possible in an aerobic environment, but does not always correlate with decolorization. Degradation of non-dye

molecules in the dye solution may be responsible for this reduction, as destruction of the chromogen is not generally observed.

In an earlier study by Brown and Laboureur (1983b), the aerobic biodegradability of aniline, o-toludine, p-anisidine, p-phenetidine, o-dianisidine, and 3,3'-dichlorobenzidine, was investigated. These compounds are all lipophilic aromatic amines and possible by-products of azo dyes. Because many aromatic structures are non-biodegradable in anaerobic environments and are not hydrophilic, they can accumulate in the adipose tissues of organisms. Many aromatics have been identified as possible carcinogens, which make their release into the environment a concern. Previous work by Brown and Laboureur (1983a) indicated that azo dyes may be broken down to their intermediate structures in a reductive environment, but were not amenable to further degradation by anaerobes.

Brown and Laboureur (1983b) concluded that aniline, p-anisidine, p-phenetidine and o-toludine were readily biodegradable by aerobes, while o-dianisidine and 3,3'-dichlorobenzidine were inherently biodegradable. They suggested that these compounds could be stabilized if released into the environment or directly from a dyehouse into a conventional wastewater treatment plant.

Shaul *et al.* (1991) conducted a study on 18 dyes to determine their fate in the activated sludge process. Of these dyes, 15 were acid azo dyes and three were direct azo dyes. The dyes were spiked into pilot-scale treatment systems, and effluent and sludge samples were collected. High performance liquid chromatography (HPLC) was used to analyze the samples. Mass balance calculations were preformed to determine the amount of the dye in the sludge and in the effluent. Eleven of the dyes passed through the activated sludge system substantially untreated, four were significantly absorbed onto the sludge, and three were apparently biodegraded.

Loyd (1992) also performed activated sludge treatment tests on two textile wastewaters. The first was a textile dyeing and finishing process water that contained Reactive Navy 106, and the second was a municipal wastewater consisting predominately of textile effluents. Both effluents were fed to laboratory-scale activated sludge reactors. The reactor effluents were analyzed for ADMI color and TOC removal, as well as toxicity. Loyd concluded that aerobic treatment of the azo dye wastewaters provided

significant biodegradation with minimal decolorization, and the biodegradation did not include the azo dyestuffs. Again, one would presume that the removal of organic compounds such as wetting agents and other process additives occurred, thus resulting in the TOC loss. Toxicity tests resulted in LC_{50} values falling outside of the 100 percent effluent concentration standard. However, Loyd does indicate that toxicity was slightly reduced following aerobic treatment.

Finally, Zissi *et al.* (1997) investigated the biological oxidation of p-aminoazobenzene (pAAB) by *Bacillus subtilis*. This was carried out in batch experiments using a suspension medium supplemented with glucose, ammonium chloride, and pAAB under sterile conditions. Cellular growth rates and inhibition, glucose utilization, pAAB degradation, and by-product formation were observed. The results proved that *Bacillus subtilis* could cometabolize pAAB in the presence of glucose, breaking the N=N double bond and producing aniline and p-phenylenediamine. Furthermore, evidence was found that suggested pAAB was inhibitory to microbial growth, and that glucose was the growth-limiting substrate. The degradation of the dye was the direct result of an oxygen-insensitive azo reductase enzyme found to be present in the soluble fraction of the biomass. This enzyme was also synthesized independently of the presence of pAAB.

The majority of previous research suggests that aerobic biodegradation of most azo dyes is not effective. While their are certainly exceptions to the case, it would appear that conventional activated sludge systems are not adequate for treating azo dye wastewaters. Evidence does show that the aerobic biodegradation of azo dye intermediates is possible and is perhaps an effective treatment process for stabilizing these compounds after anaerobic reduction.

ANA/AER Sequential Step-Treatment

Logically, after reviewing the literature regarding the anaerobic and aerobic treatment of azo dyestuffs, studies involving both types of systems will be covered in the following section. Because anaerobic bacteria are often able to reduce the azo linkages, but are generally unable to further stabilize the dye metabolites, it would seem advantageous to follow anaerobic treatment processes with an aerobic treatment step. As

mentioned previously, aerobic organisms can oxidize aromatic ring compounds to simpler molecules. A substantial amount of research has been conducted on ANA/AER sequential step-treatment systems used for degrading textile wastewaters (O'Neill *et al.*, 2000; Fitzgerald and Bishop, 1995; Seshadri and Bishop, 1994; Loyd, 1992; Brown and Hamburger, 1987).

Brown and Hamburger (1987) conducted a study on the ultimate biodegradability of various dyestuffs. This research concluded earlier research performed by Brown and Laboureur (1983a, 1983b) and Pagga and Brown (1986). Their research goal was to identify some of the amine metabolites produced from the anaerobic reduction of various dyestuffs, and to examine the biodegradation of the dyestufffs by aerobic and anaerobic organisms. Fourteen azo dyes and two other dye types were studied using lab-scale anaerobic and activated sludge reactors. The results from the anaerobic test phase are discussed in a previous section of this paper. Metabolite production was observed following anaerobic treatment, indicating the presence of eight identifiable aromatic amines. The authors could not verify that degradation of the dye intermediates occurred during anaerobic treatment. Results from the aerobic treatment phase corresponded with the work performed by Brown and Laboureur (1983b), showing reductions in DOC for most of the dyes. Some sulphonated aromatic amines were found to be non-biodegradable during this test.

Loyd (1992) also performed ANA/AER sequential step-treatment on the two textile effluents descried in the previous reviews of his work. The results generally showed a high decolorization in the anaerobic phase with little TOC, BOD_5, or COD removal. Loyd also states that there was very little methane or carbon dioxide production in the anaerobic phase, further supporting the notion that only partial dye biodegradation took place. In the aerobic phase, the anaerobic effluent showed a higher degree of TOC removal and less decolorization. These results correlate well with the findings of Brown and Hamburger (1987), as stated by Loyd.

Seshadri and Bishop (1994) investigated the fate of azo dyes Acid-Orange 7, Acid-Orange 8, Acid-Orange 10, and Acid-Red 14 in an ANA/AER sequential step-treatment system. They used a bench-scale fluidized-bed anaerobic reactor (FBR) followed by a bench-scale activated sludge reactor as a sequenced second stage treatment

step. The focus of the study was to assess the feasibility of using an FBR for the anaerobic reduction of azo dyes, and to evaluate the effects caused by altering the hydraulic retention time (HRT), influent dye concentration, and the degree of bed fluidization on the dye removal. Their results indicated that the transformation of all the dyes to intermediates was readily achieved via anaerobic reduction, and was assumed to be the result of azo bond cleavage. Complete mineralization was not observed, however. COD and color removals were greatest at HRT's of 12 and 24 hours, with one hour being the shortest HRT tested and 24 hours the longest. However, the largest cumulative removals occurred in the first two hours of treatment. This would be expected based on the higher nutrient concentrations available for biodegradation during that time period. The Acid-Orange 10 appeared to inhibit dye removal at concentrations of 15 mg L^{-1}. The authors believed the production of aromatic amines was responsible for the toxicity, citing the work of Chakrabarti *et al* (1988). All of the other dyes tested did not exhibit inhibition at a concentration of 15 mg L^{-1}. COD removal was variable depending on the dye, but reductions were seen in both the anaerobic and aerobic phases. Aerobic oxidation of dye intermediates was necessary to decrease COD levels to an acceptable range.

Fitzgerald and Bishop (1995) used three lab-scale reactor systems to study the degradation of the azo dyes Acid-Orange 10, Acid-Red 14, and Acid-Red 18. The reactor system included an anaerobic fluidized bed system in the first stage, which was followed by an aerobic Swisher reactor in the second stage. The objective of the study was to investigate the ability of this system to completely degrade the three dyes, and to identify the metabolic intermediates resulting from anaerobic treatment. Their results indicated a high degree of degradation of the Acid Red 18 and Acid Red 14 in the anaerobic stage, with decolorization greater than 90 percent. Acid Orange 10 was only decolorized by 70 percent, however. Furthermore, analysis of the dye intermediates suggested a high degree of removal in the anaerobic stage. This is interesting, as most of the literature reports poor metabolite reduction in anaerobic environments. Low color loss and COD removal was measured in the aerobic stage. The authors do state that further investigation is necessary to verify if the dye intermediates biodegraded in the anaerobic reactor.

Recently, O'Neill *et al*. (2000) conducted a study on then reactive azo dye Procion Red H-E7B. Their research investigated the degradation of Procion Red H-E7B in an ANA/AER sequential step-treatment system comprised of a lab-scale UASB reactor and an activated sludge tank. To determine the extent of dye degradation, O'Neill *et al*. used HPLC-UV methods to detect for polar compounds in the reactor effluents. This data was then compared with color and total organic nitrogen (TON) removals. Toxicity tests were also preformed on the anaerobic effluent, using respiration-inhibition tests, to further verify if aromatic amines groups were formed. The authors observed an increase in polar, UV-absorbing groups in the anaerobic effluent as compared to the reactor feed. Furthermore, respiratory–inhibition tests showed an increase in toxicity, suggesting the presence of aromatic groups. The majority of nutrient removal occurred in the anaerobic phase, however, indicating some degradation of the dye. TON levels were observed to increase after anaerobic treatment followed by a subsequent decrease after aerobic treatment. HPLC also indicated the presence of highly polar compounds in the aerobic effluent, indicating a removal or conversion of the aromatic amine groups to simpler molecules. Based on these findings, O'Neill concluded that Procion Red H-E7B could be qualitatively shown to degrade to aromatic amine derivatives after anaerobic treatment with subsequent oxidation of these derivatives following aerobic treatment.

Factors Affecting Dye Biodegradation

Due to the highly variable nature of biological treatment systems and especially textile effluents, there are a number of factors that may affect the biodegradation rate of azo dyes. Throughout the literature, researchers have discussed various problems associated with dye biodegradation that may or may not be anticipated or remedied. Non-dye related parameters such as temperature, pH, dissolved oxygen or nitrate concentrations, type and source of reduction equivalents, bacteria consortium, and cell permeability can all affect the biodegradation of azo dyes and textile effluents. Dye related parameters such as class and type of azo dye (i.e. reactive-monoazo), reduction metabolites, dye concentration, dye side-groups, and organic dye additives could also affect the biodegradability of azo dye wastewaters.

Biological WWTP have a highly variable nature. Wuhrmann *et al*. (1980) investigated the effects of pH, temperature, type and concentration of respiration substrates, and oxygen tension on the rate of biological reduction of a variety of azo dyes. A consortium of microbes was used, including *Bacillus cereus*, *Sphaerotilus natans*, and two others isolated from sewage-activated sludge. Also, activated sludge was used in experiments with mixed biocenoses. Temperatures, which are too high or too low, can result in the exclusion of a particular group of microorganisms. Using activated sludge, Wuhrmann *et al*. (1980) determined that temperature has an increasing liner relationship with the reduction rate of Orange II and Lanasylvioloet up to 28 ºC. In general however, most studies have been conducted at set temperatures, offering minimal data on temperature effects.

The wastewater pH can affect the proper functioning of both anaerobic and aerobic organisms (Grady *et al.*, 1999). Wuhrmann *et al*. (1980) also investigated the effect of pH on dye reduction rates, but was unable to conclusively establish a relationship. However, they did state that an exponential increase in the decolorization rate was observed by decreasing the pH, but this relationship depended on the dye being tested. Loyd (1992) observed an indirect increase in the rate of decolorization of Navy-106, with decreased pH values in anaerobic batch tests. No statistical data was performed to verify this result, however.

Nitrate and especially oxygen may play an important role in determining the rate of dye reduction. As seen in earlier sections of this review, the presence of oxygen generally inhibits the degradation of azo dye chromogens. Interestingly, Wuhrmann *et al*. demonstrated that obligate aerobes might actually decolorize azo compounds under temporary anoxic conditions. However, high nitrite or nitrate concentrations in the mixed liquor of activated sludge plants could significantly inhibit dye removal. Zissi and Lyberatos (1996) observed *Bacillus subtilis* to degrade p-aminoazobenzene under anoxic conditions.

Without the necessary reduction equivalents to optimize bacterial respiration and growth, dye reduction may be inhibited. Often, bacterial cultures are unable to proliferate when an azo dye is the sole carbon and nitrogen source. Therefore, additional, readily biodegradable sources may be necessary. Wuhrmann *et al*. (1980) indicated that in the

absence of oxygen an azo compound will act as the sole oxidant, and its reduction rate will be governed by the rate of formation of the electron donor. This may be a problem in WWTPs that receive a high loading of dye waste with a low, readily available, organic carbon content. Gingell and Walker (1971) state that the presence of oxygen may out-compete the azo dye as the preferred oxidizer of reduced electron carriers in the respiration chain, and thus limit the reduction of azo linkages.

The type of bacteria or consortium used for dye biodegradation will undoubtedly affect the reduction rate. Substantial support has been provided in earlier sections to elucidate this variable. In general however, aerobic microbes do not have the ability to substantially decolorize azo dyes, but can oxidize the dye metabolites. The converse applies to anaerobic microbes.

A final non-dye related factor is the cell permeability and the cell wall adsorption of azo dyes. Wuhrmann *et al*. (1980) investigated the affects of dye absorption by the cell wall and concluded the following: (1) dye adsorption follows Freundlich adsorption isotherms at low dye loads per weight of biomass, but exhibits a high variability; (2) depending on the dye, subsequent reduction may take place or the dye may remain in the cell wall; (3) adsorption does not inhibit the reduction rate of microbes that exhibit the ability to reduce azo dyes. While these conclusions are limited based on the testing performed, they do give an indication of the variability that is possible when dealing with azo dyes and biological treatment systems. Ganesh (1992) concluded that very little of the dye added to a biological reactor will be leached from the biomass when placed in a landfill. This might suggest that the dye is effectively reduced after adsorption to the cell wall or that very little dye is actually adsorbed.

Cell permeability might play an important role in dye biodegradation. In the study conducted by Wuhrmann *et al*. (1980), all dyes that were not reduced by whole cells were effectively degraded by cell extracts from both facultative anaerobes and obligate aerobes. This suggests that many cells might be capable of dye biodegradation, but are limited by the permeability of their cell walls.

The azo dye structure can play a significant role in the dye biodegradation rate. Depending on the number and placement of the azo linkages, some dyes will biodegrade more rapidly than others. In general, the more azo linkages that must be broken will

cause the reduction rate to be slower. While there are not a large number of studies that specifically address this factor, Brown and Laboureur (1983) observed that two poly-azo dyes showed only moderate to variable biodegradation as compared to four monoazo and six diazo dyes. The authors indicate that poly-azo dyes are less likely to degraded than mono- or diazo dye types.

Fiber-reactive azo dyes often contain solubilizing side groups, as well as a nucleophilic reactive group. Depending on the nature of these groups, biodegradation might be inhibited. According to Ganesh (1992), sorption of dye to sludge depends on the type, number, and position of the substituents in the dye molecule. Hydrophilic sulfo groups reduce the dye removal through sorption, and conversely, sorption is increased by the presence of hydroxyl, nitro, and azo groups in the dye molecule.

The production of toxic by-products or the presence of toxic dye additives may also inhibit biodegradation. High salt concentrations are not uncommon in textile effluents and may result in adverse conditions for biodegradation. Dispersing and solubilizing agents may also create inhibitory conditions for dye reduction (Carliell, 1994).

In various studies (O'Neill *et al*., 2000, Donlagic' and Levec, 1998, Wuhrmann *et al*., 1980) the production of inhibitory dye metabolites is cited as causing a decrease in biodegradation. O'Neill *et al*. concluded from respiration-inhibition testing that anaerobic degradation of simulated textile effluent generated metabolites that were toxic to some aerobic organisms. Donlagic' and Levec concluded that the distribution of dye intermediates plays an important role in determining the aerobic biodegradability of Orange II. They further state that low dye removal may be attributed to the presence of intermediates that are less susceptible to microbial degradation or that act as inhibitory agents. Wurhmann *et al*. attributes a decline in decolorization rates to the accumulation of reduction products in the test medium. Inhibition of various microorganisms to dye metabolites is frequently cited throughout the literature and is assumed to be a key factor when treating wastewaters containing azo dyes.

