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Soil And Water Remediation Using Controlled Release Polymer

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SOIL AND WATER REMEDIATION USING CONTROLLED RELEASE POLYMER

by

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DEDICATION

I would like to dedicate this thesis to my mom, Vinnette Nembhard, family and friends. I am grateful for all their prayers, support, continued love and encouragement as these factors have kept me focused and motivated me to complete this thesis. Most certainly not last, it wouldn't have been possible without God's continual blessing in my life.

BIOGRAPHICAL SKETCH

Jerrine Theresa Taniesha Foster was born on October 23, 1986 in Trelawny, Jamaica. She attended the Convent of Mercy Academy “Alpha” high school where she developed a passion for science and mathematics. After her matriculation at Alpha High school she attended North Carolina Agricultural and Technical State University to pursue a Bachelor of Science degree in Civil Engineering. Upon receiving her Bachelor of Science degree in 2010, she continued on to graduate school. Currently, she is a candidate for the Masters of Science degree in Civil Engineering at North Carolina Agricultural and Technical State University.

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Conclusively, I give thanks to my Lord Jesus for mental and physical strength to complete this milestone in my life.

TABLE OF CONTENTS

LIST OF FIGURES

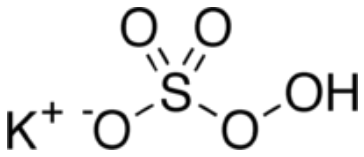
FIGURES **PAGE**

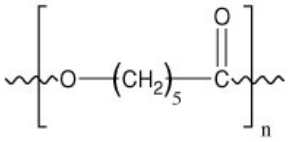
LIST OF TABLES

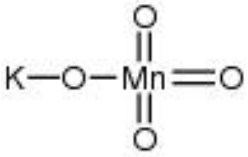
TABLES **PAGE**

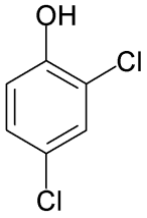
LIST OF NOMENCLATURE

DESCRIPTION **STRUCTURES**

Potassium peroxymonosulfate ……..…… 

Polycaprolactone (PCL) ……..……..… 

Potassium permanganate (KMnO4) ……..……..… 

2-4-dichlorophenol……..... 

LIST OF SYMBOLS

ABBREVIATION	DEFINITION
CFU	Colony forming unit
E. coli	Escherichia coli
HCl	Hydrochloric acid
NaOH	Sodium hydroxide
PCL	Polycaprolactone
TC	Total coliform

ABSTRACT

Foster, Jerrine Theresa Taniesha. SOIL AND WATER REMEDIATION USING CONTROLLED RELEASE POLYMER. **(Major Professor: Dr. Stephanie Luster-Teasley)**, North Carolina Agricultural and Technical State University.

Water and soil treatment for the eradication of pathogens are important today and will forever be of importance in the future. It has been noted that poor water quality poses major threat to human health and is responsible for the deaths of 1.8 million people annually worldwide, with over 90% (1.6 million) of the reported cases being children under the age of five (United Nations International Children's Emergency Fund, 2005). Fresh water can be accessed for personal use and recreation through fresh water sources, such as rivers, lakes, groundwater and springs. These sources, especially surface water sources, are exposed to high concentration of pollutants. These pollutants pose major threats to humans; hence, this issue needs to be addressed. Bacterial profiling was conducted to understand the pathogenic pollution levels in Lake A and B at the Greensboro's Country Park. The indicated levels of both lakes had values that surpassed US EPA criteria, which made it suitable for water treatment with controlled release chemical oxidant polymer (CRCOP). CRCOP was successful in the eradication of *E. coli* and Enterococci bacteria. Soil treatment experiments indicated the need for soil to be saturated for CRCOP to be effective.

CHAPTER 1

INTRODUCTION

1.0 Background

Water contamination is a global issue that needs to be addressed. It has been noted that poor water quality poses major threat to human health and is responsible for the deaths of 1.8 million people every year worldwide, with over 90% (1.6 million) of the reported cases being children under the age of five (United Nations International Children's Emergency Fund, 2005). A countries' access to water for personal use and recreation through fresh water sources, such as rivers, lakes, groundwater and springs is important for public health. These sources, especially surface water sources, need to be safe, pathogen free, and not exposed to high concentrations of pollutants. Examples of water pollutants occur in many forms, such as from microorganisms, metals, sediments and chemicals or pesticides, can end up in our water directly or indirectly. Whether these pollutants are from direct sources or indirect sources, they pose threats to the well-being and development of all human beings.

These pollutants can exist in many sources of water bodies such as groundwater and surface water, which comprise of rivers, lakes, streams and the ocean. Pollutants in variable amounts may impair water sources that people use for everyday activities such as for drinking, cooking, laundry, bathing and recreational purposes. When people are exposed to water sources that are polluted, they can contract numerous types of illnesses, due to diseases that can lead to death. Safe access to water is one of the most important

issues for both developed and developing countries. Therefore, research investigating remediation methods to ensure potable water supplies are vital.

1.1 Direct Sources of Water Contamination

Point source pollution, also referred to as direct sources, are usually described as those pollutants that can be tracked to an exact source, such as pollutants that occur due to industrial waste, sewer discharge from distribution networks or treatment plants, concentrated animal feeding operations and animal waste discharge into or near water sources. In the same regard, pathogens can be transported into our water bodies. In the United States, the highest incidents of pathogen contamination in surface and recreational water is directly connected to leaks from untreated sewage, animal or human fecal waste and storm water loading. The latter seems to be the most problematic for recreational water sources, as noted by the US Environmental Protection Agency (USEPA). Storm water also becomes a major problem during high rainfall events such that some sewer systems overflow or are redirected directly into rivers, which eventually carry pollutants and bacteria to beach waters, rivers, lakes and streams (US Environmental Protection Agency, 2011b).

1.2 Indirect Sources

A non-point source is an indirect source of pollution and is usually defined as any source of pollution that is difficult to track. In further connotation, these sources usually travel over land during runoff, which means that it is hard to locate each pollutant’s exact source. Some sources that can contribute to indirect pollution are farmlands, watersheds,

cities and their streets, and waste being washed into water bodies from animals or organisms that are a part of our ecosystem. Non-point source of pollution makes it challenging to prevent such contamination and difficult to enforce laws and regulations. This is almost impossible because if authorities cannot find the exact source of the problem then they cannot hold anyone accountable. This situation will only lead to more expenses to remediate as it cannot be stopped at the source of origin. Another dilemma is that this issue makes the problem continuous, which may eventually lead to less effort to remediate contaminated water. Remediating water can be very costly, especially if contamination always re-occurs.

1.3 Waterborne Diseases

Non-point and point source pollution are known threats to our water systems. The colossal concern is the pollution of freshwater bodies through non-point sources of pollution, as it makes remediation very difficult. This is very difficult as the pollution cannot be stopped at its source but persist for a long time. This issue may lead to public health concern and increase the cost of treating water before distribution for public use. When water is not treated or improperly treated the risk of pathogenic exposure to people that come in contact with such element increases. This exposure may develop into serious health problems, especially if ingested. Hence, it is imperative to eliminate or reduce water-borne pathogens through treatment.

Pathogens impact health by impairing body functions, which may eventually lead to death. Body functions are impaired when pathogenic microorganisms come in contact

with human or animal. Many microorganisms come in contact with the body through activities such as bathing, drinking contaminated water and using contaminated water for recreational purposes. These exposures can happen through different pathways that includes ingestion, inhalation or through infected wounds (Pond, 2005). A more detailed explanation of the impact of pathogens on human health can be accessed in chapter 2.

1.4 Project description

The goal of this work is to investigate a method to reduce pathogen levels in recreational surface water, agricultural wastewater, and agricultural soils using a controlled release chemical oxidant polymer (CRCOP) developed by Dr. Stephanie Luster-Teasley's research group. Water and soil samples for this project were taken from the Greensboro Country Park and North Carolina Agricultural and Technical State University Swine Unit respectively. Water samples were taken from Greensboro Country Park, which had a high number of waterfowl in the vicinity of the lake. Samples of water were taken on a monthly basis, when permissible, to show distribution of bacteria in the chosen lakes. Water retrieved from this lake was used to evaluate the controlled release treatment method. Preliminary work was conducted using soil samples obtained from North Carolina A&T State University's Swine unit for testing and treatment of soil and sediment.

Chapter 2 entails the literature review and will outline the literature that supports this study. It will examine and discuss other studies that were done and are somewhat

similar to this work. It will also show how this study can be achieved through the implementation of some of the ideas found in earlier work, but at the same time achieving uniqueness in this study. Chapter 3 is materials and methods and will examine and discuss the procedures taken to achieve results. Chapter 4 is described as the results and discussions and it will summarize all the data and findings for this study. Lastly, chapter 5 is the conclusions and recommendations that will use the summarized findings to make effective judgments and statements.

CHAPTER 2

LITERATURE REVIEW

2.0 Background

Water treatment is an important aspect of today's society. It is imperative to find environmentally friendly methods to remediate contaminated sites in an effort to reduce water pollution. There are many methods that have been researched and implemented for the treatment of water and soil. There are a variety of methods that are used to combat wide scale sources of contaminants, such as organic, inorganic, and pathogenic contaminants. For the remediation of inorganic and organic sources of contaminants various chemical oxidant have been used and for pathogenic sources both chemical oxidant and radiation have been applied.

2.1 Chemical Oxidation

According to the USEPA chemical oxidation is the application of chemicals, otherwise known as oxidants, to polluted soil and water in order to remediate contaminants (US Environmental Protection Agency, 2001). Chemical oxidation allows for the alteration of harmful chemicals or pathogens into less harmful ones (US Environmental Protection Agency, 2001). Some examples of the types of harmful chemicals and pathogens that oxidants breakdown and remediate are 2-4-dichlorophenol, phosphothio compounds, other organic and inorganic compounds, *E. coli* and *bacillus anthracis* (G. P. Anipsitakis & Dionysiou, 2003; G. P. Anipsitakis, Stathatos, & Dionysiou, 2005; Bandala et al., 2007; Santanu, 2008; Shang & Blatchley Iii, 2001;

Veschetti, Cittadini, Maresca, Citti, & Ottaviani, 2005; Woźniak, Koziołkiewicz, Kobylańska, & Stec, 1998). There are many chemical oxidants, such as chlorine, chloroamides, potassium permanganate and potassium peroxymonosulfate (Oxone®). All oxidant have their own unique way in which they impact remediation effort. Hence, the impact of Oxone®, with chemical formulation of $2KHSO_5.KHSO_4.K_2SO_4$, will be explored on different pollutants but will only be used to remediate pathogenic contamination for this study.

Oxone® has been implemented into many processes such as in oxidization of pulp, alternate for chlorine and chemical and microbial removal (G. Anipsitakis, 2005; Bailey, Cooper, & Grant, 2011; Woźniak, et al., 1998). The pulp industry has used Oxone® as an alternative to chlorine for bleaching pulp and paper (G. Anipsitakis, 2005; Dupont, 2008a). Peroxymonosulfate has also seen its way into the medical industry where it is used for denture cleaning as an alternative oxidizing agent to chlorine for removal of tough stains (DuPont, 2008b). This reagent has been used in swimming pools for the oxidation of pathogens and organic matter (G. P. Anipsitakis, Tufano, & Dionysiou, 2008). Based on studies conducted, Oxone® treatment ability can be enhanced by adding ultraviolet radiation and/ or a catalyst and other compounds, such as cobalt (Co^{2+}) and chlorine (G. Anipsitakis, 2005; Bandala, et al., 2007; Delcomyn, Bushway, & Henley, 2006; Do, Jo, Jo, Lee, & Kong, 2009; Sun, Song, Feng, & Pi). The coupling of Oxone® with transition metal such as Co^{2+} has been shown to reduce synthetic and organic compounds, landfill leachate and E. coli more effectively than

Oxone® alone (G. Anipsitakis, 2005; G. P. Anipsitakis & Dionysiou, 2003; G. P. Anipsitakis, et al., 2008; Sun, Li, Feng, & Tian, 2009). Another important accolade that can be associated with Oxone® is that its disinfection by products are generally deemed as safe (DuPont, 2008b) and not only can it be coupled with other oxidant but it can be combined with polymer to expand treatment phase. This combination is known as controlled release chemical oxidant polymer (CRCOP) developed in the Luster-Teasley lab.

CRCOP is the encapsulation of Oxone® oxidant into a polymer for extended remediation of polluted water and soil. The polymer used for this study was polycaprolactone (PCL). PCL is a biodegradable polymer that usually takes 2-3 years to degrade and possesses numerous application potential (Zhao et al., 2008). PCL has been tested and used in the field of agriculture and medicine for the delivery of fertilizer and drug (Vega-González, Subra-Paternault, López-Periago, García-González, & Domingo, 2008; Zhao, et al., 2008). It is important to agriculture because it allows for delivery of specific amount of fertilizer overtime without the need for regular application. This can reduce fertilizer wastage and improve soil quality over time as excess fertilizing would be reduced or eliminated. One such example of this method is the encapsulation of PCL with phosphate-solubilizing bacteria in an effort to provide an environmentally friendly way to supply phosphate to soil (Wu, Wu, & Chang, 2007). This was done by controlling the release of the bacteria, which will help to naturally stimulate and mobilize phosphate in soil without the need for synthetic fertilization. For the purpose of medicine it is used in

drug delivery over an extended period of time and does not require removal from host (Winzenburg, Schmidt, Fuchs, & Kissel, 2004). This biodegradable polymer is also used in other biomedical practices, such as tissue engineer, gene therapy, vaccine, growth and hormone delivery (Alina, 2011; Luten, van Nostrum, De Smedt, & Hennink, 2008; Nair & Laurencin, 2007; Winzenburg, et al., 2004). Hence, adopting the same idea of encapsulating biodegradable polymer with chemical oxidant can be useful in the field of environmental engineering for the remediation of pathogenic contaminated soil and water. It is this approach that will be used to treat pathogenic contaminated water and soil for this study. This kind of study in the field of environmental engineering is new and is patent pending by Dr. Stephanie Luster-Teasley. She has also explored other oxidants such as potassium permanganate and has implemented different polymer blends to control polymer degradation and slow or speed up the technology treatability.

2.2 Waterborne Pathogens

A few examples of pathogenic organisms that affect us in today's society are *vibrio cholera*, *salmonella*, *and cryptosporidium*. These organisms have their own distinct way on how they influence body functions. For example Vibrio Cholera, known today as just cholera in an infected host, is a gram negative curved rod shape mobile microorganism that belongs to the family vibrionacease (Reidl & Klose, 2002; Vanden Broeck, Horvath, & De Wolf, 2007). Cholera caused acute diarrheal illness in infected persons, which happens as a result of toxigenic vibrio cholera (Mandal, Mandal, & Pal, 2011; Reidl & Klose, 2002) and have an infectious dose ranging approximately from 10^6

to 10^{11} colony forming units. The disease caused copious watery diarrhea that leads quickly to dehydration and death if not treated fast (Mandal, et al., 2011). The primary route of transmission for this disease is through aquatic environment that are contaminated by feces from acutely infected persons (Mandal, et al., 2011; Vanden Broeck, et al., 2007). As stated by (Stine et al., 2008), the susceptible groups are those that have low gastric acidity, blood group O, poor sanitation, poor domestic and personal hygiene and limited access to safe drinking water. Table 2.1 displays some waterborne pathogens and their pathways of exposure.

Table 2.1. Waterborne pathogens and their pathways for infection (Exner & Kistemann, 2003; Pond, 2005)

Ingestion	Inhalation	Contact	Wound Infections
Vibrio cholera Salmonella spp. Escherichia coli Shigella spp. Campylobacter spp. Helicobacter spp. Enterovirus Noroviruses Hepatoviruses cryptosporidium	Legionella spp. Mycobacteria spp.	P. aeruginosa Aeromonas spp. Mycobacteria spp. Acanthamoeba spp. Naegleria spp. Schistosoma	Aeromonas spp. Pseudomonas spp. Vibrio Vulnificus Vibrio parahaemolticus

Another pathogen that is a major concern in today's society is salmonella. Salmonella spp. is a gram negative facultative anaerobic rod shape microorganism that belongs to the family enterobacteriaceae (Iowa State University & The Center for Food Security and Public Health, 2006; Steve Yan et al., 2004). There are 2500 different

serotypes for salmonella and each serotypes may possess strains (Institute for International Cooperation in Animal Biologics, Center for Food Security and Public Health, & College of Veterinary Medicine Iowa State University, 2005; Steve Yan, et al., 2004). The development of strains can be attributed to many factors including the environment in which the pathogen has to survive in and also exposures to antibiotics. The development of strains calls for costly development of new drugs to combat the effects of salmonella. According to an article published by the Center of Food Security and Public Health (Iowa State University & The Center for Food Security and Public Health, 2006), there are 40, 000 reported case of salmonellosis within the United States each year, which means that this issue is continuous and a huge concern for public health. This pathogen is transmitted via the fecal-oral pathways and then travels to the intestines of humans and animals. After transmission through the intestine it is then shed in feces, which if not handled or treated properly can end up in food and water. These bacteria can end up in our water systems and on our food by a way of overland runoffs which can impair surface, ground and irrigation water. When food and water that is contaminated are ingested there are symptoms that usual occur as a result, such as gastroenteritis, with nausea, vomiting and diarrhea with or without fever (Levantesi et al.; Steve Yan, et al., 2004). If this condition is not treated promptly then salmonella can spread throughout the host system and cause serious chronic conditions such as typhoid and paratyphoid fever, arthritis, osteomyelitis, cardiac inflammation or neural disorders (Jean-Yves, 1994; Levantesi, et al.; Touron, Berthe, Pawlak, & Petit, 2005).

Salmonella can survive in most environment and has been shown to survive for several months in feces and fecal slurries sources and 450 days on pig's meat (Institute for International Cooperation in Animal Biologics, et al., 2005). This therefore means that they pose challenge for remediation and elimination in some types of treatment systems. It has been determined by the Center of Food security and Public Health, salmonella is susceptible to many disinfectants such as 1% sodium hypochlorite and 70% ethanol (Institute for International Cooperation in Animal Biologics, et al., 2005). It requires longer contact time or more products to kill salmonella than other organisms. Salmonella can be killed by moist heat at 121^{o}C and dry heat at 160-170^{o}C for at least one hour, which might not be suitable or practical in all cases.

Cryptosporidium is a protozoan that measures 3-5 microgram and has a life cycle involving both sexual and asexual reproduction (Shun Dar, 2002; Smith & Nichols, 2010). This parasite is transmitted through the most common route of transmission, water and food. It ends up in water and food through direct contamination of such sources and through surface runoff that can affect and contaminate agricultural crops and surface water. This statement was verified by (Shun Dar, 2002), as he makes mention that cryptosporidium oocysts are found worldwide in surface waters (lakes, rivers, streams), runoff, pasture and in wastewater. This means that untreated water from these sources can infect individuals who come in contact or may have ingested such water. Cryptosporidium is isolated from feces and can infect people via the fecal-oral route (Gómez-Couso, Amar, McLauchlin, & Ares-Mazás, 2005). Once ingested it affects the

intestinal lining and is known to caused gastroenteritis, which can be associated with diarrhea, dehydration, weight loss and wasting (Jex, Smith, Monis, Campbell, & Gasser, 2008).

