Team Fungi
Northern Arizona University & Hooper Undergraduate Research Award

Remediation of *E. Coli* Contaminated Surface Water in Arizona Via Fungi Final Report

CENE486C: Engineering Design

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Table of Abbreviations

AA: Administrative Assistant
ADEQ: Arizona Department of Environmental Quality
BMP: Best Management Practice
C (-): control negative
C (+): control positive
CENE: Civil and Environmental Engineering
CFU/100mL: colony forming units per 100 mL of fluid
cm: centimeters
CWA: Clean Water Act
E. Coli: Escherichia coli
EIT: Engineer in Training
EPA: Environmental Protection Agency
HE: *Hericium erinaceous*
L: liters
LFH: laminar flow hood
LT: Lab Technician
LB: Luria-Bertani
m: meters
mm: millimeters
mL: milliliters
mL/s: milliliters per second
mg: milligrams
nm: nanometer
NAU: Northern Arizona University
NCUR: National Conference of Undergraduate Research
NPDES: National Pollutant Discharge Elimination System
PDA: Potato Dextrose Agar
PDB: Potato Dextrose Broth
PE: Professional Engineer
PO: *Pleurotus Ostreatus*
RO: reverse osmosis
RPM: revolutions per minute
SLF: Science Lab Facility
SWW: synthetic wastewater
SE: Senior Engineer
SR: *Stropharia Rugosoannulata*
TBELs: technology-based effluent limits
TNTC: too numerous to count
TAs: *Trichoderma asperellum*
TV: *Trametes versicolor*
US: United States
WQBELs: water quality-based effluent limits

Acknowledgements

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1.0 Project Introduction

This project examines four Arizona native species of fungi and their ability to remove *E. coli* from water. This research will lay the groundwork, providing a necessary engineering parameter – removal rate, for the up-scaled design of a fungal-based *Escherichia coli (E. coli)* control system, protecting Arizona's watersheds from harmful bacterial contamination using native fungi. Additionally, the project will determine a conceptual design for implementation in a local watershed, such as Oak Creek Canyon.

1.1 Project Background

Many of Arizona's rivers, streams, and lakes are contaminated to unacceptable levels with *E. coli* bacteria. The most prevalent cases of this contamination with proximity to Northern Arizona University (NAU) are Oak Creek and the Verde River [1]. *E. coli* is a known human health hazard, which causes mild to serious health impacts, including abdominal pain, nausea, diarrhea, fever, dehydration, and occasionally death [2]. During high levels of contamination, above 130 colony forming units per 100 milliliters of water (CFU/100 mL), in public waterways such as Oak Creek or the Verde River, the concentration is defined as a high risk [3]. This means public recreation must be limited during high levels of contamination to avoid outbreaks of illness due to *E. coli*.

Research shows that fungal species may be used to remediate many pollutants in water, including *E. coli* [4, 5]. Taylor's research in [4] focused on a proof of concept, where five fungal species were tested in large-diameter columns; the fungi were grown on a mixture of alder woodchips and rice straw. Thomas' study in [5] applied two species of fungi, *Pleurotus ostreatus* and *Stropharia rugosoannulata*, in a bioretention basin with plants on the Dungeness River in Washington. These studies provided high removal rates for *E. coli* and fecal coliforms, up to 90 percent [4, 5]. A non-peer reviewed study was performed within an *E. coli* contaminated watershed, but removal effectiveness of the research was not documented [6]. Limited peer reviewed research has been performed in this field of study for pilot and bench scale projects, and even fewer studies have been implemented on a field scale. Additionally, no studies were found to apply fungal-based biotechnology in watersheds for arid climates such as Arizona.

1.2 Constraints and Limitations

The project is limited by available resources such as manpower and availability of native fungal pure cultures. The project must be complete by April 30th, 2020 which limits the timeframe of the project. Additionally, team members are full-time students, further limiting their availability for the project. The selected Arizona native fungal species must be commercially available or readily purchasable, which constrains the species selection process. Although there are about 40 Arizona native fungal species documented by the US (United States) Forest Service, less than 10 of those species have readily available cultures [7, 8].

1.3 Major Objectives

The project's utmost objective is to determine the percent removal of *E. coli* from water for each fungal species tested. This includes determining the percent removal and statistical significance. With the resulting best fungi for *E. coli* removal, a field scale implementation design will be created. The field scale design will be based on the percent removal of the fungi. Finally, the project contributes to the pool of research regarding fungal-based biotechnology and aims to broaden the applicability of its use in arid climates.