A final and important factor to evaluate is the initial dye concentration of the wastewater. Seshadri and Bishop (1994) performed a study investigating the effect of different influent dye concentrations on the color removal efficiency. They concluded

that elevated dye concentrations may cause a drop in percent dye removal. Furthermore, the inhibition may be directly related to the effects of increased dye metabolite formation due to higher dye concentrations. Less pronounced reductions were seen at lower concentration levels. It should be noted that tolerable influent concentrations are likely specific to individual or related groups of dyes. Cariell *et al*. (1995) also states that toxicity assays showed that C.I. Reactive Red 141 was inhibitory to anaerobic organisms at concentrations greater than 100 mg/L, however prior biomass adaptation increased their resistance to elevated dye concentrations.

CHAPTER 3: MATERIALS AND METHODS

Introduction

The following chapter details the materials and methods used during this project. Reactor designs and operation, feed solutions, sample preparation, preservation, and analysis, and equipment specifications will be discussed. The ADMI tristimulus method and the liquid-liquid separatory funnel extraction method for acids and base/neutrals will also be described. Each phase of testing will be separately described.

Feed Solutions

Laboratory feed solutions and/or Lower Smith River POTW (POTW) influent were used for all testing except in preliminary experiments. The feed solutions used during preliminary testing varied and will be described below. All laboratory feed solutions were freshly prepared using room temperature tap water, dye, and the following mineral and organic additions:

Trace Mineral Components:

Potassium phosphate:	**K_2HPO_4**	15 mg/L
Calcium chloride:	**$CaCl_2$**	20 mg/L
Magnesium sulfate:	**$MgSO_4$**	20 mg/L
Zinc sulfate:	**$ZnSO_4$**	1 mg/L
Ferric chloride:	**$FeCl_3$**	2 mg/L
Sodium bicarbonate:	**$NaHCO_3$**	50 mg/L

Organic Components:

Peptone (Fisher Scientific) 50 mg/L

The mineral additives provided the macronutrients required for optimal biomass growth. The peptone provided additional reduction equivalents, aiding in biomass growth and dye reduction. The reactive azo dyes Cypress Green, Indigo Blue, and Sultan Red (Bassett Walker, Henry County, Virginia) were all used during this research. Only the Cypress Green dye was used following *Phase I* testing. Dye concentrations in laboratory feed solutions were volume based and varied throughout testing; however, Cypress Green dye

additions of 4ml/L and 2ml/L were primarily used. All dyes were prepared by Bassett Walker and are mixtures of several reactive azo dyes. The composite dyes are listed below and are referenced according to their proprietary name and Color Index (C.I.) classification.

Cypress Green:

Cibacron Yellow C-R	*Reactive Yellow 168*
Cibacron Red C-2G	*Reactive Red 228*
Cibacron Navy C-B	*Reactive Blue 238*

Sultan Red:

Cibacron Orange C-3R	*Reactive Orange 131*
Cibacron Red C-2G	*Reactive Red 228*
Cibacron Navy C-B	*Reactive Blue 238*

Indigo Blue:

Cibacron Red C-2G	*Reactive Red 228*
Cibacron Navy C-B	*Reactive Blue 238*
Cibacron Black LS-R	

Also, raw influent from the POTW was used as a feed solution in some tests. POTW influent grab samples were collected and analyzed for ADMI color, TOC, chemical oxygen demand (COD), TKN, and ammonia nitrogen (NH_3-N) before testing.

Standardized curves describing the ADMI color, TOC, COD, TKN, and NH_3-N concentrations for the Cypress Green dye and peptone were developed for reference purposes when preparing the feed solutions (Appendix: Figures 26-29).

Sample Preparation and Preservation

When possible, all samples were analyzed immediately after being withdrawn from the reactors; however, some samples were preserved in accordance with *Standard Methods* (1998). Excluding preliminary testing, all samples were prepared as follows: Samples with a high solids concentration were centrifuged at 1500 rpm for ten minutes. The supernatant was then vacuum filtered through a 1.0µm glass microfiber filter (Whatman Inc., Clifton, NJ). The filtrate was collected in a clean container and further prepared based on the analysis to be performed. For ADMI color measurement, samples were syringe filtered through 0.45µm membrane filters (Fisher Scientific). Prior to

ADMI color measurement, the samples were aerated for five minutes to allow reoxidation of any partially reduced dye molecules. This insured an accurate and uniform measurement among all the effluent samples. For TOC and COD analysis, samples were syringe filtered through 0.45µm membrane filters (Fisher Scientific). For TKN and NH_3-N measurement, no further sample filtration was necessary.

Sample Analysis

Sample COD, TKN, NH_3-N, TSS, and VSS were analyzed according to *Standard Methods* (1998) using methods 5220 C, 4500-N_{org} C, 4500-NH_3 C, 2540 B, and 2540 E, respectively.

TOC was determined with a Dorhmann DC-80 carbon analyzer, which utilizes ultra-violet-promoted persulfate oxidation followed by infrared detection. Prior to analysis, samples were acidified with phosphoric acid and sparged with oxygen for five minutes to remove any inorganic carbon. The instrument was calibrated before each use with standardized TOC solutions of 400mg/L and/or 10mg/L.

ADMI color was determined with a Genesis Spectrophotometer Model 5 (Spectronic Instruments, Rochester, NY) in accordance with the ADMI Tristimulus method 2120 D detailed in *Standard Methods* (1998). The ADMI color values were calculated with a computer program developed by Mr. Andrew from Severn Trent Environmental Services, Inc. The spectrophotometer was calibrated before each use with standard platinum cobalt color solutions (Fisher Scientific) of 100, 200, 300, 400, and 500 ADMI color units. The Virginia Department of Environmental Quality recognizes this method of computer assisted color calculation (Edwards, 2000).

Liquid-liquid separatory funnel extractions were performed in accordance with EPA Test Method 625 for base/neutrals and acids (Longbottom and Lichtenberg, 1982). In some tests, the method was abbreviated by using proportionally smaller sample and methylene chloride volumes. Dye degradation by-products were analyzed using a Hewlett Packard 5890 Gas Chromatograph with a 5970 Mass Selective Detector. The column used for the analysis was an HP- 5 (crosslinked 5 percent PH ME Siloxane). The analytical run time was 30 minutes per sample. Qualitative compound identification was

achieved by performing a NIST/EPA/NIH 75 K library search using HP Chemstation software.

Anion and cation concentrations in the POTW effluent were analyzed with an ion chromotograph (IC). A Dionex DX-300 IC with a conductivity detector, an AS-40 autosampler, and an AS-14 column was used for the analysis of anions. For the analysis of cations, a Dionex DX-120 with a conductivity detector and a CS-12 column was used.

Test Design and Operation

Testing was conducted in four phases. Individual testing methods are described below in following sections. *Phase I* included all preliminary testing. ADMI color removal was investigated during these tests using biomass from several treatment plants. *Phase II* includes two ANA/AER sequential step-treatment tests. The first was a 56-day test investigating ADMI color, TOC, and COD removals and the second was a batch test investigating the affects of various initial dye and TSS concentrations on the ADMI color and TOC reductions. *Phase III* involved a 15-day anaerobic test, a 15-day aerobic test, a 15-day ANA/AER/ANA/AER sequential step-treatment test, and a set of 4-day batch tests. Nitrogen removal was a primary interest during this phase of tests. *Phase IV* included two ANA/AER sequential step-treatment tests. ADMI color, TOC, and nitrogen reductions in the POTW influent were investigated using ANA/AER sequential step-treatment systems.

Phase I

Preliminary tests were aimed at assessing the ADMI color loss for several of the reactive azo dyes and also for the influent received at the POTW. All reactors were stored in darkness and room temperature, except for *Test #5*. Five separate test series were conducted as follows:

Test #1 was an anaerobic biodegradation test using activated sludge from the POTW. Forty-milliliter, amber vials were seeded with 5mL of settled sludge and filled with 35mL of test solution. All three dyes, Cypress Green, Indigo Blue, and Sultan Red, as well as the POTW influent were used for test solutions during *Phase I* testing. Peptone, a readily degradable carbon and nitrogen source, was added at a concentration

of 200 mg/L to all dye solutions. This test was conducted for 15 days. Samples were taken intermittently and ADMI color was measured.

Test #2 was an anaerobic biodegradation test using anaerobic digester sludge from Pepper's Ferry WWTP in Radford, Virginia. Test set-up and operation were the same as used during *Test #1*. This test allowed for a direct comparison between the ADMI color reductions achieved using the POTW sludge with the ADMI color losses achieved using a non-dye-acclimated anaerobic sludge.

Test #3 was an aerobic biodegradation test using activated sludge from the POTW. One-liter Erlenmeyer flasks were covered in aluminum foil, seeded with 50ml of settled sludge, and filled with 450ml of test solution. No peptone was provided as a supplement during testing. The reactors were mixed using forced aeration and sealed with ported rubber stoppers to allow air transfer while minimizing evaporative losses. This test was conducted for 18 days. Samples were taken intermittently and ADMI color was measured.

Test #4 was an anoxic biodegradation test using anaerobic digester sludge from the Pepper's Ferry WWTP. Tests were conducted using 1L amber jars with ported and stoppered lids, to allow for sampling and oxidation-reduction potential (ORP) measurement. The jars were seeded with 100mL of settled sludge and filled with 900mL of Indigo Blue dye solution. Two anoxic test reactors and one anaerobic control reactor were used. Peptone and KNO_3 were added at an initial concentration of 200 mg/L NO_3 to the test reactors. Only the peptone was added to the control reactor. Testing was conducted for seven days with intermittent sampling and KNO_3 addition on days one and three at a concentration of 100mg/L NO_3. ORP was measured using a silver-platinum electrode (Cole Parmer, Vernon Hills, IL) and ADMI color was measured as previously described.

Test #5 was an anaerobic biodegradation test utilizing anaerobic digester sludge from Pepper's Ferry WWTP. The objective of this test was to determine if biodegradation by-products were causing a toxic inhibition to the biomass. Glass cylinders (2.25L) were seeded with 250ml of settled sludge and filled with tap water. Peptone was added to the reactors at a concentration of 1g/L. The treatment systems were then allowed to sit for four days. After this time period the reactors were spiked

with a mixed solution of Cypress Green, Indigo Blue, and Sultan Red dyes. The treatment systems were allowed to react for five days, after which they were sampled and again spiked with the mixed dye solution. The test was conducted for a total of 25 days, in five 5-day intervals. Methane production was determined on day 25 using a Hewlett Packard 5890 Gas Chromatograph with a Flame Ionization Detector. The analytical column was packed with Carbosieve SIII, and the run time was 5 minutes per sample. ADMI color was measured throughout testing.

Phase II

Phase II testing was conducted with several objectives in mind. The first was to acclimate a large quantity of Pepper's Ferry WWTP anaerobic sludge to the Cypress Green dye. Furthermore, because this process involved feeding the biomass over an extended period of time, this test provided a good opportunity to characterize the anaerobic biodegradation of the Cypress Green dye. A second objective was to investigate the degradation of the dyes in a sequential step-treatment process. Therefore, aerobic reactors containing Blacksburg WWTP return activated sludge (RAS) were used to treat the effluent generated by the anaerobic reactors. Also, it was desired to examine the reactor effluents for dye metabolites. Other objectives were focused toward understanding the affect of the initial dye and biomass concentrations on the biodegradation rate. As before, each test will be separately outlined.

Test #1 was an ANA/AER sequential step-treatment test using anaerobic digester sludge from the Pepper's Ferry WWTP and RAS from the Blacksburg WWTP. Two duplicate test reactors and one control reactor were used in each treatment step.

The anaerobic portion of the test was conducted using 19L glass vessels sealed with ported rubber stoppers. The stoppers each held three glass sample tubes used for sampling, feeding, and purging of the reactor with nitrogen gas. The reactors were operated as sequencing-batch-reactors (SBRs), with a total mixed-liquor suspended solids (MLSS) volume of 18L and a hydraulic retention time (HRT) of nine days. The test reactors were fed the laboratory feed solution previously described. Cypress Green dye was added at a concentration of 4ml/L to the feed solution. The control reactors were fed the laboratory feed solution, but no dye was added. The feed and effluent were

transported into and out of the reactor using pressurized nitrogen gas. The temperature was maintained at approximately 35 °C throughout testing. Weekly sampling was conducted and the ADMI color, TOC, COD, and TSS were each measured. The pH was checked intermittently and adjusted using sodium bicarbonate ($NaHCO_3$) when necessary.

The aerobic portion of the test was conducted using 5-gallon bucket reactors with ported lids for air transfer. The reactors were mixed using forced aeration. The air supply was filtered through cotton and passed through a water tank before entering the systems. Total MLSS volume was maintained at 9L, with an HRT of 4.5 days. Again, the reactors were operated as SBRs. The effluent from the anaerobic reactors was used as the aerobic feed. The temperature was maintained at ambient conditions (20-25 °C) throughout testing. Weekly sampling was conducted and ADMI color, TOC, COD, and TSS were each measured. The pH was checked intermittently and adjusted using $NaHCO_3$ when necessary.

Effluent samples from all reactors were collected on day 56 and tested for dye metabolites using liquid-liquid extraction with methylene chloride and GC-MS analysis.

Test #2 was an ANA/AER sequential step-treatment test utilizing the acclimated sludges from *Phase II: Test #1*. The test was conducted in sets using 40mL amber vials, with six test sets used in both treatment steps.

For the anaerobic portion of the test, three laboratory feed solutions with initial Cypress Green dye concentrations of 1ml/L, 2ml/L and 4ml/L were each seeded at TSS concentrations of 4000mg/L and 8000mg/L. All samples were purged with nitrogen gas, sealed, and stored in darkness at 35 °C. Each test set was sampled and tested for ADMI color and TOC reduction following one, two, and four days of treatment.

For the aerobic portion of the test, the same test design was used; however, the final anaerobic effluents were used for the aerobic feeds. Also, the TSS concentrations were set at 1000mg/l and 2000mg/L. All samples were aerated using forced aeration and stored in darkness at ambient temperatures. Each test set was sampled and tested for ADMI color and TOC reduction following one, two, and four days of treatment.

Phase III

The focus of *Phase III* testing was to first investigate the aerobic biodegradation of Cypress Green using a non-acclimated sludge and compare these results with a similar anaerobic test using the acclimated sludge from *Phase II* testing. A further objective was to characterize the biodegradation characteristics of an ANA/AER/ANA/AER sequential step-treatment process. Lastly, a series of batch-tests were performed using a set HRT, allowing for a more direct comparison of the biodegradation rates among the different treatment system already examined. In *Phase III*, the removal of nitrogen from the dye wastewater was a primary concern that had not been previously addressed.

Test #1 included an aerobic and an anaerobic biodegradation test utilizing Blacksburg WWTP RAS and the acclimated anaerobic sludge from *Phase II* testing, respectively. The total MLSS volume was set at 10L for all reactors, with an HRT of five days. Two duplicate test reactors and one control reactor were used for each test type. Both systems were fed the laboratory feed solution with a Cypress Green dye concentration of 2ml/L. No dye was fed to the control reactors. The anaerobic systems were stored at 35 °C, while the aerobic reactors were maintained at ambient conditions. Testing was conducted for 15 days with intermittent sampling. The pH was monitored daily and adjusted using $NaHCO_3$ when necessary. The effluent TSS and VSS were monitored to determine the biomass wastage from each system. ADMI color, TOC, NH_3-N, TKN, TSS, and VSS were each measured throughout the testing period. A key objective of this test was to determine the SRT of the systems, and retro-apply this result to similar, previously conducted tests.

Effluent samples from all reactors were collected on day 15 and tested for dye metabolites, using liquid-liquid extraction with methylene chloride and GC-MS analysis.

Test #2 was an ANA/AER/ANA/AER sequential step-treatment test utilizing the sludges and reactors from *Phase III: Test #1*. The test was conducted using one test series and one control series. The systems were operated as a series of SBRs, with the initial feed injected into the first anaerobic reactor and the effluent fed into the following step. The feed, temperature, and liquid volumes were all kept constant from the previous test, *Phase III: Test #1*. The total HRT was set a 20 days, with a 5-day HRT for each

step. The test was conducted for a period of 15 days. ADMI color, TOC, NH_3-N, TKN, TSS, and VSS were each measured throughout the testing period.