Cryptosporidiosis creates serious public health issue as there is no known treatment for this disease which means that individuals have to rely on their immune system to fight illnesses and established techniques to suppress symptoms. Some techniques includes staying hydrated by drinking adequate fluid and using anti-diarrheal drug as a means to suppress symptoms (Shun Dar, 2002). Another concern is that cryptosporidium oocysts are resistant to disinfectants, such as chlorine, that are commonly used as treatment. Also, this pathogen is known to have passed through filtered and unfiltered drinking water systems (Smith & Nichols, 2010). This therefore means that even with standard drinking water treatment the pathogen can still persist and ultimately infect people.

Though many of these water borne pathogens are explained to have adverse effect on health, the USEPA recommends use of indicator bacteria to identify water potentially contaminated by water-borne pathogens such as vibrio cholera, salmonella and cryptosporidium. For safety purposes, vibrio cholera, salmonella and cryptosporidium will not be isolated for this study; instead indicator testing will be used to isolate infected water.

2.3 Biological indicators

Indicator bacteria are used to determine if water or soil sources are contaminated with bacteria that could be potential health problems. For this thesis, indicator bacteria were used to quantitatively measure soil and water for *Escherichia coli* (*E. coli*) and Enterococci. There will also be testing showing total coliform (TC). The indicator bacteria tests will be conducted for treated water and soil to determine the effectiveness of the remediation system. The results can be viewed in chapter 4.

Escherichia coli, a well-known fecal coliform, is described as a gram negative bacterium that is mobile and aerobic or facultative anaerobic (Mosaddeghi, Sinegani, Farhangi, Mahboubi, & Unc, 2010), which means that it can reproduce with or without oxygen. This bacterium has a cell diameter that ranges from 1 to 6 micro-meter (μm) (Mosaddeghi, et al., 2010) and has several strains. The most harmful strain of E. coli to human is E. coli 0157:H7 and has an infectious dose ranging from 10 to 1000 organisms (Haines & Staley, 2004). Also, E. coli is used as an indicator microorganism to determine whether a water source is contaminated with fecal matter and to assess the risk of microbial contamination of such water resources (Mosaddeghi, Sinegani, Farhangi, Mahboubi, & Unc, 2010). It is also used because it has a high detection and can be isolated from almost all fecal matter. This bacterium has a recommended count level in recreational water (freshwater) set forth by the USEPA as the geometric mean of 126 per 100 millimeters (US Environmental Protection Agency, 2000). This count level is used as a target to disallow water contact when E. coli count is over this limit.

Enterococcus is a fecal streptococcus that can be isolated from gastrointestinal tract. This is a gram negative, anaerobic and spherical bacterium that is associated with many infections such as urinary tract infections (Hach, 2000). It usually persist longer in the environment than other bacteria, hence this property makes it a good indicator bacteria (Hach, 2000). As set by the US EPA, the criteria for Enterococci bacteria in recreational water, such as fresh water, are reported as being less than the geometric mean of 33 per 100 milliliters (US Environmental Protection Agency, 2000). Anything above this level is in violation and water contact should be avoided. Conversely, total coliform are group of widespread bacteria that are in our environment naturally (US Environmental Protection Agency, 2011a). It is noted by the US EPA that all members of the Total Coliform group can occur in human; however they can be isolated from other warm blooded animals, manure, soil and water (US Environmental Protection Agency, 2011a). Therefore, total coliform is important in this study because it will help to determine the presence of other bacteria, especially when *E. coli* and Enterococci are shown to be absent from test samples. It may also be used for treated samples to determine if bacterial levels have been reduced or totally eradicated. Total coliform cannot be used to isolate specific bacteria and determine bacterial count; however it will help to show that other bacteria are present in tested water and it will indicate whether treatment has killed or reduced all bacteria.

2.4 Water contamination by pathogen from agricultural practices

Agricultural activities have caused large scale pollution to our water resources-pollution that comes in many forms such as through pollution runoff from agricultural activities. Contaminants include antibiotics in animal waste which has been applied to land, synthetic pesticides, fertilizer, sediment loading and pathogenic contamination from liquid and solid fractions of animal wastes. These contamination causes adverse environmental effects in that it affects neighboring organisms, clogged streams, reduce aesthetics of our water ways and impair viable water resources. Many of these contaminations can be traced to concentrated animal feeding operations (CAFO) due to the large amount of fecal matter that is produced (Burkholder et al., 2006). As noted by (Haines & Staley, 2004), the amount of manure that is produced by an animal farm is equivalent to the waste produced by small and medium size cities. For a clearer visualization, a farm with a 2500 herd of cows has a waste production that is similar to a city of 411, 000 people (Haines & Staley, 2004).

Manure consists of metals, antibiotics, hormones and various pathogens, which are excreted from farm animals into the environment. Manure is used on these farms as fertilizers or stored in lagoons to be biologically degraded. Using manure or animal waste for fertilizer or by storing it in lagoons can cause huge environmental problems. This is because manure consists of high number of dangerous pathogens that can affect humans and animals. Fecal matter, such as manure, may consist of pathogens such as *Escherichia coli 0157:H7, salmonella, campylobacter, Yersinia*, *listeria* and enterococci bacteria

(Haines & Staley, 2004; Unc & Goss, 2004). These pathogenic organisms can get individuals sick and should not be taken lightly. Hence, the disposal of manure needs to be treated before release or monitor closely to ensure that it does not pollute groundwater or travels over land to pollute surface water.

Soil tends to hold moisture and nutrients for crop production, however it can also contain pathogenic contaminants. Pathogens persist in soil and may become mobile during runoff, which means that it may ultimately lead to surface water and groundwater contamination (Gessel, Hansen, Goyal, Johnston, & Webb, 2004). It is important to note that pathogen survival rate is higher in moist or wet soils, hence with saturated soil the transport of pathogens are even higher. As noted by (Guimarães et al., 2010), dry soil causes lower activity for microbes by inhibiting growth and restrict microbial movement. Dauntingly, fecal bacteria can live for a long period of time after manure has been applied to soil and once it reaches groundwater or surface water the survival period can be extended for several months (Unc & Goss, 2004). This therefore means that with the right condition (pH, temperature and nutrient supply) the bacterial survival rate may be increased and their replication might impair drinking and recreational water.

Due to the fact that fecal bacteria from animals can enter the environment through pathways such as leakage from poorly constructed manure lagoons and, heavy precipitation events which may lead to overflow of lagoons and runoff from manure application on farms (Burkholder, et al., 2006) there needs to be a treatment system that kills pathogens. Efforts to remediate contaminated sites need to be a priority before

wastewater is released into the environment. Additionally, cracks in poorly maintained lagoons may cause infiltration of waste into groundwater, as macropore in soil constitute for major pathway for bacterial contamination (Warnemuende & Kanwar, 2002). It is known that bacteria generally moves one meter in unsaturated condition and 30 to 60 meters in saturated condition (Warnemuende & Kanwar, 2002), which means that pathogens in vadose zone may be transported rapidly in any field that is saturated sufficiently to fill pores on a consistent basis (Unc & Goss, 2004). Hence, in order to determine if pathogens are present in water and soil, this study will use indicator bacteria as a marker to prove the potential for higher number of pathogens that can be present in soil and water samples. The indicator bacteria that will be used to quantify bacterial levels are *E. coli*, Enterococci bacteria and total coliform to report the total group of bacteria levels. The CRCOP treatment technology will be applied in the remediation of pathogens that exists in soil and water.

CHAPTER 3

MATERIALS AND METHODS

3.0 Overview

This study used soil and water samples for treatment and analysis. Water samples were taken from a site at the Country Park in Greensboro NC and soil samples were taken from the North Carolina A&T State University swine unit. Soil and water samples were analyzed for bacterial levels using IDEXX technology and membrane filtration and treated using controlled release chemical oxidant polymer (CRCOP). Water samples were evaluated for microbial content to quantify *E. coli*, Enterococci, and Total Coliform (TC). A continuous stirred tank reactor (CSTR) was used to treat water using CRCOP and soils were treated using a batch reactor system.

3.1 Controlled Release Polymer

The active agent that was used to treat both soil and water was controlled release chemical oxidant polymer (CRCOP). CRCOP comprises of a polymer, polycaprolactone (PCL), and oxidant, potassium peroxymonosulfate, which is commercially known as Oxone®. The Oxone® is the main treatment component and was combined with polycaprolactone to form a slow releasing agent that can treat water and soil over time. PCL is also a biodegradable polymer, which means that it will not persist in the environment. To produce the CRCOP, a blend of 60% oxidant to PCL was used in this study.

3.2 Water Sampling

Water samples were taken from two locations at the Greensboro Country Park referred to as Lakes A &B. Lake A drains into Lake B and Lake B continues on to connect with other water bodies that are located at this park, Figure 3.1. Samples taken from these lakes were analyzed for *E. coli*, Enterococci bacteria and total coliform. Initially Lake B was the only lake that was being sampled dating back from October 2010, after which Lake A was incorporated into the study February 2011 to get a better understanding of the microbial activities that existed in the water at the park. Water Bacteria analysis for the projected months studied from October 2010 to October 2011 were ran using IDEXX technology and for treated water membrane filtration was used.

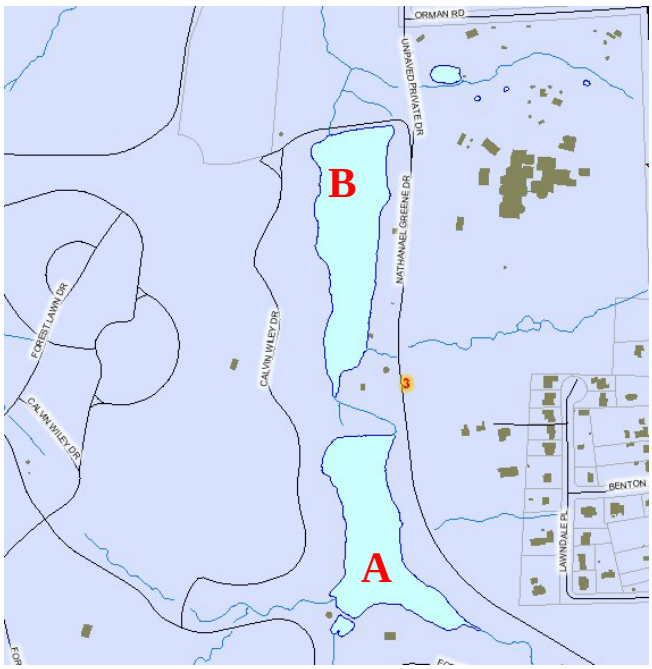


Figure 3.1. Showing Greensboro Country Park Lakes A and B

3.3 Soil Sampling

Soil samples were collected from the North Carolina A&T State University Swine Unit, located at 737 JFH Dairy Rd, Greensboro, NC 27405. Samples were retrieved from the lagoon area of the swine unit. Collected soil samples were tested for indicator bacteria as specified above and then treated to eliminate those bacteria. The IDEXX liquid based technique was used for bacteria analysis in soil, both for non-treated and treated. Two locations were sampled, below lagoon 2 (L2) labeled point A and at another point below lagoon 3 (L3) which is located to the right of the labeled point A in Figure 3.2. After each sample location the sampling apparatus were sterilized with 70% ethanol and wipe down with disposable antibacterial clothes to prevent cross contamination. The point labeled A on the map above was used as a point of reference and can be located at the following latitude and longitude 36° 3' 59.03 and -79° 43' 19.91.

Figure 3.2. Lagoons 1, 2 and 3 at NCAT Swine Unit

3.4 Microbial Analysis

3.4.1 Analysis Using IDEXX Technology

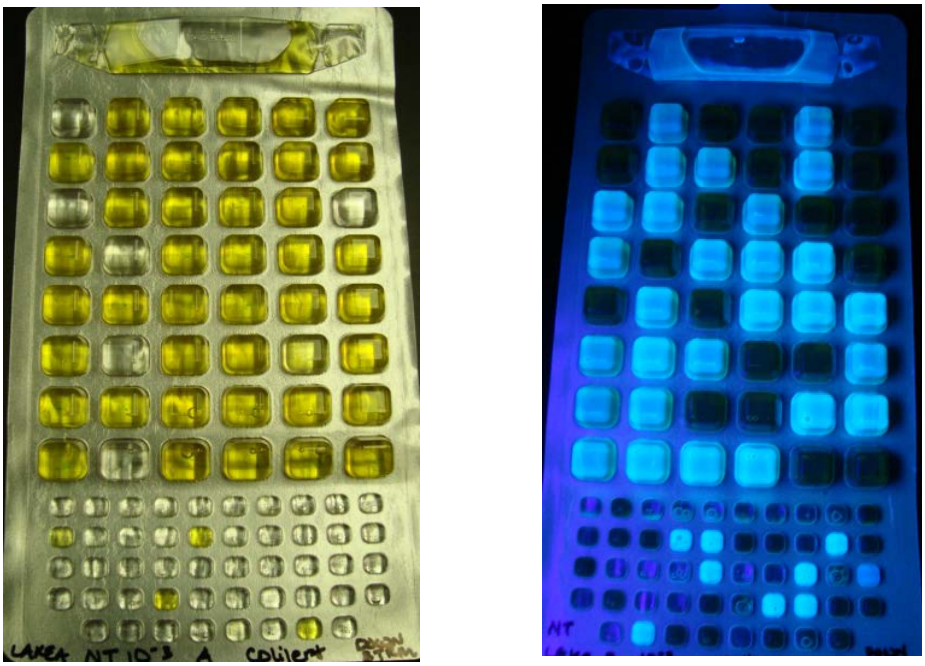
Using the text *Standard Methods for the Examination of Water and Wastewater and Methods of Soil Analysis, Part 2- Microbiological and Biochemical properties (SSSA Book Series 5)*(American Public Health Association, 2005; Weaver et al.), a dilution range was established for preliminary study of both sites. After, preliminary study was conducted on the site of interest then the numbers obtained from such study was used to predict the suitable range for the next sample period. For microbial analysis at Country Park, a dilution range of 0.001 to 1ml was used and for analysis of soil a dilution range of 0.0001 to 0.1ml was used to quantify bacteria by serial dilution. Upon the completion of dilution for each sample, they were each run for further analysis using the liquid based technique by IDEXX (Westbrook, Maine).

The media that were used for this procedure were IDEXX *Colilert*® and IDEXX *Enterolert*®. IDEXX *Colilert*® media was used to quantify both total coliform and *E. coli* and IDEXX *Enterolert*® was used to enumerate Enterococci bacteria. Using this technology for the enumeration of bacteria requires that each test sample is 100 ml. This method was used for soil analysis and water bacterial profiling at the Greensboro Country Park. The media used was dissolved into 100 milliliter of diluted sample, poured into Quanti-Trays®/2000 trays, secured by rubber inserts and heat sealed using the IDEXX Quanti-Tray sealer 2X, displays in Figure 3.3. After sealing of all trays, TC and *E. coli* samples were incubated for 24 hours at 35^{o}C and Enterococci samples were incubated for

24 hours at 41±0.5^{o}C. A Quanti-Tray®/2000 that shows a positive result for TC displays yellow color under regular light and for *E. coli* and Enterococci the wells glow under UV black light (Figure 3.4). Yellow wells in natural light and glowing wells under UV light were counted, which were reported as the number of positive big and small wells. The number of large and small wells was compared using a table representing the most probable number (MPN) to derive bacterial count. Numbers retrieved from reading the MPN table were adjusted based on dilution factor used for TC, *E. coli* and Enterococci tests.

(a) **(b)**

Figure 3.3. Photo of: (a) IDEXX Quanti-Tray sealer 2X, and (b) Rubber insert



(a) **(b)**

Figure 3.4. Photo of: (a) Quanti-Trays®/2000 displaying yellow wells, and (b) Quanti-Trays®/2000 showing glowing positive wells

It is important to note that Quanti-Tray®/2000 trays provide bacterial count up to 2419 coliform per 100 ml water sample. The Quanti-Tray®/2000 consist of 49 big wells and 48 small wells. Based on the number of wells read after the incubation period the bacterial counts were evaluated using the IDEXX Quanti-Tray®/2000 MPN table. The table consisted of numbers based on the number of small wells and big wells that existed on each tray. Numbers on the y-axis are representative for the number of positive large wells and the numbers on the x-axis corresponds for the number of positive small wells. The number retrieved from reading the trays after incubation are cross-referenced on the MPN table and wherever both of them meet represents the number of bacteria in the

tested sample per 100 ml. For example, a reading from 49 big wells and 47 small wells would give an enumeration of 2419.6 MPN per 100 ml.

3.4.2 Analysis Using Membrane Filtration

Membrane filtration was performed on treated water samples retrieved from the Country Park Lake B. Membrane filtration was used because it required less volume to be removed from treatment versus IDEXX liquid based technique that required a volume of 100 ml for each bacterium analyzed. The continuous stirred tank reactor (CSTR) requires a volume of at least 1000 ml and only holds up to 2200 ml in volume. In addition, using IDEXX would require at least 600 ml to be removed daily for a triplicate run. Hence, this testing technique was adopted versus using IDEXX because it required a smaller sample volume for microbial analysis. Membrane filtration was used to detect *E. coli* and Enterococci bacteria using the membrane –Thermotolerant *Escherichia coli* (mTEC) and Membrane-Enterococcus Indoxyl-β-D-glucoside (mEI) agar. The mTEC agar was used as a medium for *E. coli* and mEI was used for Enterococci. For the enumeration of these bacteria using this method there are certain procedures that must be followed both for making the medium and for filtering water for bacterial analysis.

Agar plates were produced for Enterococci and *E. coli* using a standard operating procedure based on EPA method 1600 and EPA method 1603, respectively (United State Environmental Protection Agency, 2002; US Environmental Protection Agency, 2002). To produce mTEC agar plates for *E. coli* enumeration, plates were produced in 60 plate batches by adding 13.68g of the mTEC powder to 300 ml of distilled water. The agar was

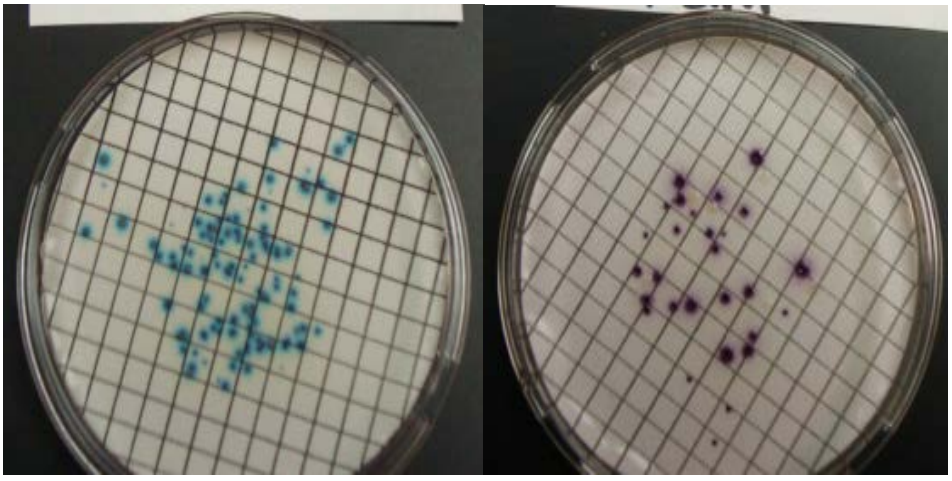
heated until fully dissolved or clear enough to see through and pH calibrated using sodium hydroxide (NaOH) or hydrochloric acid (HCL) to achieve a pH of 7.3±0.2. The solution was autoclaved for at least 15 minutes at 121^{o}C and then cooled in a water bath at 50-56^{o}C. Using aseptic conditions, each plate received 5-7 ml of the solution and the plates were covered to protect the agar from light.

