Fall 10-24-2016

E. Coli Removal by Pleurotus Ostreatus Mycofilter in Simulated Wet Environmental Pond

Savannah E. Martinez
University of New Mexico - Main Campus

Savannah Martinez
Candidate

Civil Engineering
Department

This thesis is approved, and it is acceptable in quality and form for publication:

Approved by the Thesis Committee:

Dr. Mark Stone, Chairperson

Dr. Kerry Howe

Dr. Andrew Schuler

E. Coli Removal by Pleurotus Ostreatus Mycofilter in Simulated Wet Environmental Pond

by

Savannah Elizabeth Martinez

B.S., Civil Engineering, University of New Mexico, 2014

THESIS

Submitted in Partial Fulfillment of the
Requirements for the Degree of

Master of Science
Civil Engineering

The University of New Mexico
Albuquerque, New Mexico

December, 2016

Acknowledgments

I would like to thank my advisor and mentor, Dr. Mark Stone, for his continued guidance and support throughout my entire college career. I would also like to acknowledge my committee members, Dr. Kerry Howe and Dr. Andrew Schuler, for providing technical assistance and allowing me to access and use of supplies in the Civil Engineering Laboratory.

This research would not have been able to be completed without the never-ending support and encouragement from my family and friends. You all have truly made the process of graduate school a great experience. I am so thankful for all of you.

This research was made possible by funds provided by the Albuquerque Metropolitan Arroyo Flood Control Authority. As well as the donation of P. Ostreatus Mycelium from Exotic Edibles of Edgewood; this research would not have been possible without your generous donation.

E. Coli Removal by Pleurotus Ostreatus Mycofilter in Simulated Wet Environmental Pond

by

Savannah Elizabeth Martinez

B.S., Civil Engineering, University of New Mexico, 2014

M.S., Civil Engineering, University of New Mexico, 2016

Abstract

Stormwater runoff has been referred to as the water quality issue of the day. Contamination of surface waters is an environmental concern for both human and ecosystem health. In Albuquerque, New Mexico, it is a common practice to pond stormwater runoff before it is released into the Rio Grande. Ponding stormwater works as a best management practice (BMP) to help remove floatable debris and contaminants. Mycofiltration is a BMP that has recently been introduced into the stormwater quality community. Mycofiltration is the use of fungal mycelium as a natural mitigation approach to stormwater pollution. Mycofiltration has been proven to reduce Escherichia Coli (E.Coli) in previous research studies testing limited variables such as contact time and mycelium species. The objectives of this study were to: (1) investigate the treatment effectiveness of mycofiltration to reduce the concentration of E. Coli after repeated exposure to synthetic stormwater in a wet environmental pond setting; and (2) analyze the long-term potential use of the P. Ostreatus mycelium in a wet environmental pond setting. The objectives of this study were met by simulating wet environmental ponds in a

laboratory where each pond contained a floating Pleurotus Ostreatus inoculated mycofilter. The results of this study showed: (1) the mycofilter reduced the concentration of E.Coli at the water surface compared with controls; and (2) the long-term potential use of Pleurotus Ostreatus mycelium in a pond setting is promising. On average, the mycofilter reactors removed an overall amount of 98% of the E. Coli concentration. The amount of E. Coli removed increased week after week of testing; the Mycofilters removed 97% week 2 and 98% in week 3. Although there were complications with false positive results for E. Coli and unequal distributions of concentrations in the simulated ponds, the potential for using mycofilters as a real-life BMP is still supported by this study.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1 INTRODUCTION

1.1 Potential Stormwater Application

Stormwater quality has been a growing topic of concern for many agencies over the past several decades. The United States Environmental Protection Agency (USEPA) considers nonpoint source pollution, including stormwater runoff, to be one of the most important sources of contamination of the nation's waters (source). Some of the principal contaminants found in stormwater runoff include heavy metals, toxic chemicals, organic compounds, pesticides and herbicides, pathogens, nutrients, sediments and salts. All of these contaminants are discharged into surface water areas having gone through little to no treatment.

The Clean Water Act (CWA) of 1972 established a basic structure for regulating the discharges into the Nation's waters. The CWA made it illegal to discharge any pollutants from point and nonpoint sources unless a National Pollutant Discharge Elimination Permit (NPDES) was obtained. The NPDES permit has limitations on what can be discharged, monitoring and reporting requirements to ensure discharge does not affect the water quality of the Nation's waters. The NPDES permit specifies acceptable levels of pollutants allowed for discharge. In the most recent Summary of Urban Stormwater Quality in Albuquerque, New Mexico (USGS, 2015) the median concentrations for E. Coli bacteria in stormwater samples was above the New Mexico water quality standard. Table 1 shows the current standard of high loading values of the New Mexico water quality permit. This standard is based on an E. Coli loading per area basis (cfu/sq mile/day) and target load calculations (cfu/day) based on a 3.5 square mile jurisdictional area within the NPDES permit area (Permit No. NMR04A000, Appendix B, Section B.2.1).

Table 1: Standard High E.Coli Loading Rates for Middle Rio Grande (USGS, 2015)

	E. Coli Loading (cfu/sq. mi/day)	**Target Load** (cfu/day)
Alameda to Isleta	1.79E+09	6.26E+09
Angostura to Alameda	3.25E+09	1.14E+10

USEPA requires a Stormwater Management Program (SWMP) as part of the CWA. The SWMP is a comprehensive program that is written by NPDES Permit holders. The Stormwater Management Program's purpose is to help implement and enforce the reduction of quantity and increase quality of stormwater runoff by developing effective best management practices (BMPs). A BMP is a practice that involves pollution control and can be structural or non-structural (such as street sweeping). Typically a BMP uses actions such as water detention, evapotranspiration and biological or chemical controls.

Currently there are very few BMPs that have proven effective at consistently reducing E. Coli concentrations. Sand filtration and retention ponds are two common examples of BMPS that are not effective. Sand filtration is the only BMP known to consistently remove bacteria. However, sand filtration requires a low loading rate and requires regular maintenance because of clogging issues (Bright et al. 2010). Retention ponds, a common BMP, create habitats for wildlife, which can potentially exacerbate the E. Coli concerns.

With the high concentrations of E. Coli and mandated USEPA permits, mycofiltration has the potential to serve as an effective BMP to comply with these permits. As reported in a study completed by Tetra Tech (2013), "Compared to typical stormwater BMPs or other proprietary filtration systems that require large capital

investments and significant maintenance costs, mycofiltration is extremely low cost, low impact and requires minimal treatment area and may be able to be added to existing stormwater structures or at least not increase their footprint." The objective of this study was to determine treatment effectiveness of mycofiltration to reduce the concentration of E. Coli after repeated exposure to synthetic stormwater in a wet environmental pond setting and providing more insight for the long-term potential use of P. Ostreatus mycelium in a pond setting.

CHAPTER 2 BACKGROUND

2.1 Mycofiltration

Mycofiltration is the process of using networks of fungal mycelium (also known as hyphae or branching structure of fungal vegetative growth) to facilitate improved water quality (Stamets, 2005). Paul Stamets discovered the technique of runoff management using mycofiltration while performing several treatment studies that documented bacteria removal from agriculture runoff. Stamets installed outdoor woodchip beds of Storpharia rugoso annulata species mycelium and other mushroom species in an area about 50 ft wide and 200 ft long. This garden of mycelium was downstream of his livestock farm. A year after planting the garden, analysis of Stamets outflowing water showed "a hundred-fold drop in coliform levels despite the fact that I had more than doubled my population of farm animals" (Stamets, 2005). This discovery drew the attention of laboratories and research has ensued ever since.

It should be noted that mycofiltration has very limited field experiments but has been used for removal of E. Coli in the Dungeness Watershed, WA. Thomas (2009) used mycoremediation (a form of conditioned native fungi and fungal mycelium applied to surface soils to remove contaminants) treatment for two field sites; one field site was a control biofilter without fungi and the other was a biofilter with fungi. Thomas (2009) looked at fecal coliform and nutrient concentrations in source water and two outflow pipes from the two field sites. This study saw a 66% reduction in fecal coliform in the control biofilter and a 90% reduction in their biofilter containing fungi. Thomas concluded that the benefits of mycoremediation treatment application to a bioretention cell or other type of site were many and included: reducing fecal coliform and nutrients when properly designed, applicability to a variety of other contaminants (e.g. Polycyclic

Aromatic Hydrocarbons (PAHs), Polychlorinated biphenyl (PCBs) and metals), minimal handling, and low maintenance.

At Evergreen State College, Rogers (2012), completed a similar lab experiment that used Pluerotus Ostreatus mycelium inoculated sawdust in a column test. Rogers (2012) loaded the inoculated sawdust and non-inoculated sawdust with an E. Coli solution and observed that the effects of mycofiltration significantly reduced the E. Coli in solution. This study supported the evidence that Thomas (2009) and Stamets (2005) had found in that mycofiltration did reduce E. Coli in a lab and field study.

2.2 Mycelium Species

Different species of mycelium have been tested for bacteria removal but this study focuses on Pleurotus Ostreatus. Pleurotus Ostreatus has been researched in a few different studies and was found to be effective in bacterial removal: "the presence of P. Ostreatus mycelium causes a reduction in bacterial abundance within the solution" (Rogers, 2012). P. Ostreatus mycelium are easy to grow and very adaptable to their environment. This species is grown locally in Albuquerque, NM and is available to be used for future research in this area.

The P. Ostreatus mycelium was found to "attack and destroy bacterial colonies, which then serve as a nutrient source for the fungus" (Barron, 1987). Barron (1987) noticed fungal secretions from the mycelium (hyphae) stopped the colonies from growing and seemed to use the bacteria as an intermediate nutrient source to reach a higher nutrient content food source. The P. Ostreatus produces a nematoxin that is contained in the fungal secretions. When the bacteria came in contact with the secretion, the bacteria

was immobilized and the cell walls were under destruction and served as a nutrient source for the fungus.

P. Ostreatus is a species not typically found in an aquatic setting and has not been tested in such conditions. Gulis and Suberkropp (2003) have a theory that the P. Ostreatus might not work well in an aquatic setting because the natural fungal secretions become diluted and inhibits the effects on bacteria. "The fact that inhibition of bacterial growth was demonstrated in culture experiments and not observed in microcosm experiments points to the assumption that inhibitory fungal secretions become too diluted to be effective and/or wash away in aquatic environments, though this relationship requires further clarification (Rogers, 2012)." This study attempts to clarify this relationship by observing the adaptation of P. Ostreatus mycelium in an aquatic setting and the E. Coli concentration removed in this laboratory experiment.

2.3 Project Objectives:

The objectives of this study were to: (1) investigate the treatment effectiveness of mycofiltration to reduce the concentration of E. Coli after repeated exposure to synthetic stormwater in a wet environmental pond setting; and (2) analyze the long-term potential use of the P. Ostreatus mycelium adaption in a wet environmental pond setting. The objectives were met by simulating wet environmental ponds in a laboratory where each pond contained a floating P. Ostreatus inoculated mycofilter. E. Coli was chosen for this study because it is a pathogen that causes harm to the natural environment and because the New Mexico stormwater samples often exceed the standard concentration levels of E. Coli. The overarching goals of this study were determine treatment effectiveness of mycofiltration to reduce the concentration of E. Coli after repeated exposure to synthetic

stormwater in a wet environmental pond setting and providing more insight for the long-term potential use of P. Ostreatus mycelium in a pond setting.

CHAPTER 3 METHODOLOGY

3.1 Laboratory

The objectives were met by testing the mycofilters in a laboratory experiment. The laboratory experiments were conducted in the Environmental Engineering labs of the Department of Civil Engineering (CE) at the University of New Mexico (UNM) using scaled models of standard wet environmental ponds (reactors). Laboratory experiments were preferable for this study over field experiments in order to control for the wide range of environmental variables and to allow for replication and controls. The procedures of Flatt (2013) were used as a guideline and incorporated into the design of this study. Flatt (2013) and this study used the same strain of E. Coli (E.Coli ATCC 11775), stormwater solution, and E. Coli testing method (Coliscan Membrane Filter Chromogenic Method).

3.2 Experimental Design

3.2.1 Scaled Wet Environmental Reactors

Mycofilters were placed in scaled down models of a standard wet environmental pond (reactors). The dimensions of a local wet environmental pond were used as the upscaled model for this study. The process of calculating the downscaled wet environmental reactor dimensions is shown below. The calculated dimensions for the laboratory included a 0.88 liters mycofilter in a volume of 28.3 liters of water.

Real Life Dimensions

Volume of Actual Pond

Length X Width X Depth = Volume

65.2 m X 43 m X 1.2 m = ***3,364 m^3***

For this study, the efficiency of the mycofilters was tested for covering 25% of the surface area of the wet environmental pond. Future studies could incorporate testing more or less surface area covered by mycofilters.

25% Surface Area of Actual Pond

$$65.2\ m\ X\ 43\ m\ X\ 0.25 = \mathbf{701\ m^2}$$

Volume of Mycofilter in Actual Pond

$$Area\ X\ Thickness\ of\ Mycofilter = Volume$$

$$701\ m^2\ X\ 0.15\ m = \mathbf{105.15\ m^3}$$

Lab Dimensions

Volume of Model Reactor = 2.8×10^{-2} m^3 or 28.3 L

Volume of Mycofilter

$$105.15\ m^3 \div 701\ m^3 = \mathbf{0.15\ m^3}$$

Mycofilter = 15 cm wide and 23 cm long and 2.5 cm thick *(see Figure 1)*

Once per week, synthetic stormwater was released into the reactors. For the upscaled model pond, a typical rainstorm would replace about 10% of the pond's volume. This was based on preliminary documents and actual construction plans from the Albuquerque Metropolitan Arroyo Flood Control Authority (AMAFCA). This data came from discussions with AMAFCA and descriptions of actual flows that have been observed over the past 14 years. A typical storm would produce approximately 1.42 to 2 m^3/s (cubic meter per second) of flow for duration of 10 minutes on average with a volume of 3,364 m^3. The typical storm producing a flow of 1.42 m^3/s for 10 minutes would replace a volume of approximately 840 m^3, which is 25% of the total volume. For the 28 L laboratory reactors, 25% of the total volume was 0.007 m^3, which was applied at 1.1×10^{-6} m^3/s or 0.0011 L/s over 10 minutes.