Test #3 was a series of 4-day batch-tests. The tests included an anaerobic, an aerobic, an ANA/AER sequential step-treatment, and an ANA/AER/ANA/AER sequential step-treatment test. Glass cylinders with a 2.25L volume were used for reactor vessels, and were designed in the same manner as the larger reactors used in *Phase III: Test#2*. Biomass was taken from the larger reactors, and the VSS concentration set at 2000mg/L for all tests. Proportional volumes of MLSS from the larger reactors were settled, centrifuged at 1500 rpm for ten minutes, and the biomass resuspended at the desired VSS concentration. The laboratory feed solution, with a Cypress Green dye concentration of 2ml/L, was used for all tests. As before, in step-treatment tests the effluent from a preceding step was fed into the following step. All anaerobic reactors were sparged for ten minutes to minimize the initial oxygen concentrations. ADMI color, TOC, NH_3-N, and TKN were measured throughout the testing period.

Phase IV

Phase IV concluded the experimental testing for this research project. The tests conducted during this phase were based on previous results and were focused toward effectively biodegrading the POTW influent. Two ANA/AER sequential step-treatment tests were conducted.

Test #1 utilized the acclimated sludges developed during *Phases II* and *III*. The reactor design and operation was identical to the ANA/AER sequential step-treatment system used in *Phase III: Test #3*. The HRT was shortened to two days for this test. POTW influent was used for the feed solution. The VSS concentration was set at 2000mg/L, using the procedure developed in *Phase III: Test #3*. ADMI color, TOC, NH_3-N, and TKN were measured throughout the testing period.

Test #2 design and operation was identical to *Phase IV: Test #1*; however, POTW activated sludge was used in the aerobic step.

CHAPTER 4: RESULTS

Introduction

In this chapter, the experimental results from each phase of testing will be discussed. In general, the experimental results correspond well with the findings reported in the literature. Maximum color removal was achieved with anaerobic biodegradation. Minimal color, carbon and nitrogen removal was seen in aerobic biodegradation tests. Of all the tests conducted, ANA/AER sequential step-treatment provided the best color and carbon removal. ANA/AER/ANA/AER sequential step-treatment did not yield greater reductions than the ANA/AER systems.

Phase I

The results of this phase are from preliminary tests conducted at the beginning of this project. These tests helped to delineate the focus and direction of future tests. Anaerobic and aerobic biodegradation systems were used to treat the Cypress Green, Indigo Blue, and Sultan Red dyes and the POTW influent. These dyes were provided by the textile plant and are representative of the dyes they use. Anaerobic digester sludge and POTW activated sludge were both used for testing. Also, an anoxic system, utilizing anaerobic digester sludge, was used to degrade the Indigo Blue dye in this phase. *Phase I: Test #1* utilized an anaerobic treatment system with POTW activated sludge. The results presented in Figure 4 show a high reduction in the ADMI color of the Cypress Green, Indigo Blue, and Sultan Red dyes. Total ADMI color reductions were 82, 87, and 88 percent, respectively. The ADMI of the POTW influent was reduced to a lesser extent, only 69 percent. Most of the total color loss occurred by day six of testing, but continued reductions occurred over the entire test period. *Phase I: Test #2* utilized an anaerobic treatment system using dye-unacclimated Pepper's Ferry WWTP anaerobic digester sludge. The results were similar to *Phase I: Test #1*, however, most of the color loss occurred by day three of testing (Figure 5). ADMI color reductions for Indigo Blue and Sultan Red were both 83 percent. Cypress Green and the POTW influent showed similar ADMI color reductions of 68 and 67 percent on day seven. The color increase on

day 16 for the influent cannot be explained, but may have been due to an air leak in the system, and reoxidation of the dye.

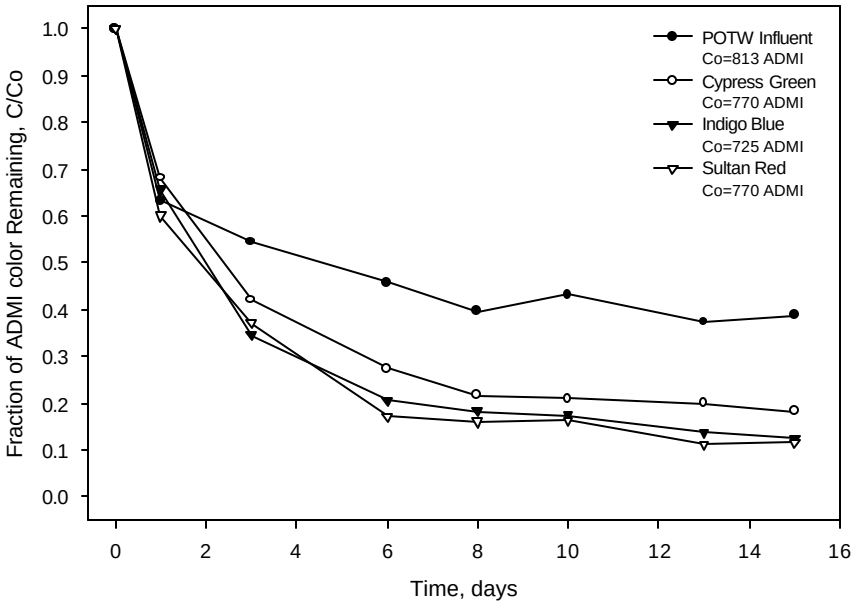


Figure 4: Phase I: Test #1: Anaerobic biodegradation of Cypress Green, Indigo Blue, Sultan Red, and POTW influent ADMI color using POTW activated sludge.

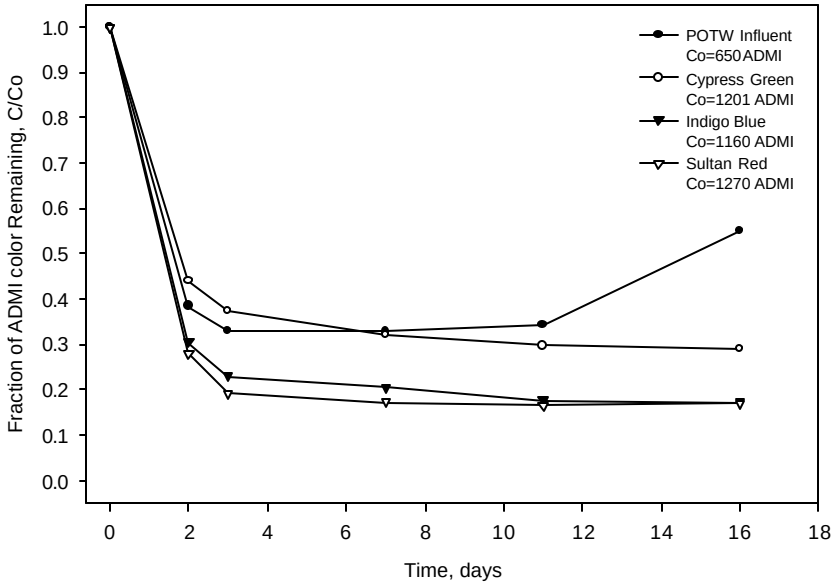


Figure 5: *Phase I: Test #2*: Anaerobic biodegradation of Cypress Green, Indigo Blue, Sultan Red, and POTW influent ADMI color using Pepper's Ferry digester sludge.

Phase I: Test #3 utilized an aerobic treatment system with POTW activated sludge. The results showed high ADMI color reductions for the Cypress Green, Indigo Blue, and Sultan Red dyes. Total reductions were 82, 88, and 85 percent on day six, respectively (Figure 6). The POTW influent ADMI color was reduced less, with a total loss of 51 percent.

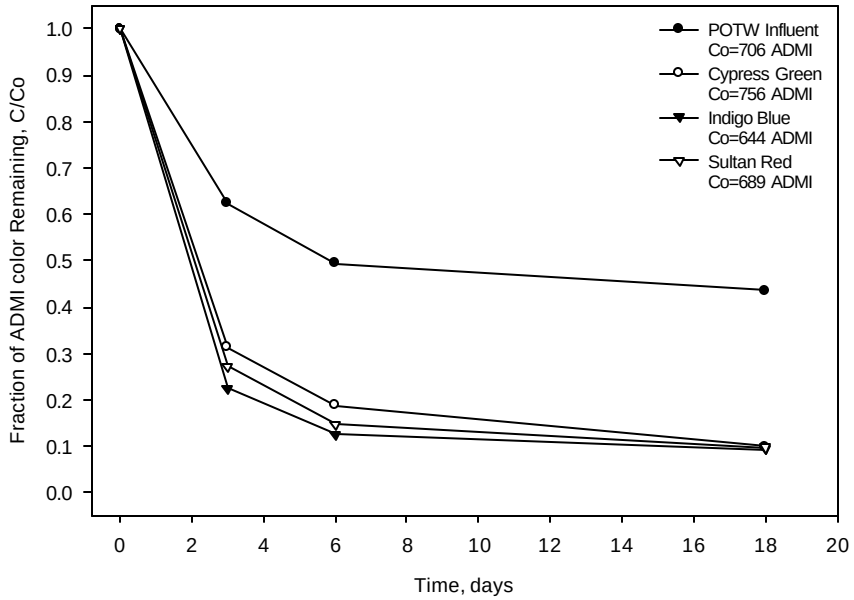


Figure 6: *Phase I: Test #3*: Aerobic biodegradation of Cypress Green, Indigo Blue, Sultan Red, and POTW influent ADMI color using POTW activated sludge.

Phase I: Test #4 utilized an anoxic treatment system using Pepper's Ferry WWTP anaerobic digester sludge. KNO_3 was added periodically to maintain anoxic conditions in the test reactors. The results showed that anoxic color removal of Indigo Blue dye is similar to, but slightly less than, anaerobic color removal, with ADMI color reductions of 75 and 85 percent, respectively (Figure 7).

Phase I: Test #5 utilized an anaerobic treatment system with Pepper's Ferry WWTP anaerobic digester sludge. The results indicated that the mixed-dye solution of Cypress Green, Indigo Blue, and Sultan Red contained a non-biodegradable fraction. Repeated spiking of the dye showed a general increase in the residual ADMI color with each dye addition step (Figure 8). Toxic inhibition of the biomass from the production of dye metabolites could not be discerned. All reactors tested positive for methane production.

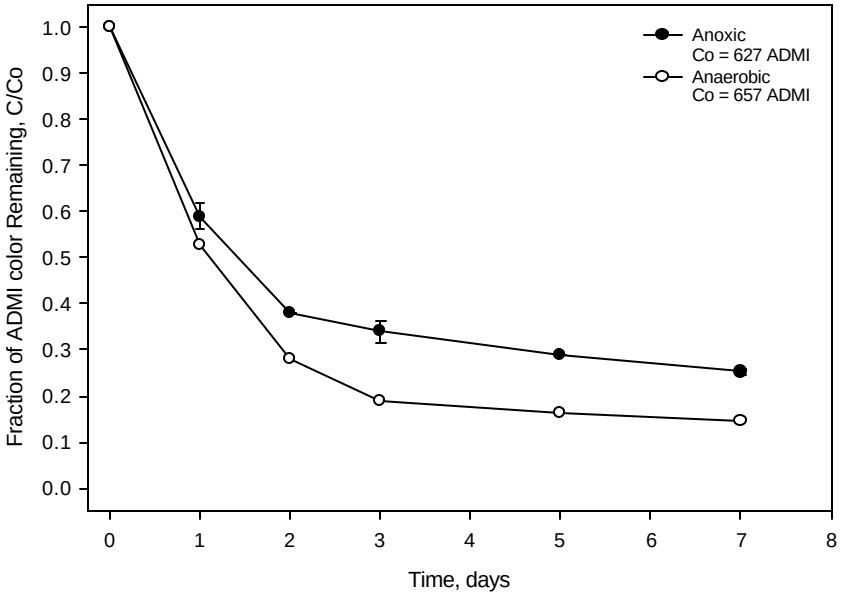


Figure 7: *Phase I: Test #4*: Anoxic versus anaerobic biodegradation of Indigo Blue ADMI color using Pepper's Ferry digester sludge. (Standard deviation, n=2)

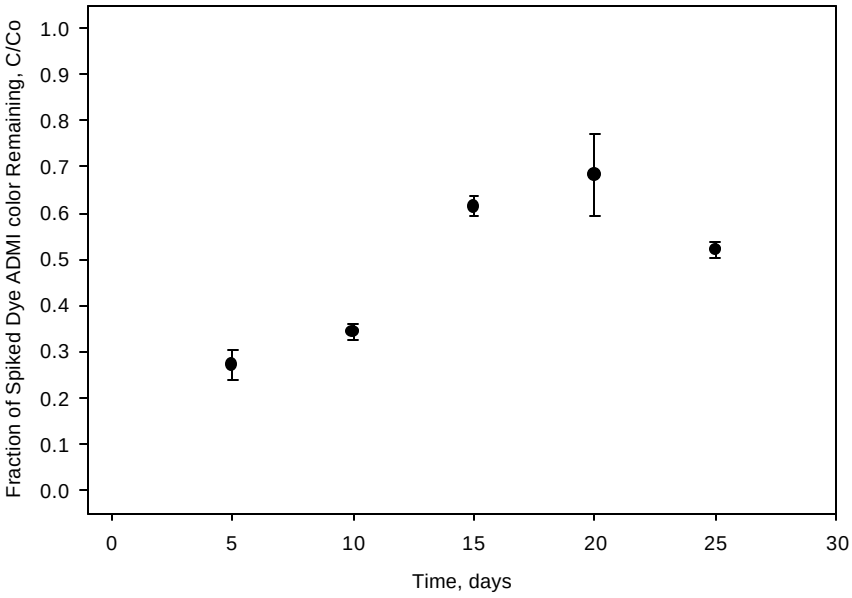


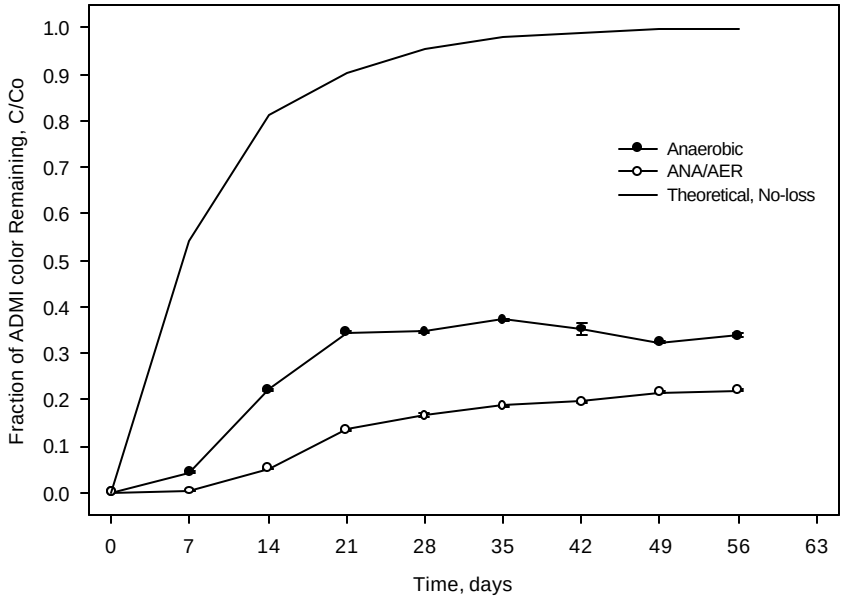
Figure 8: *Phase I: Test #5*: Anaerobic ADMI color removal of Sultan Red/Cypress Green/Indigo Blue mixed-dye solution using Pepper's Ferry digester sludge. Reactors were spiked with dye on days 0, 5, 10, 15, and 20. (Standard deviation, n=3)

Phase II

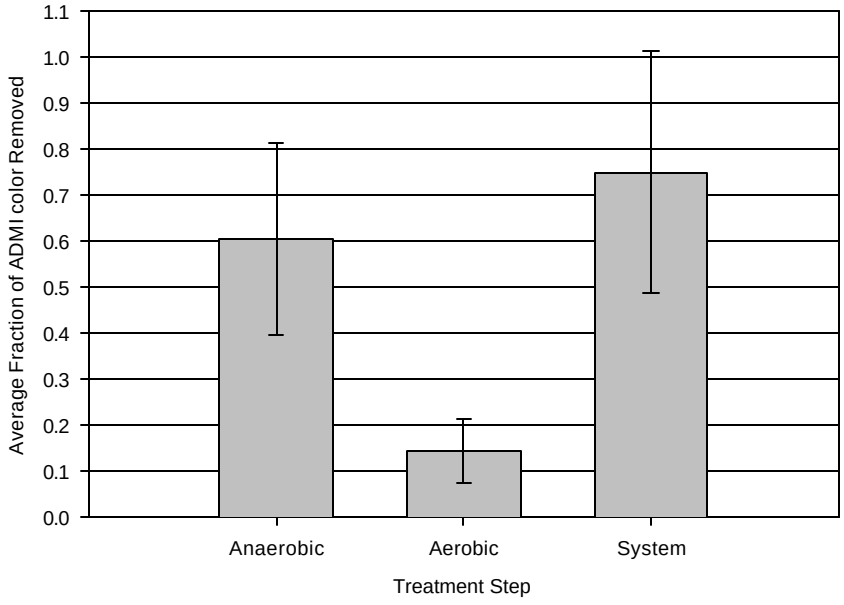
The results of this phase are from are from two ANA/AER sequential step-treatment systems. *Phase II: Test #1* was run for 56 days at 35 °C using Pepper's Ferry WWTP digester sludge in the anaerobic step and Blacksburg WWTP RAS in the aerobic step. The MLSS volume was 18 liters in the anaerobic reactors and nine liters in the aerobic reactors. The HRTs were nine and 4.5 days in the anaerobic and aerobic reactors, respectively. The laboratory feed solution, with a Cypress Green dye concentration of 4ml/L, was fed to the anaerobic systems. The anaerobic effluent was withdrawn and fed to the aerobic systems. *Phase II: Test #2* was designed using microcosms and utilized the acclimated sludges from *Phase II: Test #1*. This test investigated the affect of the TSS and the initial dye concentrations on the Cypress Green ADMI color and TOC removal rates.