The mEI plates were produced in 60 plate batches using 21.6g of mEI agar powder mixed with 300 ml of distilled or nanopure water. The agar powder and water was mixed using magnetic hotplate and metal rod until the solution boiled. The content was boiled until all the powder was completely dissolved or appeared translucent. When the mixture completely dissolved, the temperature was regulated in a water bath until it was 50 - 56°C then the pH was adjusted to 7.1 ± 0.2 using 1N NaOH or 1N HCl. Subsequently the mixture was transferred to the autoclave for 15 minutes at 121^{o}C. After Autoclaving 0.006g triphenyltetrazolium chloride and 1.5ml of naliddixic acid was added to the content. For the Naliddixic solution, 0.072g of naliddixic acid was added to 1.50 ml water. To dissolve, the mixture a few drops of 0.1N NaOH was used to dissolve the content. After the mEI agar solution was cool enough to where it could be held for at least 5 seconds, 5ml proportion of the solution was added to the 47-mm small petri plate using aseptic conditions. The plates for both mEI and mTEC were wrapped in foil and stored in a 4^{o}C refrigerator. All plates were used within 30-days.

A Millipore filtration apparatus, as seen in Figure 3.5, included filter paper, filter heads, a flask for collecting water and a filter duct that channels the water from the filter

head to the flask. A part of this set up was a laboratory vacuum pump that was used to withdraw water from the filter duct. In addition, a 70% alcohol burner was used to decontaminate the tweezers that were used to handle filter paper. This was done by placing the tip of the tweezers in 70% alcohol for a few seconds then passing it through the flame for about 3 second. This process was standard throughout the entire procedure that involved handling of filter paper. The dish were covered after the filter paper was placed on the agar medium within the dish for mTEC, as mTEC is sensitive to light and reading might be affected due to this exposure.

Figure 3.5. Filtration Apparatus setup



(a) ***(b)***

Figure 3.6. Photo of: (a) Petri dish of MEI showing blue colonies and (b) MTEC showing red to magenta color

After preparation, mEI batches were placed in an incubator at 41^{o}C and mTEC batches were placed in an incubator at 35 ± 0.5 °C for 2 hours before transferred into a water bath for 24 hours. The batch with mTEC media were tied in zip lock bags and secured to prevent water from entering then afterwards they were completely submerged in water. The water bath was kept at a temperature of 44.5 ± 0.2 °C. Following the incubation and water bath period the red or magenta colonies were counted for mTEC and blue colonies were counted for mEI. Plates that contains 20-80 colonies were counted and were used to calculate the number of *E. coli* and Enteroccoci colonies per 100ml (US Environmental Protection Agency, 2002):

$$E.coli\ or\ Enterococci/100mL = \frac{Number\ of\ E.coli\ or\ Enterococci\ colonies}{Volume\ of\ sample\ filtered\ (mL)} \times 100 \qquad (1)$$

3.5 Dilutions

In order to develop soil samples that can be analyzed using the IDEXX technology, sample dilution was required. Typically, when analyzing water samples with the IDEXX technology, a dilution of 10^0 is the collected water sample retrieved during field sampling at the lake. Following that, 10^{-1} dilution is produced by mixing 10 ml of the collected sample with 90 ml of the deionized (DI) or distilled water and 10^{-2} dilution is the combination of 1 ml of 10^0 with 99 ml of DI water, see Figure 3.7.

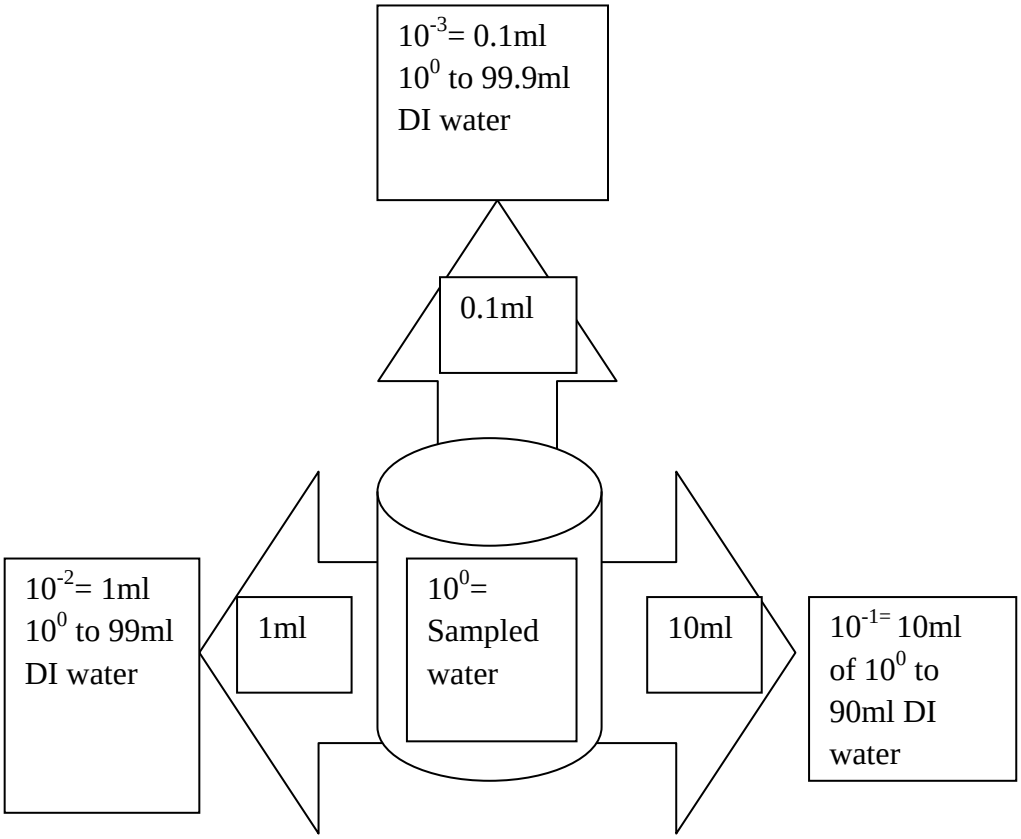


Figure 3.7. Schematic showing dilution setup for water analysis

For soil analysis, the same method for liquid serial dilutions was used, Figure 3.8. The 10^0 dilutions consisted of soil recovered from the farm during field sampling without any addition of water. The 10^{-1} dilution consisted of 10 grams (g) of field soil sample to 90 ml of DI or distilled water. A 10^{-2} dilution was produced using 10 ml of 10^{-1} diluted sample to 90 ml of DI water and to achieve a dilution of 10^{-3} 10 ml of the previous was extracted and combine with DI water to make up the remaining of total volume to 100 ml.

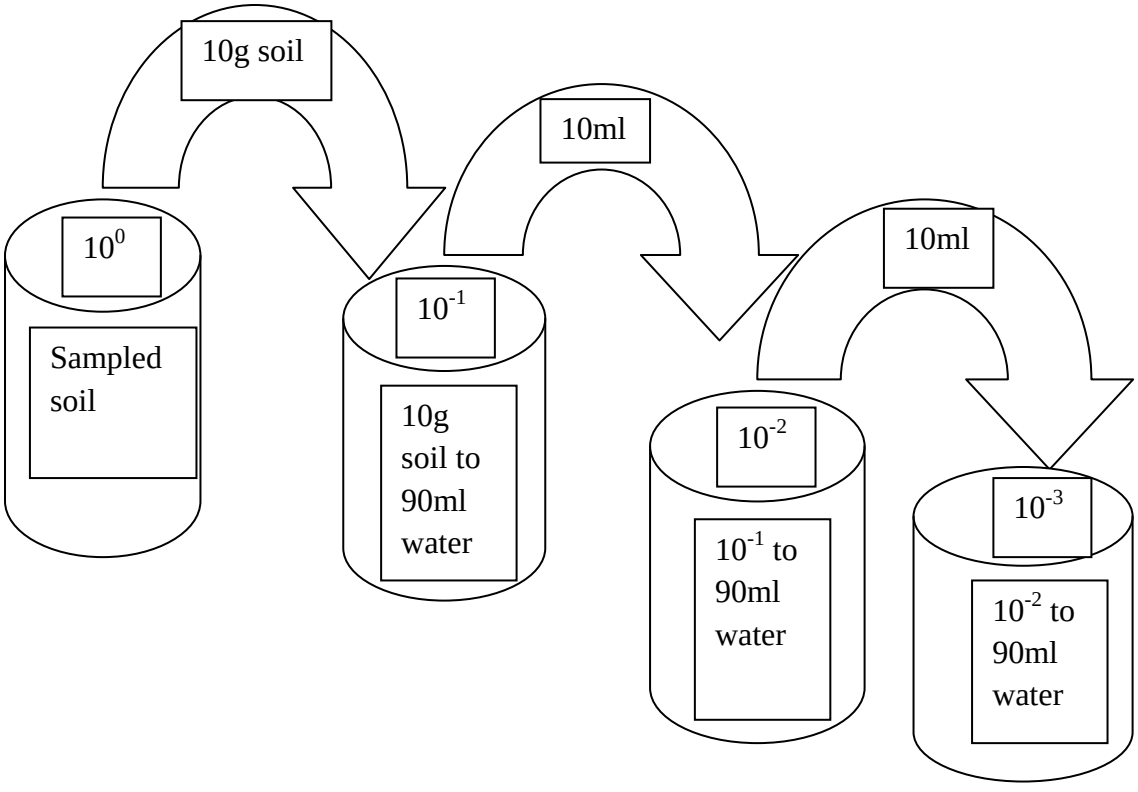


Figure 3.8. Schematic showing serial dilution setup used during soil analysis

The IDEXX system requires all test samples to be 100 ml in total liquid content; therefore, 25g of soil was combined with 225 ml of DI water to provide the same 10^{-1} dilution factor for bacteria analysis. All of the dilutions were made up of a total of 250 ml. This allowed for both *E. coli* and Enterococci to be tested from the same soil that initially started the experiment on any given occasion.

3.6 Treatment

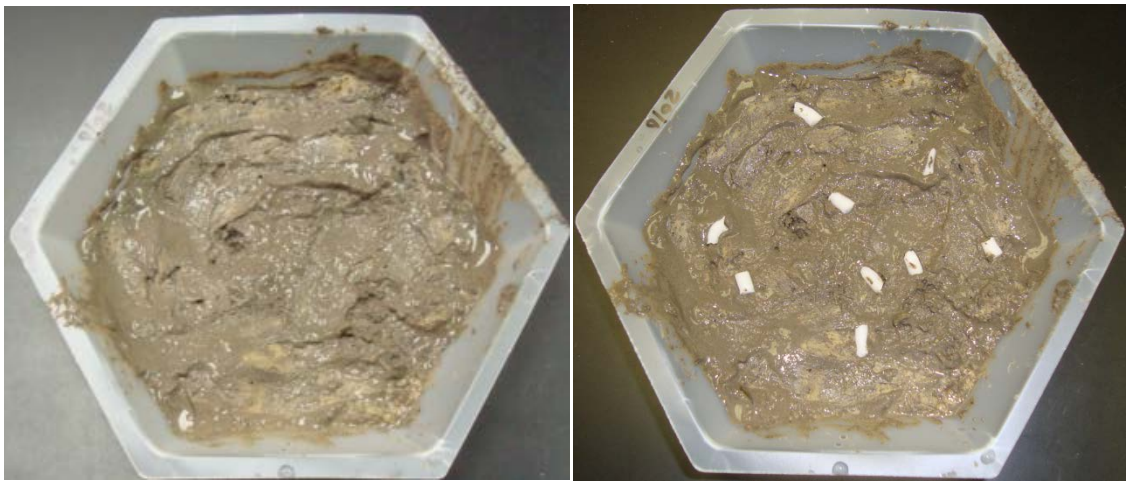
Water was treated using a CSTR with a revolution of 77 rpm and soil was treated by increasing the water content in the soil and inserting the CRCOP pellets into the soil, an example of both methods can be viewed in Figure 3.9 and Figure 3.10. During preliminary studies, the CSTR was heated to 28^{o}C; however, this was discontinued because the polymer was heat sensitive and released the oxidant faster than was expected. Even though this was the case it is still a great observation as treatment using this method would be favorable during the summer for rapid but control treatment. Once the water was collected from the lake and brought back to the laboratory it was combined with the polymer blend at a certain volume and then set in the CSTR for treatment (Figure 3.9). The volumes used were 1000, 1200, 1400, 1600, and 2000ml. There was also attempts made to observe how well the CRCOP would treat when recycle after treatment periods. Finally, water treatment was carried out using polymer pellets with no chemical oxidant to determine its effect on the treatment method.

(a) **(b)** **(c)**

Figure 3.9. Photo of water treatment mechanism: (a) CRCOP pellets, (b) wastewater treatment setup, and (c) CSTR water treatment system

Water treatment setup was considered day zero of treatment during the first day that the water was placed in the CSTR for remediation effect and there after the days were counted forward until the end of the treatment cycle. The treatment cycle is considered completed if there were no bacterial colonies found during analysis. Treated water was analyzed for bacteria using the membrane filtration method as described above. During membrane filtration a volume of 5-25ml of the treated water and controlled water sample were filtered through for both the enumeration of *E. coli* and Enterococci. Following the filtration the filter paper was placed onto the appropriate dish then after all the dishes where completed they were placed in their appropriate incubator. Soil was treated by the insertion of the CRCOP pellets into the soil. Before the pellets

were inserted the soil was weighed and recorded, after which water was added to it to allow CRCOP to diffuse into the soil, as depicted in Figure 3.10.



(a) **(b)**

Figure 3.10. Soil treatment mechanism: (a) Soil setup without CRCOP (b) soil treatment setup with CRCOP

The water content of the soil used in treatment was calculated from the weights that were recorded without pellets added. This sample was allowed to be oven dry so that the water content of the field soil could be determined. The water content of soil was measured using equation 2. For soil treatment the batch reactor concept was adopted by setting up treatment for each day, where day zero is the first day that the experiment was ran and does not include treatment pellets (control). There were four batch setup for four days and each were tested according to the day that they fall on after pellets were installed for remediation, for example day one was the day after the initial setup. Each

day the appropriate samples were tested using the IDEXX technology setup and the dilution range and procedures that were discussed for soil previously were used.

$$\theta_m = \frac{wet\ soil\ weight - dry\ soil\ weight}{dry\ soil\ weight} \times 100 \quad (2)$$

The IDEXX technology method of bacterial analysis was carried out using the dilution of 10^{-1} to 10^{-3}. It is important to note that for soil experiment a concentration of 10^{0} is the pure soil that was retrieved from the field. The soil retrieved from the field was used in the dilution process, as explained above. After each dilution was setup then the appropriate media, Colilert® and Enterolert® was added and poured into quanti-trays®/2000. Each tray was heat sealed using IDEXX Quanti-Tray sealer 2X and placed in the appropriate incubator for 24 hours.

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CHAPTER 4

RESULTS AND DISCUSSIONS

4.0 Bacteria profiling at Greensboro Country Park

Bacterial analysis was conducted for this study in order to fully understand the contamination level in the park's lakes. The water at Country Park was tested for *E. coli*, Enterococci and total coliform (TC). This park initially was designed for recreational activities and the lakes were to be used for water recreational activities such as swimming, fishing and boat ride. However, due to the high presence of pathogenic organism in the lakes activity such as swimming is no longer permitted. There are seasonal boat rides available for public access and park patrons have been observed fishing in both lakes. The profiling study was carried out from October 2010 to October 2011 and sampling was performed monthly. As an example of the bacteria levels enumerated during a monthly sampling event, Figure 4.1 and Table 4.1 show *E coli*, Enterococci, and TC levels in Lake B for October 2010. Analysis for October 2010 is marked by sample location denoted by a one and samples taken from February to September 2011 are denoted 2 after the location lettering. The inclusion of Lake A into the study during February 2011 incorporated water sample for analysis from 2 locations on the lake, upstream (A2) and downstream (B2), Figure 4.2 and Figure 4.3. Figure 4.4 depicts initial sample points for Lake B, referred to as A1 for testing done October 2010 and C2 throughout 2011. Figure 4.5 shows the boat ride dock and sampling location D2 and F1 for Lake B. Sample point F1 is the last point to be tested in 2010 and D2

represents the fourth sample point for all 2011 monthly analysis. The final test samples were always retrieved from location E2 for test done in 2011, Figure 4.6. Table 4.1 summarizes the bacterial levels at each sample point of Lake B during October 2010. The levels displayed in this report were the geometric mean of the results derived from testing each sample location and all plates were run in triplicate.

Table 4.1. Bacterial projection in Lake B, 2010

	Distance	TC	E. Coli	Enterococci
Location	feet	MPN/100 ml	MPN /100 ml	MPN /100 ml
A1	0	874	100	1057
B1	96	1050	144	1167
C1	236	1180	95	65
D1	339	620	29	360
E1	493	2110	225	250
F1	606	2728	1140	148

The levels of bacteria during this sample period proved to be high, as some areas of the lake exceeded the EPA's maximum required levels. The maximum acceptable levels for *E. coli* and Enterococci in freshwater sources use for recreational purposes are 126 and 33 colonies per 100 ml of test sample or approximately 2.1-log and 1.5-log per 100 ml, respectively (US Environmental Protection Agency, 2000). As seen in Table 4.1 and Figure 4.1, B1, E1 and F1 reported levels above 2.1-log for *E. coli* and Enterococci exceeded 1.5-log in all areas of Lake B during the month of October 2010. The

Enterococci levels were highest at B1, which could have been contributed by the storm water inlet that is located just before sample point B1. During October most of the water birds that were present during the summer migrated elsewhere, however bacterial levels are still high in the lakes. This could mean that bacteria are persisting for a long time in the environment, which could be the case for enterococci. This may have been the case as the USEPA used enterococci as indicator bacteria as they tend to exist in the environment for a long time (Hach, 2000).