The mycofilters were placed in their respective stormwater reactors for three weeks and once a week a simulated stormwater flow was introduced into 9 out of 12 reactors (See 3.3 Replicates and Controls). The mycofilters were designed to cover 25% of the surface area in the simulated wet environmental ponds. The mycofilters were located 0.5 m from the input and 0.2 m from the output. The location was relevant to this study because in a real life study the mycofilters would be floating around the output of the pond and not near the stormwater input (Figure 1). Other details of procedures in this study are included in Appendix C and D.

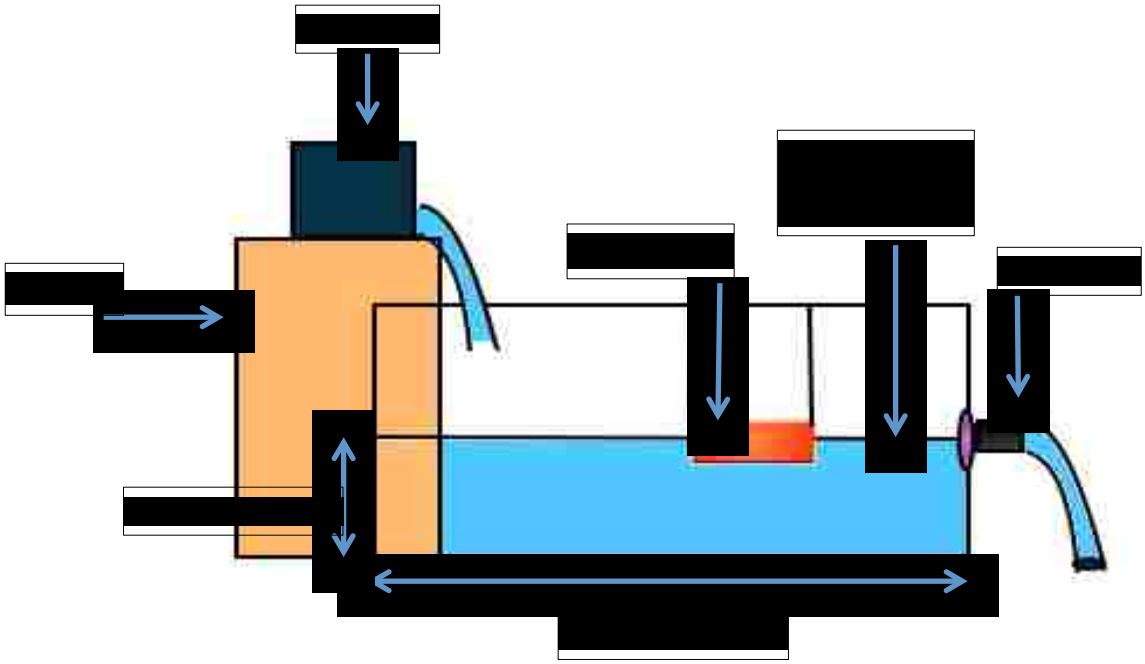


Figure 1: Mycofilter Reactor Schematic with dimensions of reactor 0.7m X 0.47m X 0.14m (Length, width and height)

3.2.2 Mycofilter Design

All mycofilters followed the same design and were assembled by filling a sterilized burlap sack with P. Ostreatus inoculated barley straw. The design of the

mycofilter came from Stamets (2005) where he suggested multiple filling options for the mycofilters depending on the mycelium species, but the burlap sack was the most effective material for all mycelium. This is effective because the mycelium are adaptable to the structure and breathability of the burlap sack and are able to quickly grip and colonize the fabric. Barley straw was chosen as the packing material for the burlap sack. Barley straw is one of the most common substrates for P. Ostreatus to grow upon.

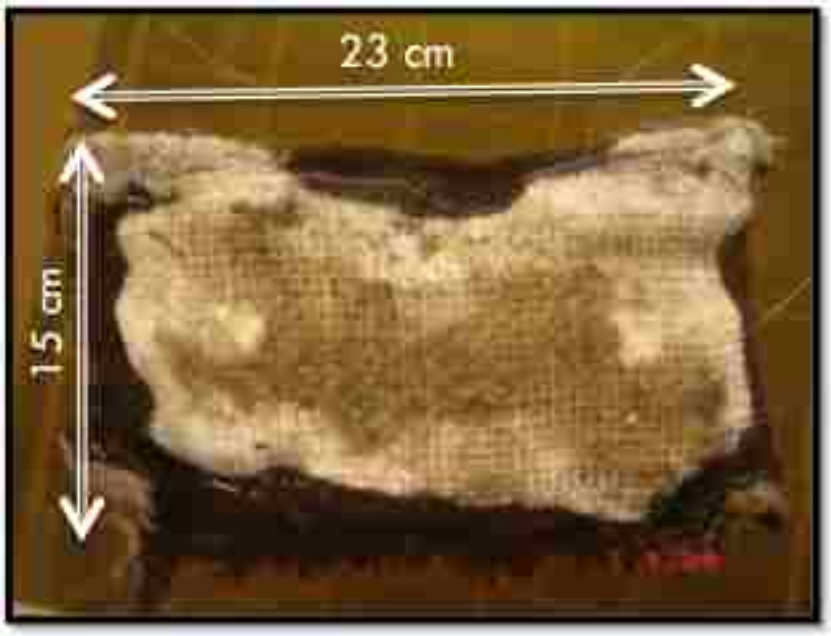


Figure 2: Mycofilter Design Dimensions: Width: 15cm, Length: 23 cm and Thickness: 2.5 cm

3.3 Replicates and Controls

The experimental design included twelve reactors. Each reactor was filled with 28.3 L of dechlorinated tap water to begin the study. The twelve reactors were separated into four different testing scenarios (treatments): (1) three controls (Blank reactors with dechlorinated tap water); (2) three mycofilters without mycelium; (3) three mycofilters with mycelium; and (4) three mycofilters with mycelium but not dosed with synthetic

stormwater. Henceforth, these treatments are referred to as control, filter, mycofilter, and mycofilter (N), respectively. The reactors were developed for comparison of E. Coli decay between treatments. The twelve reactors were set up side by side on a workbench with a lid on top of each reactor to minimize other bacteria from entering (Figure 3).

Figure 3: Mycofilter reactor triplicates during Week 1 of the experiment

3.4 Experimental Protocol

Plastic water jugs were disinfected and used only on rainstorm days for the influent. Discharge from each reactor was released after 20 minutes from initial stormwater input. 7.07 liters of influent was introduced every 7 days using synthetic stormwater. Once a week, synthetic stormwater was created for the weekly "storm". The synthetic stormwater was created by mixing dechlorinated tap water with an E. Coli inoculated solution. E. Coli ATCC 11775 Culti Loop was chosen for the E. Coli bacteria

source based on Flatt (2013). The E. Coli grew overnight in 5 mL Tryptic Soy Broth and was mixed into 95 liters of dechlorinated tap water. Other common nutrients such as nitrogen and phosphorus were not added to this stormwater solution. The concentration of E.Coli varied per reactor. This is described in more detail in Chapter 4 Results. The study was focused on the removal of E. Coli and other nutrients would have caused too many unknowns for the cause of E. Coli removal. The methods of preparation are detailed in Appendix D.

3.5 Sampling Protocol

Sampling was focused on the effluent because this is the concern from a permit compliance perspective. Daily samples from reactors were taken at 2.5 cm depth and 7.6 cm in length from the discharge point/outlet in order to represent reactor effluent (Figure 1). The sample was collected at this location because the effluent of the actual pond is only released during a storm event and this study wanted to replicate the collection of samples taken from the actual pond. Collection of samples involved taking 1 mL of reactor water using a sterilized pipette. The 1mL sample was then diluted with 99 mL of DI water in a purchased sterilized sample bottle; the procedure of dilution was taken from Flatt (2013). On simulated storm days, samples were collected by taking 50 mL of influent, reactor water (same as daily samples), and effluent (through tap outlet) and stored in sterilized sample bottles. The influent was taken immediately at time of input, the reactor water was taken 10 minutes after input and effluent was taken 20 minutes after input. The time separation was taken into consideration to allow for immediate treatment by mycelium. From the sterilized sample bottles, 1 mL of sample was taken by

sterilized pipette and diluted with 99 mL of DI water. All samples were placed into a 4° C refrigerator and tested for E.Coli within 6 hours of collection.

All diluted samples were tested in triplicate using the Coliscan Membrane Filter Chromogenic Method (Coliscan MF Method). This method was used because it is an EPA approved method (9222c) and it was used in prior mycofiltration studies. The Coliscan MF Method is a widely used method of obtaining E. Coli and General Coliforms from liquid samples. The Coliscan MF Method uses a nutrient medium that contains two color-producing chemicals. The two color producing chemicals are for detecting the enzyme glucuronidase (enzyme produce by E.Coli) and galactosidase (enzyme produce by general coliforms and E.Coli). The actual detailed instructions of the Coliscan MF Method are attached in Appendix C. To summarize the process, 100mL of a diluted water sample was vacuum filtered onto a 0.45 μm filter pad and then transferred to a petri dish containing an absorbent pad soaked with 1.75 mL of the Coliscan MF medium. The petri dish was then inverted and incubated at 35°C for 24 hours. After 24 hours, the cells grew into colony forming units on the surface of the filter. When E.Coli colonies are present, glucuonidase reacts with a specific color producing a substrate in the medium and a water insoluble teal-green pigment will color the colony. However, E. Coli produces both galactosidase and glucuronidase, those colonies appear as a combination of the teal-green and pink pigments and will appear as a shade of blue-purple.

Prior to testing, it was anticipated that there might be false positive E. Coli as reported in previous studies (Flatt, 2013). The false positive colonies are commonly found from wood substrate or straw and are a product of non-fecal bacteria called

Klebsiella (Caplenas and Kanarek, 1984). An indole presence test can be used to verify a particular colony as E. Coli or as a false positive. The product, Kovac's Solution, was used in this study to verify questionable colonies. This process is described in more detail in Appendix C.

3.6 Data Analysis

Data analysis involved counting the colonies that grown after incubation occurred. Pictures were taken of each incubated filter and blue-purple colonies were counted one by one. For incubated filters that exceeded 1000 colonies, an average of colonies per gridded square was taken as the results.

CHAPTER 4 RESULTS

Overall the results of this study showed that the mycofilter reactors lowered the E. Coli concentration of the surface water. All of these results are discussed and graphed below. For the results that are graphed, a semi-log plot was used to better show the high values of E. Coli concentration. The spikes on the graphs represent the simulated storm event (days 2/15, 2/22, and 2/29). The initial spike for week 1 is not shown because the concentration was too numerous to count. For the following weeks of study a vertical dashed line represents stormwater spikes.

Standard deviation was calculated for each set of reactors by using the following equation:

$$Standard\ Deviation = \sqrt{\frac{\sum(x - \bar{x})^2}{(n-1)}}$$

*Where x is daily sample, $\bar{x}$ is mean of daily samples and n is the number of daily samples.

Percent concentration removal was calculated by using the following equations:

$$Percent\ Concentration\ Removal\ A = \frac{C_{in} - C_R}{C_{in}}$$

*Where C_{in} is Concentration of Influent and C_R is Concentration of daily Reactor water.

For week two and three, percent reduction was calculated by using the following equation:

$$Percent\ Concentration\ Removal\ B = \frac{(C_{in} + C_{pw}) - C_R}{C_{in} + C_{pw}}$$

*Where C_{in} is Concentration of Influent, C_{pw} is Concentration of Previous Week Reactor water and C_R is Concentration of daily Reactor water. Week two and three were calculated differently because the reactor water of the previous week was not cleaned out before every storm. More stormwater was added to each reactor and the percent reduction would be more accurate if the previous week concentration was added to the new influent. For a few samples, the previous

week sample was too numerous to count and therefore unable to be incorporated into the calculation.

4.1 Control Reactor Results

Table 2 shows the daily E. Coli coliform concentration for each of the control reactors as a function of time over the experimental period (21 days). A threshold of 1,600 E. Coli cfu/100 mL was used to designate concentrations that were effectively zero because concentrations below this level are present at ambient background conditions (see mycofilter (N) results table 8). The threshold, 1,600 E. Coli cfu/100 mL was established based on the taking the daily average E. Coli enumerations from the Mycofilter (N) reactors for all three weeks (table 8). The life cycle of E. Coli without nutrients was 3 days for the first week. The second and third week showed E. Coli concentration was not present after two days. The early non-detect of E. Coli concentration was a result of the E. Coli falling to the bottom of the reactors; this is described in more detail in Appendix B. On days, 2/20,2/21 and 2/27, the controls showed an increase in E. Coli concentration after a few non-detect days. This could be from contamination of the filter apparatus from previous samples. The standard deviations for most of the control reactors were high and showed that the E. Coli colonies were more widely distributed around the mean (shown in figure 4). The storm day results were tabulated and graphed below. The results of the control storm day showed that the there was very little deviation around the mean. Overall, the control reactors showed a 100% removal of E.Coli concentration.