1.4 Exclusions

The project will not apply fungi in the field. Therefore, the only project exclusion is field implementation. It is recognized that to fully understand the capabilities of the best fungi to remove *E. coli* from real surface waters, the fungi would need to be tested in the field. However, due to project constraints, the fungi will only be tested under laboratory conditions.

2.0 Determining Fungal Test Species

Selecting which fungal species to test was a primary initial task of the project, as seen in Appendix A: Gantt Chart, Task 1: Select Fungi. This task began with an intensive literature review where multiple fungal species were assessed for their ability to remediate *E. coli*. With the research findings, potentially viable test species were evaluated with a weighted decision matrix. Five criteria were utilized in the decision matrix, which include Arizona native (abundance), reasonable growth time, human/environment hazard, cost, and supporting research, as seen in Table 2-1. The criteria "Arizona Native (Abundance)" considered if the species grows naturally in Arizona, and the abundance of its appearance in nature. "Reasonable Growth Time" referred to the time it takes the fungi to mature. If a fungi takes more than one to two months, then the growth time was considered unreasonable. This criteria was important because the project was constrained by time. "Human/Environment Hazard" evaluated if the fungal species posed a threat to humans or the environment. "Cost" referred to the amount of money to purchase pure cultures for the species. Finally, "Supporting Research" referred to whether the fungi have been researched in the past to remove *E. coli* from water. Each potential test species was evaluated for the criteria, and scored one through 10, where a higher number was better. Those species were *Trametes Versicolor, Pleurotus Ostreatus, Hericium erinaceous, Armillaria mellea, Inonotus arizonicus* and *Stropharia rugosoannulata*. The criteria were weighted based on their importance for the project, where Arizona Native (Abundance), Reasonable Growth Time, and Supporting Research were each weighted at 20 percent. Human/Environment Hazard was weighted the highest, at 30 percent, because fungi which pose a threat to the public or welfare will have a low chance of being implemented in the field. Finally, the cost was weighted the lowest, at 10 percent, because the project, to a reasonable extent, was not highly constrained by funding. Explicitly, reasonable cost meant that the cost was less than $200.00 per pure, live culture.

Table 2-1: Fungal Species Weighted Decision Matrix

Criteria Description	Arizona Native (Abundance)	Reasonable Growth Time	Human & Environment Hazard	Cost	Supporting Research	Criteria Total
Weight	0.20	0.20	0.30	0.10	0.20	1.00
Fungi Options	Score	Score	Score	Score	Score	Weighted Score
Trametes versicolor	10	7	10	10	1	7.6
Pleurotus ostreatus	8	10	10	10	10	9.6
Hericium erinaceous	6	6	10	10	1	6.6
Armillaria mellea	2	6	1	10	1	3.1
Inonotus arizonicus	8	6	8	1	1	5.5
Stropharia rugosoannulata	1	8	10	10	10	7.8

According to [7], each fungi option grows in Arizona; however, the abundance of each species was further evaluated based on the number of documented observations in Arizona [9]. *Trametes Versicolor* was observed most in Arizona, which is why it received a 10 [9]. The growth times were determined with the help of mycologist, Dr. Catherine Gehring [10]. *Pleurotus ostreatus* was stated as having the fastest growth time, thus it was given the highest score [10]. All fungi options, except *Inonotus Arizonicus*, produce known edible mushrooms, and therefore posed little threat to humans. The *Armillaria mellea*, is a "virulent species" which is known to cause white rot of tree root systems [7]. Furthermore, Dr. Gehring said that the species is parasitic to forests [10]. Therefore, *Armillaria mellea* was given the lowest score of one due to its hazard to trees in the environment. Each species evaluated were readily available for purchase for approximately the same price, except *Inonotus Arizonicus*. Therefore, all fungi options were scored ten except *Inonotus Arizonicus*, which was not readily available for purchase [8]. Finally, only two fungi amongst the options were shown in past research to remediate *E. coli* in water [4, 5, 11]. Consequently, the other fungi options without supporting research were given the lowest score possible.

Based on the outcomes of the decision matrix above, the best fungi options for testing are: *Pleurotus Ostreatus, Stropharia rugosoannulata, Trametes versicolor, and Hericium Erinaceous*. During the interview with Dr. Gehring, another fungi option arose – *Trichoderma asperellum* [10]. According to Dr. Gehring, this species of fungi was found growing in a water treatment process of the Wildcat Water Reclamation Plant in Flagstaff, AZ, and a pure culture was available in the Science Lab Facility (SLF) [10]. Most fungi do not grow directly in water, usually near water such as on a riverbank. Because *Trichoderma asperellum* was growing directly in water, and the contaminant, *E. coli*, being studied lives in water, it was decided to add

this species to the test group. The final test group of species included *Pleurotus Ostreatus*, *Stropharia rugosoannulata*, *Trametes versicolor*, *Hericium Erinaceous*, and *Trichoderma asperellum*.