Phase II: Test #1 results showed a high ADMI color reduction. The average ADMI color removal was 75 percent for the entire system, with 60 percent occurring in the anaerobic step (Figure 9). TOC decreased by 18 percent in the anaerobic step, with an additional 28 percent removed in the aerobic step (Figure 10). COD losses corresponded well with the TOC removals. A COD reduction of 49 percent was observed for the entire treatment system, with 22 percent removed during the anaerobic step (Figure 11). All reduction values were calculated from the theoretical no-loss plot. The TOC/COD ratio reached an average value of 0.37 in both treatment steps (Figure 12.a).

Based on the initial input concentrations of biological solids, the TSS concentrations for the anaerobic and aerobic reactors were maintained at approximately 4000mg/L and 1500mg/L, respectively (Figure 12.b). Additional tests were performed in *Phase III* to better quantify the biomass concentrations in these systems. These tests indicated a low biomass yield and wastage rate for the anaerobic systems. Data in Figure 12.b suggests that the biomass may have been washing out of the aerobic systems.

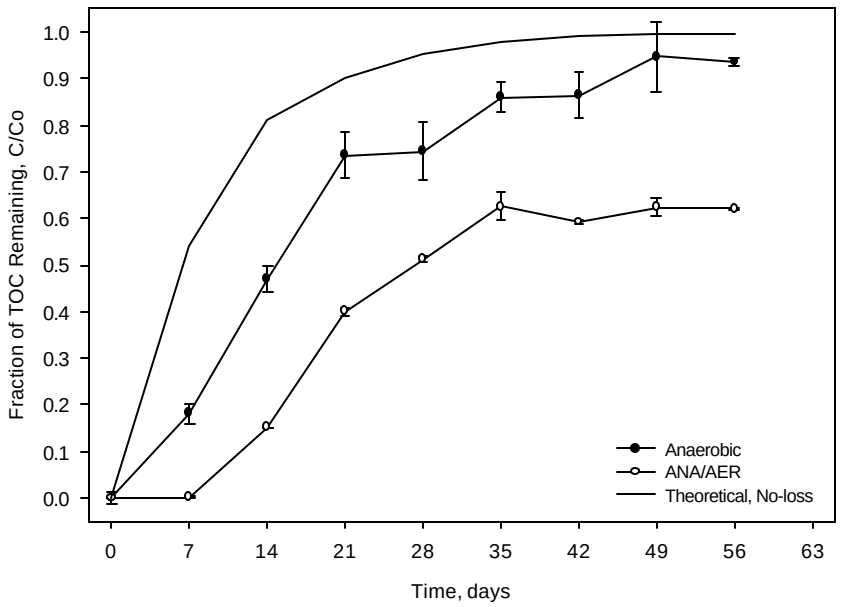


(a)

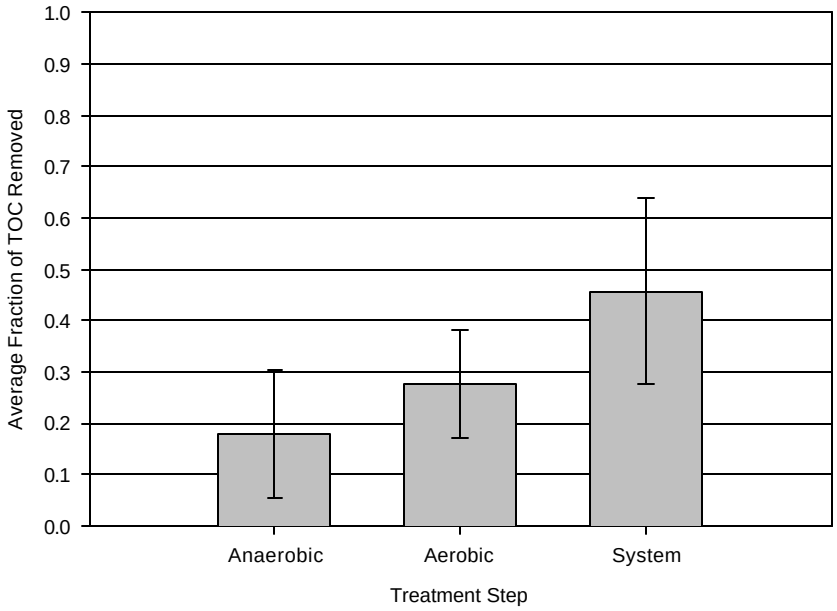


(b)

Figure 9: *Phase II: Test #1*: (a) ADMI color reduction of Cypress Green dye following each step of ANA/AER sequential step-treatment. (b) Average fraction of ADMI color removed during each step of ANA/AER sequential step-treatment. (Standard deviation, n=2)

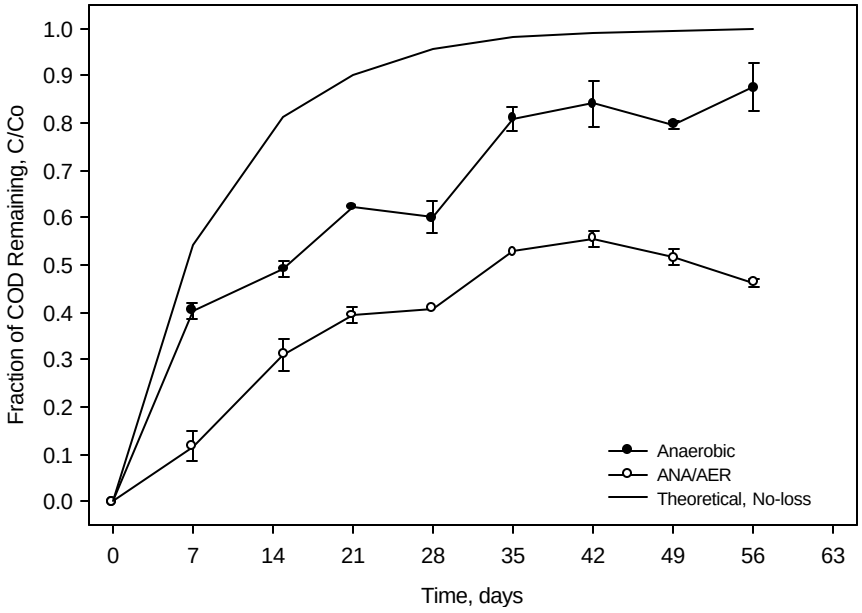


(a)

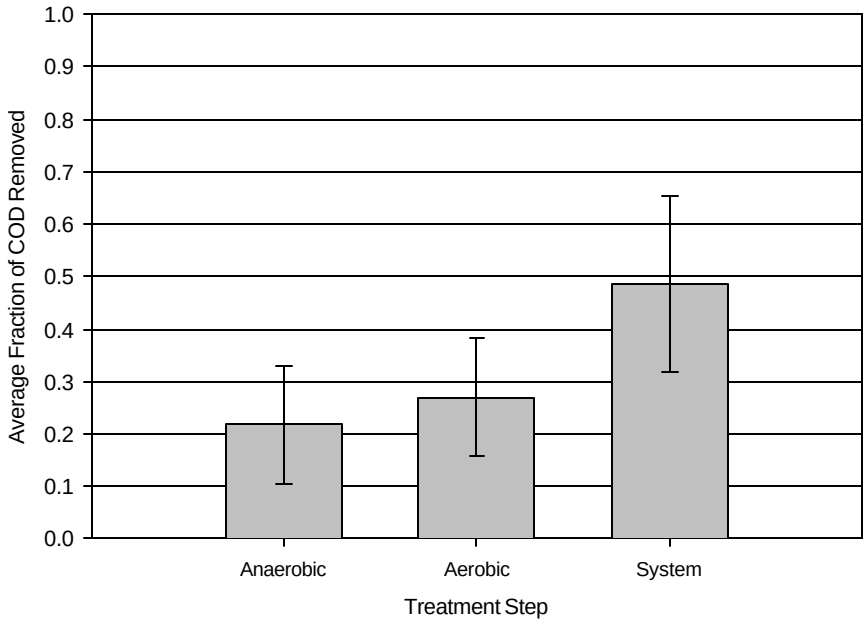


(b)

Figure 10: *Phase II: Test #1*: (a) Cypress Green dye TOC removal following each step of ANA/AER sequential step-treatment. (b) Average fraction of TOC removed during each step of ANA/AER sequential step-treatment. (Standard deviation, n=2)

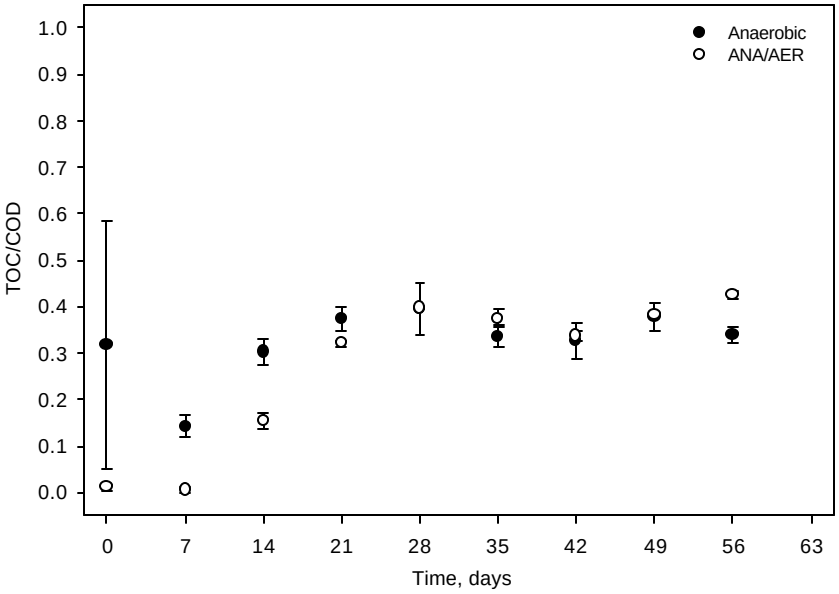


(a)

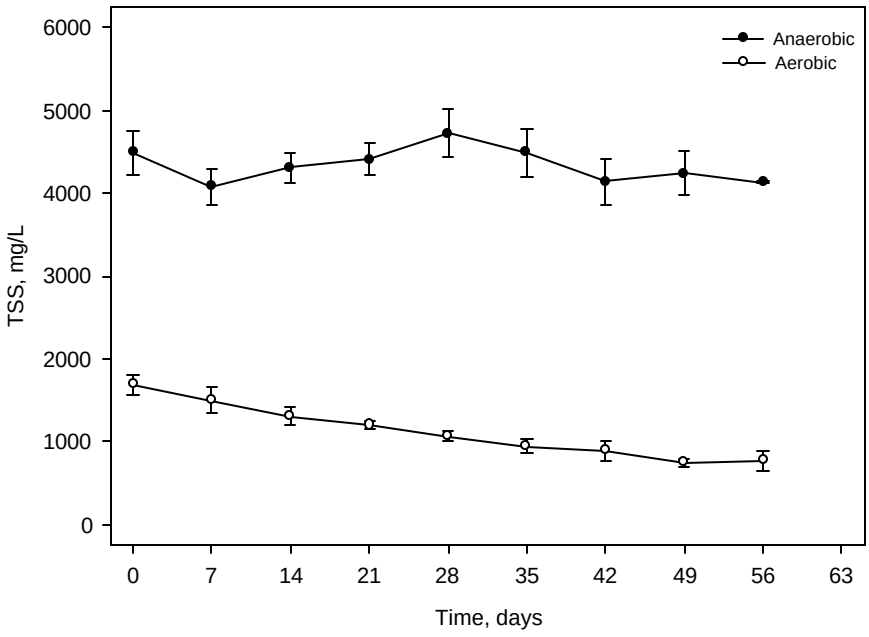


(b)

Figure 11: *Phase II: Test #1*: (a) Cypress Green dye COD removal following each step of ANA/AER sequential step-treatment. (b) Average fraction of COD removed during each step of ANA/AER sequential step-treatment. (Standard deviation, n=2)



(a)



(b)

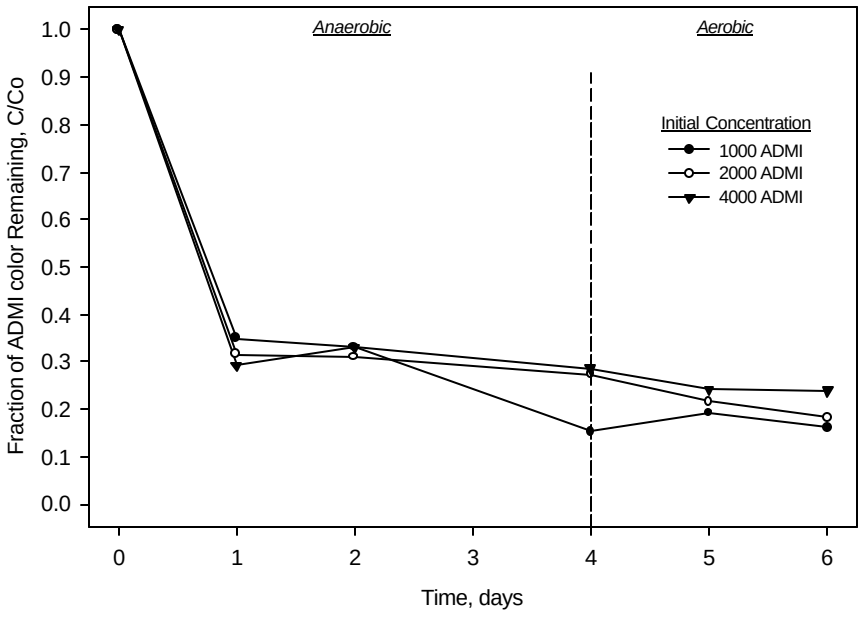
Figure 12: *Phase II: Test #1*: (a) TOC/COD ratio for anaerobic and ANA/AER effluents. (Standard deviation, n=2) (b) TSS in anaerobic and aerobic reactors during ANA/AER sequential step-treatment. (Standard deviation, n=3)

During *Phase II: Test #2*, different TSS concentrations were used to treat the Cypress Green dye. These concentrations were 4000mg/L and 8000mg/L in the anaerobic systems, and 1000mg/L and 2000mg/L in the aerobic systems. A range of initial dye concentrations was also tested. The initial ADMI color values were approximately 1000, 2000, and 4000 units. These color values were achieved by using the laboratory feed solution with Cypress Green dye concentrations of 1ml/L, 2ml/L, and 4ml/L. The results show a high ADMI color reduction, but a low TOC loss. The ADMI color removal rate was initially highest in the anaerobic system with the TSS concentration of 8000mg/L. However, the fractional ADMI color reduction for both anaerobic systems was not very different following four days of treatment. ADMI color removal was approximately 70 to 80 percent in both anaerobic systems (Figure 13). Following anaerobic treatment, the ADMI color removal was negligible in the aerobic systems. TOC reduction was low in all of the treatment systems (Figure 14). The treatment systems fed the 2ml/L and 4ml/L dye concentrations exhibited the highest TOC reductions. The TOC reduction rate was slightly higher in the anaerobic step, as compared to the aerobic step. The fraction of TOC remaining in the anaerobic and aerobic effluents was similar at both biomass concentrations.