Table 2: Civil Engineering Laboratory Control Reactor Results

	Control1				Control2				Control3			
Date	Mean	Std Deviation	Percent Std Deviation (+/-)	% Conc Removal	Mean	Std Deviation	Percent Std Deviation (+/-)	% Conc Removal	Mean	Std Deviation	Percent Std Deviation (+/-)	% Conc Removal
2/16/16	2033	1882	93%	*N/A	6600	3027	46%	*N/A	5733	3101	54%	*N/A
2/17/16	333	252	75%	*N/A	267	208	78%	*N/A	133	153	115%	*N/A
2/18/16	1000	781	78%	*N/A	333	231	69%	*N/A	0	0	0%	*N/A
2/19/16	67	58	87%	*N/A	0	0	0%	*N/A	0	0	0%	*N/A
2/20/16	667	1155	173%	*N/A	0	0	0%	*N/A	100	173	173%	*N/A
2/21/16	200	173	87%	*N/A	467	635	136%	*N/A	133	153	115%	*N/A
Average Mean				717				1278				1017
Deviation				4600				0				0
Overall Average Percent Std Deviation (+/-)				**642%**				**0%**				**0%**
Mean												**1003.70**
Concentration												***N/A**
2/23/16	233	252	1	100%	267	462	2	112%	533	751	141%	100%
2/24/16	33	58	2	100%	100	173	2	100%	0	0	0%	100%
2/25/16	133	153	1	100%	33	58	2	100%	0	0	0%	100%
2/26/16	133	58	0	100%	67	58	1	100%	0	0	0%	100%
2/27/16	0	0	0	100%	0	0	0	100%	67	115	173%	100%
2/28/16	67	115	2	100%	33	58	2	100%	0	0	0%	100%
Average Mean				100				83				100
Average Standard Deviation				7600				13524				11858
Overall Percent Std Deviation (+/-)				**7600%**				**16229%**				**11858%**
Overall Average Mean												**94**
Overall Average Concentration Removal												**100%**
3/1/16	2033	874	0	99%	33	58	2	100%	0	0	0%	100%
3/2/16	0	0	0	100%	0	0	0	100%	0	0	0%	100%
3/3/16	0	0	0	100%	0	0	0	100%	0	0	0%	100%
3/4/16	0	0	0	100%	0	0	0	100%	0	0	0%	100%
3/5/16	0	0	0	100%	0	0	0	100%	0	0	0%	100%
3/6/16	0	0	0	100%	0	0	0	100%	0	0	0%	100%
Average Mean				339				6				0
Average Standard Deviation				5191				3204				4941
Overall Percent Std Deviation (+/-)				**1532%**				**57672%**				**0%**
Overall Average Mean												**115**
Overall Average Concentration Removal												**100%**

*TNTC = Too Numerous to Count, *N/A: Results not applicable

Table 3: Civil Engineering Laboratory Control Reactor Storm Day Results

	Control1			Control2			Control3		
	Mean (cfu/100mL)	Std Deviation	Percent Std Deviation (+/-)	Mean (cfu/100mL)	Std Deviation	Percent Std Deviation (+/-)	Mean (cfu/100mL)	Std Deviation	Percent Std Deviation (+/-)
Storm 1 2/15/16									
Influent	TNTC	*N/A	*N/A	TNTC	*N/A	*N/A	TNTC	*N/A	*N/A
Reactor Water	TNTC	*N/A	*N/A	TNTC	*N/A	*N/A	TNTC	*N/A	*N/A
Effluent	TNTC	*N/A	*N/A	TNTC	*N/A	*N/A	TNTC	*N/A	*N/A
Average Influent Standard Deviation			*N/A			*N/A			*N/A
Average Reactor Water Standard Deviation			*N/A			*N/A			*N/A
Average Effluent Standard Deviation			*N/A			*N/A			*N/A
Storm 2 2/22/16									
Influent	233767	9757	4%	262200	9873	4%	233767	9757	4%
Reactor Water	102600	0	0%	74100	19745	27%	91200	19745	22%
Effluent	68400	0	0%	68400	0	0%	68400	17100	25%
Average Influent Standard Deviation			2%			2%			2%
Average Reactor Water Standard Deviation			0%			13%			10%
Average Effluent Standard Deviation			0%			0%			12%
Storm 3 2/29/16									
Influent	83367	20384	24%	131100	26121	20%	148200	9873	7%
Reactor Water	37167	24480	66%	39900	9873	25%	62700	9873	16%
Effluent	45600	9873	22%	57000	9873	17%	51300	17100	33%
Average Influent Standard Deviation			12%			9%			3%
Average Reactor Water Standard Deviation			31%			12%			7%
Average Effluent Standard Deviation			10%			8%			16%

*TNTC = Too Numerous to Count, *N/A: Results not applicable

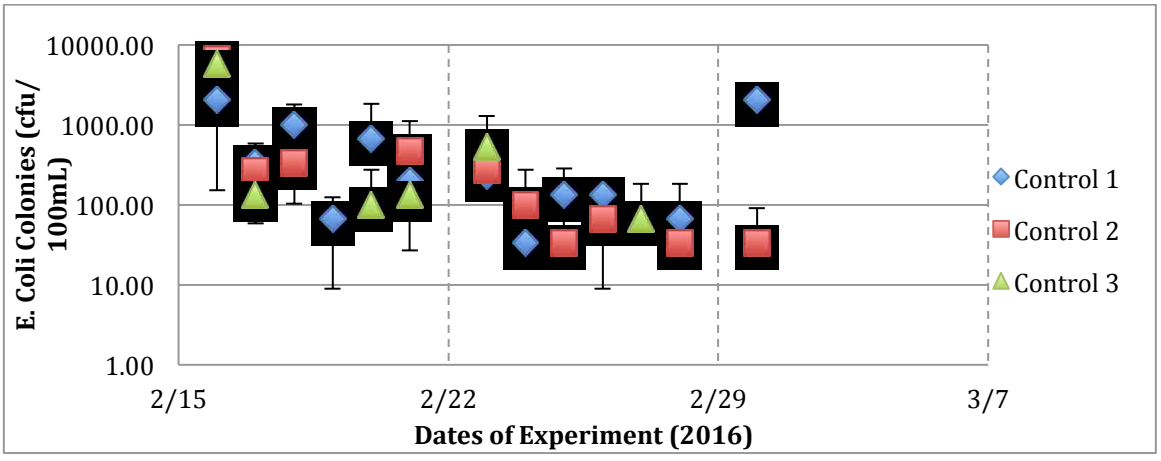
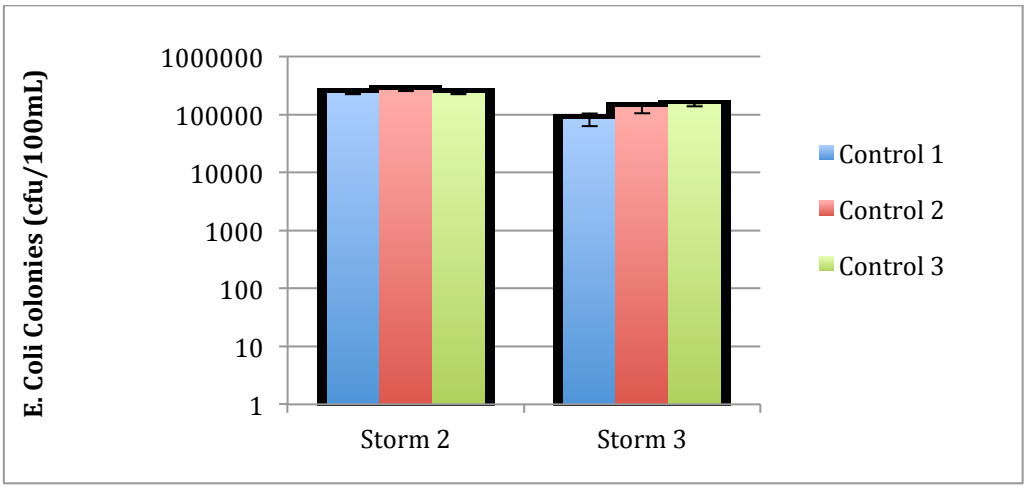
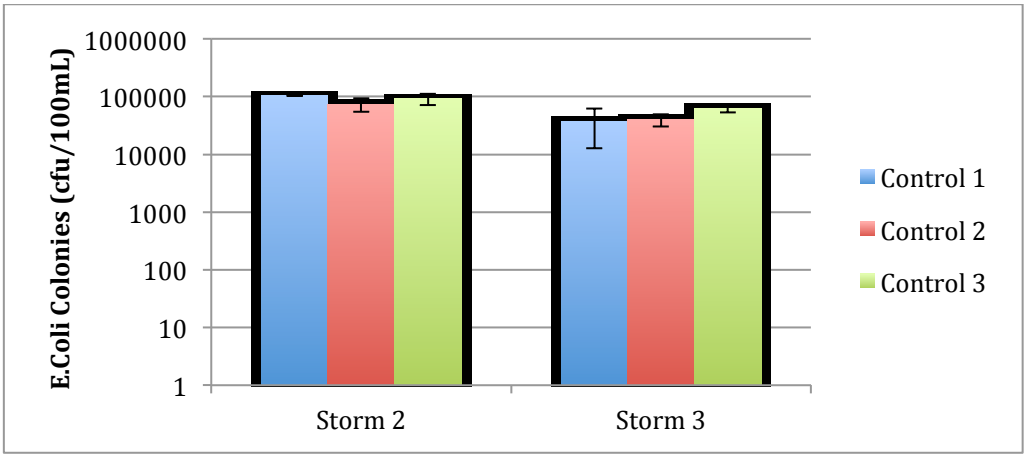


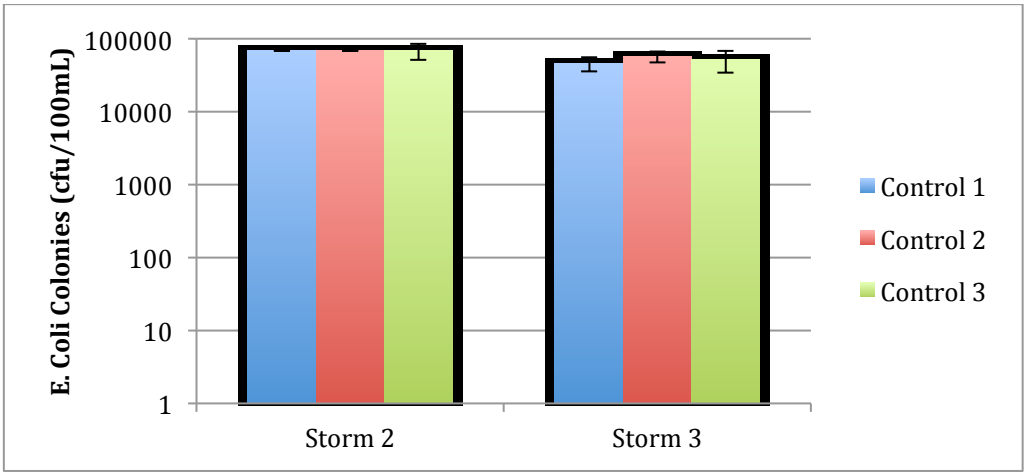
Figure 4: Control Reactor Results, E. Coli concentrations as a function of time for the control treatment reactor with Standard Deviation (+/-)



A: Control Reactor Storm Day Influent Results



B: Control Reactor Storm Day Reactor Water Results



C: Control Reactor Storm Day Effluent Results

Figure 5: E. Coli concentrations as a function of time for the control treatment reactor with Standard Deviation (+/-)

4.2 Filter Reactor Results

Table 4 shows the mean E. Coli coliform concentration for each of the filter reactors. During week 1, the filter reactor results were too numerous to count and therefore not plotted on the graph. The filter reactors presented a different trend than the other reactors. The E. Coli concentration showed signs of decay and it steadily decreased in concentration. The E. Coli concentration never completely dropped below the E. Coli threshold of 1600 cfu/100mL. Without the mycelium present in these filters the percent reduction for Week 2 was 56% and Week 3 was 81%.

Table 4: Civil Engineering Laboratory Filter Reactor Daily Results

	Filter1				Filter2				Filter3			
Date	Mean	Std Deviation	Percent Std Deviation (+/-)	% Conc Removal	Mean	Std Deviation	Percent Std Deviation (+/-)	% Conc Removal	Mean	Std Deviation	Percent Std Deviation (+/-)	% Conc Removal
2/16/16	TNTC	*N/A	*N/A	*N/A	TNTC	*N/A	*N/A	*N/A	TNTC	*N/A	*N/A	*N/A
2/17/16	TNTC	*N/A	*N/A	*N/A	TNTC	*N/A	*N/A	*N/A	TNTC	*N/A	*N/A	*N/A
2/18/16	TNTC	*N/A	*N/A	*N/A	TNTC	*N/A	*N/A	*N/A	TNTC	*N/A	*N/A	*N/A
2/19/16	TNTC	*N/A	*N/A	*N/A	TNTC	*N/A	*N/A	*N/A	TNTC	*N/A	*N/A	*N/A
2/20/16	TNTC	*N/A	*N/A	*N/A	TNTC	*N/A	*N/A	*N/A	TNTC	*N/A	*N/A	*N/A
2/21/16	TNTC	*N/A	*N/A	*N/A	TNTC	*N/A	*N/A	*N/A	TNTC	*N/A	*N/A	*N/A
Average Mean				TNTC				TNTC				TNTC
Average Standard Deviation				*N/A				*N/A				*N/A
Overall Average Mean												**TNTC**
Overall Average Concentration Removal												**TNTC**
2/23/16	TNTC	*N/A	*N/A	*N/A	TNTC	*N/A	*N/A	*N/A	TNTC	*N/A	*N/A	*N/A
2/24/16	118266.67	20608.09	17%	48%	136100.00	39090.79	29%	40%	113333.33	30550.50	27%	52%
2/25/16	59400.00	32947.84	55%	74%	182100.00	13683.20	8%	20%	83166.67	15002.78	18%	64%
2/26/16	22133.33	6841.30	31%	90%	151800.00	42205.21	28%	33%	145000.00	43789.04	30%	38%
2/27/16	12466.67	416.33	3%	95%	144000.00	0.00	0%	37%	90000.00	0.00	0%	61%
2/28/16	18933.33	1361.37	7%	92%	111933.33	37973.85	34%	51%	119866.67	26839.77	22%	49%
Average Mean				46240.00				145186.67				110273.33
Average Standard Deviation				7600.00				13524.00				11858.00
Overall Percent Std Deviation (+/-)				**16%**				**9%**				**11%**
Overall Average Mean												**100566.67**
Overall Average Concentration Removal												**56%**
3/1/16	43500.00	19284.45	44%	76%	32000.00	6471.48	20%	89%	59266.67	16354.00	28%	78%
3/2/16	79800.00	9872.69	12%	56%	35700.00	14906.71	42%	88%	91200.00	9872.69	11%	66%
3/3/16	82666.67	11547.01	14%	55%	17466.67	1365.04	8%	94%	77000.00	0.00	0%	71%
3/4/16	30300.00	11153.03	37%	83%	14433.33	862.17	6%	95%	68400.00	0.00	0%	74%
3/5/16	100.00	173.21	173%	100%	16733.33	2371.36	14%	94%	68400.00	17100.00	25%	74%
3/6/16	17433.33	665.83	4%	90%	14933.33	2200.76	15%	95%	39633.33	1422.44	4%	85%
Average Mean				42300.00				21877.78				67316.67
Average Standard Deviation				5191.00				3204.00				4941.00
Overall Percent Std Deviation (+/-)				**12%**				**15%**				**7%**
Overall Average Mean												**43831.48**
Overall Average Concentration Removal												**81%**