The high levels of bacteria in the lake may also mean that runoff from precipitation has loaded unwanted pathogens in the water. In addition the small number of birds or other animal that exist in the area during October may have caused fecal contamination. It should also be noted that fecal contamination during summer periods could persist throughout winter, especially in North Carolina where temperatures are inconsistent during winter periods. During winter warmer temperatures may exist occasionally and this condition could cause bacteria to replicate and live longer. The levels of bacteria in the lake will always be inconsistent; however levels will give an idea of how bad the contamination in the lake is. From Table 4.1, the E. coli levels ranged from 29 to 1140 MPN per 100 ml and Enterococci ranged from 65 to 1167 MPN per 100 ml. This therefore signifies that some parts of the lake were in safe range however on a larger scale it would not be healthy for swimming and other recreational activities.

TC distribution ranges from 874 to 2728 MPN per 100ml for point A1 to F1. The bacterial levels increased downstream, however the level decreased at point D1. The increase that occurred at B1 and C1 may have been because of contribution from the

storm water inlet just before B1 and the geese that love to hangout between B1 and C1. Contrary, Enterococci bacteria were recorded at their highest upstream of the lake, A1 and B1. However, there was a significant decrease at C1 with more than 1-$\log_{10}$ reduction as compared with previous. Contrary, E. coli had the highest recorded levels at F1 and the lowest at D1, which might be due to E. coli's flagellant structure that allows them to be mobile. E. coli may be able to move with water downstream, hence high levels maybe recorded depending on where lake water moves.

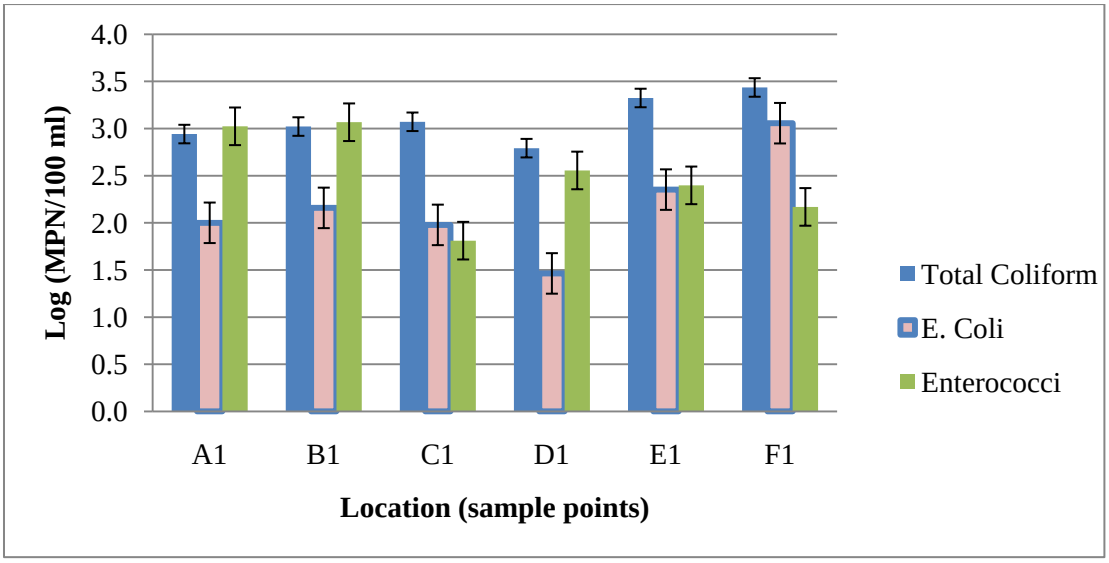

Figure 4.1. Bacterial distribution in Lake B, October 2010

Lakes A and B and the surrounding area are habitat for various organisms, such as birds, fishes and a vast amount of microorganisms. Areas around the lakes have high

numbers of water fowl using the river bank, Figure 4.7 and 4.8. These geese produced a significant amount of feces in the lake and along its shoreline, Figure 4.9. The fecal matter produced by the geese could be a potential source of contamination for this lake; however, it does not mean that this is the only source of pollution. It was also observed that several stormwater ducts drain directly into Lake B and as explained before this stormwater can add pathogens to this surface water. However, the level of bacteria can be reduced through proper treatment of shorelines and stormwater that empties into both lakes. Bacteria can attach to moist soil sediments, which allow them to persist in the environment longer. The binding of bacteria to sediments will also mean that if stormwater is treated then the problem will persist if the soil is not treated. Hence it is imperative to do a combination treatment in situation where there is fecal pollution, especially when it involves both components (soil and water).

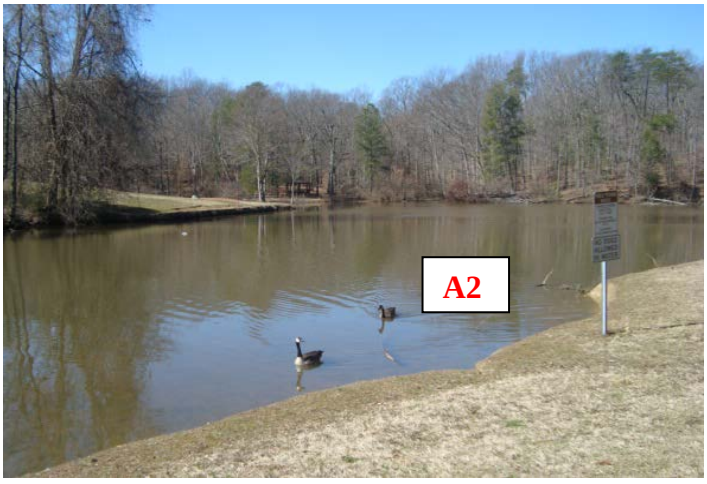

Figure 4.2. Sample point A2 at Lake A

Figure 4.3. Sample point B2 at Lake A

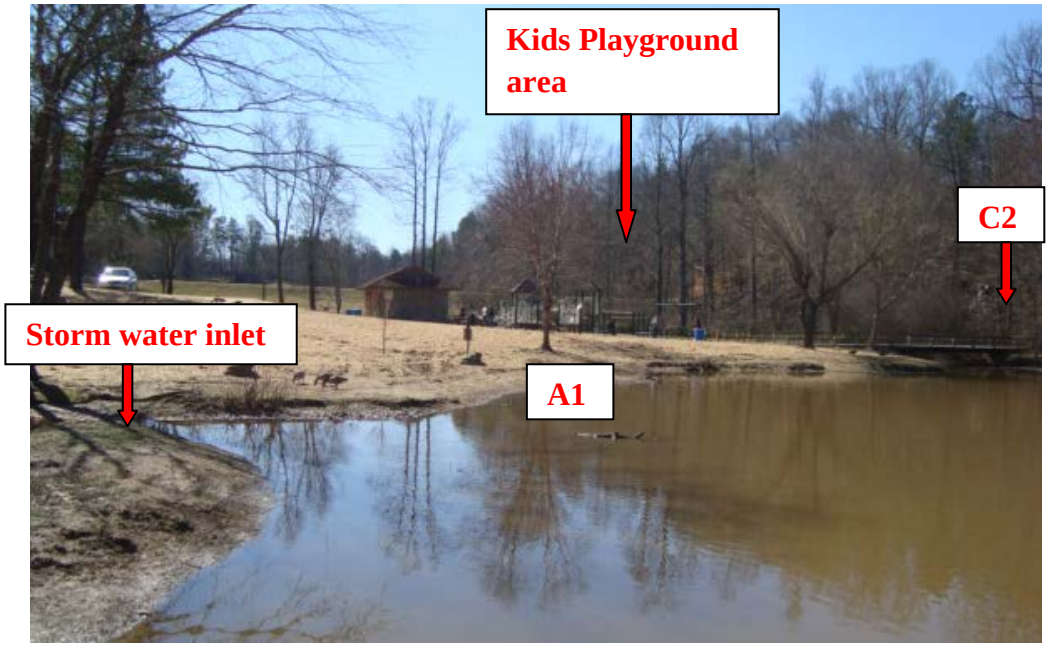

Figure 4.4. Sample points A1& C2 and playground

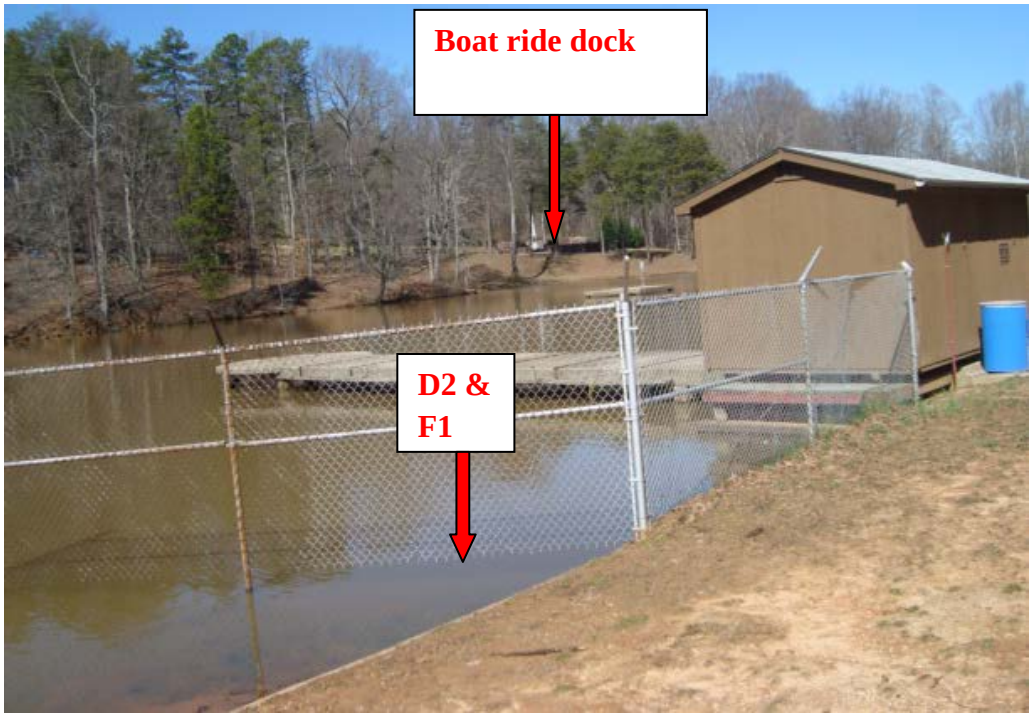

Figure 4.5. Boat dock area and sample points D2 & F1 at Lake B

Figure 4.6. Sample point E2, downstream Lake B

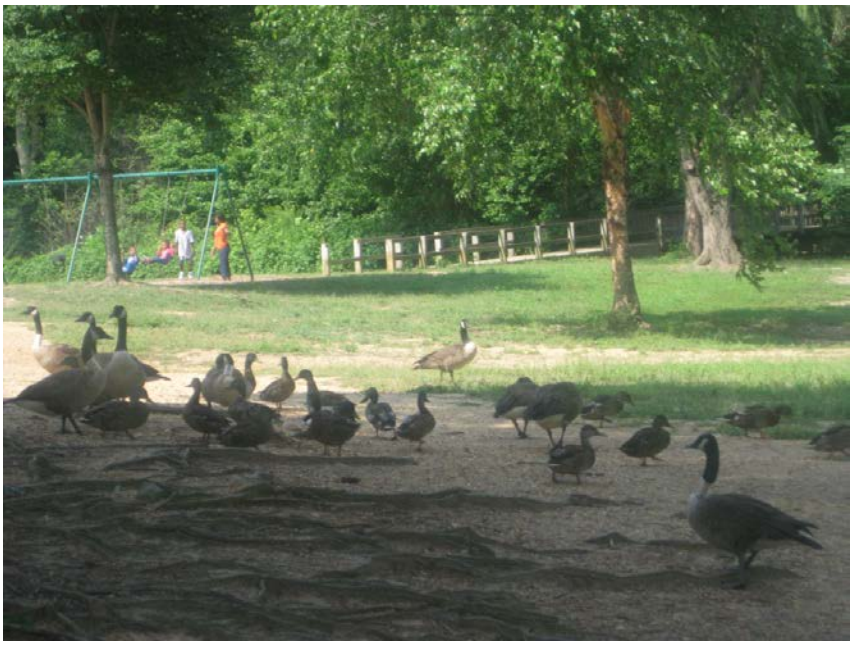

Figure 4.7. Congregation of geese and play area above C2 sample point

Figure 4.8. Geese in Lake water and on shoreline

Figure 4.9. Fecal matter on shorelines, depicted by dark discolorations on soil surface

The extended study done on both lakes in 2011 gave a quantitative understanding of the bacterial levels that exist. Based on this study it was observed that bacterial growth was higher during summer than winter periods. The average monthly temperatures for 2011, Figure 4.10, were used to determine the months where the highest bacterial levels might be reported. Table 4.2 explains the total coliform content in both lakes in MPN per 100ml and Figure 4.11 give a visual understanding of the bacterial variation at each sample point of the lakes on a monthly basis. Figures 4.10 and 4.11 shows the months that recorded the highest levels for total coliform occurred during the summer and early fall. The highest count for TC occurred during the months of May, June, July and September. On the contrary the months that recorded the lowest levels were during early spring where average temperatures were at its lowest, February to April. It is important to

note that the bacterial levels recorded at these lakes are not based solely on temperatures but also on other factors such as, adequate food for bacteria, precipitation, and the number of geese or warm blooded organism that exist and excrete in the area. TC count consists of mostly harmless and intestinal bacteria and is usually used to determine the overall quality of water and the possibility for fecal contamination, hence its level is important to monitor and control. Positive TC test samples require additional testing for fecal coliform, such as *E. coli* and Enterococci.

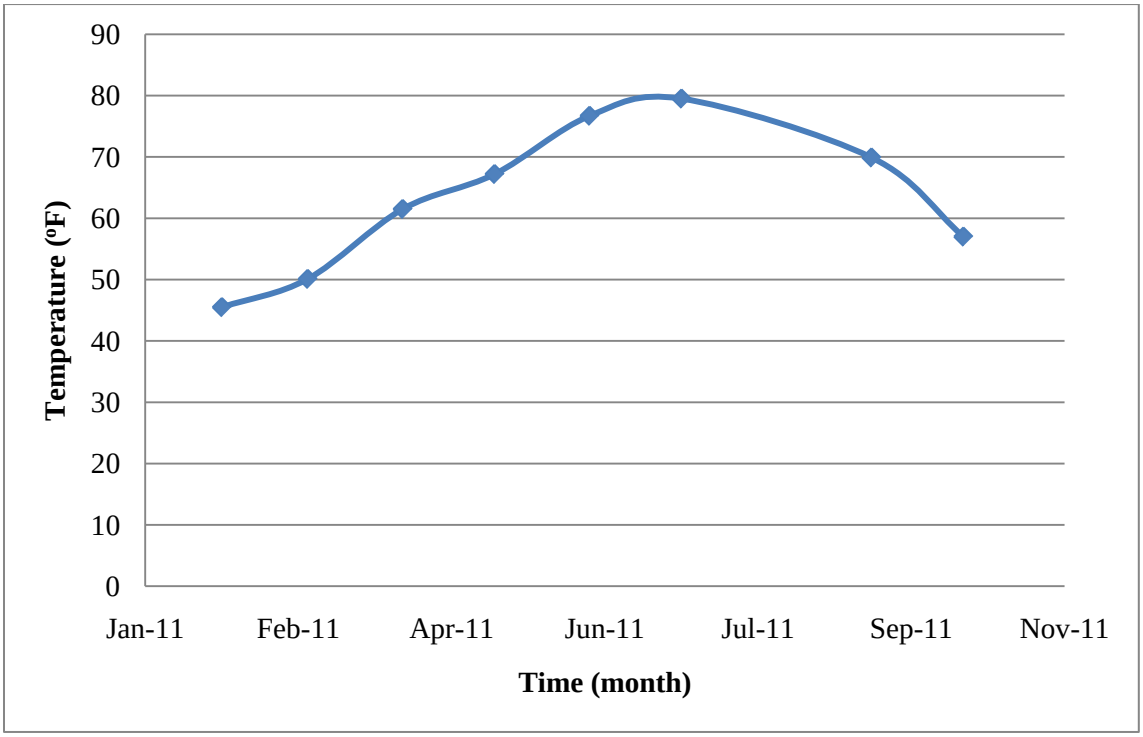

Figure 4.10. Average temperature for Greensboro, 2011 (NCAT Agricultural Research Service Weather Station)

Table 4.2. Total Coliform monthly projection in MPN/100 ml, 2011

Location	February	March	April	May	June	July	September	October
A2	284	836	2564	12385	4519	16053	5680	4979
B2	302	1809	3208	8551	9243	14978	3559	625
C2	148	>2419	8626	48672	15362	30423	5462	1645
D2	306	867	5522	24746	3699	11346	5392	4919
E2	251	1795	857	19862	1798	8673	10324	6746

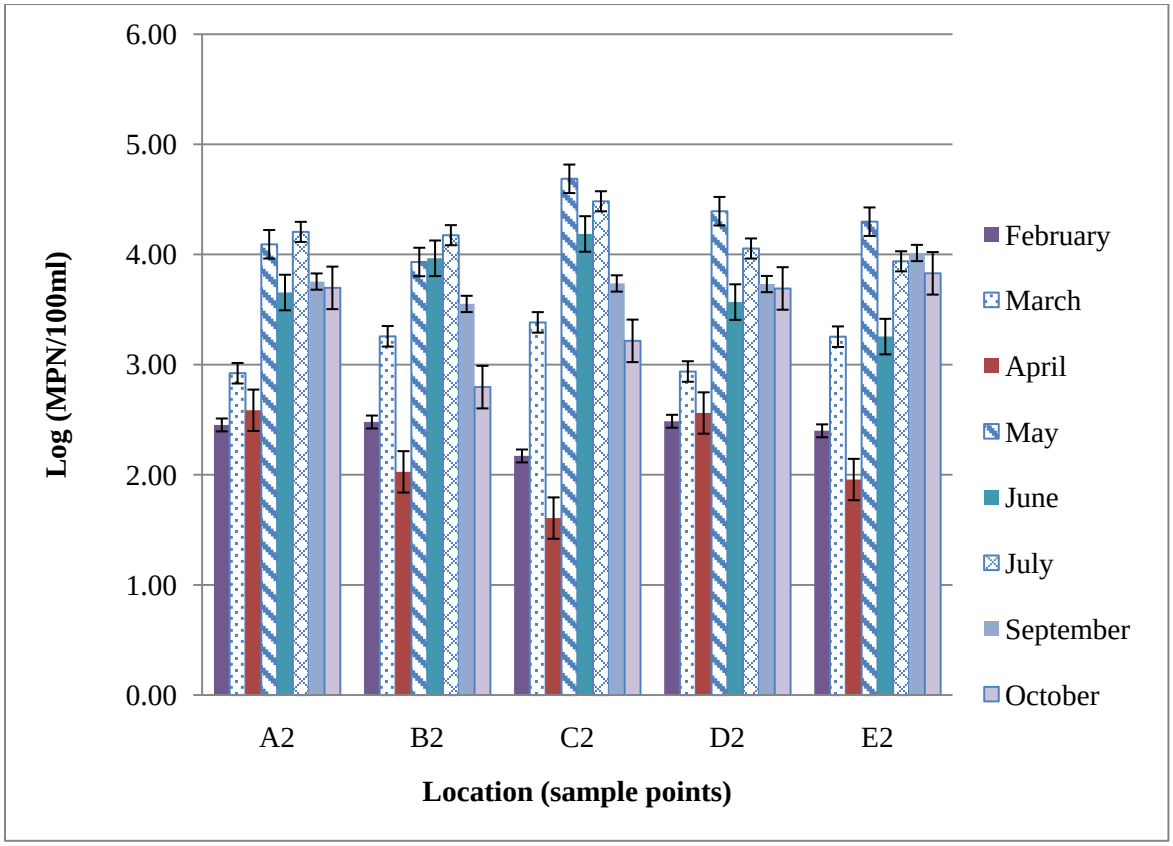

Figure 4.11. Total Coliform monthly projection, 2011

E. coli was recorded at its highest during the month of May with levels reaching 20,478 MPN per 100ml (4.31-$\log_{10}$), Table 4.3 and Figure 4.11. The bacteria levels vary considerably at each sample points for each month and the lowest level reported was at point A2 and B2 in the month of March and February respectively. During those months majority of the geese relocate to warmer climates. Furthermore, the geese mostly hangout in Lake B, hence bacterial count in Lake A might be lower. The *E. coli* levels that were discovered from this experiment were high in some areas over the months tested. However, the month of May had the highest count of E. coli and surpassed the EPA recommended level of 126 MPN per 100ml for freshwater by 2.05-log MPN per 100ml. The highest number that was recorded for May was 20,478 MPN per 100ml (4.31-log) of *E. coli*. The numbers could have been high on the month of May due to temperature changes for that week and on the day of sampling. In Figure 4.10 above, the average temperatures for each month are displayed, which does not mean that throughout the entire month the temperature were all the same. The average temperature for the month of May was lower by 9.5^{o}F and 12.3^{o}F than the month of June and July respectively. However, there were more rainfalls experienced between June and July. This association could have affected the number of quantify bacteria for June and July, hence those months were lower than May even though they recorded the highest average temperature during that period. Winter periods had lower bacterial levels compared to summer, as can be seen during February and March. Also, the fluctuating levels of E. coli on a monthly basis in the lakes could be due to a decrease in the number of geese or perhaps because of migration.