3.0 Testing and Analysis Methods

Testing and analysis methods consist of seven subsections outlined below.

3.1 Lab Work: Filter Media and Fungal Prep

To prepare for lab testing, the team created culture plates of the *Stropharia rugosoannulata*, *Pleurotus ostreatus*, *Trametes versicolor*, *Hericium erinaceous*, and *Trichoderma asperellum*. Each species was given its own abbreviation for use in the laboratory, as seen in Table 3-1. This allowed for easier labeling on culture plate lids.

Table 3-1: Fungal Species Abbreviations

Species Name	**Abbreviation**
Hericium erinaceous	HE
Pleurotus ostreatus	PO
Stropharia rugosoannulata	SR
Trametes versicolor	TV
Trichoderma asperellum	TAs

For the experiment to be as standard as possible, the same gene expression of fungi should be used for each species. To do this, samples from the NAU mycology lab were used, taking small slices of each fungus which were placed on a petri dish with Potato Dextrose Agar (PDA). These petri dishes were left for four weeks, after which the process was repeated, taking samples from the culture plates and bulking them up to five dishes per species. In Figure 3-1, one can see the slices of each sample in their respective

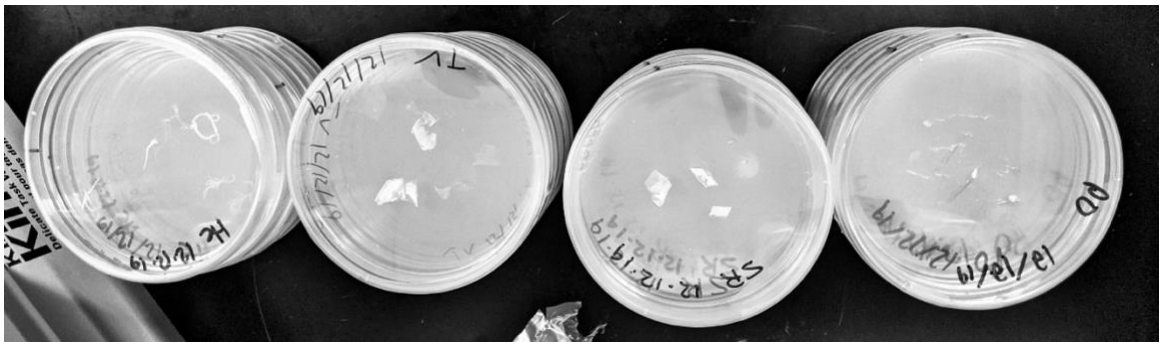

Figure 3-1: Culture plates for (from left to right) Hericium e., Trametes v., Stropharia r., and Pleurotus o.

petri dishes, and the dishes are in piles of five. The petri dishes were also labeled with the inoculation date. Although not in Figure 3-1, TAs was also bulked up.

After four to five weeks, each culture plate was filled from growth of its respective fungi, as seen in Figure 3-2. However, *Hericium erinaceous* (HE) was removed from the experiment because of two reasons. The first was the culture plates were not as filled as the ones for other species in Figure 3-2. The second was that according to the team's mycologist advisor, Dr. Ron Deckert, HE was the least compatible with what the experiment was looking for.

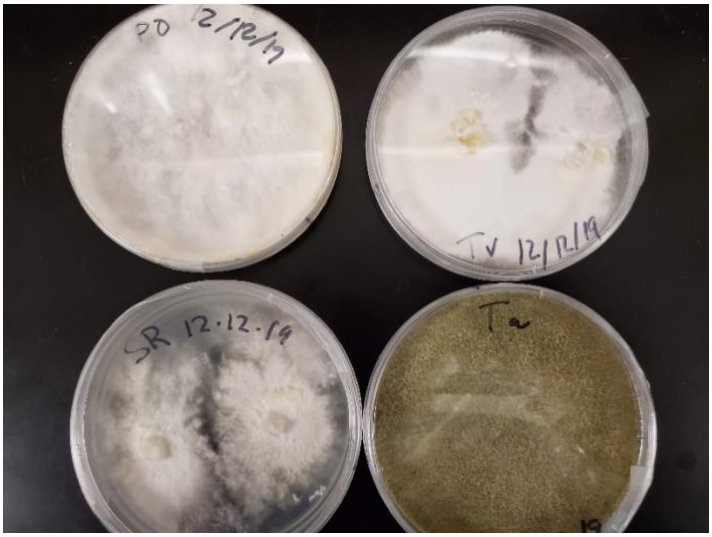

Figure 3-2: Fully Bulked Petri Dishes: (Left to right, top to bottom) Stropharia, Pleurotus, Trichoderma, and Trametes