*TNTC = Too Numerous to Count, *N/A: Results not applicable

Table 5: Civil Engineering Laboratory Filter Reactor Storm Day Results

	Filter1			Filter2			Filter3		
	Mean (cfu/100mL)	Std Deviation	Percent Std Deviation (+/-)	Mean (cfu/100mL)	Std Deviation	Percent Std Deviation (+/-)	Mean (cfu/100mL)	Std Deviation	Percent Std Deviation (+/-)
Storm 1 2/15/16									
Influent	TNTC	*N/A	*N/A	TNTC	*N/A	*N/A	TNTC	*N/A	*N/A
Reactor Water	TNTC	*N/A	*N/A	TNTC	*N/A	*N/A	TNTC	*N/A	*N/A
Effluent	TNTC	*N/A	*N/A	TNTC	*N/A	*N/A	TNTC	*N/A	*N/A
Average Influent Standard Deviation			*N/A			*N/A			*N/A
Average Reactor Water Standard Deviation			*N/A			*N/A			*N/A
Average Effluent Standard Deviation			*N/A			*N/A			*N/A
Storm 2 2/22/16									
Influent	228000.00	39490.76	17%	228000.00	9872.69	4%	233700.00	9872.69	4%
Reactor Water	233766.67	9757.22	4%	TNTC	*N/A	*N/A	199500.00	9872.69	5%
Effluent	TNTC	*N/A	*N/A	TNTC	*N/A	*N/A	239400.00	0.00	0%
Average Influent Standard Deviation			8%			2%			2%
Average Reactor Water Standard Deviation			2%			*N/A			2%
Average Effluent Standard Deviation			*N/A			*N/A			0%
Storm 3 2/29/16									
Influent	163866.67	25081.93	15%	186700.00	48025.72	26%	146766.67	4907.48	3%
Reactor Water	22933.33	4105.28	18%	29866.67	4239.50	14%	41866.67	17854.22	43%
Effluent	20666.67	1101.51	5%	21866.67	17022.73	78%	43600.00	10346.01	24%
Average Influent Standard Deviation			7%			12%			2%
Average Reactor Water Standard Deviation			8%			7%			20%
Average Effluent Standard Deviation			3%			37%			11%

*TNTC = Too Numerous to Count, *N/A: Results not applicable

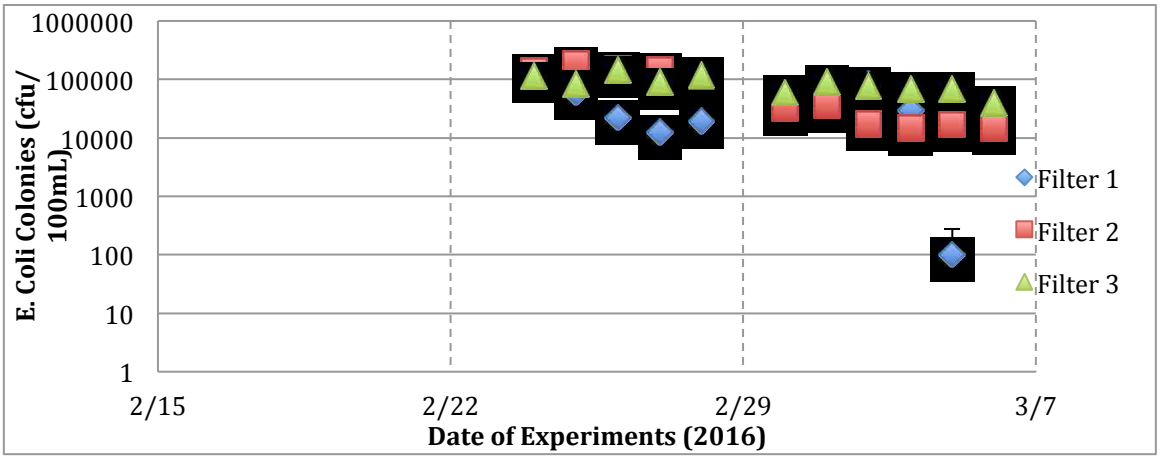
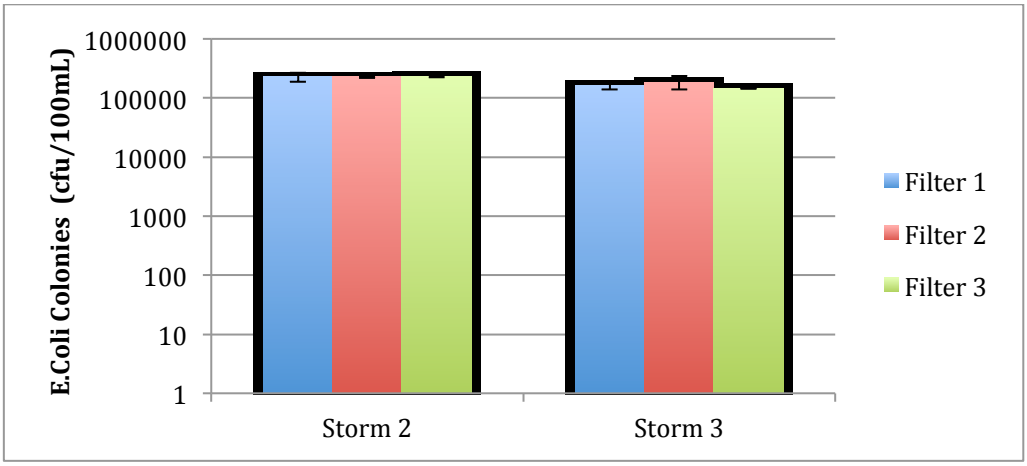
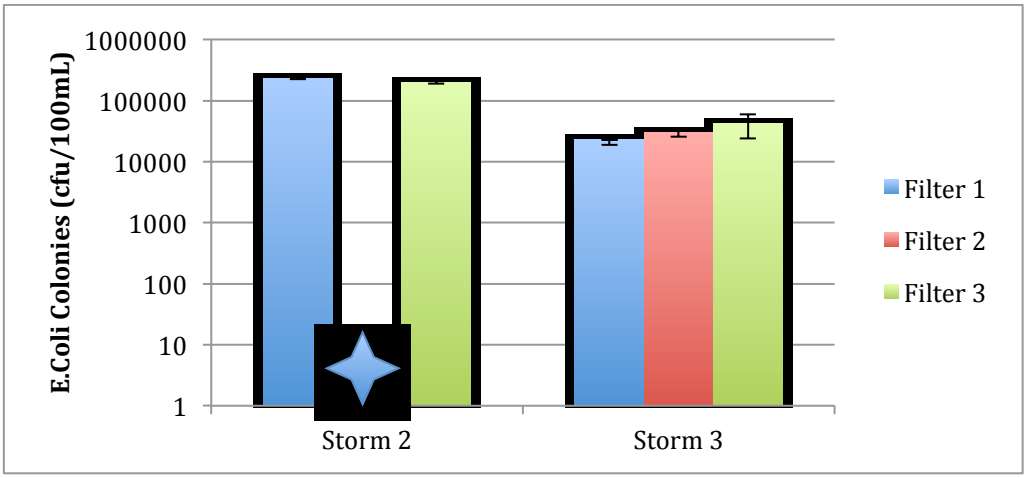


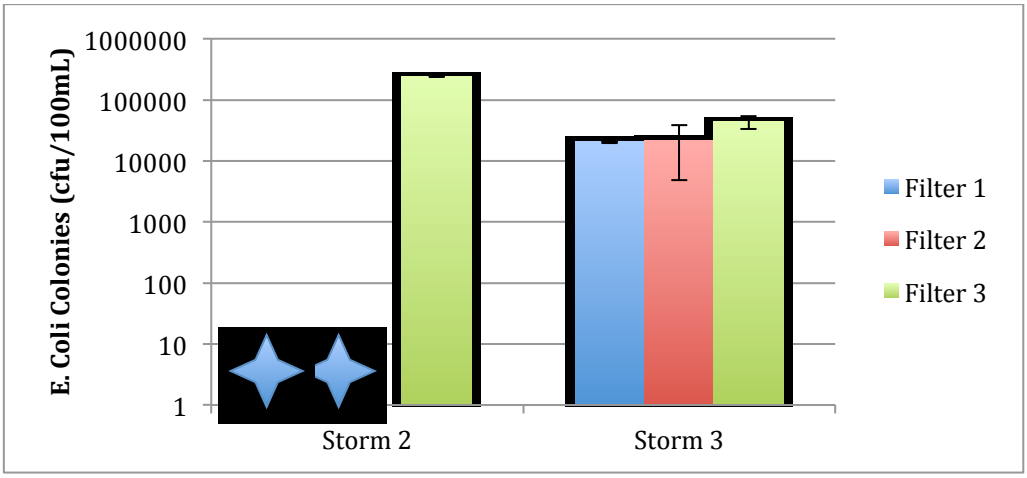
Figure 6: Filter Reactor Results, E. Coli concentrations as a function of time for the filter treatment reactor with Standard Deviation (+/-)



A: Filter Reactor Storm Day Influent Results



B: Filter Reactor Storm Day Reactor Water Results



C: Filter Reactor Storm Day Effluent Results

Figure 7: E. Coli concentrations as a function of time for the filter treatment reactor with Standard Deviation (+/-). * The blue four-point star represents TNTC data

4.3 Mycofilter Reactor Results

Figure 8 shows the daily E. Coli coliform concentration for the mycofilter reactor experiments. The mycofilter results showed a decreasing trend in E.Coli concentration over time. Once again, the results during week 1 were too numerous to count. However, the second week showed a very smooth decrease in E. Coli concentration. A 97% reduction in E. Coli concentration was observed in Week 2 and a 98% reduction was observed in Week 3 (Table 6).

Table 6: Civil Engineering Laboratory Mycofilter Reactor Results

	Mycofilter1				Mycofilter2				Mycofilter3			
Date	Mean	Std Deviation	Percent Std Deviation (+/-)	% Conc Removal	Mean	Std Deviation	Percent Std Deviation (+/-)	% Conc Removal	Mean	Std Deviation	Percent Std Deviation (+/-)	% Conc Removal
2/16/16	TNTC	*N/A	*N/A	*N/A	TNTC	*N/A	*N/A	*N/A	TNTC	*N/A	*N/A	*N/A
2/17/16	393300.00	17100.00	4%	*N/A	TNTC	*N/A	*N/A	*N/A	TNTC	*N/A	*N/A	*N/A
2/18/16	483033.33	71480.09	15%	*N/A	TNTC	*N/A	*N/A	*N/A	TNTC	*N/A	*N/A	*N/A
2/19/16	395300.00	36845.49	9%	*N/A	TNTC	*N/A	*N/A	*N/A	TNTC	*N/A	*N/A	*N/A
2/20/16	359900.00	160605.76	45%	*N/A	TNTC	*N/A	*N/A	*N/A	TNTC	*N/A	*N/A	*N/A
2/21/16	222300.00	17100.00	8%	*N/A	TNTC	*N/A	*N/A	*N/A	TNTC	*N/A	*N/A	*N/A
Average Mean				370766.67				TNTC				TNTC
Average Standard Deviation				34878.00				*N/A				*N/A
Overall Average Mean												**TNTC**
Overall Average Concentration Removal												**TNTC**
2/23/16	38733.33	3477.55	9%	92%	159600.00	35596.49	22%	28%	TNTC	*N/A	*N/A	*N/A
2/24/16	6300.00	3740.32	59%	99%	33533.33	6986.65	21%	85%	TNTC	*N/A	*N/A	*N/A
2/25/16	3733.33	472.58	13%	99%	14800.00	1216.55	8%	93%	TNTC	*N/A	*N/A	*N/A
2/26/16	1800.00	556.78	31%	100%	5533.33	585.95	11%	98%	TNTC	*N/A	*N/A	*N/A
2/27/16	1100.00	458.26	42%	100%	3366.67	650.64	19%	98%	121800.00	38870.55	32%	*N/A
2/28/16	1066.67	416.33	39%	100%	3000.00	1969.77	66%	99%	89466.67	6316.82	7%	*N/A
Average Mean				8788.89				36638.89				105633.33
Average Standard Deviation				1000.00				6997.00				7627.00
Overall Percent Std Deviation (+/-)				**11%**				**19%**				**7%**
Overall Average Mean												**50353.70**
Overall Average Concentration Removal												**97%**
3/1/16	19800.00	1473.09	7%	86%	5733.33	288.68	5%	97%	7200.00	1757.84	24%	97%
3/2/16	6700.00	1835.76	27%	95%	2066.67	808.29	39%	99%	2466.67	723.42	29%	99%
3/3/16	1600.00	556.78	35%	99%	900.00	100.00	11%	99%	3200.00	1081.67	34%	99%
3/4/16	466.67	305.51	65%	100%	1633.33	152.75	9%	99%	7300.00	1539.48	21%	97%
3/5/16	500.00	173.21	35%	100%	1800.00	600.00	33%	99%	1800.00	100.00	6%	99%
3/6/16	100.00	100.00	100%	100%	433.33	152.75	35%	100%	2100.00	346.41	16%	99%
Average Mean				4861.11				2094.44				4011.11
Average Standard Deviation				470.00				207.00				519.00
Overall Percent Std Deviation (+/-)				**10%**				**10%**				**13%**
Overall Average Mean												**3655.56**
Overall Average Concentration Removal												**98%**