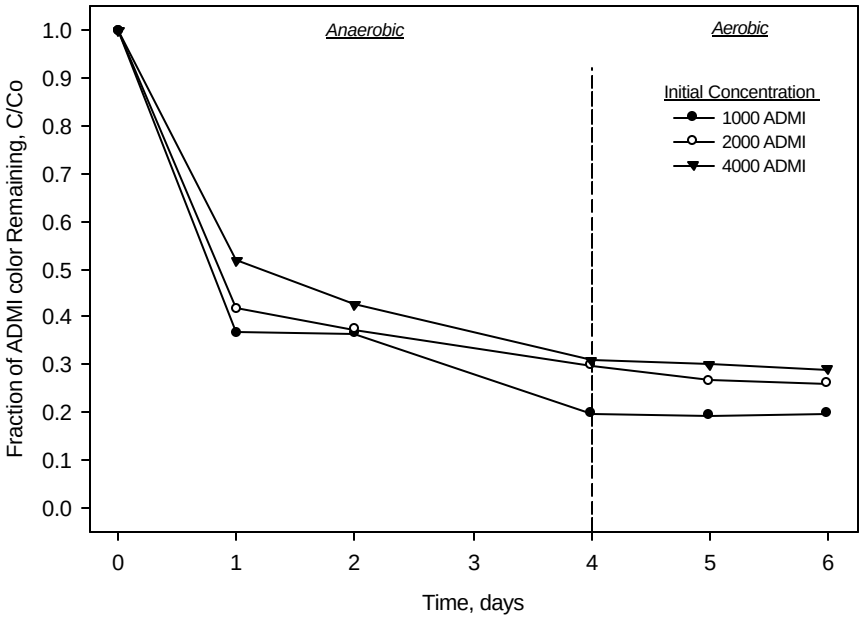
Phase III

Several different systems were tested during *Phase III*. A primary objective of this phase was to examine the removal of nitrogen from the wastewaters. Three separate test series were conducted, and are described below.

Phase III: Test #1 involved separate anaerobic and aerobic treatment systems. Both sets of systems had MLSS volumes of ten liters and HRTs of five days. The acclimated sludge from *Phase II: Test #1* was used in the anaerobic systems and Blacksburg RAS was used in the aerobic systems. The laboratory feed solution, with a Cypress Green dye concentration of 2ml/L, was fed to all the reactors. The results showed a medium to high ADMI color reduction in the anaerobic systems, but low removal in the aerobic systems (Figure 15). After reaching steady-state, the average ADMI color reduction was 59 percent in the anaerobic reactors and 28 percent in the aerobic reactors. TOC losses were low in both systems. At steady-state, TOC reductions

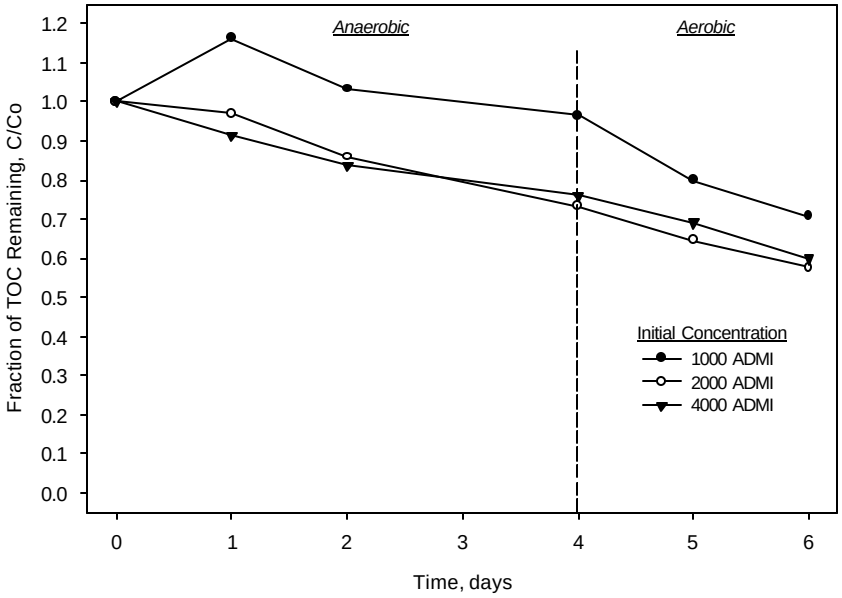


(a)

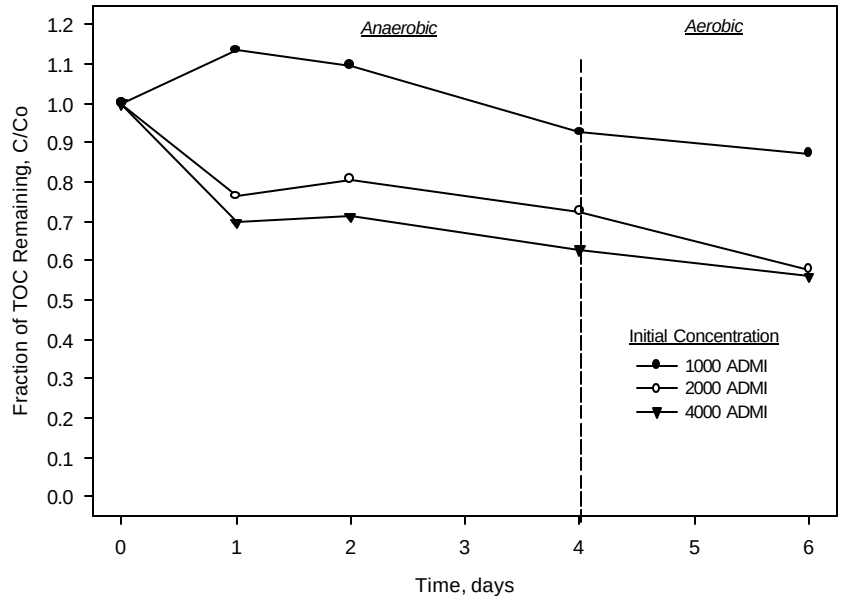


(b)

Figure 13: *Phase II: Test #2*: (a) Cypress Green dye ADMI color loss using anaerobic TSS concentration of 8000 mg/l, followed by aerobic TSS concentration of 2000 mg/L. (b) Cypress Green dye ADMI color loss using anaerobic TSS concentration of 4000 mg/l, followed by aerobic TSS concentration of 1000 mg/L.



(a)



(b)

Figure 14: *Phase II: Test #2*: (a) Cypress Green dye TOC reduction using anaerobic TSS concentration of 8000 mg/l, followed by aerobic TSS concentration of 2000 mg/L. (b) Cypress Green dye TOC reduction using anaerobic TSS concentration of 4000 mg/l, followed by aerobic TSS concentration of 1000 mg/L.

were 32 and 25 percent in the anaerobic and aerobic systems, respectively (Figure 16). The average NH_3-N production in the anaerobic reactors was 0.80mg/L, while no NH_3-N was measured in the aerobic reactors. In the anaerobic system, TKN levels were fairly constant following day five of treatment. However, on average, no TKN reductions were seen. A 28 percent TKN reduction was seen in the aerobic system between days 10 and 15 of treatment. All reduction values were calculated from the theoretical no-loss plot.

The TSS and VSS concentrations for the anaerobic and aerobic reactors, as well as the effluents, were monitored throughout the testing period. The average biomass concentration in the anaerobic reactors was 2200mg/L. The SRT was very high based on a wastage rate of approximately 0.001 percent. In the aerobic reactors, the average biomass concentration was also 2200mg/L, with an SRT similar to the anaerobic system.

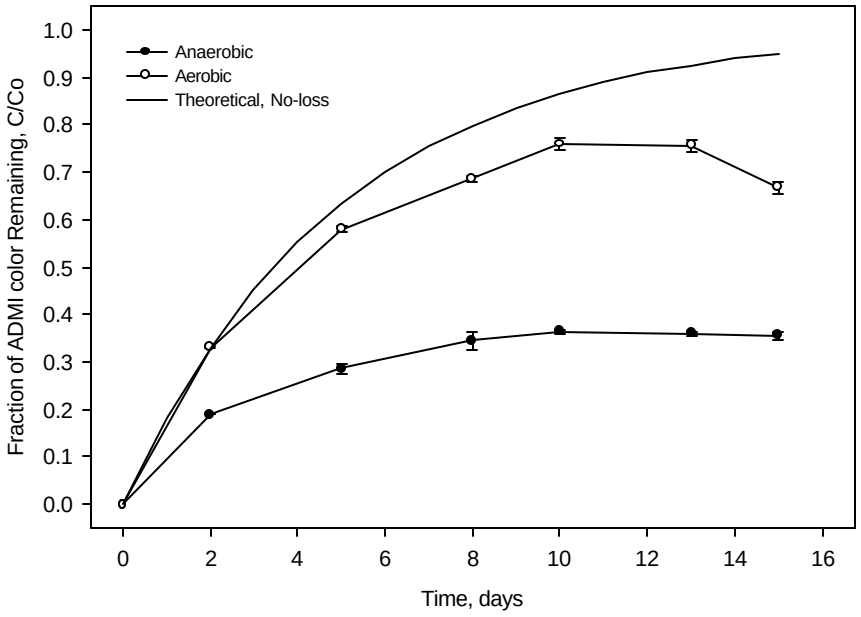


Figure 15: *Phase III: Test #1*: Anaerobic versus aerobic Cypress Green dye ADMI color loss. (Standard deviation, n=2)

Phase III: Test #2 utilized an ANA/AER/ANA/AER sequential step-treatment system design. The reactors and biomass from *Phase III: Test #1* were used for this test. The laboratory feed, with a Cypress Green dye concentration of 2ml/L, was fed into the first anaerobic reactor. The effluent from each reactor was withdrawn and then fed into the next sequential reactor. The HRT was set at five days for each of the treatment steps.

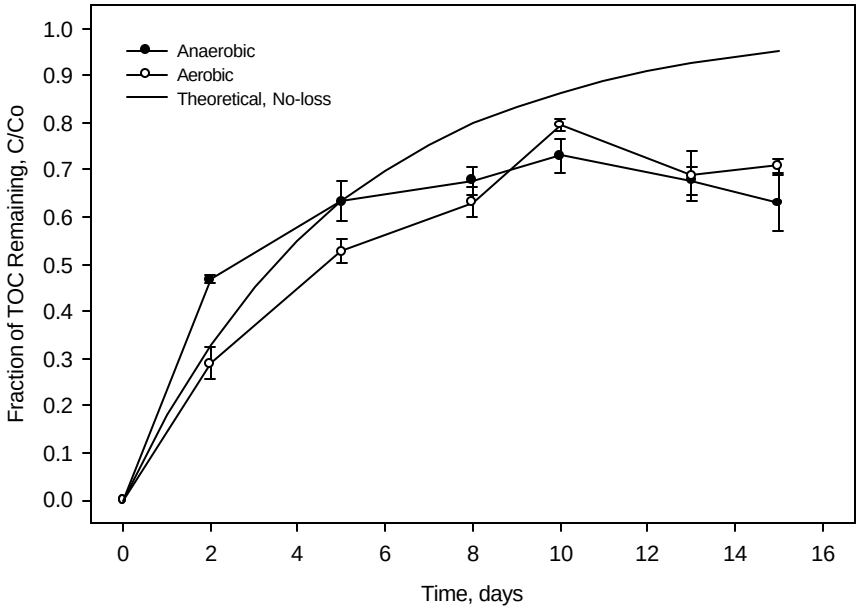


Figure 16: *Phase III: Test #1*: Anaerobic versus aerobic Cypress Green dye TOC removal. (Standard deviation, n=2)

The results (Figure 17) were, in part, similar to the ANA/AER sequential step-treatment systems from *Phase II.* All of the reported measurements were taken following 15 days of treatment. The ADMI color was 60 percent removed in the first anaerobic step, with an additional 21 percent removed in the first aerobic step. No change in the ADMI color was seen in the second anaerobic and aerobic steps. TOC removal was highest following the first aerobic step, with a 68 percent reduction. A small increase in TOC was measured following the second aerobic step. NH_3-N production of 1.36mg/L of was measured in the first anaerobic reactor, with a high removal occurring in the first aerobic reactor. NH_3-N was not detected in the final aerobic reactor. TKN was 69 percent reduced for the entire system. The low TKN value measured in the second anaerobic reactor is unexplained.

Phase III: Test #3 involved a set of four different treatment systems. They included an anaerobic system, an aerobic system, an ANA/AER sequential step-treatment system, and an ANA/AER/ANA/AER sequential step-treatment system. All of the systems were designed as batch reactors with a 4-day HRT. The sludges from *Phase III: Test #2* were used in all of the systems, with the VSS concentrations set at approximately

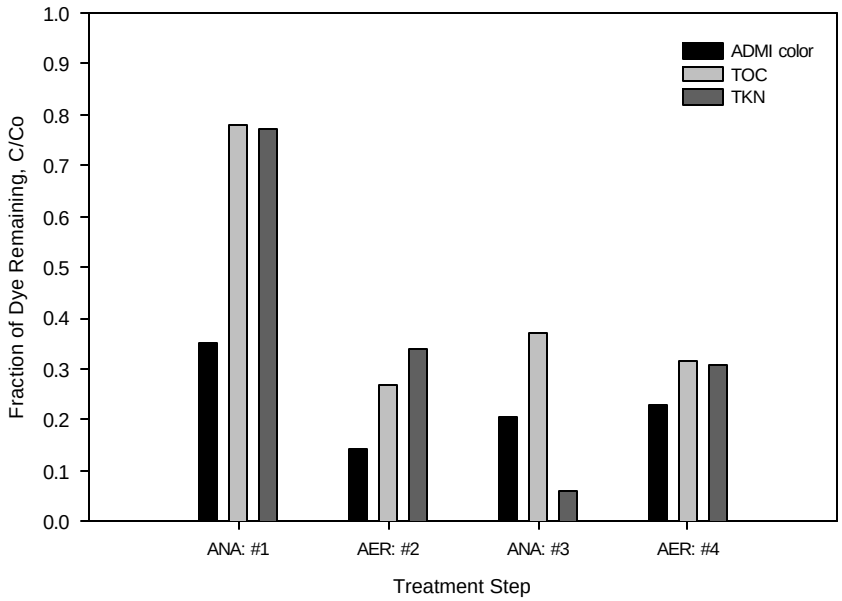


Figure 17: *Phase III: Test #2*: ANA/AER/ANA/AER sequential step-treatment of Cypress Green dye. The reported ADMI color, TOC, and TKN reductions were taken on day 15 of treatment system operation.

2000mg/L. The laboratory feed, with a Cypress Green concentration of 2ml/L, was fed to all of the test reactors.

The results from the anaerobic treatment system (Figure 18) showed an average ADMI color reduction of 62 percent between days two and four. TOC removal was higher than in the previous anaerobic systems, with an average reduction of 42 percent between days two and four. NH_3-N production was low, with a maximum concentration of 0.36mg/L measured on day two (Table 2). TKN biodegradation was also low, with an average reduction of six percent between days two and four.

The results from the aerobic treatment system (Figure 19) showed low to no removals for all of the parameters measured. The ADMI color was reduced by an average of nine percent between days two and four. TOC and TKN reductions did not occur, and no NH_3-N production (Table 2) was measured.