Table 4.3. E. coli monthly projection in MPN/100 ml, 2011

Location	February	March	April	May	June	July	September	October
A2	10	5	385	10067	134	85	108	108
B2	5	12	106	20478	337	97	34	54
C2	15	8	40	12068	87	27	30	383
D2	250	40	364	12493	138	137	140	630
E2	34	296	91	7593	105	282	123	238

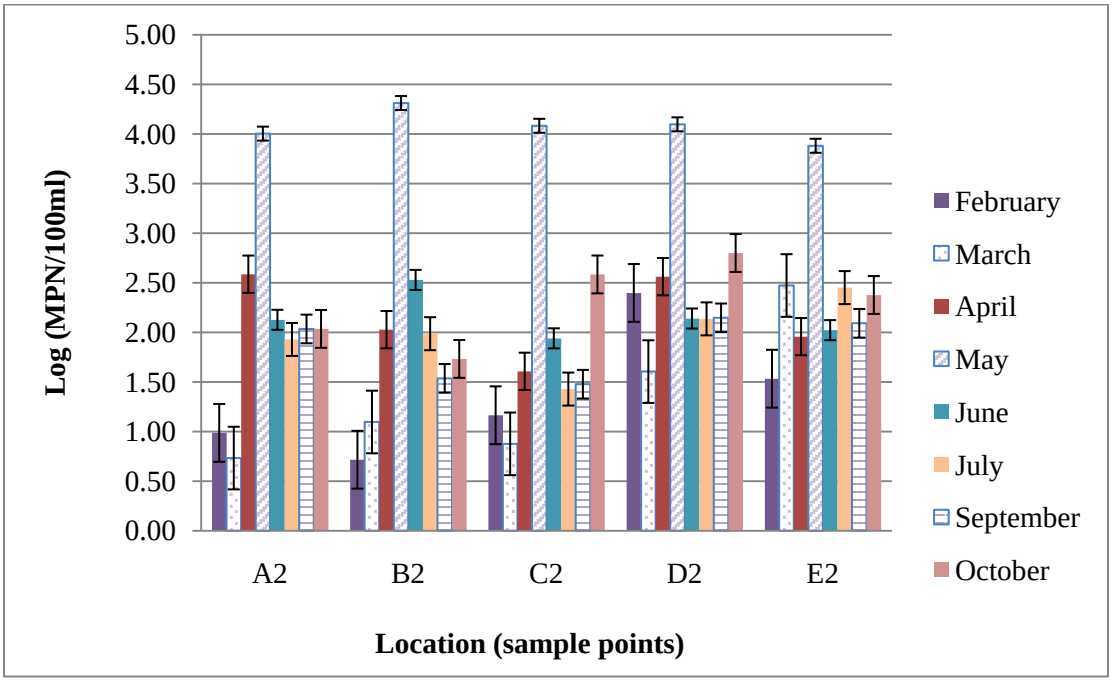

Figure 4.12. E. coli monthly projection levels, 2011

Enterococci levels were consistently above 33 colonies per 100ml, as set forth by the USEPA, for all months tested, Table 4.4 and Figure 4.13. The months that consistently recorded the highest levels were May and June and all the rest of reported months consistently fluctuate. It can be observed that A2 and B2, Lake A, for the month of October recorded levels lower than that of sample points analyzed at Lake B (C2-E2), which might have been due to less water fowl hangout in Lake A. In Table 4.4 the month of May had counts exceeding 2419 (3.8-log10) which is due to the dilution factor that was setup. Hence, to get an actual Enterococci count further dilution would have to be setup. The standard error bars shown on each graph can be used to determine the bacterial variation for each month based on the mean of all dilutions for each sample location.

Table 4.4. Enterococci monthly projection in MPN/100 ml, 2011

Location	May	June	July	September	October
A2	>2419	2978	141	121	26
B2	>2419	2637	86	329	15
C2	>2419	315	261	208	114
D2	>2419	71	52	46	80
E2	>2419	148	128	159	46

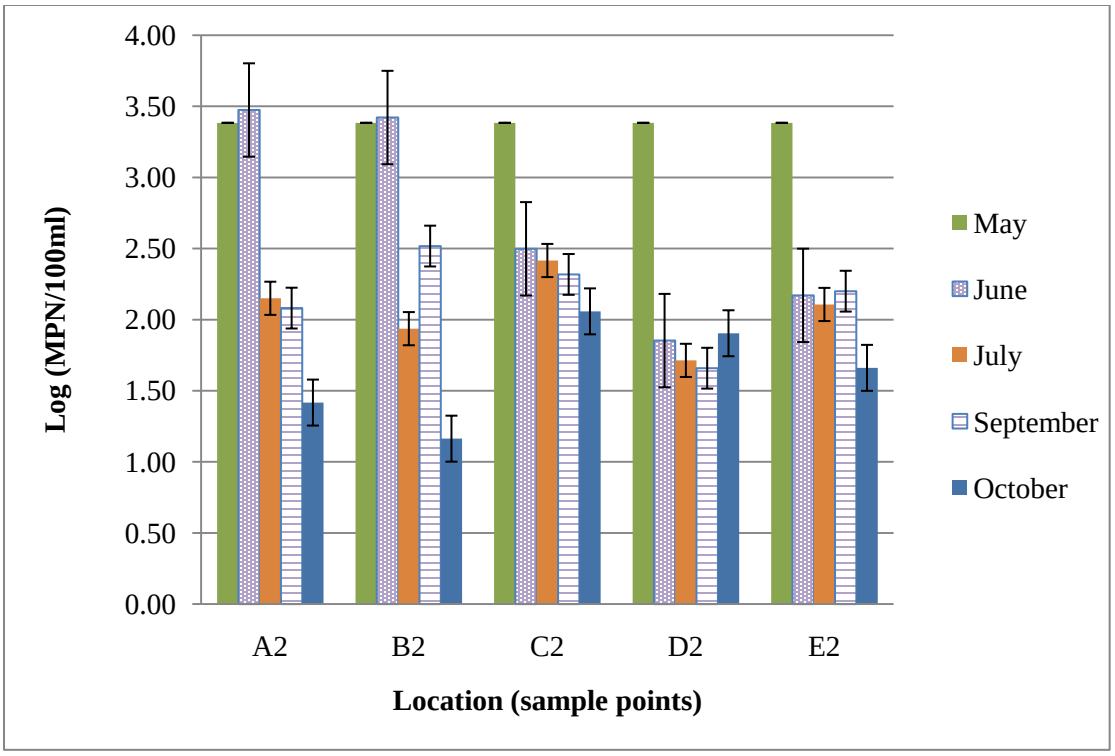

Figure 4.13. Enterococci bacteria monthly projection levels, 2011

The bacterial projection over a period of time will in many times be different as the conditions each day are different. The temperature varies and climatic conditions are never constant and are always unexpected. From doing this experiment the data retrieved displays this type of inconsistency. Nevertheless, important information can be obtained from these findings to warn people or protect public health at the Park.

4.1 Water treatment

Based on bacterial profile for the months investigated it was clear that the water at the site exceeded the acceptable levels for recreational water. Hence, the lake water would be classified as impaired, which would make this location suitable for remediation.

Hence, a CSTR system for water treatment was investigated. The CSTR was setup to mix at a rate of 77 Rev per minute with water from the lake and CRCOP pellets. There were 7 treatment periods with 5 of those being samples tested at different volume and the remaining two included recycled CRCOP and polymer blend without Oxone®, *Figure 4.14* to Figure 4.27. A treatment period typically last a day or two after all bacteria has been killed in treatment. Treatment period one was done using 1000 ml the water from the Park's Lake and was calibrated to maintain a treatment temperature of 28^{o}C, however, all other treatment periods were monitored at room temperature. This was done over a seven day period and tested to detect *E. coli* and Enterococci levels in the water.

As seen in Table 4.5 and Figure 4.14, the *E. coli* levels were reduced (1.96-log$_{10}$ reduction) on day one of treatment compared to the control and it was fully eradicated on day two of treatment. On the other hand, Enterococci levels were completely inactivated after one day of treatment, Figure 4.15. Compared to the other test done without the temperature gage, the *E. coli* levels in treatment period one took a longer time to be reduced than treatment period 2 to 5. This could mean that *E. coli* replicate faster at that temperature in treatment, which made it harder to reduce colonies in the CSTR using CRCOP. Also, controls showing count greater than 2419 colonies were samples that required further dilution in order to get an exact number.

Table 4.5. Treatment of 1000 ml wastewater, treatment period 1

E. coli (CFU/ 100 ml)					Enterococci (CFU/ 100 ml)			
Time (day)	Control	Control st. dev.	Treated	Treated st. dev.	Control	Control st. dev.	Treated	Treated st. dev.
0	15337	1771	15337	1771	>2419	-	>2419	-
1	4554	1909	169	171	9944	495	0	0
2	1249	71	0	0	2298	141	0	0
3	110	103	0	0	218	35	0	0
7	160	-	0	0	0	0	0	0

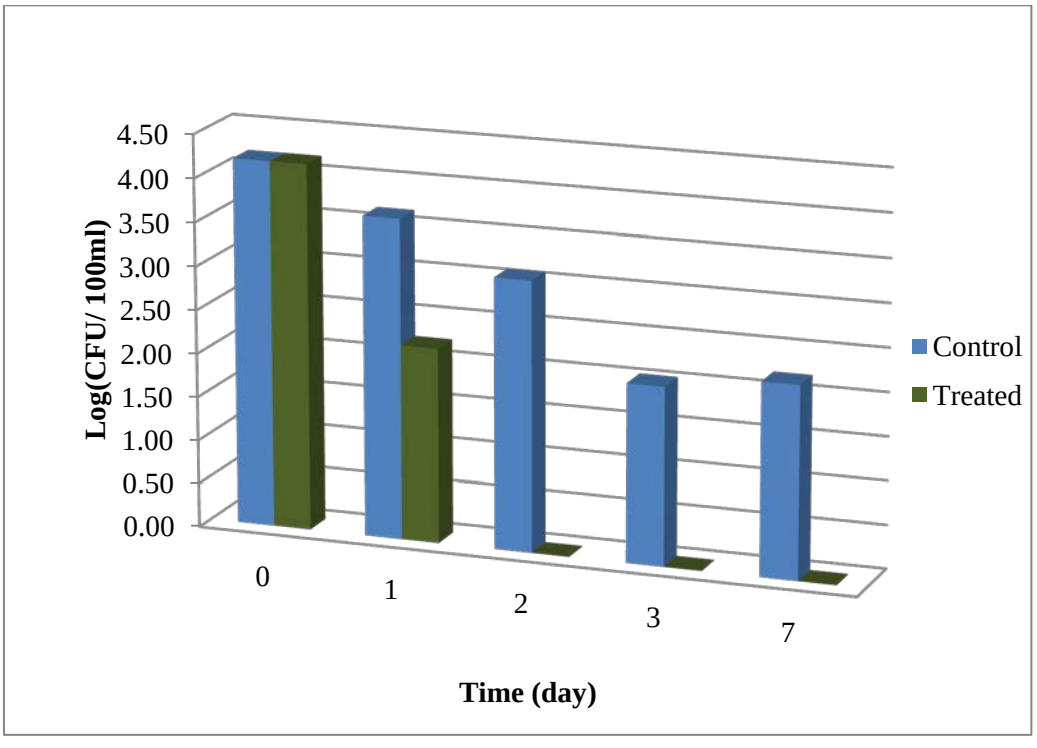

Figure 4.14. E. coli degradation in 1000 ml of wastewater, treatment period 1

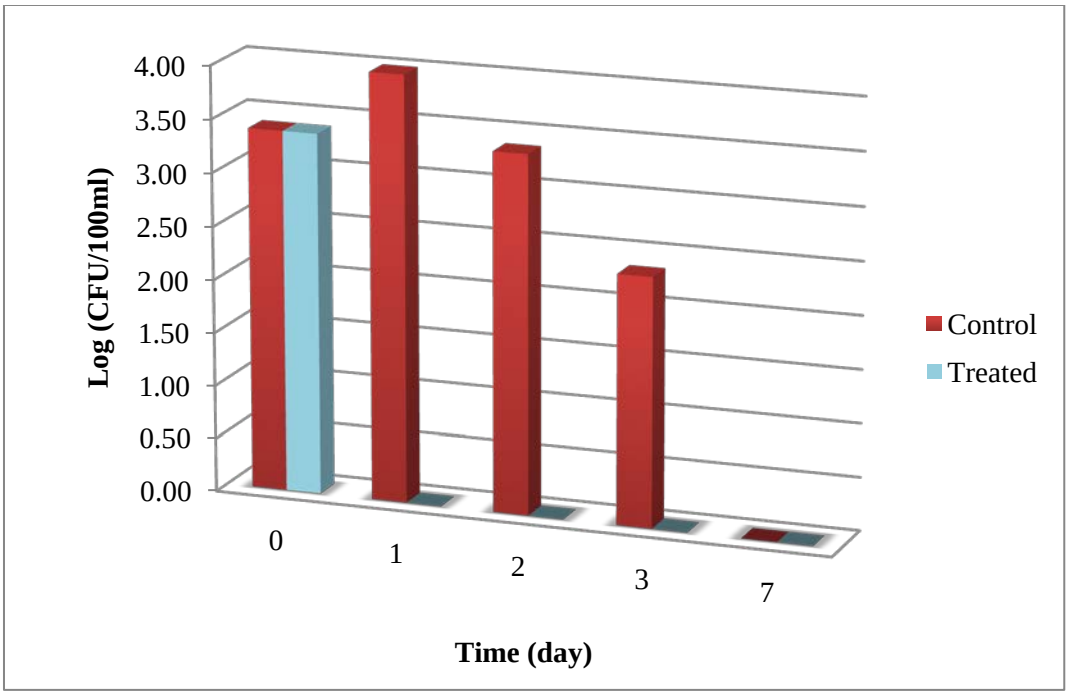

Figure 4.15. Enterococci degradation in 1000 ml of wastewater, treatment period 1

As explained before, the *E. coli* and Enterococci levels were removed completely in treatment period 2 to 5 after one day of treatment as compared to their controls, Figure 4.16 to Figure 4.23. The control was done to determine the levels of bacteria that were present in the same wastewater without treatment. In treatment period 2, *E. coli* and Enterococci had an approximate reduction of 2.7-$\log_{10}$ after a day of treatment, Figure 4.16 and 4.17. Table 4.6 displays the geometric mean of colonies for *E. coli* and Enterococci and the standard deviation for both treatment and untreated (control) water tested. The standard deviations are large, as they were done based on the amount filtered

through at their specific dilution. Hence depending on the amount filtered through and dilutions used the numbers will vary considerably.