The team decided to use Aspen wood chips as the fungal media; an organic bedding for the fungi to grow on. Aspen wood chips were readily available for purchase, they had a proper particle size, and several species of fungi enjoy growing on hardwood, such as Aspen [10, 4]. The wood chips were filtered to a size in-between 2.0 and 9.5 mm using sieves. To achieve a constant particle size distribution, after sieving the media through the 9.5 mm sieve seen in Figure 3-3, the media was filtered through the 2.0 mm sieve for 60 seconds. The sieved media was placed in metal pans at a 6.4 cm depth. The media was then tightly covered in tinfoil and autoclaved for two "Solid 30-Minute" cycles for solid contents, to ensure total sterilization of the woodchip media. For liquid contents, there was a separate autoclave setting, such as "Liquid 30-Minute" cycle.

Figure 3-3: Sieve size 9.5 mm With Aspen Media

3.2 Filter Apparatus Prep

Each filter was created using a 25.4 cm long, one and one eighth inch outer diameter, one and one sixteenth inch inner diameter clear polycarbonate tube. The base of each tube was sealed with a black 2.9 cm rubber cap. An image of the tubes with their caps can be seen in Figure 3-4. The white 3.8 cm caps were purchased for the top of each filter. The tubes and caps were sterilized in a 50 percent bleach solution.

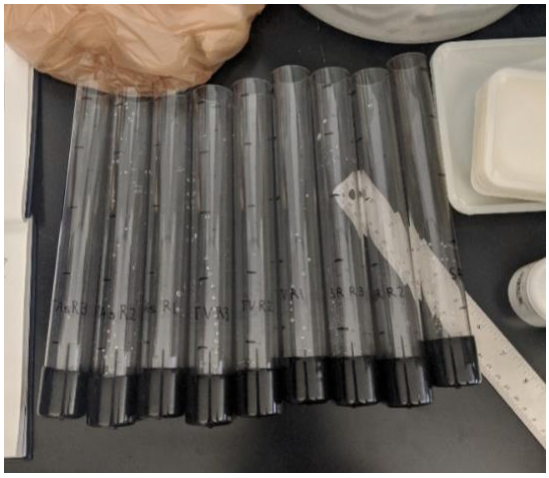

Figure 3-4: Polycarbonate Tubes and Caps

Figure 3-5 is a schematic design of how the filter will work. Included in the design is an experimental set up with a ring stand and burette, a cross section of the filter tube, and a cross section of one layer of filter media. The biofilter was designed with five layers of fungi plugs and broth aliquots, spaced evenly throughout the filter.