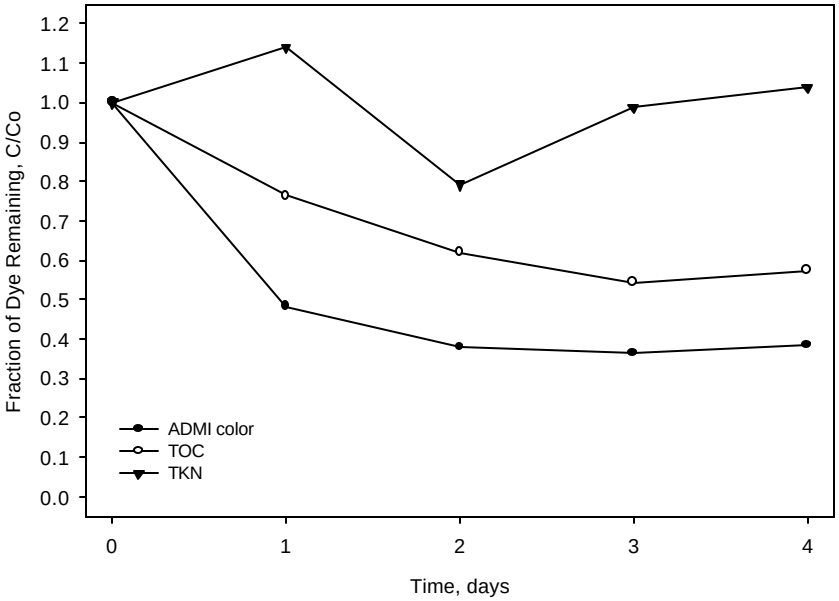


Figure 18: *Phase III: Test #3*: Anaerobic reduction of Cypress Green dye ADMI color, TOC, and TKN using batch reactors with an initial dye concentration of 2ml/L.

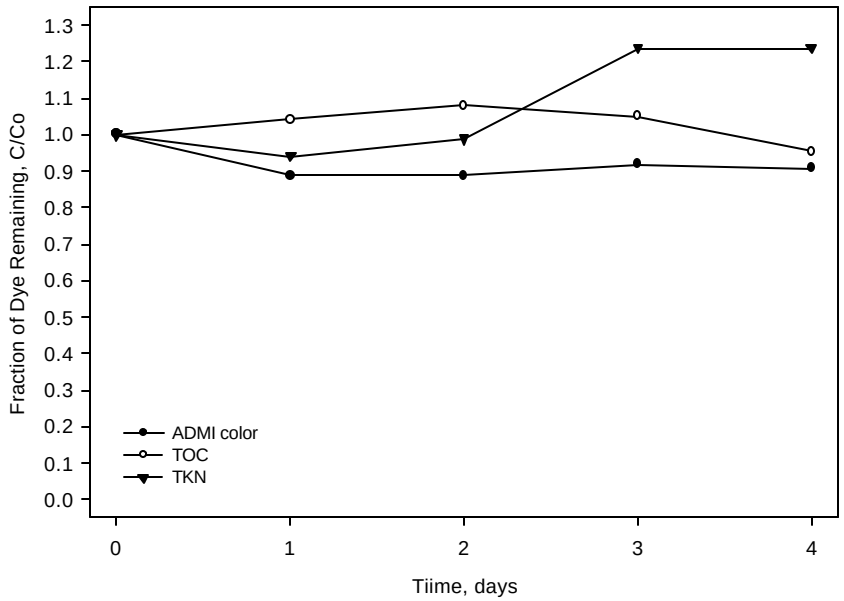


Figure 19: *Phase III: Test #3*: Aerobic reduction of Cypress Green dye ADMI color, TOC, and TKN using batch reactors with an initial dye concentration of 2ml/L.

In the ANA/AER system (Figure 20), the ADMI color was reduced 57 percent in the anaerobic step, with an additional reduction of two percent in the aerobic step. TOC removal was 12 percent in the anaerobic step. No additional TOC reduction occurred in

the aerobic system. An NH_3-N concentration of 1.12mg/L was measured following the anaerobic step, with none detected following the aerobic step (Table 2). TKN increased in both steps.

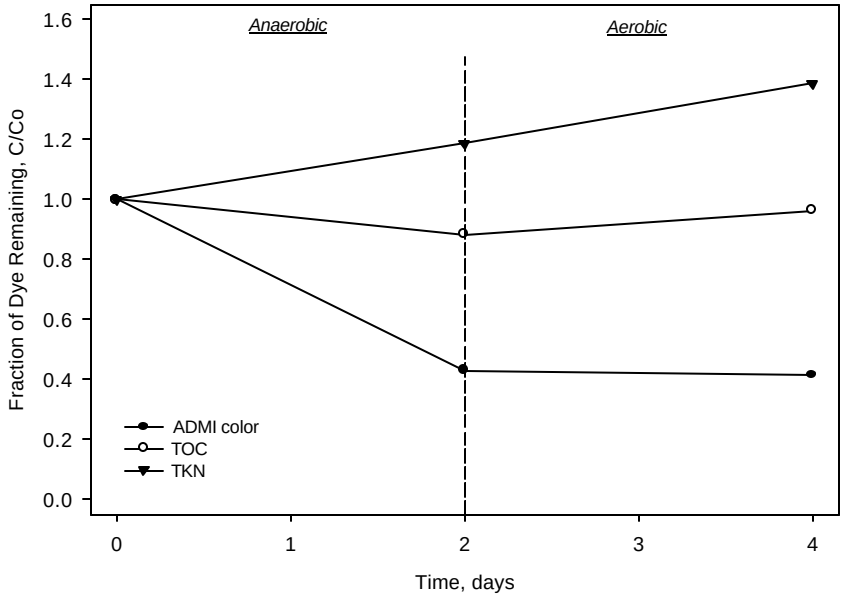


Figure 20: *Phase III: Test #3*: Reduction of Cypress Green dye ADMI color, TOC, and TKN using ANA/AER sequential step-treatment batch reactors with an initial dye concentration of 2ml/L.

In the ANA/AER/ANA/AER sequential step-treatment system (Figure 21), the ADMI color was 41 percent reduced, with 36 percent of the removal occurring in the first ANA/AER phase. TOC and TKN both increased over the duration of the test. NH_3-N production reached a maximum of 0.11mg/L in the first anaerobic step and was not detected in the subsequent aerobic and anaerobic steps (Table 2).

Treatment System	**Maximum NH_3-N, mg/L**	**Final NH_3-N, mg/L**
Anaerobic	0.36	0
Aerobic	0	0
ANA/AER	1.12	0
ANA/AER/ANA/AER	0.11	0

Table 2: Final and maximum NH_3-N concentrations detected in the different treatment systems used during *Phase III: Test #3*.

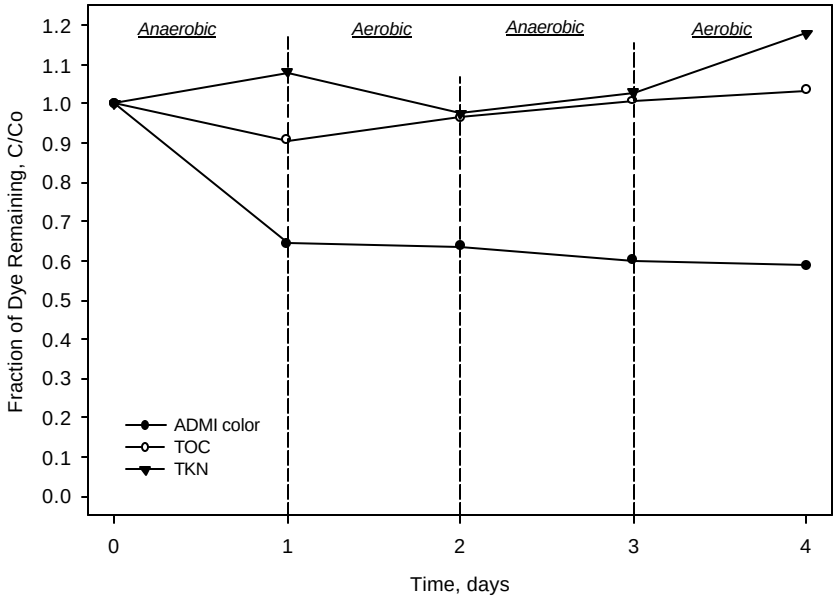


Figure 21: *Phase III: Test #3*: Reduction of Cypress Green dye ADMI color, TOC, and TKN using ANA/AER/ANA/AER sequential step-treatment batch reactors with an initial dye concentration of 2ml/L.

Phase IV

Phase IV: Test #1 utilized an ANA/AER sequential step-treatment system to degrade the POTW influent. The anaerobic and aerobic sludges from *Phase III* were used for this test. The effluent from the anaerobic reactor was withdrawn and then fed into the aerobic reactor. The HRT was one day in each of the reactors, which were operated in a batch-system. Test results (Figure 22) showed intermediate ADMI color, TOC, and TKN reductions for the POTW influent, with a complete NH_3-N removal. Influent ADMI color and TKN were reduced by a total of 52 and 57 percent, respectively. TOC was reduced by a total of 66 percent, with 22 percent occurring in the anaerobic step. NH_3-N was reduced from an initial 4.60mg/L to 0.00mg/L following aerobic treatment. *Phase IV: Test #2* was conducted exactly like *Phase IV: Test #1*. However, POTW activated sludge was used in the aerobic treatment step. This was done to allow for a comparison between the laboratory acclimated sludge and the POTW sludge. The results (Figure 23) showed high ADMI color, TOC, TKN, and NH_3-N reductions. Influent TOC and TKN were both 83 percent reduced and no NH_3-N was detected following aerobic treatment. The ADMI color was 67 percent reduced in the anaerobic step, with a slight increase experienced in the aerobic step.

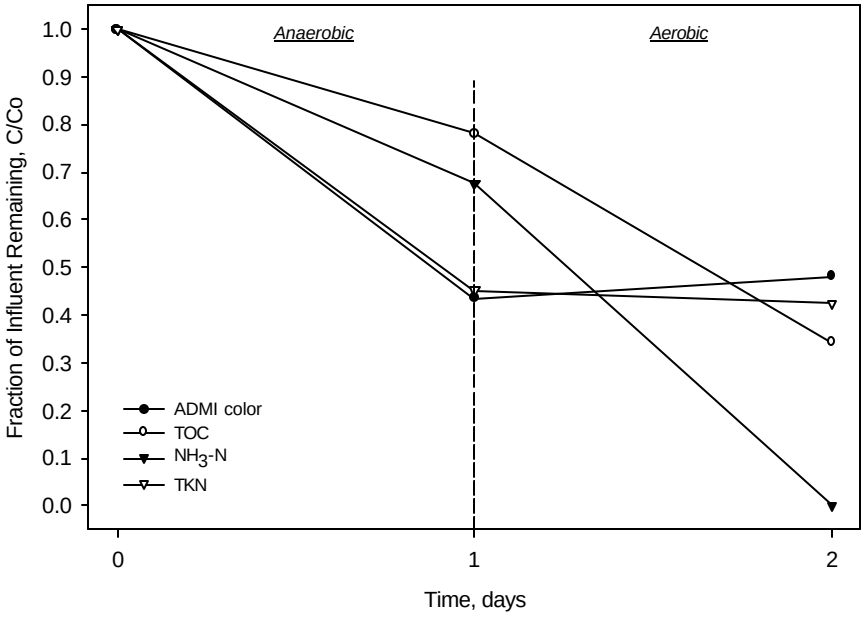


Figure 22: *Phase IV: Test #1*: Biodegradation of POTW influent using ANA/AER sequential step-treatment batch reactors with laboratory acclimated sludges.

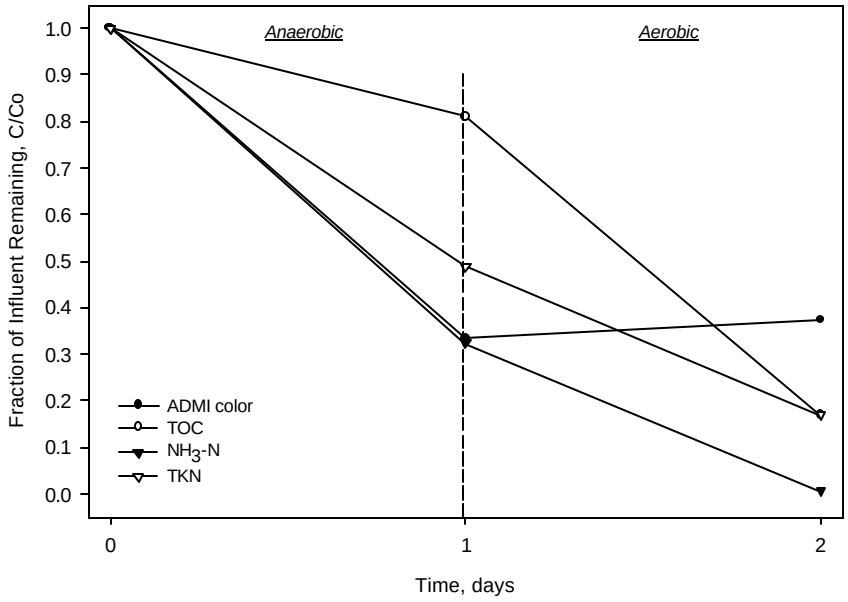


Figure 23: *Phase IV: Test #1*: Biodegradation of POTW influent using ANA/AER sequential step-treatment batch reactors with laboratory acclimated anaerobic sludge and POTW activated sludge.

POTW Data

The average ADMI color of the POTW influent during the months of February 2001 and April 2001 was 1252 units. POTW color removal data from these months (Figure 24) shows an average ADMI color reduction of approximately 80 percent for the entire treatment system. Most of the ADMI color loss, 63 percent, occurs in the first treatment basin, with smaller reductions occurring in the second treatment basin and the chlorine contact-tank (CCT).

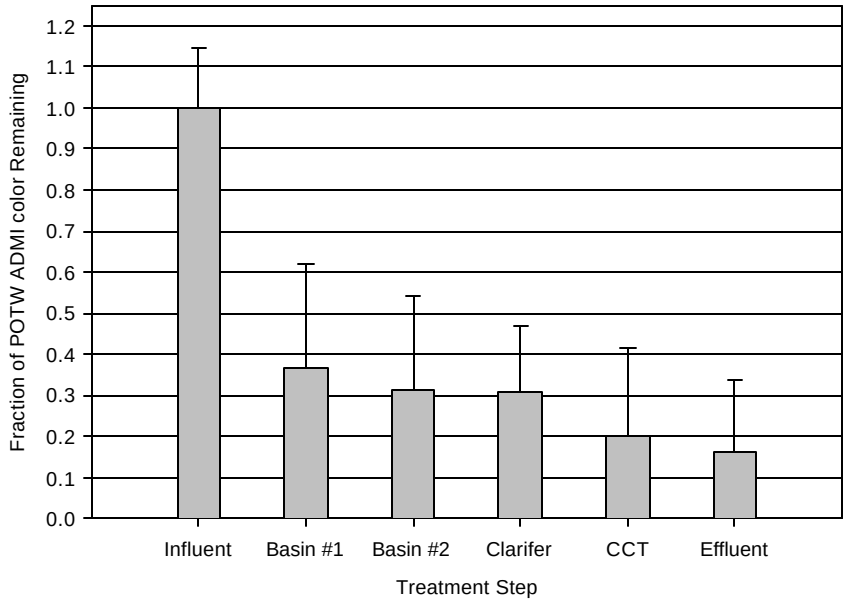


Figure 24: POTW influent ADMI color remaining in wastewater following various treatment steps. Data represents an average of the values recorded during the months of February 2001 and April 2001. ADMI color values were recorded daily for most treatment steps. (Standard deviation, n=53)

Metabolite Identification

Liquid-liquid extraction followed by GC-MS analysis was used to identify dye metabolites produced during anaerobic and aerobic biodegradation. Test results were variable, with a number of different compounds being identified. Several of the likely by-products from each type of treatment system are listed in Table 3. No further tests were conducted to verify these results. Table 3 should only be considered as a reference of possible metabolites produced by the biodegradation of Cypress Green, Sultan Red, and Indigo Blue reactive azo dyes.

POTW Effluent Cation and Anion Identification

IC analysis was used to identify selected cation and anion concentrations in the POTW effluent. Test results showed an extremely high concentration of chlorides, and moderately high nitrate and sulfate concentrations. Sodium was also detected at a high concentration. Additional results can be seen in Table 4.

Azo Dye Metabolites

Anaerobic Biodegradation	*Aerobic Biodegradation*
2-methylphenol	2-methylphenol
3-methylphenol	3-methyl-benzonitrile
Indole	Indole
1,4-dione-2,5-cyclohexadiene	Benzeneacetonitrile
Butylated Hydroxytoluene	

Table 3: Possible compounds produced during the anaerobic and aerobic biodegradation of Cypress Green, Sultan Red, and Indigo Blue dyes.