*TNTC = Too Numerous to Count, *N/A: Results not applicable

Table 7: Civil Engineering Laboratory Mycofilter Reactor Storm Day Results

	Mycofilter1			Mycofilter2			Mycofilter3		
	Mean (cfu/100mL)	Std Deviation	Percent Std Deviation (+/-)	Mean (cfu/100mL)	Std Deviation	Percent Std Deviation (+/-)	Mean (cfu/100mL)	Std Deviation	Percent Std Deviation (+/-)
Storm 1 2/15/16									
Influent	TNTC	*N/A	*N/A	TNTC	*N/A	*N/A	TNTC	*N/A	*N/A
Reactor Water	TNTC	*N/A	*N/A	TNTC	*N/A	*N/A	TNTC	*N/A	*N/A
Effluent	TNTC	*N/A	*N/A	TNTC	*N/A	*N/A	TNTC	*N/A	*N/A
Average Influent Standard Deviation			*N/A			*N/A			*N/A
Average Reactor Water Standard Deviation			*N/A			*N/A			*N/A
Average Effluent Standard Deviation			*N/A			*N/A			*N/A
Storm 2 2/22/16									
Influent	233700.00	9872.69	4%	222300.00	0.00	0%	TNTC	*N/A	*N/A
Reactor Water	153900.00	0.00	0%	267900.00	9872.69	4%	TNTC	*N/A	*N/A
Effluent	239400.00	0.00	0%	273600.00	0.00	0%	TNTC	*N/A	*N/A
Average Influent Standard Deviation			2%			0%			*N/A
Average Reactor Water Standard Deviation			0%			2%			*N/A
Average Effluent Standard Deviation			0%			0%			*N/A
Storm 3 2/29/16									
Influent	143933.33	6524.82	5%	161033.33	40433.94	25%	155333.33	6568.36	4%
Reactor Water	33600.00	2884.44	9%	12266.67	2663.33	22%	44400.00	13546.96	31%
Effluent	50400.00	11711.53	23%	36666.67	9411.34	26%	34400.00	10740.58	31%
Average Influent Standard Deviation			2%			12%			2%
Average Reactor Water Standard Deviation			4%			10%			14%
Average Effluent Standard Deviation			11%			12%			15%

*TNTC = Too Numerous to Count, *N/A: Results not applicable

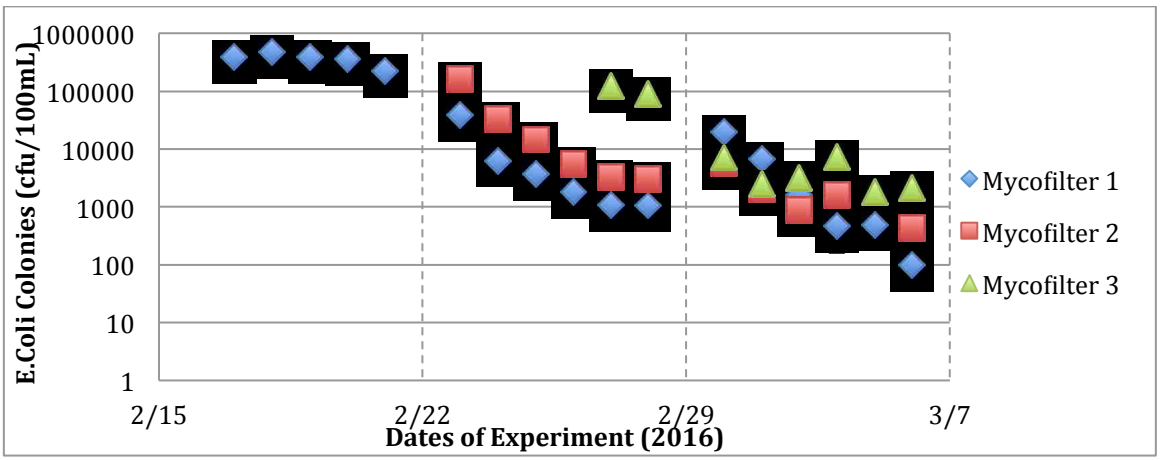
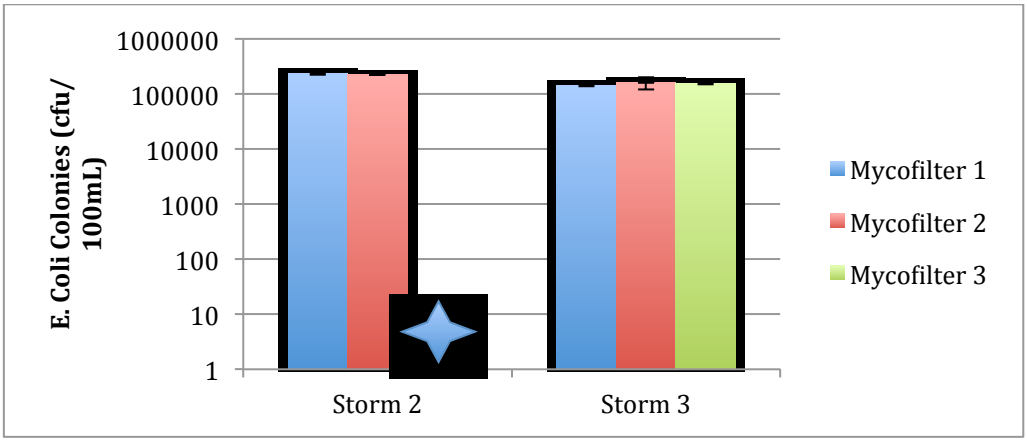
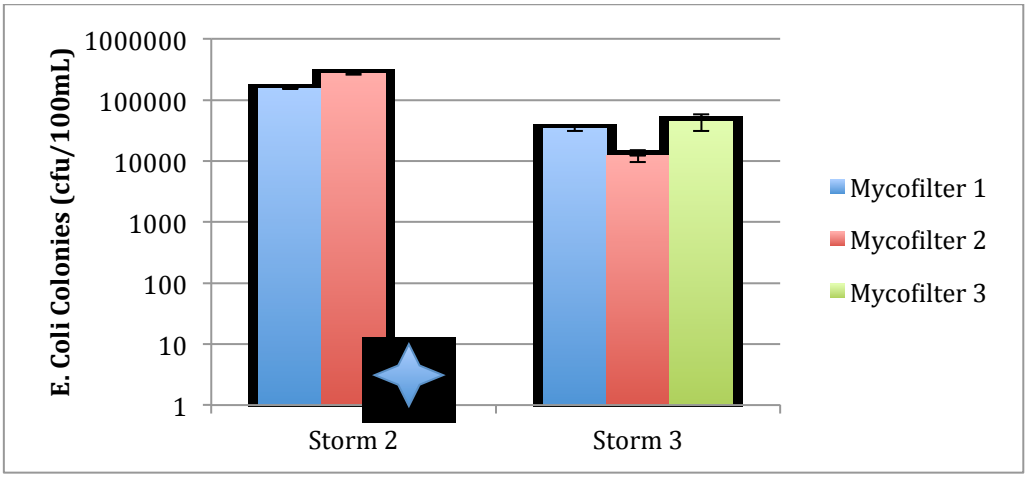


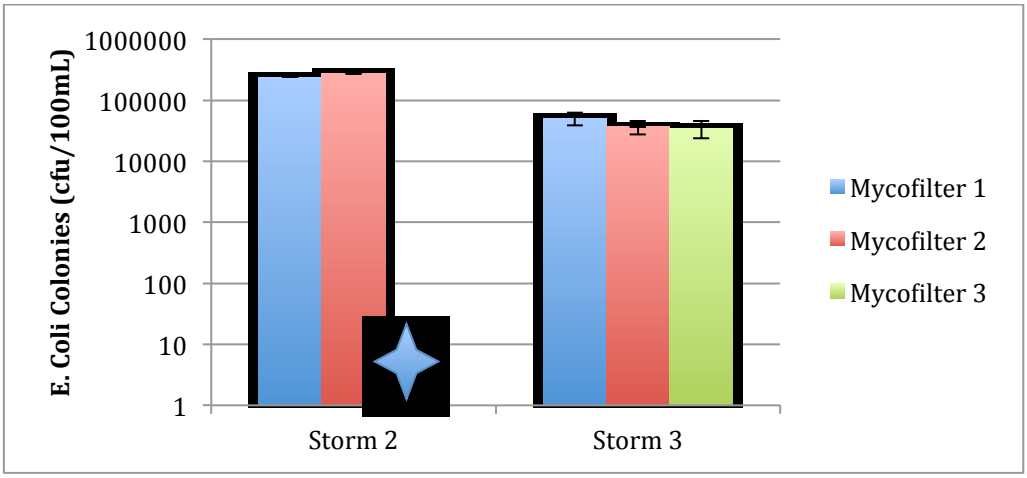
Figure 8: Mycofilter Reactor Results, E. Coli concentrations as a function of time for the mycofilter treatment reactor with Standard Deviation (+/-)



A: Mycofilter Reactor Storm Day Influent Results



B: Mycofilter Reactor Storm Day Reactor Water Results



C: Mycofilter Reactor Storm Day Effluent Results

Figure 9: E. Coli concentrations as a function of time for the mycofilter treatment reactor with Standard Deviation (+/-). * The blue four-point star represents TNTC data

4.4 Mycofilter [No Dosing] Reactor Results

Table 8 represents the mean E. Coli coliform concentration for each of the mycofilter (N) reactors. Mycofilter (N) reactors were never dosed with E. Coli stormwater nor any other substance. The results revealed a consistently low concentration of approximately 1600 E. Coli cfu/100 mL throughout the experiment.

Table 8: Civil Engineering Laboratory Mycofilter (N) Reactor Results

	Mycofilter(N)1				Mycofilter(N)2				Mycofilter(N)3			
Date	Mean	Std Deviation	Percent Std Deviation (+/-)	% Conc Removal	Mean	Std Deviation	Percent Std Deviation (+/-)	% Conc Removal	Mean	Std Deviation	Percent Std Deviation (+/-)	% Conc Removal
2/16/16	0.00	0.00	0%	*N/A	366.67	404.15	110%	*N/A	100.00	173.21	173%	*N/A
2/17/16	100.00	100.00	100%	*N/A	333.33	57.74	17%	*N/A	733.33	230.94	31%	*N/A
2/18/16	5666.67	1401.19	25%	*N/A	2233.33	1201.39	54%	*N/A	3500.00	916.52	26%	*N/A
2/19/16	5200.00	2783.88	54%	*N/A	3266.67	850.49	26%	*N/A	4533.33	665.83	15%	*N/A
2/20/16	6833.33	1703.92	25%	*N/A	933.33	1137.25	122%	*N/A	2700.00	346.41	13%	*N/A
2/21/16	466.67	115.47	25%	*N/A	933.33	550.76	59%	*N/A	2800.00	1113.55	40%	*N/A
Average Mean				3044.44				1344.44				2394.44
Average Standard Deviation				684.00				382.00				318.00
Overall Percent Std Deviation				**22%**				**28%**				**13%**
Overall Average Mean												**2261.11**
2/23/16	733.33	305.51	42%	*N/A	1966.67	57.74	3%	*N/A	2233.33	152.75	7%	*N/A
2/24/16	366.67	321.46	88%	*N/A	1666.67	152.75	9%	*N/A	2133.33	611.01	29%	*N/A
2/25/16	2800.00	556.78	20%	*N/A	1066.67	896.29	84%	*N/A	1033.33	929.16	90%	*N/A
2/26/16	2300.00	300.00	13%	*N/A	3066.67	550.76	18%	*N/A	1666.67	907.38	54%	*N/A
2/27/16	733.33	416.33	57%	*N/A	933.33	808.29	87%	*N/A	1366.67	585.95	43%	*N/A
2/28/16	166.67	288.68	173%	*N/A	1033.33	208.17	20%	*N/A	833.33	850.49	102%	*N/A
Average Mean				1183.33				1622.22				1544.44
Average Standard Deviation				178.00				260.00				342.00
Overall Percent Std Deviation				**15%**				**16%**				**22%**
Overall Average Mean												**1450.00**
3/1/16	533.33	351.19	66%	*N/A	1733.33	702.38	41%	*N/A	1933.33	251.66	13%	*N/A
3/2/16	533.33	416.33	78%	*N/A	333.33	57.74	17%	*N/A	466.67	461.88	99%	*N/A
3/3/16	833.33	57.74	7%	*N/A	1933.33	208.17	11%	*N/A	2500.00	793.73	32%	*N/A
3/4/16	266.67	208.17	78%	*N/A	433.33	115.47	27%	*N/A	800.00	700.00	88%	*N/A
3/5/16	233.33	404.15	173%	*N/A	733.33	321.46	44%	*N/A	2000.00	781.02	39%	*N/A
3/6/16	833.33	378.59	45%	*N/A	2000.00	400.00	20%	*N/A	2333.33	757.19	32%	*N/A
Average Mean				538.89				1194.44				1672.22
Average Standard Deviation				155.00				174.00				309.00
Overall Percent Std Deviation				**29%**				**15%**				**18%**
Overall Average Mean												**1135.19**
Three Week Total Overall Average Mean												**1615.43**