Table 4.6. Treatment of 1200 ml wastewater, treatment period 2

	E. coli (CFU/ 100 ml)				Enterococci (CFU/ 100 ml)			
Time (day)	Control	Control st. dev.	Treated	Treated st. dev.	Control	Control st. dev.	Treated	Treated st. dev.
0	493	419	493	419	447	263	447	263
1	493	419	0	0	447	263	0	0
2	223	42	0	0	80	18	0	0
3	-	-	-	0	80	18	0	0

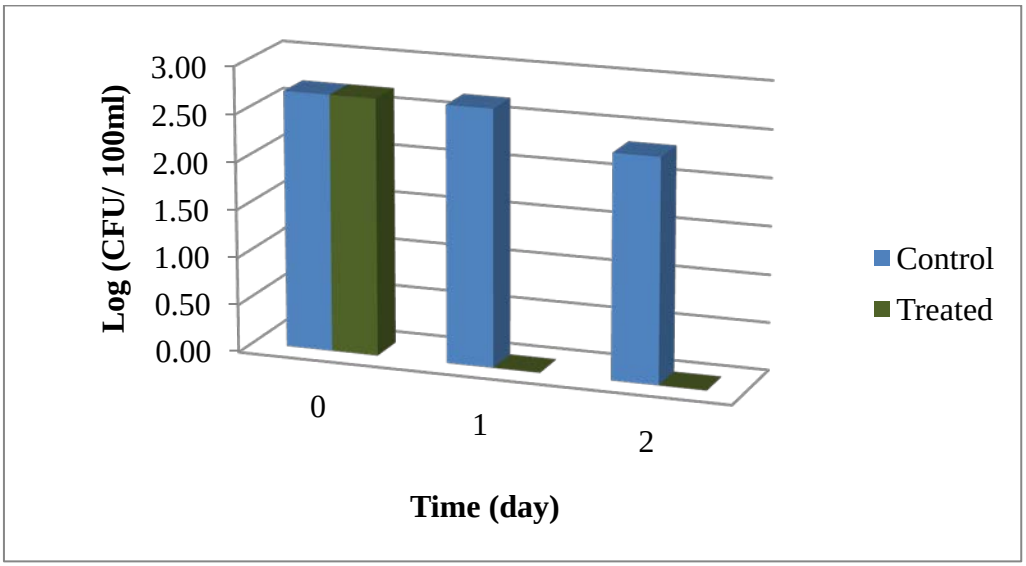

Figure 4.16. E. coli degradation in 1200 ml of wastewater, treatment period 2

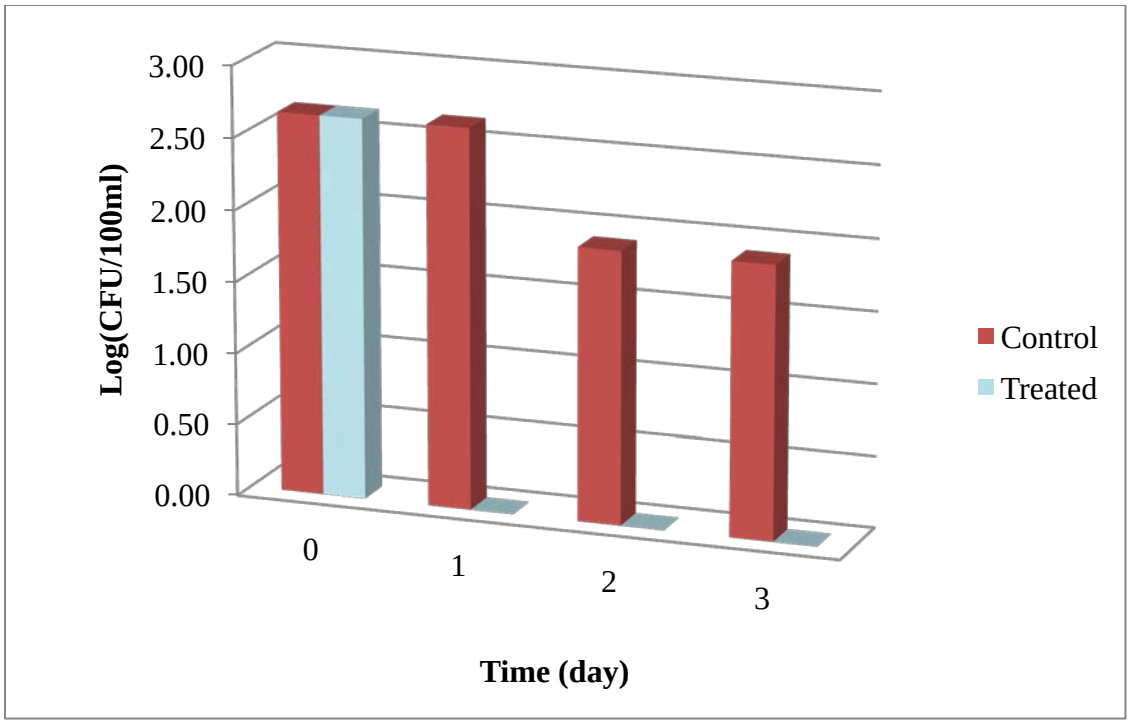

Figure 4.17. Enterococci degradation in 1200 ml of wastewater, treatment period 2

Treatment period 3 had a volume increase to1400ml and the same consistency of CRCOP, 60% oxidant to polymer ratio, was used. Table 4.7 displays the standard deviation and the geometric mean of bacterial levels in treated and control water. CRCOP oxidation treatment done for treatment period 3 was successful in the inactivation of *E. coli* and Enterococci bacteria after a day of treatment, Figure 4.18 and Figure 4.19. It is important to note that there could have still been bacteria in treated water but the amount filtered through might have been too small to detect.

Table 4.7. Treatment of 1400 ml wastewater, treatment period 3

E. coli (CFU/ 100ml)					Enterococci (CFU/ 100ml)			
Time (day)	Control	Control st. dev.	Treated	Treated st. dev.	Control	Control st. dev.	Treated	Treated st. dev.
0	279	195	279	195	210	122	210	122
1	330	-	0	0	160	-	0	
2	310	-	0	0	120	-	0	
3	300	-	0	0	80	-	0	

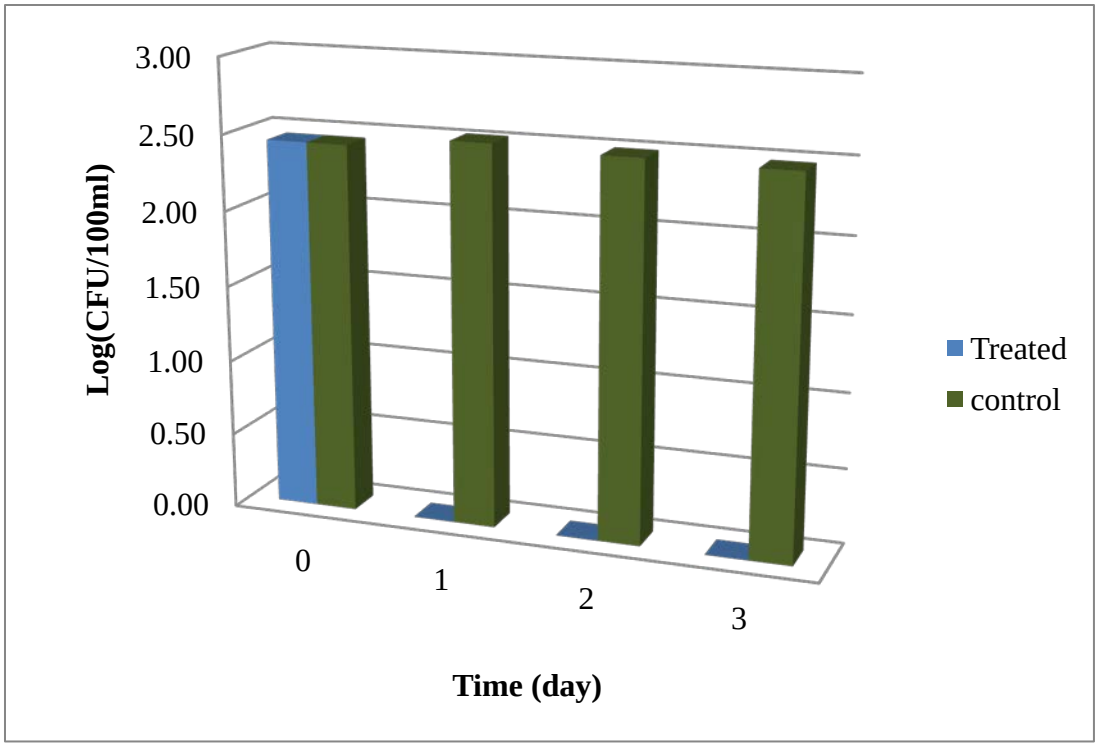

Figure 4.18. E. coli degradation in 1400 ml of wastewater, treatment period 3

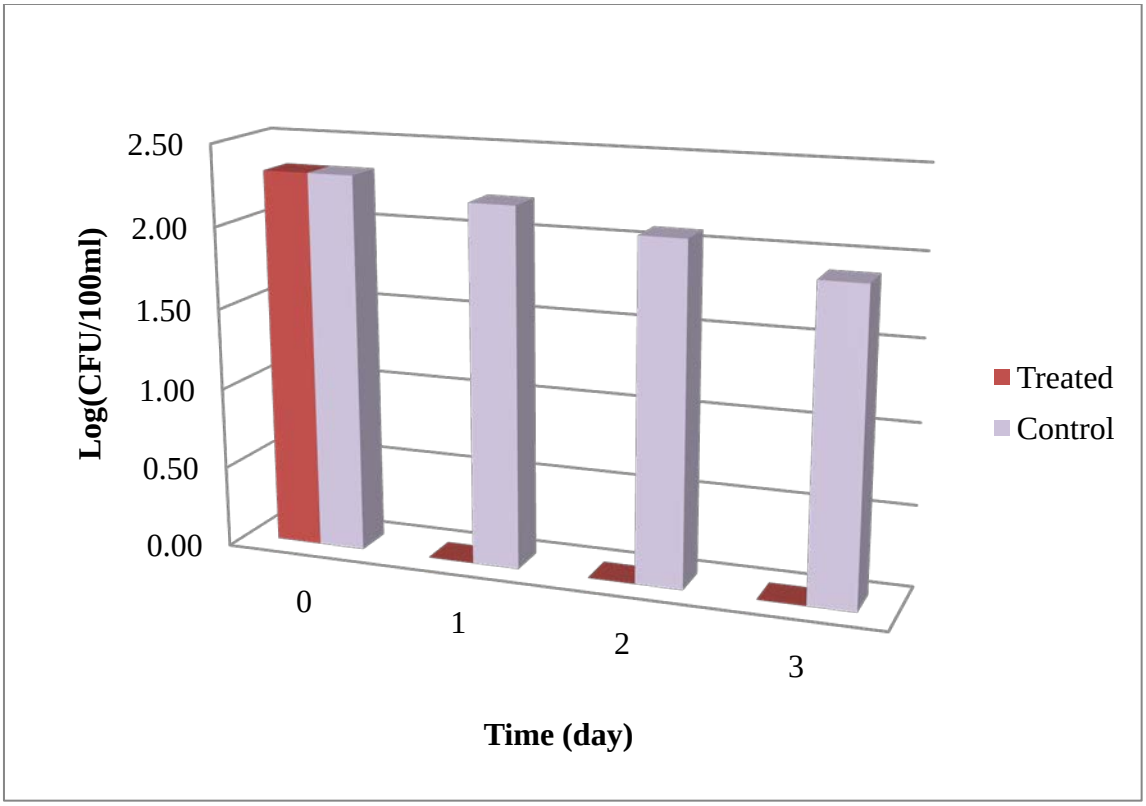

Figure 4.19. Enterococci degradation in 1400 ml of wastewater, treatment period 3

Wastewater was increased to 1600ml and treatment progress as did with treatment periods 2 and 3. Table 4.8 shows the colonies that were present in control and treated water. The standard deviation found showed that they were higher than the geometric mean of all the controls through treatment period. The standard deviations vary as they were taken using the counts retrieved per volume filtered with respect to its dilution. It was observed that the CRCOP was effective in treatment period 4 as all *E. coli* and Enterococci bacteria were completely eliminated after a day of treatment, Figure 4.20 and 4.21. *E. coli* had a 2.2-$\log_{10}$ reduction and Enterococci 2.4-$\log_{10}$ after a day of treatment.

Table 4.8. Treatment of 1600 ml wastewater, treatment period 4

E. coli (CFU/ 100ml)					Enterococci (CFU/ 100ml)			
Time (day)	Control	Control st. dev.	Treated	Treated st. dev.	Control	Control st. dev.	Treated	Treated st. dev.
0	167	277	167	277	261	255	261	255
1	185	201	0	0	179	335	0	0
2	188	165	0	0	204	190	0	0
3	132	155	0	0	140	200	0	0

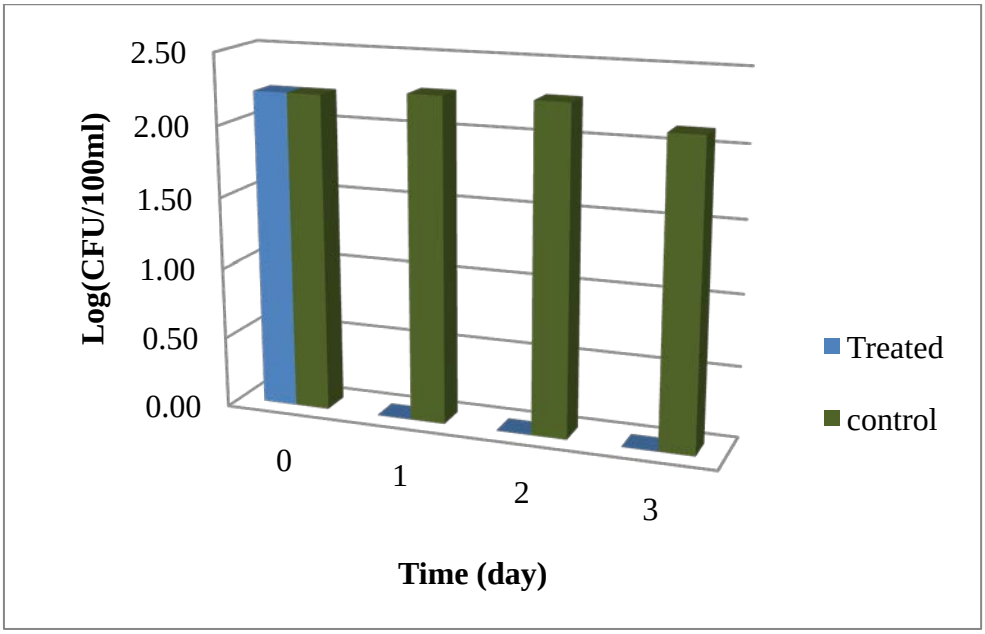

Figure 4.20. E. coli degradation in 1600 ml of wastewater, treatment period 4

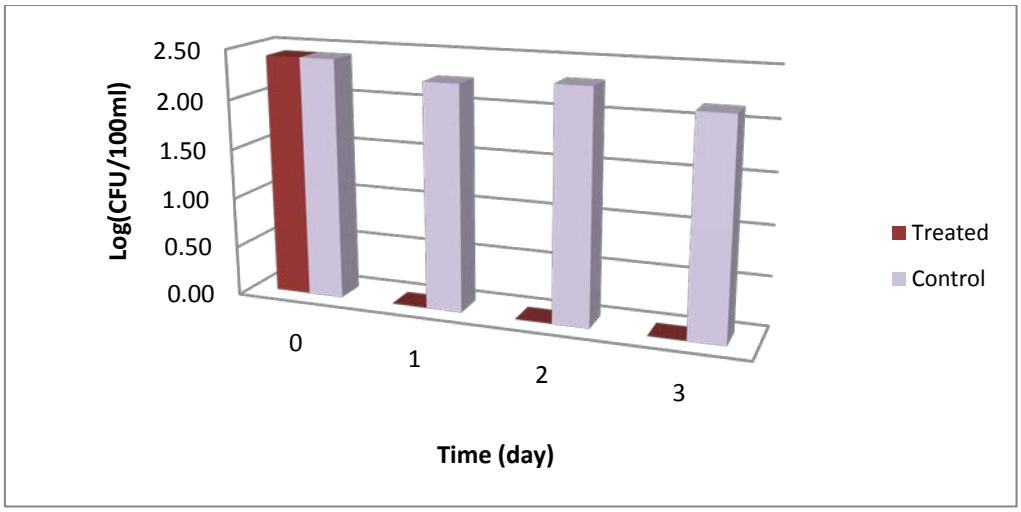

Figure 4.21. Enterococci degradation in 1600 ml of wastewater, treatment period 4

Figure 4.22 and 4.23 display the complete eradication of bacterial levels in 2000ml of wastewater for treatment period 5. In Table 4.9 the standard deviations and the colony count that exist in treatment and control are shown. An approximate 2.5-$\log_{10}$ reduction was observed for *E. coli* and 2-$\log_{10}$ reduction for Enterococci.

Table 4.9. Treatment of 2000 ml wastewater, treatment period 5

E. coli (CFU/ 100ml)					Enterococci (CFU/ 100ml)			
Time (day)	Control	Control st. dev.	Treated	Treated st. dev.	Control	Control st. dev.	Treated	Treated st. dev.
0	307	296	307	296	110	64	110	64
1	259	262	0	0	58	96	0	0
2	312	499	0	0	144	116	0	0
3	245	210	0	0	38	38	0	0

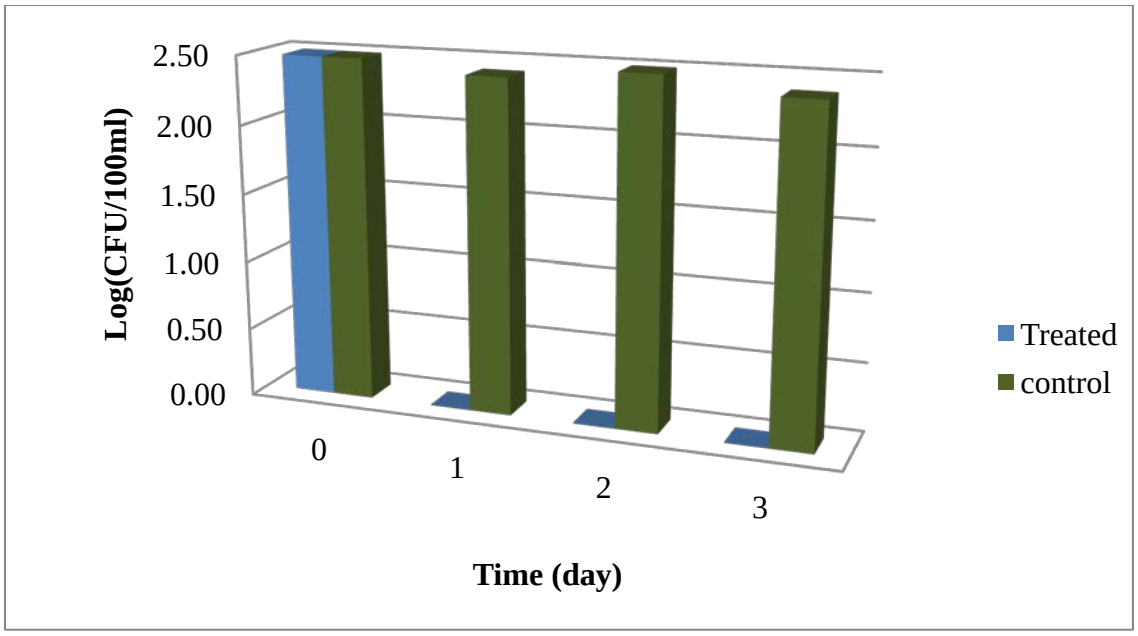

Figure 4.22. E. coli degradation: 2000 ml of wastewater, treatment period 5

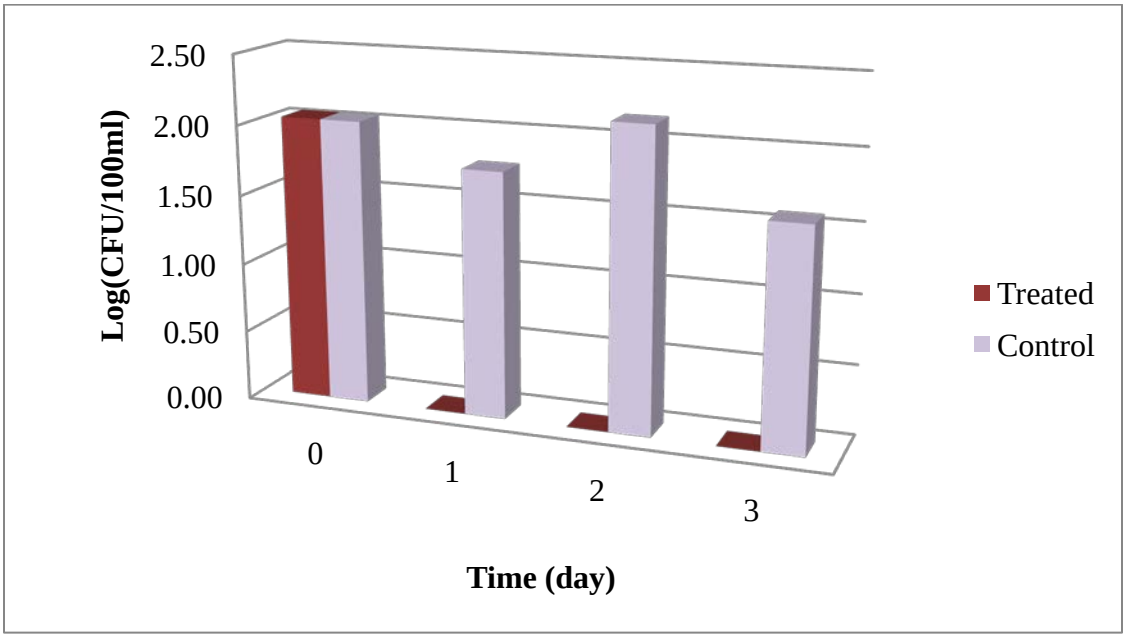

Figure 4.23. Enterococci degradation: treatment 2000 ml of wastewater, treatment period 5

Figure 4.24 and 4.25 depict the result of treatment with recycled CRCOP. The CRCOP pellets that were used in treatment period 5 were reused to treat a fresh set of wastewater retrieved from the Country Park Lake. On day one of treatment all the *E. coli* were eradicated, however after one day of treatment Enterococci levels were higher than what initially went into treatment, Figure 4.25. This could therefore mean that the concentration released into treatment was not enough breakdown Enterococci. Enterococci persist longer in the environment (Hach, 2000), which may mean that structurally they take a longer time to decompose.