Cations		**Anions**	
Compound	*Concentration, mg/L*	*Compound*	*Concentration, mg/L*
Sodium, Na^+	630	Chloride, Cl^-	854
Potassium, K^+	18	Nitrate-N, NO_3-N	53
Magnesium, Mg^{++}	7	Phosphate-P, PO_4-P	15
Calcium, Ca^{++}	69	Sulfate, SO_4^{-2}	93

Table 4: POTW effluent cation and anion concentrations.

CHAPTER 5: DISCUSSION

The focus of this research was to determine if an improved treatment system could be developed to reduce the Lower Smith River POTW effluent ADMI color. This facility receives approximately 80 percent of its influent flow from a textile dyeing and finishing plant. Treatment systems utilizing anaerobic and aerobic microbiological sludges in several different sequences were tested for their ability to degrade the POTW influent and laboratory dye solutions. ADMI color reduction was the primary treatment goal, however, carbon and nitrogen removals were also measured. Previous research performed by Edwards (2000) and preliminary tests from this study both confirmed that the reactive azo dye, Cypress Green, has a color reduction rate similar to the POTW influent. Most of the treatment systems were fed a solution of Cypress Green dye. Data from these tests were used to determine the treatment design bested suited for degrading the textile mill effluent. The findings from this research are discussed below; the reader is referred to Chapter 4 for individual test results. The findings from *Phase I* through *Phase III* tests are discussed first, followed by the final tests performed on the POTW influent in *Phase IV*.

Treatment of Cypress Green Dye

The results from preliminary testing confirmed that anaerobic reduction of the POTW influent ADMI color was possible, but less than the color loss experienced by different laboratory dye solutions. Indigo Blue and Sultan Red azo dyes both exhibited greater reductions in ADMI color than the POTW influent during preliminary testing. The Cypress Green dye had an ADMI color reduction similar to the POTW influent following anaerobic treatment with Pepper's Ferry anaerobic digester sludge in *Phase I: Test #2* (Figure 5). For this reason, it was decided that the Cypress Green dye would be used as a surrogate solution for the POTW influent.

Anaerobic Treatment

During anaerobic treatment, it is expected that the dye molecules would cleave via reduction of the azo bonds (Chinwetkitvanich, 2000; Razo-Flores *et al.*, 1997; Loyd, 1992, Ganesh, 1992; Brown and Hamburger, 1987; Brown and Laboureur, 1983, Chung

et al., 1978). While this is often effective for reducing the color of the dye, it does not usually lower the carbon content (Loyd, 1992, Ganesh, 1992). Further breakdown of the dye molecule may be inhibited by the complex aromatic ring structures found in many azo dyes.

All of the anaerobic treatment systems utilizing Pepper's Ferry anaerobic digester sludge and fed with the standardized Cypress Green dye feed solution ADMI color removals of approximately 60-75 percent of the initial ADMI values, except for *Phase I* tests, which were slightly higher. While some minor differences were seen in *Phase II: Test #2*, it appears that the initial dye concentration does not largely dictate the fraction of color removal. This result does not agree with the results of Seshadri and Bishop (1994) who performed a study investigating the effect of different influent dye concentrations on the color removal efficiency. They concluded that elevated dye concentrations might cause a drop in color loss. *Phase II: Test #2* does indicate some variance in the ADMI color reduction efficiency with increased dye concentrations; however, the difference is only 10 percent among dye concentrations varying from 1000 to 4000 ADMI units (Figure 13).

Carbon and nitrogen removals were typically low for all of the anaerobic treatment systems. TOC reductions varied from 17 to 42 percent with an average of 27 percent for all of the anaerobic systems tested. Compared to the ADMI color loss, the TOC reduction was much smaller, indicating loss of color is due to partial rather than complete degradation of the dye molecule. This was expected based on the findings of Loyd (1992), Ganesh (1992), and Brown and Hamburger (1987) who all reported good ADMI color losses, but low carbon removals following the anaerobic treatment of various azo dyes. It is hypothesized that the dye molecules undergo only partial degradation, limiting the carbon loss, but not the color removal.

Furthermore, a small, but proportionate fraction of the Cypress Green dye TOC and COD are removed during anaerobic treatment. This is based on an average 0.37 TOC/COD value seen in the *Phase II: Test #1* effluent, as compared to the 0.32 TOC/COD value for the standardized feed. An average 22 percent COD reduction was seen in this test (Figure 12.a).

Deamination was not appreciable in any of the anaerobic systems tested for

NH_3-N production. NH_3-N concentrations ranged between 0.11mg/L and 1.4mg/L, with an average production of 0.75mg/L. Assuming all of the NH_3-N was produced via deamination of Cypress Green dye, this would represent 17 percent of the total dye nitrogen fed into the systems. The average anaerobic TKN reduction was only 8 percent. Combining the average NH_3-N production and TKN reduction, 25 percent of the total dye nitrogen is either incorporated into the biomass or made readily available for further use by the microorganisms during anaerobic treatment.

Aerobic Treatment

Except for Phase I: Test #3, aerobic treatment of Cypress Green did not greatly reduce ADMI color, carbon, or nitrogen levels. During preliminary testing with the POTW activated sludge, a high reduction in ADMI color was seen; however, this test does not correlate well with later tests. In *Phase III: Test #1*, the average ADMI color loss following aerobic treatment was 28 percent, while the average TOC reduction was 25 percent. Compared to anaerobic treatment, the percentage of ADMI color removed during aerobic treatment is more highly correlated with the TOC loss. The TOC loss may be, in part, due to the degradation of dye additives; however, no tests were performed to confirm this. Somewhat similar results were seen in *Phase III: Test #3*, with a nine percent reduction in ADMI color and zero percent TOC loss. These were expected results based on the findings reported in the literature. Pagga and Brown (1983) concluded, “As expected from their structures and function, dyestuffs are most unlikely to [biodegrade] in short-term aerobic tests". They do state however, that carbon removal is possible in an aerobic environment, but does not always correlate with decolorization.

Nitrogen removal was similar to the ADMI color and TOC losses. No NH_3-N was detected during aerobic treatment, while TKN removals varied from 0 to 28 percent in *Phase III: Test #1* and *Phase III: Test #3*, respectively.

ANA/AER Sequential Step-Treatment

ANA/AER sequential step-treatment of Cypress Green provided the greatest reductions in ADMI color and carbon, but no nitrogen removal was observed. Three separate test series were conducted using ANA/AER sequential step-treatment, with an

average ADMI color removal of 71 percent. The TOC reduction was also high, with an average 38 percent removal for *Phase II: Test #1* and *Phase II: Test #2*. The zero percent TOC loss observed in *Phase III: Test #3* is unexplained.

Theoretically, the greatest ADMI color loss is expected to occur during anaerobic treatment, with little accompanying carbon removal. Once the azo chromogen is destroyed, the dye metabolites are subject to further biodegradation under aerobic conditions (O'Neill *et al.*, 2000; Seshadri and Bishop, 1994; Loyd, 1992; Brown and Hamburger, 1987). Looking at the results for *Phase II: Test #1* (Figures 9-11) this type of sequential degradation is seen. Most of the ADMI color loss occurs during the anaerobic step, with a higher fraction of the TOC removed during the aerobic step. Loyd's results (1992) also support this finding.

According to Oniell *et al*. (2000), total organic nitrogen (TON) levels may increase following anaerobic treatment, but subsequently decrease after aerobic treatment. Nitrogen removal was measured only in *Phase III: Test #3*, and no losses were seen. Admittedly, most of the parameters tested in *Phase III: Test #3* showed low reduction percentages compared to the other ANA/AER sequential step-treatment systems. NH_3-N production was moderate during this test; a concentration of 1.12mg/L was measured following the anaerobic step. The NH_3-N was subsequently removed following aerobic treatment of the anaerobic effluent. Based on the NH_3-N concentrations produced during the anaerobic treatment of Cypress Green, toxicity is not believed to be a concern, especially if the wastewater is aerobically polished before discharge.

ANA/AER/ANA/AER Sequential Step-Treatment

ANA/AER/ANA/AER sequential step-treatment of Cypress Green did not provide reductions beyond the first ANA/AER treatment steps. High ADMI color and TOC removals were observed following the first ANA/AER treatment steps of *Phase III: Test #2*. It was expected that further reductions would be seen in the second ANA/AER treatment steps, once the dye molecules were prone to further anaerobic degradation. This hypothesis was not met as indicated by the data shown in Figure 17 for ANA/AER/ANA/AER sequential step-treatment. The results do support the idea that

there is a recalcitrant fraction within the Cypress Green dye that biodegrades very slowly or not at all.

Nitrogen removal was high, with an average 61 percent TKN reduction in *Phase III: Test #2* (Figure 17). The increased TKN removal is unexplained. NH_3-N production was higher than in previous tests, with a 1.46mg/L concentration detected in the first anaerobic step of this test. No NH_3-N was detected following the second aerobic treatment step. Again, aquatic toxicity from an elevated NH_3-N concentration in the final effluent is not a concern.

Treatment of the POTW Influent

After using different systems to treat the Cypress Green dye, it was determined that the ANA/AER sequential step-treatment design provided the greatest ADMI color reduction. Because color removal from the POTW influent was the primary objective of this research, the fate of the POTW influent was studied in two ANA/AER sequential step-treatment systems.

The results from *Phase IV: Tests #1* and *#2* were, in part, similar to previous ANA/AER treatment systems. POTW activated sludge was used in the second test for comparison with the laboratory acclimated sludge. As expected, an intermediate to high reduction in the ADMI color was measured during both tests, with no losses occurring in the aerobic steps. The slightly higher anaerobic reduction in *Test #2* cannot be explained, as both systems were identical. An increase in the ADMI color was observed following anaerobic treatment in the aerobic steps. Loyd (1992) also observed a similar increase in ADMI color following aeration of an anaerobically treated azo dye effluent. It is hypothesized that a fraction of the dye molecules in the anaerobic effluent are not completely reduced, and after extended aeration are reoxidized to a darker color.

TOC removal was higher in *Test #2* than in *Test #1*, with reductions of 66 and 83 percent, respectively. The TOC fractions removed during anaerobic treatment were very similar, with an average of 21 percent. As before, the majority of the TOC was lost during aerobic treatment. Comparing *Test #1* and *Test #2*, the POTW activated sludge provided a greater TOC removal than the laboratory acclimated sludge. This suggests

that extended biomass acclimation periods may be necessary to achieve optimal dye reduction.

Total nitrogen removal was higher than in previous ANA/AER sequential step treatment systems. Anaerobic TKN removal was approximately 54 percent in both tests. However, TKN loss was greatest in *Test #2*, using the POTW activated sludge. Again, this may indicate that extended biomass acclimation periods may be necessary for optimal dye reduction. Based on the TKN removal measured in *Test #2*, it seems possible that achieving future permit standards for effluent TON concentrations may not be a problem. However, this assumption largely relies on the influent TON concentration. In both tests, the initial 4.60mg/L NH_3-N concentration in the POTW influent was reduced to zero following ANA/AER sequential step-treatment. As before, permitted that the textile wastewater is aerobically treated before discharge, and the treatment system supports nitrification, the chance of aquatic toxicity from an elevated NH_3-N concentration is minimized.

Recommendations for the POTW

Using their current treatment system design, the POTW is capable of achieving an 80 percent ADMI color reduction, and is generally able to meet the VPDES permit level of 300 ADMI color units. Most of the ADMI color loss, 63 percent, occurs in the first treatment basin, with smaller reductions occurring in the second treatment basin and the chlorine contact-tank (CCT) (Figure 25). The POTW system does not employ an anaerobic treatment step. By varying the mixing speed of their mechanical surface aerators, the POTW attempts to create an anoxic/aerobic/anoxic/aerobic (ANO/AER/ANO/AER) sequential step-treatment system. Based on this research, the POTW treatment system is operating in the most effective feasible manner. Preliminary tests confirmed that anoxic and anaerobic treatment produce similar reductions in ADMI color. Anaerobic treatment of Indigo Blue dye provided only a slightly greater reduction than anoxic treatment. Results from Zissi and Lyberatos (1996) support this finding. Their work confirmed that the anoxic biodegradation of p-aminobenzene, a simple azo dye, is effective in removing its apparent color.

The findings from this research suggest that an ANA/AER sequential step-treatment system is the most effective method for biologically treating textile wastewaters. Furthermore, based on these findings, the POTW may lower operating costs and maintain optimal color removal by reducing the mixing speed in the first

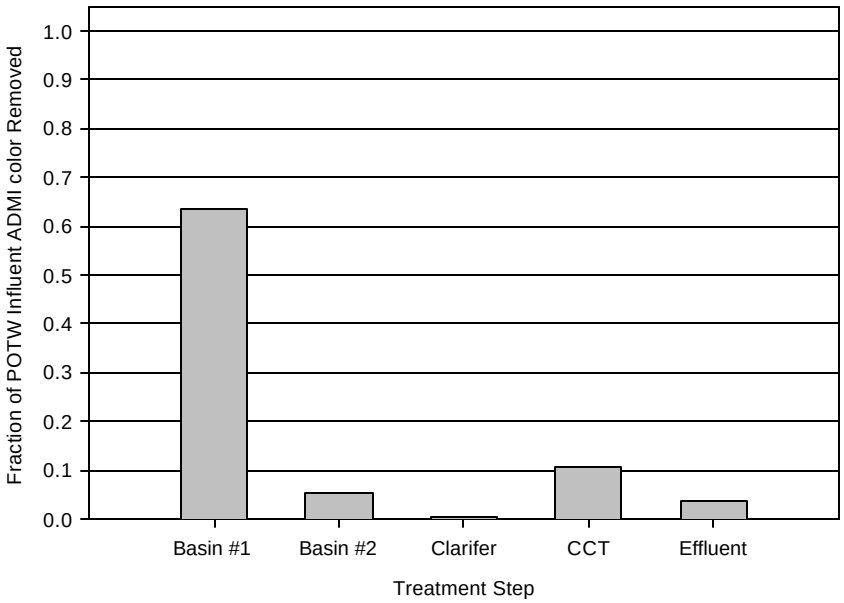


Figure 25: Fraction of POTW influent ADMI color removed from wastewater following various treatment steps. Data represents an average of the values recorded during the months of February 2001 and April 2001.

treatment basin, while maintaining a medium mixing speed in the second treatment basin. By doing so, the POTW will essentially operate as an ANO/AER sequential step-treatment system. Physical alteration of the POTW would be necessary to achieve anaerobic conditions in the first treatment basin.

CHAPTER 6: SUMMARY AND CONCLUSIONS

Evidence from this study suggests that biological color removal of textile wastewaters is sufficient to meet a required level of 300 ADMI units. Furthermore, the carbon and nitrogen concentrations within these wastewaters may also be biologically treated and reduced, but these losses may be from the degradation of dye additives. The findings of this research correspond well with the results of similar studies found in the literature. The data presented in this text applies most aptly to the wastewater received by the Lower Smith River POTW in Henry County, Virginia.

In this research, various biological treatment systems were evaluated for their ability to degrade textile wastewaters containing reactive azo dyes. ADMI color reductions were seen in most of the treatment systems tested; however, ANA/AER sequential step-treatment produced the greatest removals for both ADMI color and TOC. Anaerobic treatment generally produced the highest ADMI color reductions, but was not effective for removing carbon and nitrogen. Without anaerobic pretreatment, aerobic treatment typically was inadequate for reducing ADMI color, carbon, and nitrogen. In one preliminary test the ADMI color of several dye solutions and the POTW influent were successfully reduced, but this test proved to be an exception compared to later results. ANA/AER/ANA/AER sequential step-treatment did not yield reductions greater than ANA/AER sequential step-treatment alone. These findings compare well to the results found in the literature.

The conclusions from this study are as follows:

- The Sultan Red, Indigo Blue, and Cypress Green reactive azo dyes may be partially decolorized using biological anaerobic and aerobic treatment, but the Cypress Green dye is most resistant.

- The POTW influent is slightly more resistant to biological color loss compared to the reactive azo dyes tested, but generally exhibits a greater reduction in TKN and TOC following ANA/AER treatment.

- Anaerobic treatment of the POTW provides the greatest reduction in ADMI color, while aerobic post-treatment of the anaerobic effluent provides higher reductions in TOC, with a possibility for additional TKN removal.

- ANA/AER sequential step-treatment is the most efficient method for removing textile wastewater ADMI color, TOC, and nitrogen concentrations.