*TNTC = Too Numerous to Count, *N/A: Results not applicable

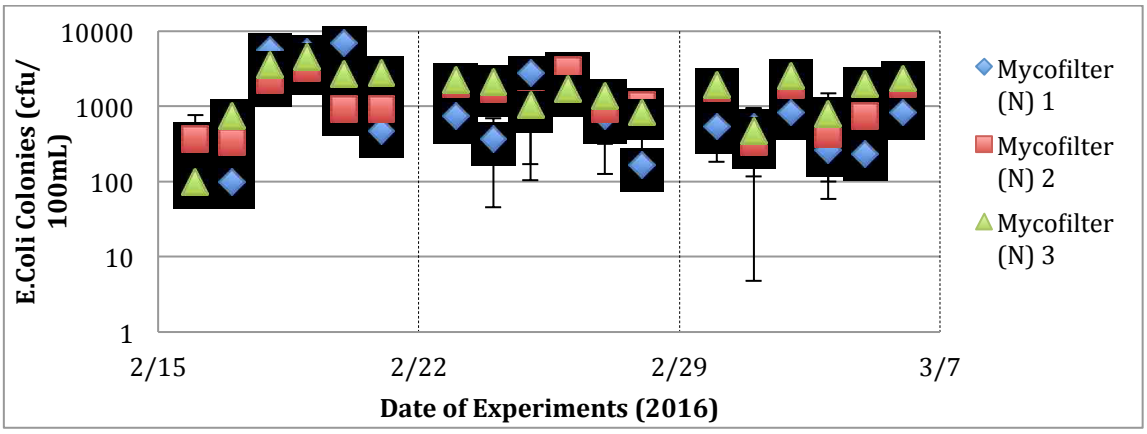


Figure 10: Mycofilter (N) Reactor Results, E. Coli concentrations as a function of time for the mycofilter (N) treatment reactor

The effluent of each reactor was taken to a certified lab for verification of E. Coli concentration after each simulated storm. The results of this are shown in Appendix A. The results of each simulated storm for the filter reactors were too high for the lab to count and the best results were estimated to be greater than 2419.6 cfu/100mL. This estimate complies with the certified lab methods and analysis procedures in reporting results (Hall Environmental Analysis, 2015). As well the certified laboratory results for the Mycofilter (N) Reactors showed to be much lower than the Coliscan MF Method results. The reason for the lower results could be the false positive that were appearing in the Coliscan MF Method and the certified lab was able to ignore the false positive bacteria.

4.5 Second Week Observations

During Week 2, a mucus-like substance was growing underneath the burlap sacks of the mycofilters. The mucus-like substance resembled a mesh/net in the water but when pulled out of the water, the substance clumped together acting as a solid substance. As well as, after the simulated rainstorm, this substance rose from under the mycofilter, and started spreading itself like fingers in the water, as if it were reaching for the nutrients (Figure 10). The mucus substance movement began approximately thirty minutes after the simulated rainstorm and no water movement was present. Dr. Don Natvig, a mycologist at the University of New Mexico, was contacted to examine the mucus and growth of the mycofilters. A sample of the mucus substance was obtained and resulted that it was an extension of the P. Ostreatus mycelium. The substance is a filament known as a clamp connection, which is a feature of the Basidiomycota. The clamp connection is a structure that is formed by growing mycelium cells of fungi. Dr.

Natvig mentioned that it was unusual to find a basidiomycete growing in a pond environment but the clamp connection shows the adaptability of this species. Dr. Natvig suggested that the growth of the clamp connection would most likely have the same properties of the non-aquatic mycelium. Meaning the secretions of enzymes would mostly be present when in contact with E. Coli. The research of the clamp connection was beyond the scope of this thesis but should be studied further for future pond application of mycofilters.

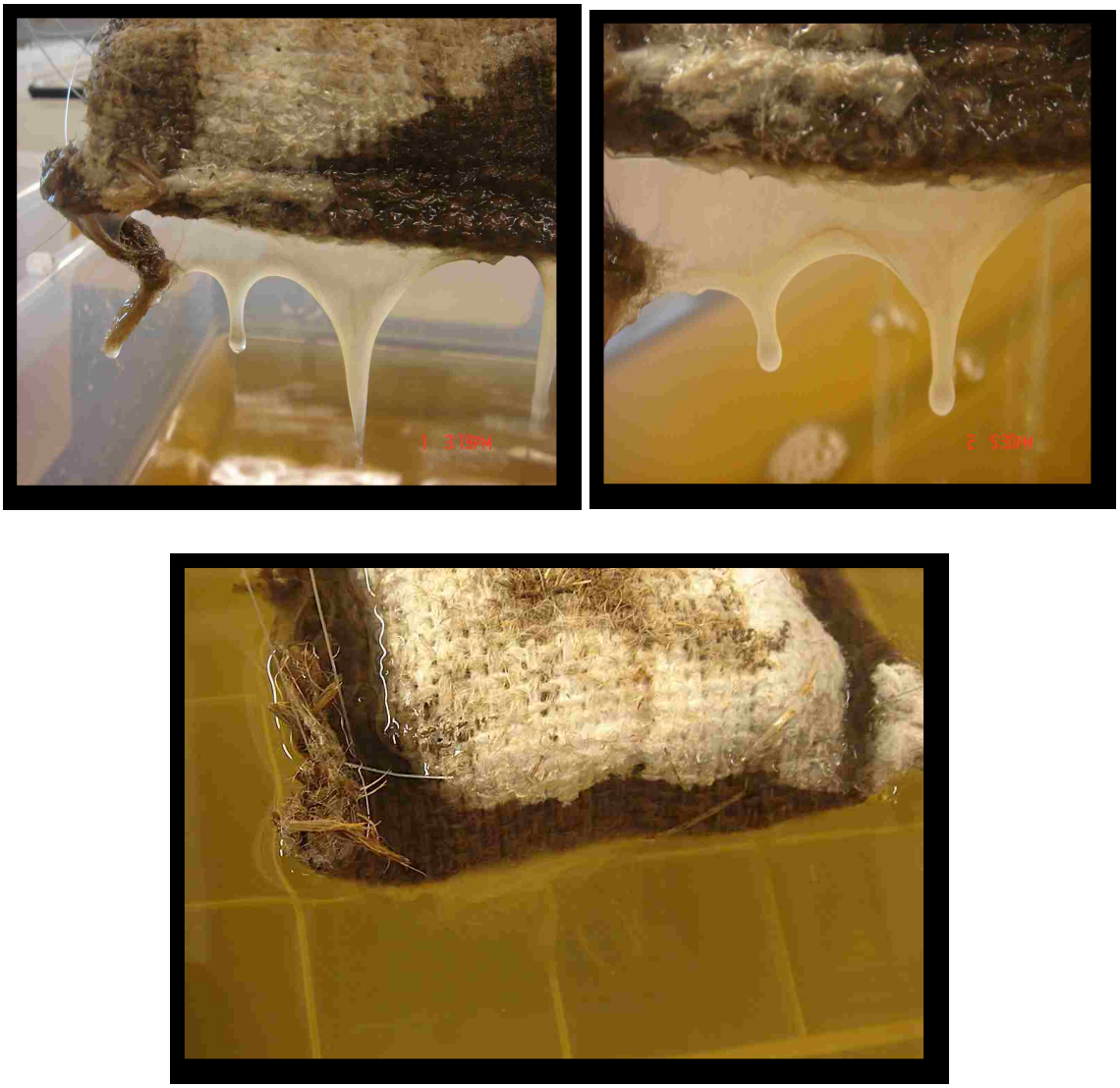


Figure 11: Mycofilter 1, during week 2 showing Growth of Clamp Connections

CHAPTER 5 DISCUSSION AND CONCLUSION

5.1 Discussion

One objective of this study was to investigate the treatment effectiveness of mycofiltration to reduce the concentration of E. Coli after repeated exposure to synthetic stormwater in a wet environmental pond setting. From analyzing week three of the data above, the control reactors showed the most E.Coli concentration removal of 100%, the mycofilters were at 98% removal and filter reactors were 81%.

The mycofilter reactors resulted in very high concentration removal percentages. Even though this result is encouraging, it is an uncertain result because it was only based on surface testing. In week 3, it was discovered that some E. Coli coliforms had fallen to the bottom of the mycofilter reactors (Appendix B). This unexpected finding brings uncertainty to the results. It is unclear whether during week 1 and week 2, the E. Coli had also fallen to the bottom of the pond and not been consumed by the mycelium. It was also found that the filter reactors had an evenly distributed amount of E. Coli throughout the entire simulated pond. The findings of these results can be described to say that the filter reactors grew other bacteria that were competing with the E. Coli for nutrients; whereas with the mycofilters, the secretion and clamp connections caused a barrier for the E. Coli and the clamp connections were most likely growing downwards toward the food source. These finding results were not included with the surface testing results because it was beyond the scope of this study and there were very few tests done.

The control reactors would not be a realistic option for a BMP in a stormwater system. This laboratory study had controlled variables and controllable environment, in a realistic situation, there would be no control over the environment for the control reactor and the results would differ in that situation compared to this study. The control reactors

purpose in this study was to witness how the E.Coli reacts to a situation of dechlorinated tap water and the results showed E.Coli was non-detected after three days in those reactors. The mycofilter reactors showed the potential of this BMP in a pond setting. The amount of E. Coli removed increased week after week of testing in the filter and mycofilter reactors. With the addition of mycelium in the filters, the reduction of E. Coli increased, as seen in Table 6. The results of this study suggest that after week 1, the mycelium was growing and adapting to the nutrient source of E. Coli contaminated stormwater.

Adapting the mycelium to E Coli contaminated water (the main nutrient source) before using it as a remediation technique impacts its potential growth (Stamets 2005). For future studies, it would be beneficial to see if the P. Ostreatus has a growing limit of E. Coli removal in a wet environmental pond, given the presence of other nutrient sources.

The second objective of this study was to analyze the long-term potential of the P. Ostreatus mycelium adaption in a wet environmental pond setting. P. Ostreatus mycelium is not known for being found in an aquatic environment but is grown locally in Albuquerque, New Mexico (location of study). The P. Ostreatus proved to be adaptable in this particular setting. As discussed earlier, the mycelium grew downward into the water by way of clamp connection. Other long-term studies have not noted the growth of mycelium due to complications of combining other plants with the mycofilters. This study is the first of its kind to document the growth of mycelium in a simulated wet environmental pond in a controlled laboratory setting. Due to this, future research is

recommended to examine the clamp connection and the secretions of enzymes that could potentially be present when in contact with E. Coli.

The results of the current study coincide with both Flatt (2012) and Thomas (2009) mycofilter reductions at comparable percent reductions as shown in Table 6. Thomas (2009) study saw a 66% reduction in fecal coliform in the control biofilter and a 90% reduction in their biofilter containing fungi. Flatt (2012) Pluerotus inoculated mycofilters had an average removal of 60-80% removal rates. The current study saw overall removal rates of 98% for the mycofilters and 81% for the filters. These results encourage the other study results that mycofiltration can reduce E. Coli concentrations in a simulated wet environmental pond. The other studies showed the mycofilters can remediate during inflow of contaminated water and this current study shows the same amount of remediation is possible in a wet environmental pond. Both in laboratory and field research the mycofiltration reductions prove to be promising for actual BMP usage.

Similar to the Flatt's 2012 study, there were false positives that grew on the sample petri dishes. The false positives are due to different bacteria, such as Klesbiella, growing in the reactors (Appendix B). Identifying the bacteria through DNA testing did not occur in this study. The false positives and using the Kovac solution made examining and counting the results time consuming. Flatt (2012) suggested using a different material (not barley straw) to avoid the false positive results. Due to funding constraints, donated P. Ostreatus mycelium was used which had already been grown on barley straw. P. Ostreatus mycelium grows well on broadleaf hardwoods, and on composting bales of straw (Stamets, 2005). Therefore, using a different material source for inoculating with mycelium might cause problems in the growth and health of the mycelium. Even though

false positives appeared, this is most likely a common occurrence for this type of wood/straw and could be found in a natural system. The problem of false positives makes it difficult for researchers to interpret results based on the Coliscan MF Method. Perhaps for future research, a different E. Coli enumeration method should be used.

There were a few limitations in this study. Verification of E. Coli concentration by a certified laboratory was limited based on budget constraints. Verified measurements of daily E. coli concentrations would have reduced uncertainty. Also, testing different layers of the pond would have been helpful in determining the distribution of E. Coli in the simulated pond. This study is the beginning of showing that the reactor and mycofilter system could work in a field application but further research is needed to prove the E.Coli removal effects of the clamp connections in a pond setting.

5.2 Implications for Stormwater Management

With the mycofilter reactor results of 98% at the end of week 3, there is encouragement of using mycofiltration as a BMP. The economical value of using the mycofilter is worth investing into further research. Using all recycled materials to create the mycofilters, this technology would be low cost and easily maintained. For NPDES permit holders, mycofiltration would be a way of complying with the measurable goals to protect water-bodies from polluted runoff. This study showed that after repeated exposure to synthetic stormwater, the removal rates increased with time. This has yet to be done by any current BMP used in the field. Mycofiltration has the capability of removing PCB's, metals and other contaminants in addition to lowering E. Coli concentrations. This technology is ready for field-testing after witnessing the results of

the laboratory testing. Involving more variables, such as weather and other contaminants, can further show potential usage of mycofiltration as a BMP.