Table 4.10. Treatment of 1000 ml wastewater with recycled CRCOP, treatment period 6

E. coli (CFU/ 100ml)					Enterococci (CFU/ 100ml)			
Time (day)	Control	Control st. dev.	Treated	Treated st. dev.	Control	Control st. dev.	Treated	Treated st. dev.
0	40	23	40	23	182	268	182	268
1	437	452	0	0	265	291	1093	50

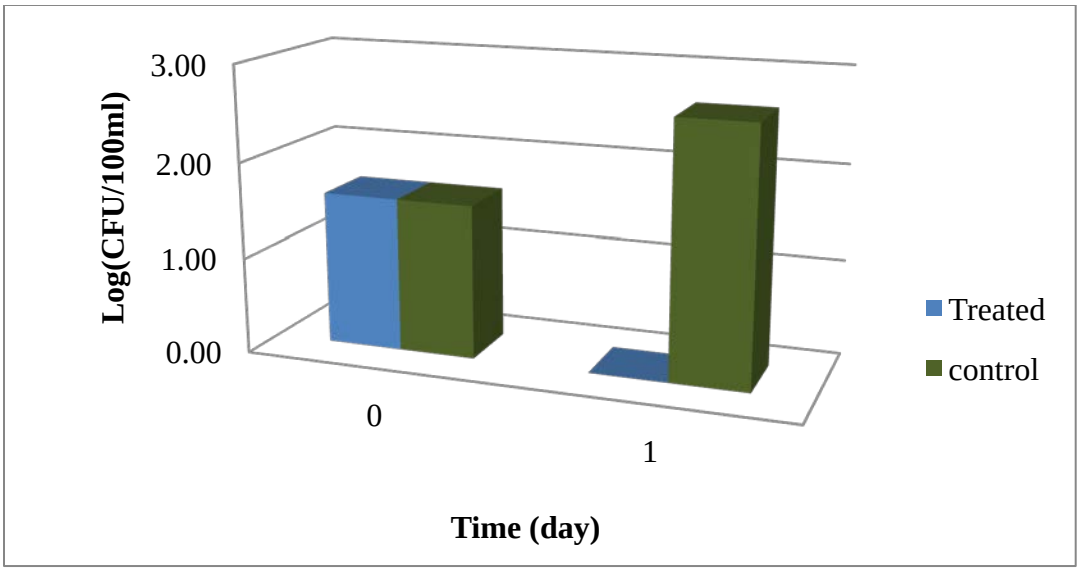

Figure 4.24. E. coli reduction in 1000 ml of wastewater using recycled CRCOP, treatment period 6

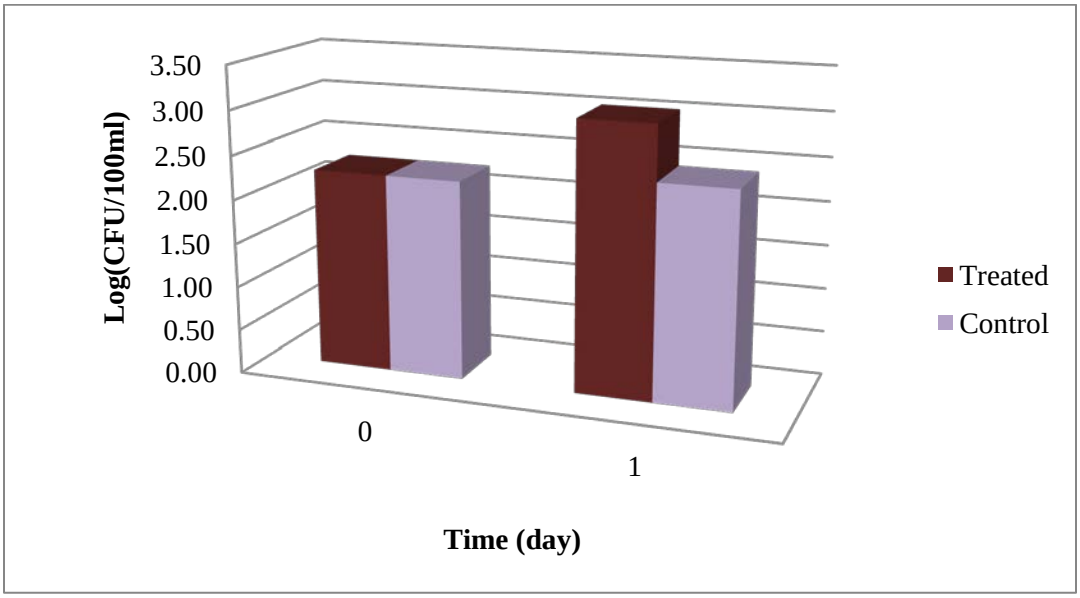

Figure 4.25. Enterococci reduction in 1000 ml of wastewater using recycled CRCOP, treatment period 6

Treatment period 7 was conducted to determine whether the polymer had any effect on the eradication of *E. coli* and Enterococci. There was no *E. coli* present in water before test with polymer only was conducted and at the end of the experiment the result remained the same, Figure 4.26. However, after a day of treatment control showed that E. coli was present, this may have been because of cross contamination during the experiment. Enterococci was present in the water before treatment attempt with polymer only and after testing the treated level was higher than the control, hence showing bacteria replication, Figure 4.27. This therefore, proves that solely polymer did not eliminated the bacteria in treatment previously but the Oxone® that was combined with the polymer was acting as the active ingredient in the removal of Enterococci and *E. coli*.

Table 4.11. Treatment wastewater using polymer only, treatment period 7

E. coli (CFU/ 100ml)					Enterococci (CFU/ 100ml)			
Time (day)	Control	Control st. dev.	Treated	Treated st. dev.	Control	Control st. dev.	Treated	Treated st. dev.
0	0	0	0	0	858	500	858	500
1	20	12	0	0	140	81	1093	50

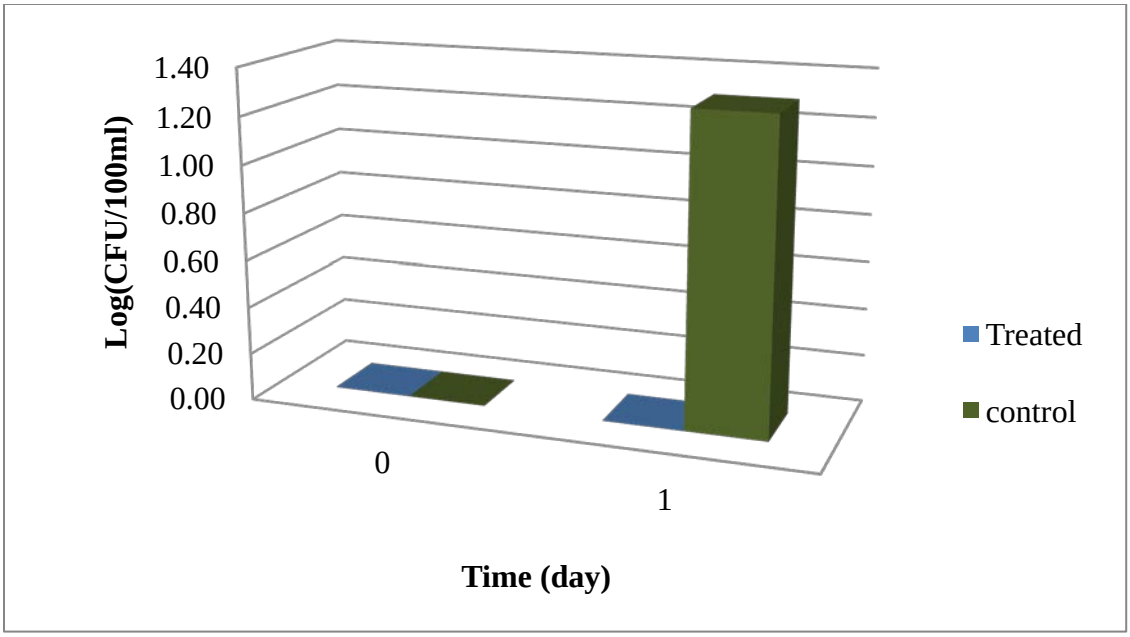

Figure 4.26. E. coli degradation in 1000 ml of wastewater using polymer only, treatment period 7

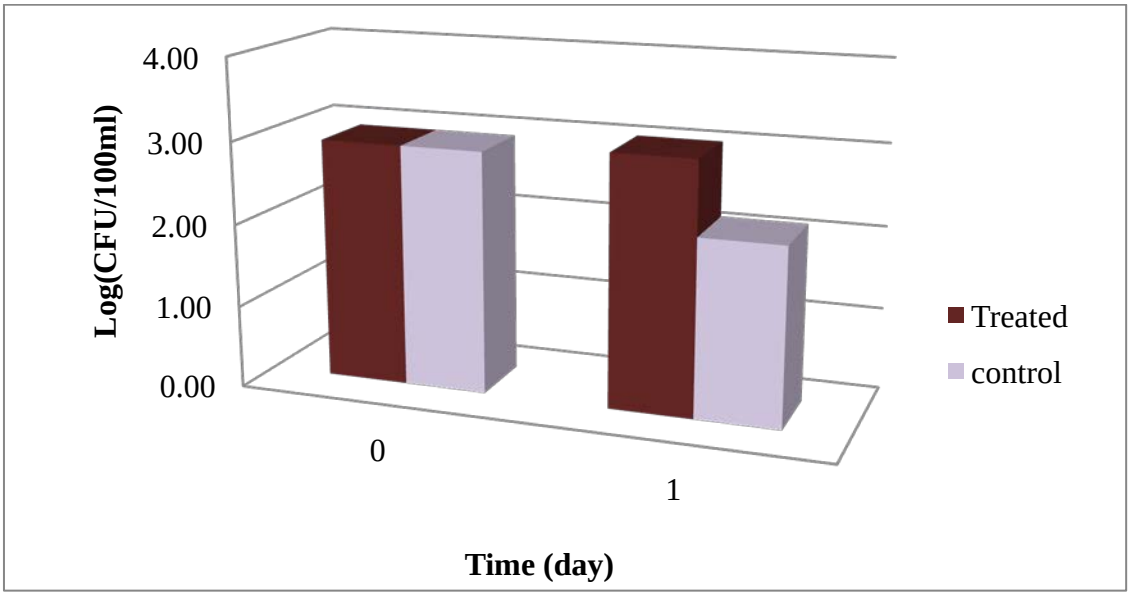

Figure 4.27. Enterococci reduction in 1000 ml wastewater using polymer only, treatment period 7

4.2 CRCOP Release Trajectory in a CSTR

The amount of Oxone® released from the CRCOP pellets during treatment was tracked. A calibration curve was established using 5 grams of Oxone® to 1000 milliliters of deionized (DI) water and dilutions from this volume were set up. Oxone® solution of specific dilution was analyzed for absorbency using a UV spectrometer at a wavelength of 254 nano-meters (nm). The stock used began at a concentration of 5g Oxone® per 1000ml of DI water and the remaining were produced as dilutions from stock solution using serial dilutions. The absorbency readings recovered per dilution were plotted against known concentrations, see Figure 4.28. Based on the plot developed from the calibration curve, an equation y = 0.0248x – 7E-05 was determined with a R^2 value of 0.9889. This equation was used to derive the concentrations of Oxone® released in treatment water per day by substituting "x" for the absorbency recorded.

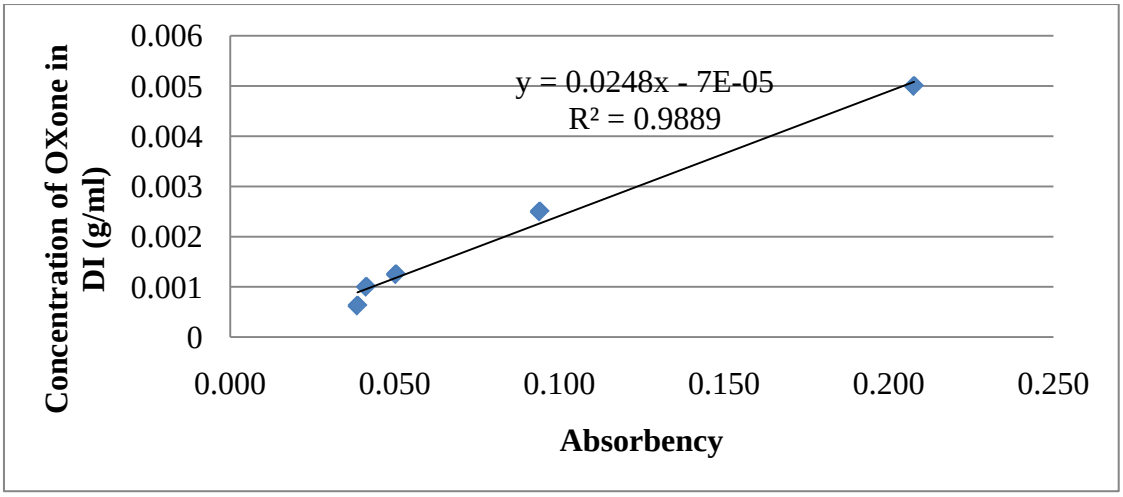

Figure 4.28. Calibration curve for Oxone® in gram per ml

There were two treatment periods that were incorporated into this study, treatment period one and two. Treatment period one was setup to treat 1000ml of wastewater and regulated at a temperature of 28^{o}C/82.4^{o}F and 77 revolutions per minute. Treatment period two had the similar setup except treatment was run at room temperature and 1200ml wastewater for treatment. Both treatment setups were done with the CRCOP consistency of 0.3 grams Oxone® and 0.5 grams PCL blends. The release data were calculated and plotted using the Oxone® concentrations against the absorbencies, Oxone® concentrations released were plotted against time and the amount of Oxone® that was remaining in the pellets per day of treatment was measured. Figure 4.29 depicts the Oxone® released per absorbency recorded for treatment period one, Figure 4.30 shows the daily concentration of Oxone® released in treatment water and Figure 4.31 displays the amount of Oxone® remaining in pellets daily.

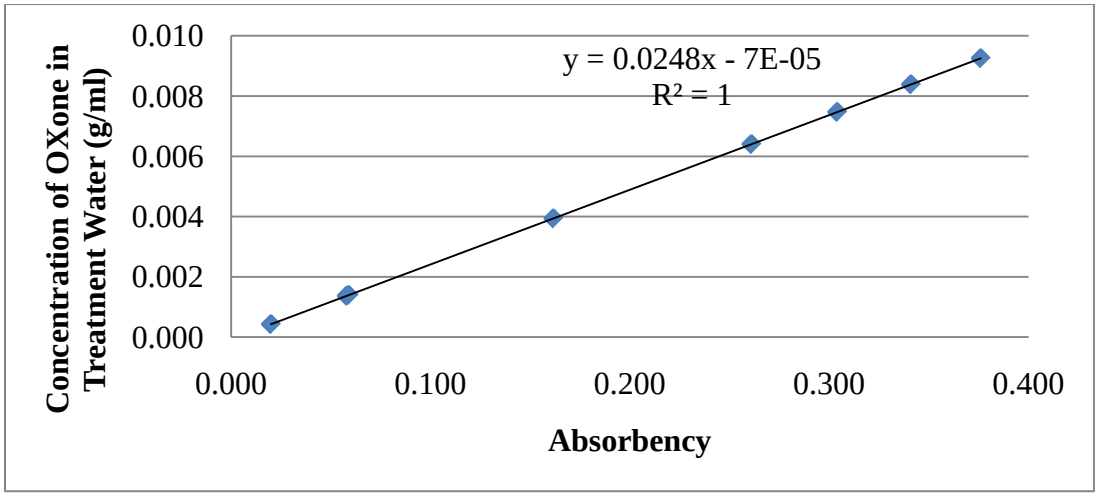

Figure 4.29. Treatment period one CRCOP release curve in gram per ml

An initial rapid release of Oxone® occurred at the beginning of treatment and gradually the diffusion rate slowed over time. This is because Oxone®, on or near the surface of PCL mixture, tended to release the fastest into treatment. Oxone® release slows down as the surface oxidant is removed and further degradation is required for the release to occur. Also, the CRCOP mixture is not necessarily homogeneous and further research is needed to develop the technology for blend consistency. Figure 4.30 gives a graphical explanation of this release mechanism, where the concentration of Oxone® in the treatment water was monitored for 8 days of treatment.