APPENDIX

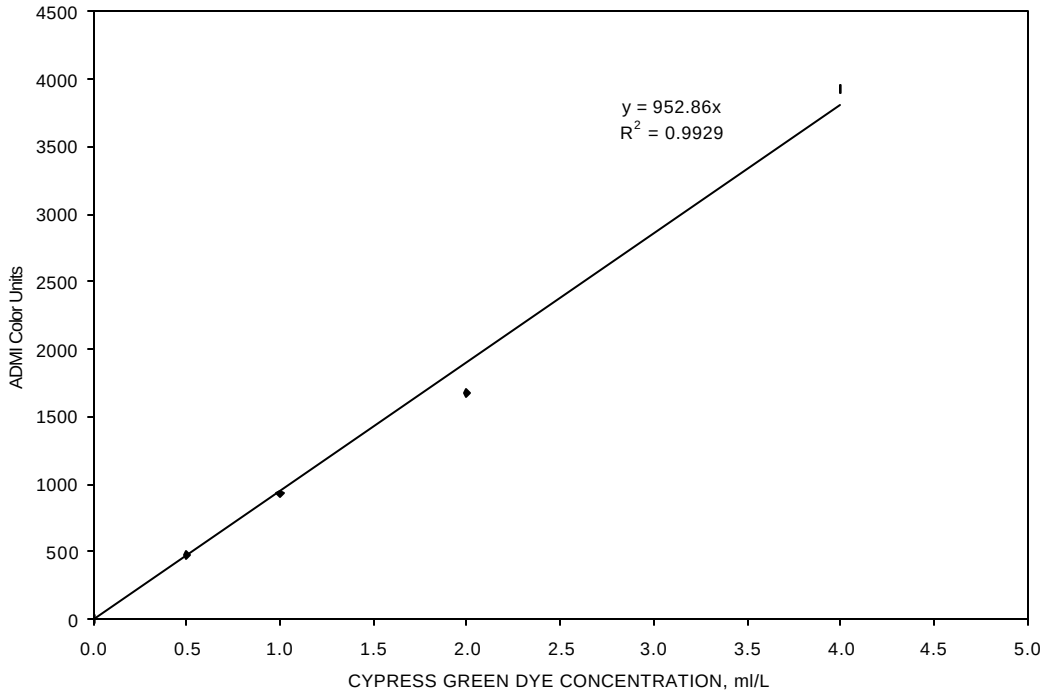
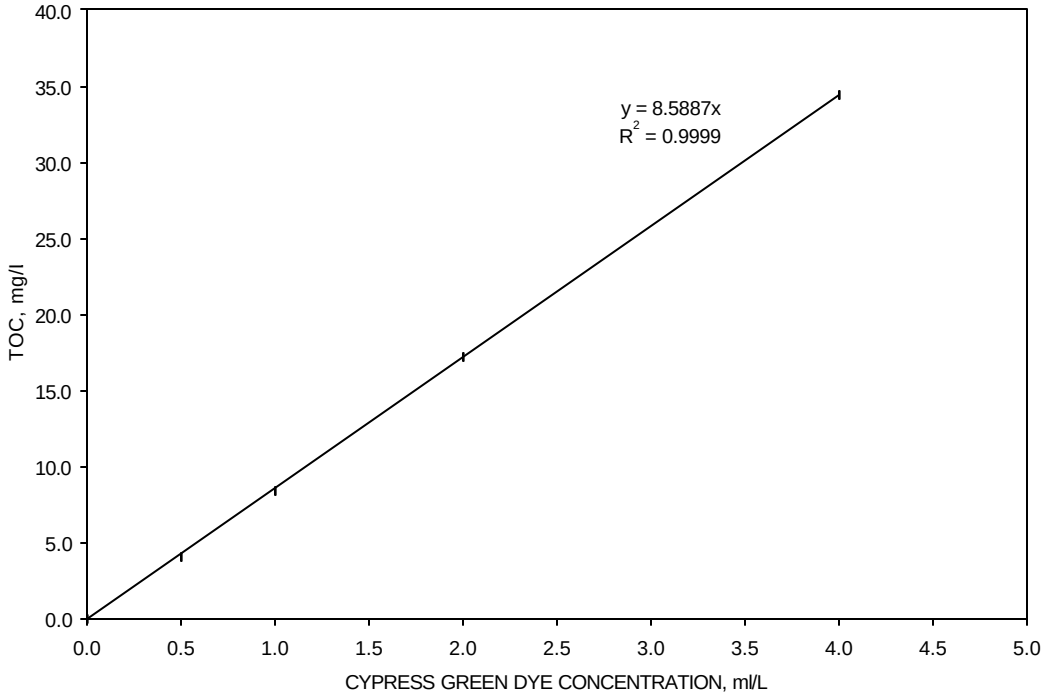
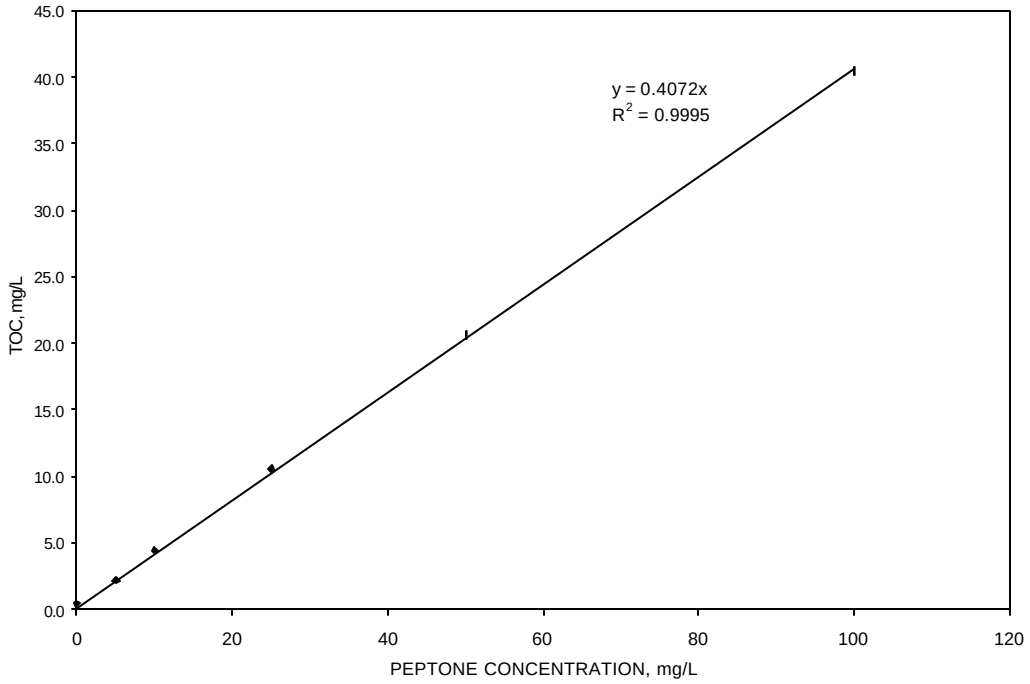


Figure 26: Standardized curve for Cypress Green dye ADMI.

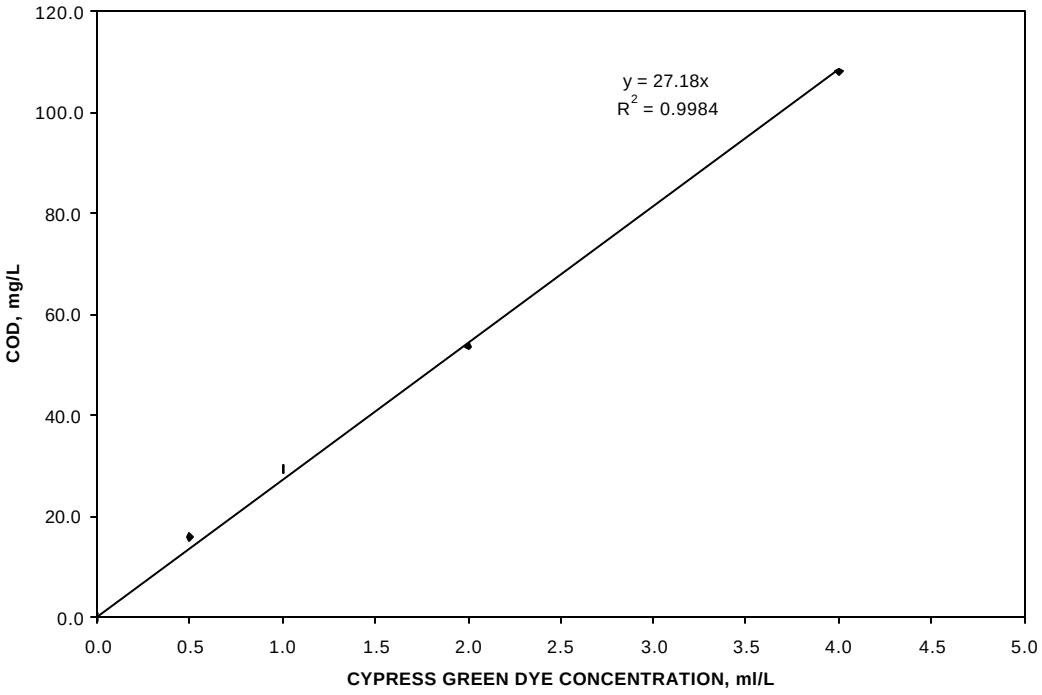


(a)

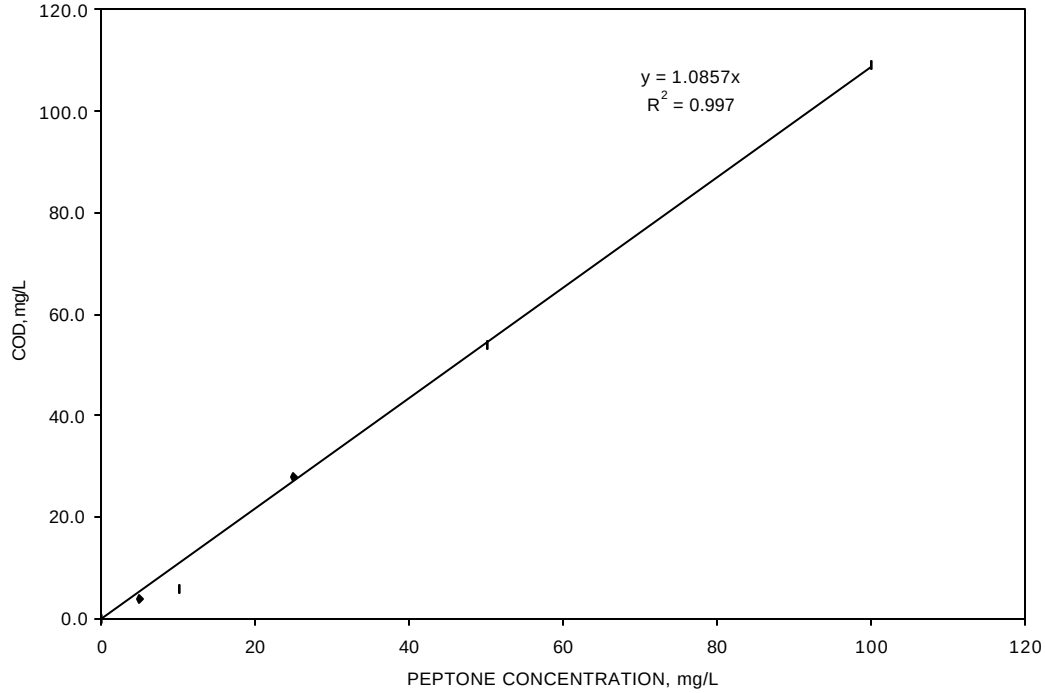


(b)

Figure 27: (a) Standardized curve for Cypress Green dye TOC. (b) Standardized curve for Peptone TOC.

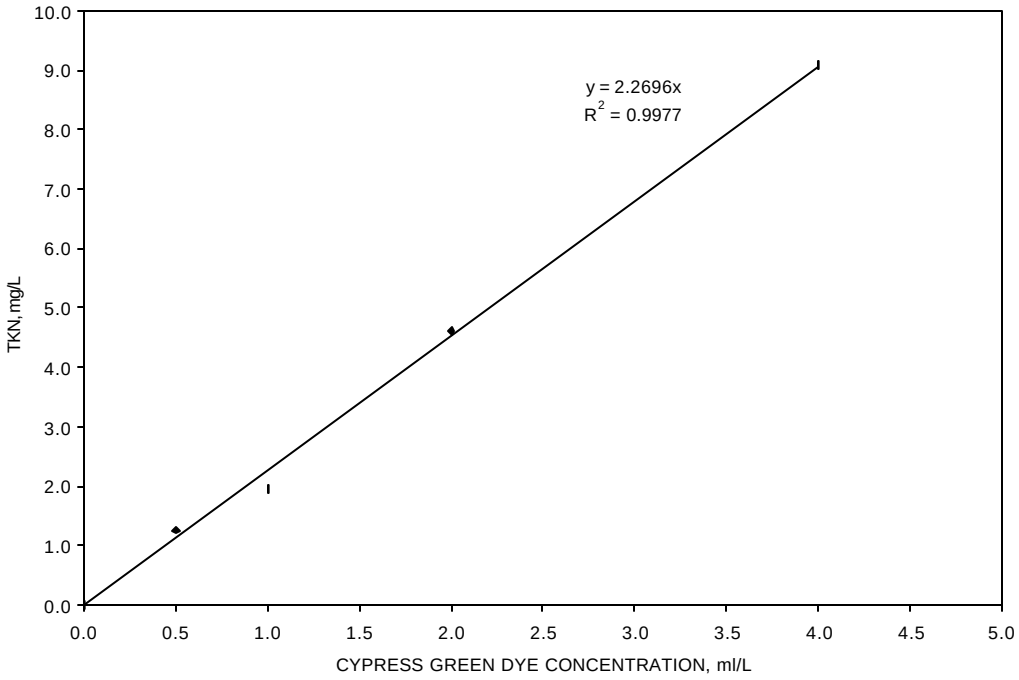


(a)

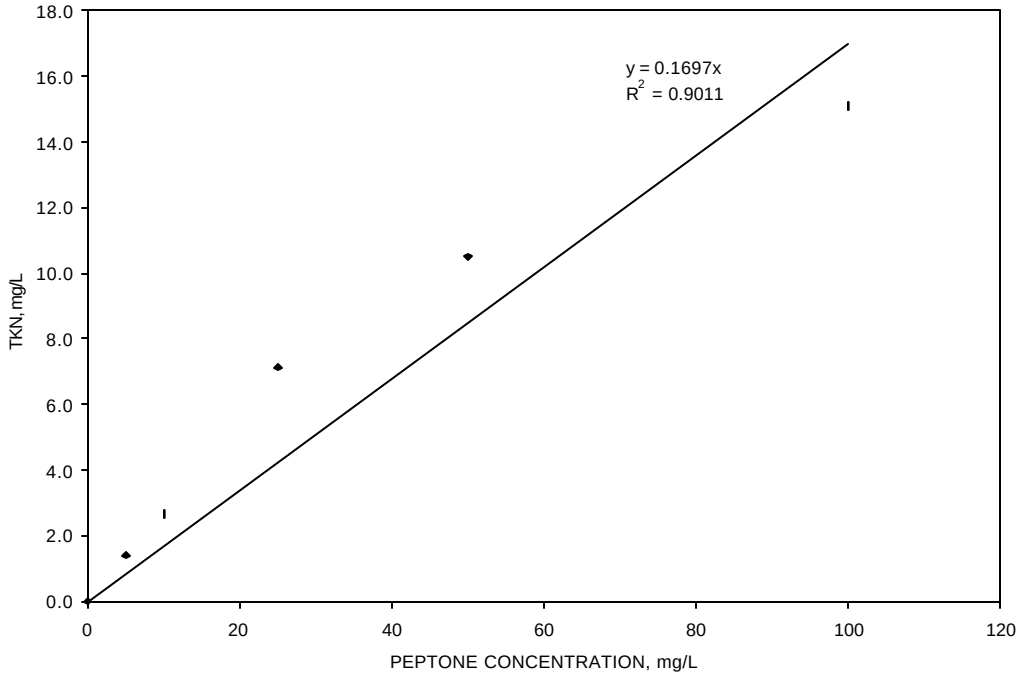


(b)

Figure 28: (a) Standardized curve for Cypress Green dye COD. (b) Standardized curve for Peptone COD.



(a)



(b)

Figure 29: (a) Standardized curve for Cypress Green dye TKN. (b) Standardized curve for Peptone TKN.

VITA

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