5.3 Implications for Future Research

Suggestions for future research should include testing the mycofilter clamp connections for enzyme excretion. The clamp connections have the potential for underwater treatment and the interaction of E. Coli on these clamp connections would help further show the adaptability of the P. Ostreatus species. Bacterial identification of each reactor would have been helpful in identifying the different bacteria present. Bacterial identification would be helpful in future field research to know which bacteria is being added to the system and whether that type of bacteria is common in wet environmental ponds.

Growing the P. Ostreatus mycelium in a laboratory setting would have also reduced the different bacteria growths. With the reduction of different bacteria growths, it would have made an impact on the E. Coli results. There would have been less false positives and a more accurate representation of the E. Coli concentration.

5.4 Conclusion

The current study tested the treatment effectiveness of mycofiltration to reduce the concentration of E. Coli after repeated exposure to synthetic stormwater in a simulated wet environmental pond setting. The results of this study showed: (1) the mycofilter reduced the concentration of E.Coli at the water surface compared with controls; and (2) the long-term potential of Pleurotus Ostreatus mycelium adaption in a pond setting is promising. The potential for P. Ostreatus mycofilters to be used in an actual BMP is a definite possibility. Based on the research of this study, the mycofilter is

ready to be deployed into a field environment. The field environment will have more variables that will be uncontrollable. From the evidence provided from this research study and previous studies, the P. Ostreatus mycelium can remediate a water system that was heavily loaded with E. Coli. The next step is to see how this species will react in a natural environment. Future efforts should be placed on learning more about the clamp connection and if the enzyme secretion is present under the water surface - as well as studying the removal of other pollutants that is existent in stormwater. The promise of using a natural remediation for an environmentally introduced problem is an idea that is worth continuing to study.

References

Barron, G. L., and Thorn, R. G. (1987). Destruction of nematodes by species of *Pleurotus*. *Botany,* 65 (4), 774-778.

Flatt, Alicia Ann. "Removal of Escherichia Coli from Stormwater Using Mycofiltration." Thesis. Washington State University, 2013. Print.

Gulis, V. and Suberkropp, K. (2003). Interactions between stream fungi and bacteria associated with decomposing leaf litter at different levels of nutrient availability. Aquatic Microbial Ecology. 30:149-157.

Humphrey, Guy and Kuethe, Jeffrey (2005). Practical Methodologies for the Synthesis of Indoles, *Chemical Reviews* 2006 *106* (7), 2875-2911 DOI: 10.1021/cr0505270

Micrology Laboratories, LLC. Coliscan MF. Retrieved from Http://www.micrologylabs.com/page/94/Coliscan-MF

Rogers, Tim. "Experimental Evaluation of Mycoremediation of Escherichia Coli." Thesis. The Evergreen State College, 2012. Print.

Summary of Urban Stormwater Quality in Albuquerque New Mexico. Rep. Albuquerque: USGS, 2015. Print.

"Source Water Protection Practices Bulletin Managing Storm Water Runoff to Prevent Contamination of Drinking Water." *United States Environmental Protection Agency*. N.p., July 2001. Web. May 2015.

Stamets, Paul. *Mycelium Running: How Mushrooms Can Help Save the World*. Berkeley, CA: Ten Speed, 2005. Print.

Tetra Tech. North Carolina. *Introduction to Mycofiltration: Biotechnology for Stormwater Treatment*. N.p.: n.p., 2013. Print.

Thomas, S.A., L.M. Aston, D.L. Woodruff, V.I. Cullinan. (2009). Field demonstration of mycoremediation for removal of fecal coliform bacteria and nutrients in the dungeness watershed, Washington. Final Report. *Pacific Northwest National Laboratory.* PNWD-4054- 1.

Appendix A

Reactor Data

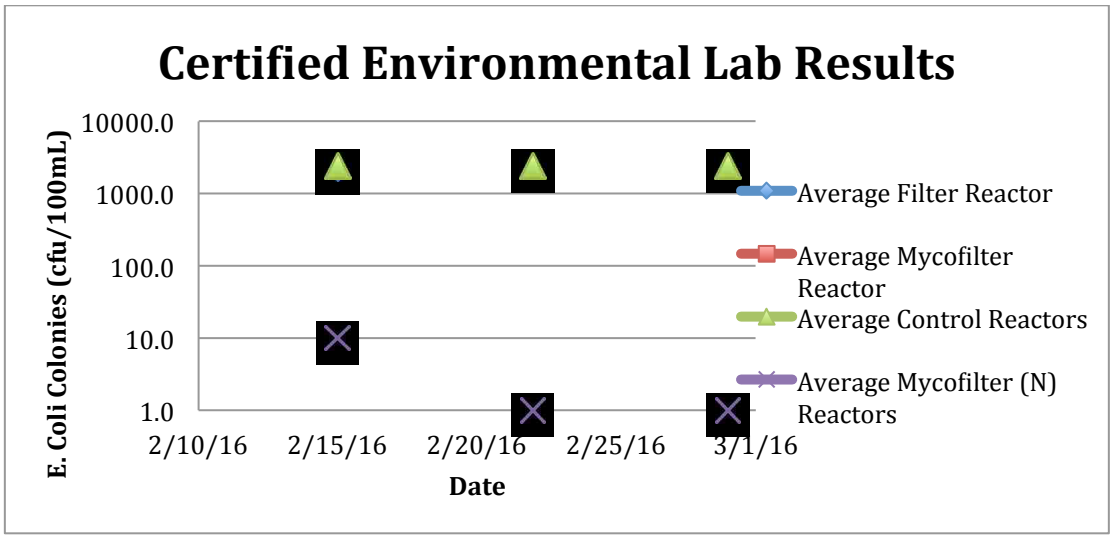


Figure 1: Certified Environmental Lab Results

Table 1: HALL Environmental Lab Results

Date Taken	Sample Name	E. Coli Colonies (cfu/100mL)
2/15/16	F1-S1-E	1986.3
	F2-S1-E	>2419.6
	F3-S1-E	>2419.6
	M1-S1-E	>2419.6
	M2-S1-E	>2419.6
	M3-S1-E	>2419.6
	MN1-S1-E	<10
	MN2-S1-E	<10
	MN3-S1-E	<10
	C1-S1-E	>2419.6
	C2-S1-E	>2419.6
	C3-S1-E	>2419.6
2/22/16	F1-S2-E	>2419.6
	F2-S2-E	>2419.6
	F3-S2-E	>2419.6
	M1-S2-E	>2419.6
	M2-S2-E	>2419.6
	M3-S2-E	>2419.6
	MN1-S2-E	<1
	MN2-S2-E	<1
	MN3-S2-E	<1
	C1-S2-E	>2419.6
	C2-S2-E	>2419.6
	C3-S2-E	>2419.6

2/29/16	F1-S3-E	>2419.6
	F2-S3-E	>2419.6
	F3-S3-E	>2419.6
	M1-S3-E	>2419.6
	M2-S3-E	>2419.6
	M3-S3-E	>2419.6
	MN1-S3-E	<1
	MN2-S3-E	<1
	MN3-S3-E	<1
	C1-S3-E	>2419.6
	C2-S3-E	>2419.6
	C3-S3-E	>2419.6

Appendix B

Additional Observations in Data

First Week of Testing Observations

During the first week of testing two of the mycofilter reactors had developed an oily layer on the surface of the water. This oily layer created problems when using the Coliscan MF method; the oily layer did not allow the E. Coli to grow on the filter pad properly causing a sample error that looks like the sample below.

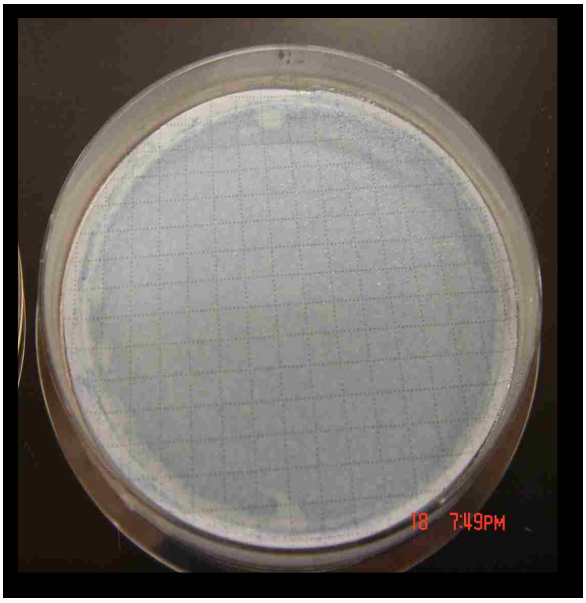


Figure 7: Oily Layer Sample

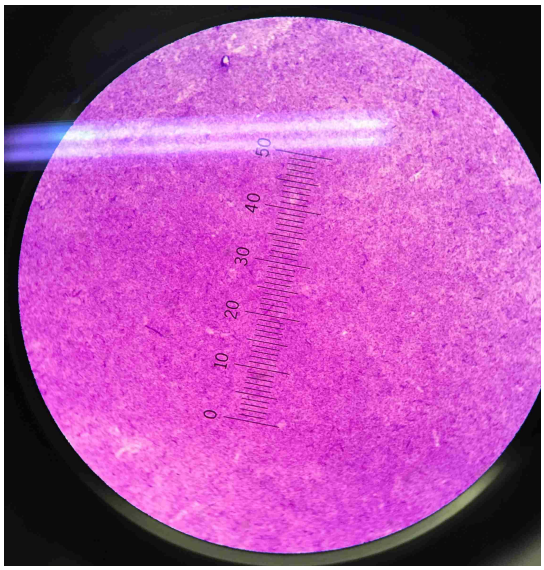


Figure 8: Oily Sample at 1000X

Water samples of the oily substance and the other reactors were taken to a biology lab to conduct testing. The samples were placed onto a culture plate and grown for 24 hours. The colonies that grew were then placed onto slide plates for crystal violet staining. The results of this staining showed several different types of bacteria growing inside the reactors. The microbiologist was unsure of the source causing the oily substance but suggested it could be the P. Ostreatus secretion. Due to financial and time constraints, further testing was unable to occur.

Third Week of Testing Trend

During the third week of testing, an interesting observation was made when analyzing the results of the first couple of days. The Coliscan MF Method petri dishes showed the E. Coli to be non-detectable unusually quicker than in the previous week 1 and week 2. The reduction in E. Coli was unusual based on the results of the control reactor during week 1 and week 2. The control reactor had shown the E. Coli concentration was unable to be spotted after three to four days without nutrients. Week 3 was showing the E. Coli concentration was non-detect within two days. Nearing the end of testing, there were only a few extra Coliscan MF testing kits and due to financial reasons, more tests could not be purchased. Using the few extra tests, the bottom of each reactor was tested for E. Coli. The results of these test showed the Mycofilter reactors displayed that the majority of the E. Coli was present at the bottom of the reactor. The Filter reactors showed the E.Coli was well mixed throughout the reactor (same concentration at surface as was at the bottom). The Control reactors showed E. Coli present at the bottom but the concentration was similar to day three of week 1 and week 2. Due to lack of testing, conclusions are difficult to be made. A hypothesis that the filters grew other bacteria in the reactors that were competing with the E. Coli for nutrients, whereas with the Mycofilters, the secretion and clamp connections caused a barrier for the E. Coli and the clamp connections were most likely growing downwards toward the food source. For future research, it would be beneficial to test the different levels of the water column for the duration of the study.

Common Weekly Testing Trend Observed

Due to the anticipation of false positives showing on the results of the Coliscan MF Method the Kovac's Solution was used throughout the duration of testing. E. Coli is a positive indole bacteria species, which means the bacteria species can convert tryptophan into an indole. Indole is an organic compound that is widely distributed in the environment and can be produced by a variety of different bacteria (Humphrey, 2006). This study was only concerned with the concentration of E. Coli in the reactors and other bacteria could cause a false positive for E. Coli. Based on the color of the colonies present in the Coliscan test, determining the difference between E. Coli colonies and false positive bacteria was the challenge. Due to the financial budget, the bacteria identification of the growth in these filters was unable to be completed, the actual indole negative bacteria is unknown. The Kovac Solution was used on the lighter blue colonies that did not resemble the dark blue colonies of E. Coli (based on details found in Appendix C). The Kovac Solution was also used on the dark blue colonies as verification of the E. Coli bacteria. A droplet of the Kovac solution is applied to the questionable colony and the colony will develop a color, cherry red for positive indole and yellow for a negative indole. Figure 10 and 11 are examples of the results that were tested using the Kovac Solution. All Kovac results were taken into account during counting of E. Coli Colonies.

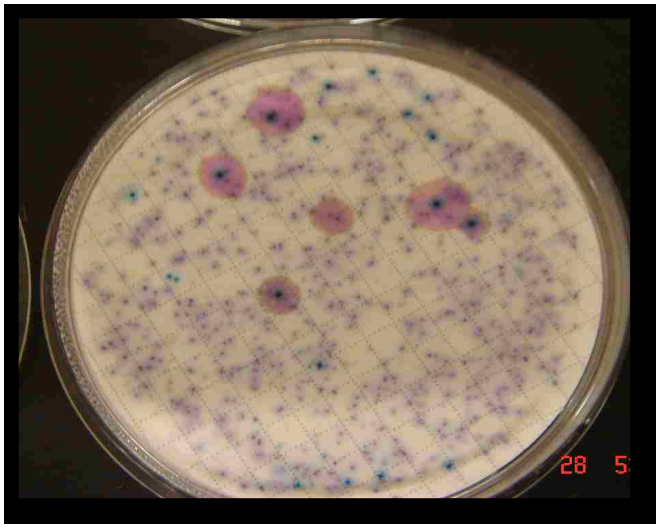


Figure 10 E. Coli Positive Colonies

Figure 11 False Positive Colonies

Appendix C

Coliscan MF Method Details

Coliscan Membrane Filter Method

The Coliscan Membrane Filter Method is a widely used method of obtaining E. Coli and General Coliforms from liquid samples. The Coliscan MF method uses a nutrient medium that contains two color-producing chemicals. The two color producing chemicals are for detecting the enzyme glucuronidase (enzyme produce by E.Coli) and galactosidase (enzyme produce by general coliforms and E.Coli). The actual detailed instructions of the Coliscan MF Method are attached at the end of this appendix. To summarize the process, 100mL of a diluted water sample was vacuum filtered onto a 0.45 µm filter pad and then transferred to a petri dish containing an absorbent pad soaked with 1.75mL of the Coliscan MF medium. The petri dish was then inverted and incubated at 35°C for 24 hours. After 24 hours, the cells grew into colony forming units on the surface of the filter. When E.Coli colonies are present, they produce an enzyme called glucuonidase. Glucuonidase reacts with a specific color producing a substrate in the medium and a water insoluble teal-green pigment will color the colony. However, E. Coli produces both galactosidase and glucuronidase, those colonies appear as a combination of the teal-green and pink pigments and will appear as some shade of blue-purple. Figure 12 shows an example of an incubated Coliscan filter looked like after 24 hours.