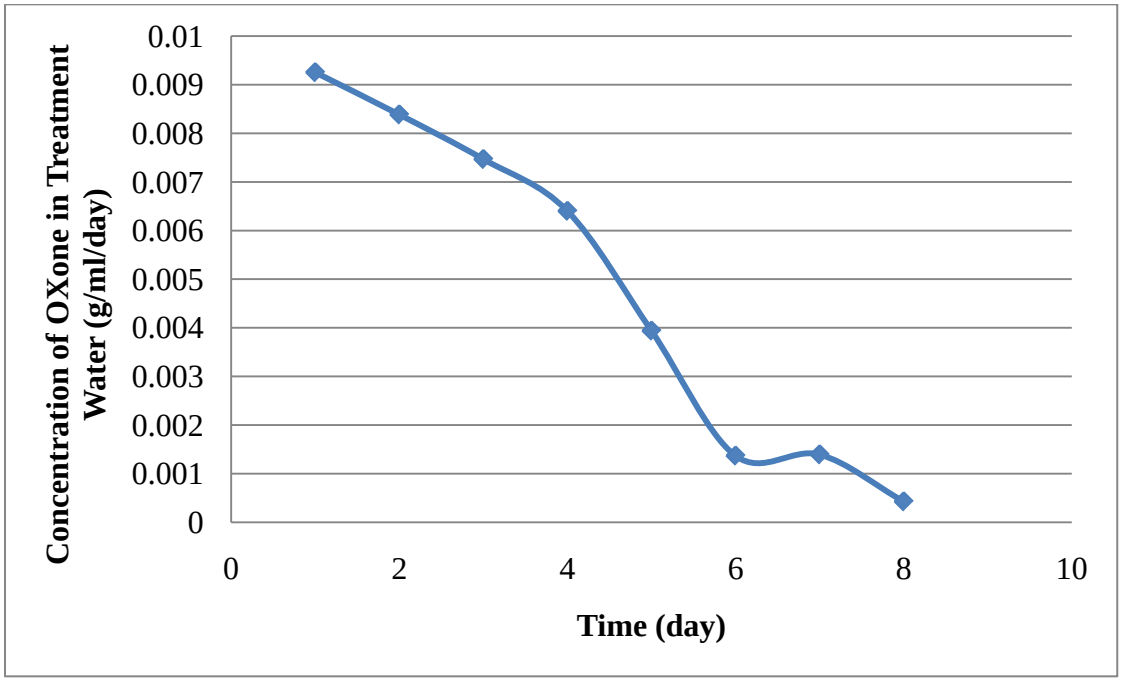

Figure 4.30. Oxone® concentration in treatment water, treatment period 1 release

Treatment using a controlled temperature gave a curve that was consistent in releasing Oxone®. In Figure 4.31 the amount of Oxone® in CRCOP pellets declined from 0.3 grams to approximately 0.26 grams after 8 days.

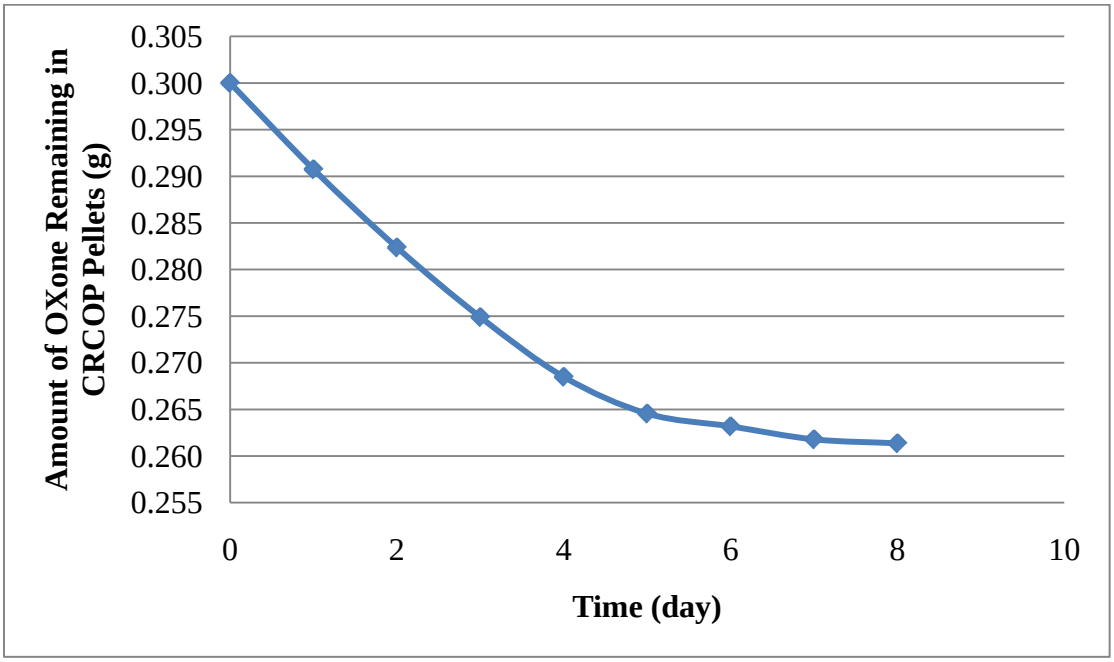

Figure 4.31. Amount of Oxone® remaining in CRCOP pellets per treatment day

Treatment period two had absorbencies that were reported and fitted to achieve a R^2 value of 1, see Figure 4.32. Figure 4.32 shows the concentration of Oxone® released in CSTR versus its absorbencies. Figure 4.33 portrays the Oxone® release per day and Figure 4.34 illustrated the amount of Oxone® remaining in the CRCOP pellets per day of treatment.

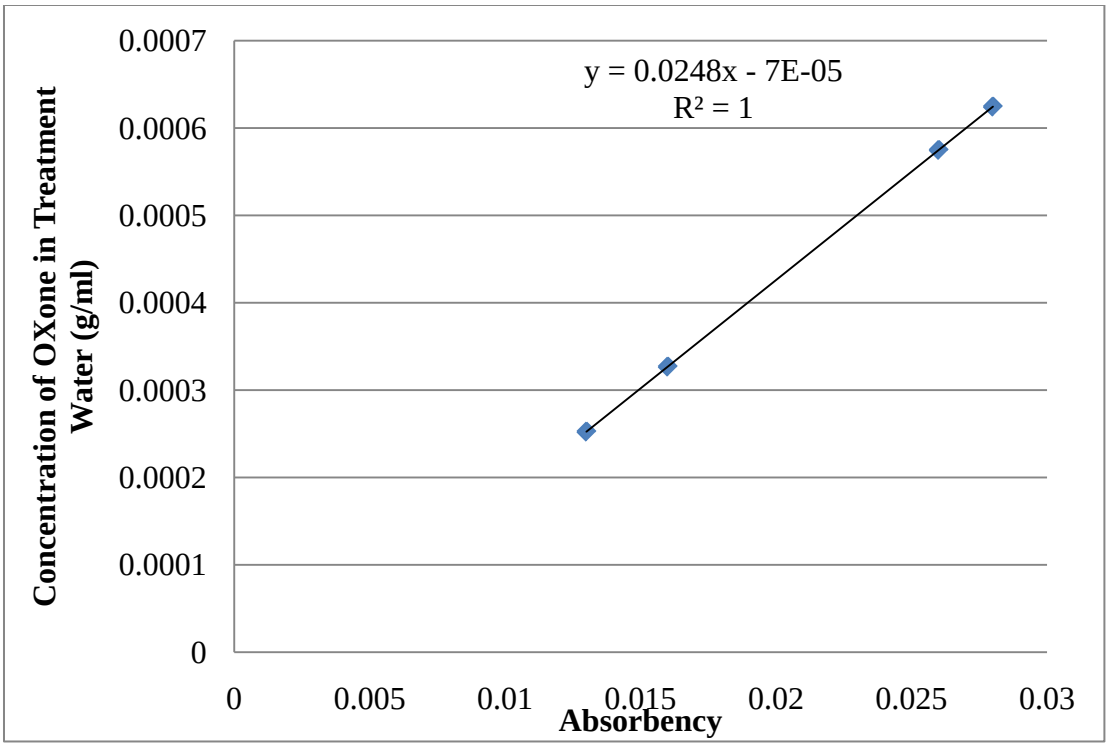

Figure 4.32. Treatment period two CRCOP release curve in gram per ml

The Oxone® release was high after one day of treatment compared to day 2 and day 3, Figure 4.33. This could be a result of the discharge of surface Oxone® that was initially released during the treatment setup. On day 2, however the levels decreased and then gradually increased as treatment progressed. This may have been due to gradual degradation of CRCOP, hence releasing more oxidant over time. Also, the water used for this treatment was a bit cloudier than water used in treatment period one. Hence, this cloudiness might have affected absorbency readings as the spectrometer is very sensitive to water that are not crystal clear.

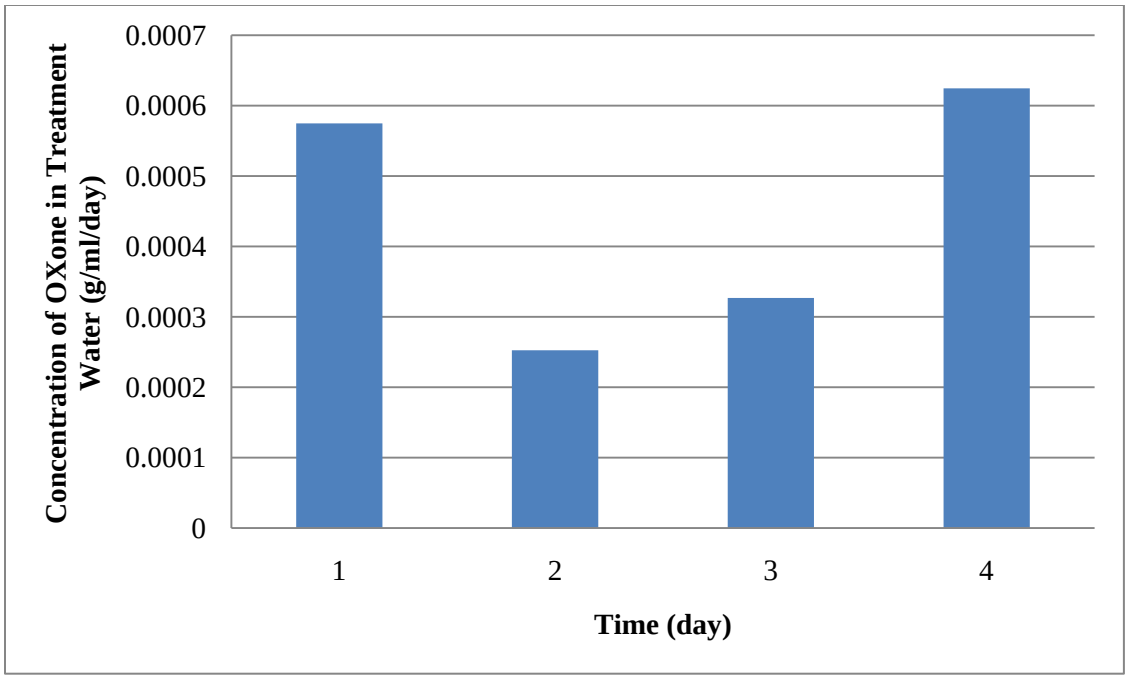

Figure 4.33. Oxone® concentration in treatment water, treatment period 2 release

Figure 4.34 shows that the amount of Oxone® remaining in CRCOP pellets gradually declined from 0.3 grams to 0.298 grams. This type of release method is important to predict how well CRCOP pellets release oxidant for treatment, especially setup exceeding 7 days. This can help to monitor Oxone® levels when treated water is above room temperature. As seen from the studies done, PCL release oxidant faster at 28^{o}C than at room temperature at about 23^{o}C. Hence, it can be predicted that oxidant will be released quicker as temperature increase.

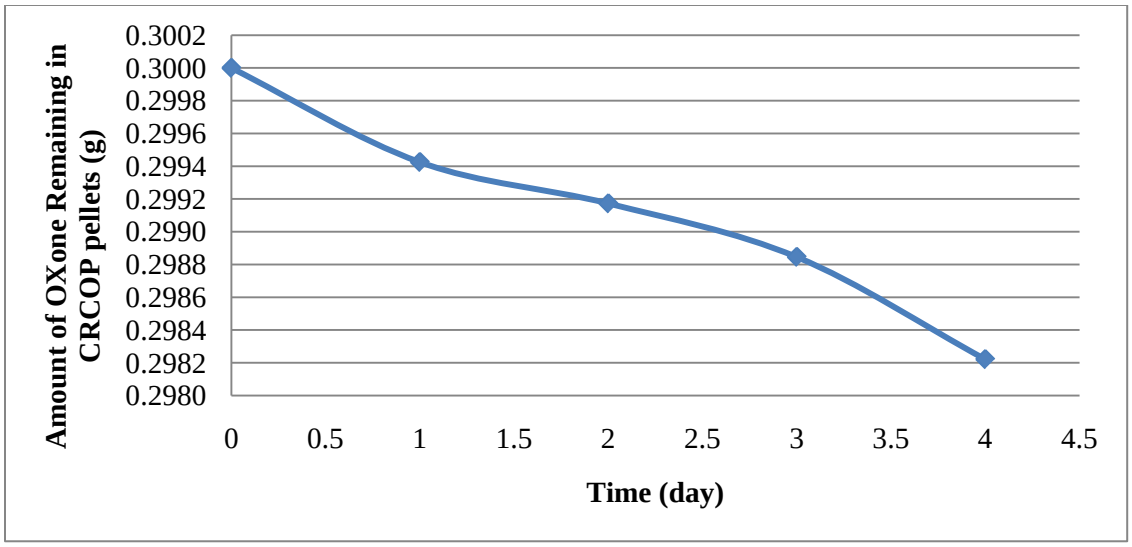

Figure 4.34. Amount of Oxone® remaining in CRCOP pellets per treatment day

4.3 Soil treatment

Soil treatment was performed using a batch reactor setup, meaning that a treatment sample was prepared initially (day zero) for each of the 4 days. Each sample was setup by increasing the water content of the soil before the insertion of the CRCOP pellets for treatment. The water content was increased to aid in the activation of the CRCOP treatment in releasing the Oxone® from the pellets and degrading the polymer. In Figure 4.35, only one testing was able to be done using this treatment method due to time constraint. The water content of the soil collected from lagoon 2 used was 19% and approximately 5 grams of water was added to 40 grams of soil for each batch for treatment. The 5 grams of water added to this mass of soil increased each batch water content to 43% before treatment. In Figure 4.35, the treatment did not dramatically decrease the level of Enterococci and E. coli.

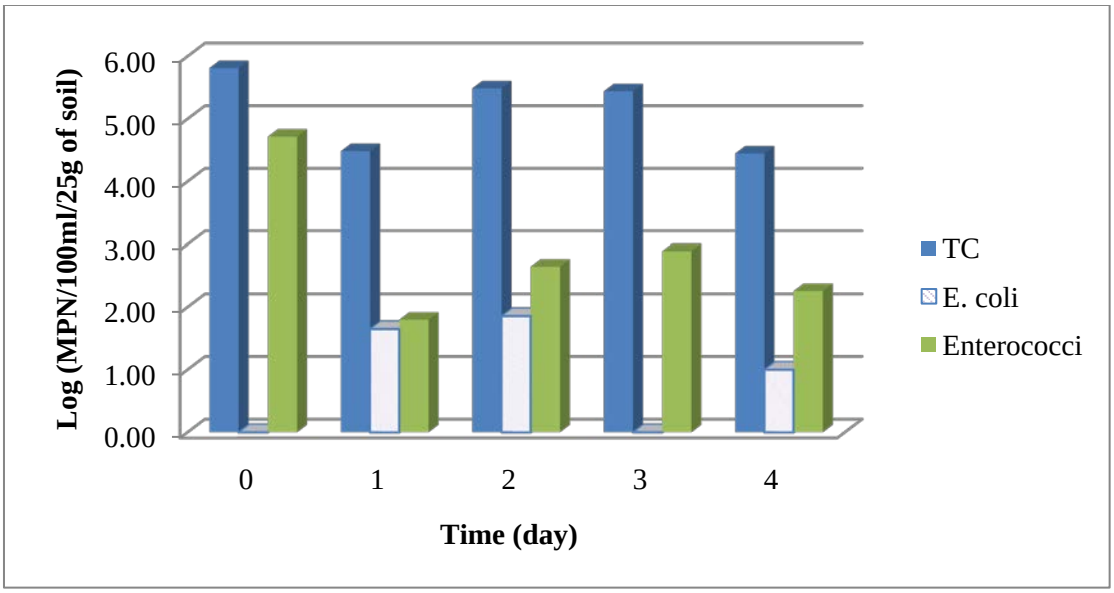

Figure 4.35. Eradication of bacteria in soil through CRCOP treatment

From observation the soil was not wet enough for the CRCOP to breakdown and kill the bacteria. Hence, in a future study, it is recommended that the water content should be increase to higher than 43%. Also, it was observed that even though the samples that were treated were covered, evaporation of water was evident and appears to have an impact on adjusted water content for extended treatment period. In an ideal situation in the field, when there is a lot of rainfall or simply irrigation, the soil will be saturated hence aiding in the activation of CRCOP for treatment. Therefore, this application will only be effective when soil is well saturated.

CHAPTER 5

CONCLUSIONS AND RECOMMENDATIONS

It has been determined through experimentation that Greensboro Country Park Lake A and B exhibit levels of *E. coli* and *Enterococci* that exceed permissible limits set forth by the USEPA for recreational water. The bacterial levels are even more significant during summer periods, as temperatures are usually at its peak during that time, as seen between May and July. Interestingly, summer periods are when the Lakes are in use for recreational activities, such as boat ride and fishing. Caution should be exercised when participating in activities that involve the lake, as contact through ingestion, open wounds or inhalation can pose potential health problems. This could impact children the most as they are still developing and are susceptible to many diseases, especially through waterborne organisms. Hence, parents should monitor children closely while at the park, especially during summer periods.

Experiments conducted with CRCOP revealed that this technology was effective in the remediation of low strength wastewater. Using the CSTR, it was shown that the CRCOP eradicated all tested bacteria at room temperature in one day. At a temperature of 28^{o}C wastewater undergoing treatment were completely eradicated of *E. coli* and *Enterococci* after two days of exposure. Consequently if this method is used during summer periods on farm it might take more treatment to degrade within specified time. Also, treatment method that employs the reuse of CRCOP did not remove *Enterococci* bacteria; instead there was an increase in their levels. In addition, results revealed that the

use of the polymer only, without Oxone®, did not fully eliminate bacteria in CSTR treatment system. It is important to note that if bacteria are not exposed to the appropriate conditions they will undergo stress and die naturally; however it is not as fast as a treatment system that contain chemical oxidant. CRCOP has the potential to be effective in treatment system or applied to the soil for removal of pathogens.

The soil treatment experiment revealed that for soil remediation the soil must be saturated. The embedding of the pellets in contaminated sites will help to reduce pathogenic organism as they become viable in soil, at a given soil moisture content. The perspective of CRCOP is to eliminate or reduce bacterial contact with groundwater, or reduce contamination through infiltration or runoff. In order to reduce contaminated wastewater from CAFO a CSTR system with CRCOP can be set up to treat for pathogens before release into the lagoons. Treated water can also be recycled and use to flush animal units. This oxidation method is very promising for existing and for lagoons abandoned. Also, treatment system that mimics the CSTR may be implemented at stormwater drains that are connected directly to lakes or other surface water in order to reduce pathogenic loading. Another system that may be implemented is the connection of CRCOP technology to the outlet of contaminated water bodies, such as lakes, to prevent or reduce contamination downstream.

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