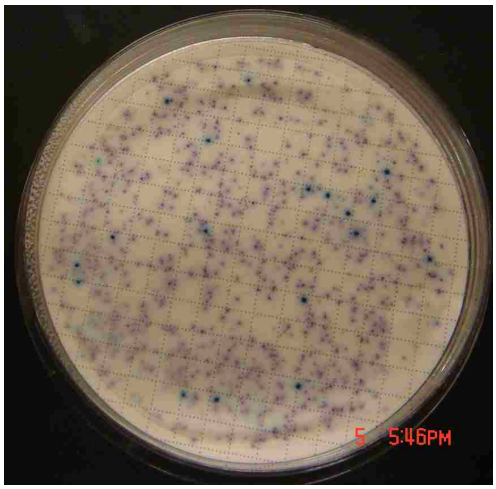


Figure 12: Incubated Coliscan Filter

Coliscan® MF/Coliscan® MF Plus Procedure

For use with Micrology Laboratories filter apparatus only. Read entire instructions before beginning.

Items needed (minimum):

- 1 Filter Apparatus (with vacuum device)
- 10 Membrane Filters
- 10 3 mL Calibrated Droppers (or pipette, any size)
- 1 Coliscan MF (Plus) bottle
- 10 50 mm dishes w/ pads

Preparation and setup

1. Thaw the desired number of bottle(s) of Coliscan® MF (Plus) by leaving at room temperature overnight. For rapid same-day thawing, stand in warm water until liquid. All unused bottles should be left in freezer. Collect the water to be tested in the appropriate volume and dilution (see table below). It is best to do this within a couple hours prior to filtering or, if this is not possible, water may be stored in refrigerator for no more than 24 hours.

Water amount to be collected

Water Sources	Amount to collect
Environmental:	
River, lake, pond, stream, ditch	1.0 to 5.0 mL added to sterile dilution water (10 to 90 mL)
Drinking water:	
Well, municipal, bottled	100 mL

2. Open a dropper or pipette and sterilely add 1.75 to 2 mL Coliscan® MF (Plus) to each pad in each dish that is to be used.

3. Filter apparatus setup. The filter apparatus comes in a sterile pack. Open the pack and remove the apparatus. The clear top of the apparatus is the funnel, which is calibrated for 100 and 150 mL samples and is covered with a lid. It fits on the bottom collection container and is sealed with an O-ring. There is a side port with a tip for the attachment of the vacuum syringe. Twist it and it can also be removed. It contains a plug in its tip which can be removed. The contents of the bottom collection container are most easily poured out when the tip is removed. It is easily replaced by twisting back on.

4. To prepare the apparatus for use, remove the funnel and using a clean forceps place a sterile pad on the top grid-work (in the blue circle) of the container.

5. Open a sterile filter envelope and with the clean forceps, carefully remove the membrane filter from the pack. **Be sure to separate the filter from the protective backing and handle the filter carefully so it is not torn or damaged.** Place the filter, grid side up, on top of the sterile pad. Push the funnel down so that it is held and sealed by the O-ring and the filter and pad are held firmly in place. The funnel must be pushed down as far as possible to obtain a good seal.
6. Attach the syringe to the filter apparatus by pushing the end of the hose on to the side port tip of the funnel contained. Be sure that the syringe plunger is not pulled out.

Filtering the water

7. Pour the water sample into the funnel, swirl to mix and create a vacuum by pulling out the plunger of the syringe. The water will be pulled through the filter, depositing any microoganisms present onto the filter surface.

8. When the water sample has been completely passed through the filter, disconnect the syringe, remove the funnel and with the clean forceps remove the filter and place grid side up directly on top of the pad of a dish prepared earlier. Make sure that there are no air spaces (bubbles) between the pad and the membrane filter. Place the lid back on the dish.

9. The filtered water in the collection container should be emptied and the filter apparatus prepared for repeat use. Before the funnel is used again it should cleaned. This may be done by rinsing with alcohol or radiated for 1 minute with germicidal UV if desired. The absorbent pad can generally be reused as it will only contain filtered water (sterile).

Incubation and interpretation

10. Incubate in an incubator or a warm place. If using an incubator, incubate at 35° for 18- 24 hours. If an incubator is not available, find a place that will be warm for a 24 hour period. **DO NOT** place in direct sunlight or over a direct heat source, radiator, furnace duct etc. You may place them near one of these sources or in a warm spot in the kitchen. Allow 24-48 hours for growth to begin. Once growth begins you can incubate another 24 hours for complete growth to take place.

11. Once the incubation period is complete, a count of the colonies can be done. Count all **blue** colonies as ***E. coli*** **(fecal coliform)** and all **red colonies** as **general coliforms**. The sum of these two is the **total coliform** population.

    Additionally, with Coliscan® MF Plus, verification of *E coli* is accomplished by shining a long wave (366 nanometer) UV light on the back of the dishes (do this in a dark room). If any of the colonies are *E coli*, the area around the colonies will fluoresce a bright bluish color. This fluorescence can also be used as proof for the presence of *E. coli* in a sample, thus making the medium an effective P/A test for *E. coli* if quantitative results are not needed.

If you have any questions, call 1.888.327.9435.

Micrology Laboratories, LLC.
PO Box 340 Goshen, Indiana 46527-0340
Phone: 574.533.3351 Fax: 574.533.3370
Call toll free 888.327.9435
Email: info@micrologylabs.com

Indole Presence Test for *E. coli* Confirmation

Traditionally, the identification of *E. coli* in environmental samples has involved the use of selective and differential media such as MacConkey, VRB and EMB agars and EC, BGLB, Lactose or other broths. Confirmation could be through various additional biochemical tests on cultures isolated from the primary media.

One set of biochemical tests which has been used is the IMViC series, where I=indole, M=methyl red, Vi= voges proskaur, C=citrate and the typical *E. coli* culture results in ++-- for these four tests, while the typical *Enterobacter aerogenes* gives --++ for the series. Of the four tests in this series, it is generally conceded that the production of indole is a very useful test to confirm *E. coli* identification. There are numerous published methods for the performance of the indole test. The most commonly used is probably the isolation of the colony in question by inoculating a tube of tryptone broth, allowing growth for 48 hrs, and then adding several drops of Kovac's reagent to the broth, agitating slightly and checking for color in the reagent that collects at the top of the broth. A cherry red color is positive for indole, and a yellow color is negative. There are also spot tests described in the literature.

The use of Coliscan® MF or Coliscan® Easygel® eliminates the necessity of doing all of the above procedures to confirm *E. coli* in a sample. Blue/purple colonies on these media are confirmatory for *E. coli* and indicate the production of glucuronidase and galactosidase. If the investigator has a problem being sure whether a colony is teal green, pink (magenta) or blue/purple, we recommend they use our Confirmation Media to clear up their confusion of interpretation. We only recommend going to the additional trouble of the indole test for persons who feel that they need reassurance of their final identification.

However, for persons using the Coliscan® MF method, it is extremely easy to check individual colonies growing on the membrane for indole production. This provides an additional verification and confirmation that a blue/purple colony is *E. coli.* Following are the instructions for doing this test.

1. Choose a colony growing on the membrane filter and add a small drop of Kovac's reagent on or at the edge of the colony. (Use a small wire or plastic loop, or a new toothpick to pick up the Kovac's reagent and transfer it.)

2. If the colony is indole positive, a bright red zone will develop within 5 seconds where the Kovac's has spread. (The reagent will likely kill the colony, so any transfer of the colony should be made before testing.)

3. You should read the results within the first minute of the application of the reagent as the red color will be replaced with green/blue later as the solvents work on the chromogens.

Micrology Laboratories LLC, Goshen IN 46526 USA www.micrologylabs.com P 574.533.3351 F 574.533.3370

Appendix D

Set Up and Procedures Details

Mycofilter Construction

All filters were constructed the same way; a sterilized burlap pouch filled with pasteurized barley straw or with P. Ostreatus inoculated barley straw (mycofilters). Creating the burlap pouches, a burlap sack was cut into dimensions of 6 inches by 9 inches and made into a pouch. The burlap pouch was then rinsed thoroughly with tap water and placed into the Autoclave at 121°F for 30 minutes. Once sterilized, the burlap pouch was placed onto a sterilized metal tray to cool down. During this process, the barley straw was pasteurized. The procedure to pasteurize the straw is as follows.

1. Partially fill a metal drum (pot) with water and heat to a stable 160°F.
2. Cut straw into 3 inch pieces, this helps mycelium colonize the straw faster. Put straw into nylon mesh bag.
3. Put straw bag into water bath and make sure entire bag is completely submerged. Leave bag inside bath for 1 hour. Keep an eye on temperature and water level.
4. After an hour, remove bag and strain out water.
5. Let bag drain and get to room temperature. Once at room temperature place into burlap sack and sew together with sterilized nylon fishing string.

Each burlap pouch was filled to a 1 inch thickness with either pasteurized straw or mycelium inoculated straw. Each filter sat in a disinfected plastic storage bin that was 2.3ft x 1.5ft x 2ft (length, width, height) scaled down. Inside of these reactor bins each filter was submerged 0.5 inches in dechlorinated tap water. Tap water was used because in comparison to de-ionized water, tap water contains some minerals and is more representative of stormwater runoff. Typical pond nutrients were not added in this simulated pond experiment because focus was only on E. Coli removal and the addition of other nutrients would cause too many unknowns in what might actually be consuming the E.Coli.

Calculated Flow Rates

For this particular pond, a typical rainstorm would replace about 10% of the volume in the pond. This is based on preliminary documents and actual construction plans from a local flood control agency. This data came from discussions with the owners of the pond and descriptions of actual flows that have been seen for the past 14 years. A typical storm would see about 50- 70 cfs of flow for duration of 10 minutes on average. For this model, a storm at 50 cfs for 10 minutes would replace about 30,000 ft^3 and that would directly correlate to 0.248 ft^3 in model size or $4.1x10^4$ cfs.

Procedures:

The P. Ostreatus Mycelium was delivered a few weeks before testing began and was three months old. They were delivered frozen and immediately placed into a walk-in fridge that is kept at a constant 4°C. Mycelium was taken out 48 hours prior to being placed into sterilized burlap sacks. This was to allow for any defrosting and equalizing of the temperature of mycelium to approximately 70° F. Mycofilters were then placed into reactors a day prior to first to storm flush, to acclimate to new settings.

Stormwater Recipe:

Once a week, synthetic stormwater was created for the weekly "storm". The synthetic stormwater was created by mixing dechlorinated tap water with a E. Coli inoculated solution. E. Coli ATCC 11775 Culti Loop was chosen for the E. Coli bacteria source based on another study using this same source (Flatt, 2013). The E. Coli grew overnight in 5mL Tryptic Soy Broth and was mixed into 25 gallons of dechlorinated tap water. Other common nutrients such as nitrogen and phosphorus were not included in this stormwater solution. The study was focused on the removal of E. Coli and other nutrients would have caused too many unknowns for the cause of E. Coli removal. The methods of preparation are written below.

Preparing Tryptic Soy Broth

The Tryptic Soy Broth solution was created by mixing De-Ionized water with 9 grams of solid Tryptic Soy Broth composition. Solution was mixed until dissolved and then autoclaved for 15 minutes at 121 ° C. Once sterilized, 300mL broth was placed inside fridge at 4 °C to be used for later use. Test tubes were filled with 5mL of broth and warmed to 35 °C. One E. Coli Culti-loop was warmed up in a test tube and slowly dissolved in four different tubes. An agar plate was also streaked with the E.Coli loop. For other storms, a sterilized loop was used and wiped with cultured agar plate. This loop was then stirred into a test tube with 5 mL of broth and allowed to grow for 16-18 hours. Broth was then mixed into dechlorinated tap water and synthetic stormwater was finished. Sodium Thiosulfate was used to prepare the dechlorinated tap water with the ratio of 25 mg/ L per 30 L of tap water was used.

Rainstorm Day Procedure:

24 hours before rainstorm, stormwater solution was prepared. From the 5mL test tube of inoculated broth, 30 drops were mixed into a 30 gallon plastic barrel filled with 25 gallons of dechlorinated tap water. A sterilized disposable inoculating loop (50 x 1 µL) was used to gather each drop from the test tube. A hand pump was used to mix and pump out 1.87 gallons of storm water in the disinfected water containers.

Disinfection

All buckets and materials that were used were disinfected with bleach. Stormwater effluent was sterilized by dumping ½ cup of bleach per 1 gallon of stormwater. A mixture of the same ratio was mixed into a spray bottle and made once every three days. Clorox disinfecting wipes were used to wipe down handles, knobs and any other hard to reach areas. Precautions for contamination were held in high regard for this experiment, lab coats and gloves were worn.