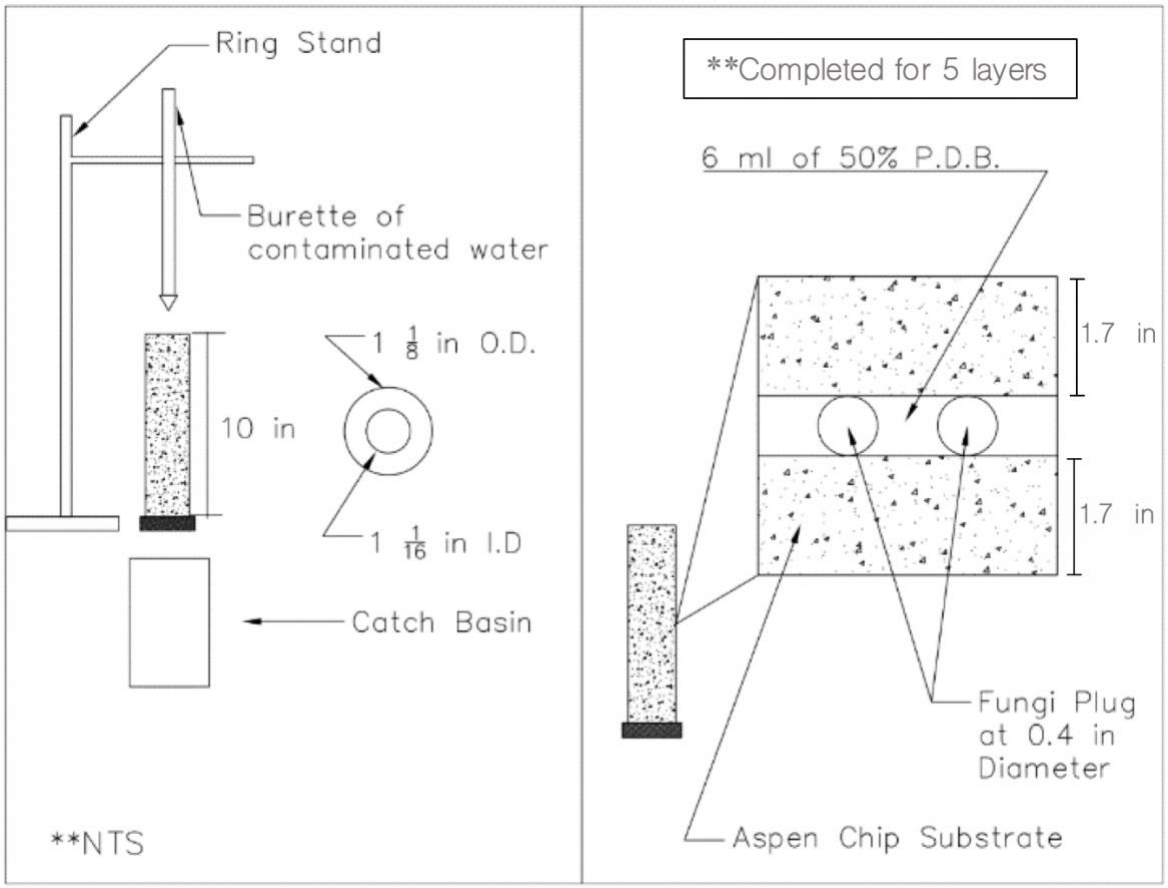

Figure 3-5: Model Rendering of Biofilter and Test Setup

3.3 Filter Creation

Once the media and biofilter apparatus components were sterilized, the filter creation process was continued. The process was completed in a laminar flow hood. The flow hood set up can be seen in Figure 3-6. The filter making process was broken into three tasks: filling the filter with Aspen media, pipetting half-strength Potato Dextrose Broth (PDB) onto the substrate, and placing plugs of each fungi pure culture onto the broth moistened media. Each filter had five layers of: 4.8 cm of media, six mL of broth, and two one-cm diameter plugs of its respective fungi. Each filter contained a single fungal species, and was filled by passing from the first position, to the second, third, then back to the first position until the filter was filled to the fifth layer. A model of this design can be seen in Figure 3-5.

Figure 3-6: Laminar Flow Hood Setup

The filter creation process was repeated for 18 filters. Three filters only contained plugs of PDA and PDB aliquots, which acted as the negative control (C-), as seen in Figure 3-7. This provided information on how much removal was done by the media alone. Six filters were filled with *Pleurotus ostreatus* (PO), three of which were sterilized to kill the fungi before testing the filters. The dead PO provided data on the nonbiological removal of the mycelium alone. The remaining nine filters were filled with *Stropharia rugosoannulata* (SR), *Trametes versicolor* (TV), and *Trichoderma* (TAs): in that order.

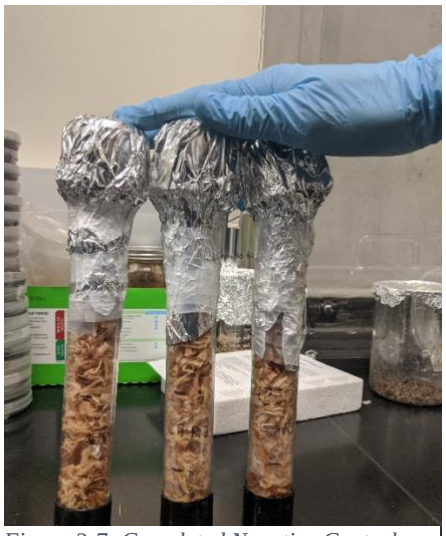

Figure 3-7: Completed Negative Control Filters

Once the filters were filled, white 3.8 cm caps were placed on top, covered in a square of aluminum foil, and then sealed with paraffin film. The paraffin film was used to keep spores from getting in or out of the filter and let the fungi breath. A completed batch of three filters can be seen in Figure 3-7. The filters were left for five weeks for the fungi to mature. Immediately before testing, each filter had a screen rubber-

banded to the base to help hold in any larger particulates, such as the Aspen media. The screen served as an underdrain for the filters. This can be seen in Figure 3-11.

3.4 *E. coli* Contaminated Water Supply

The *E. coli* contaminated water supply was created with the procedures outlined in the following subsections.

3.4.1 Stock *E. coli* Cultivation

The *E. coli* strain used for the project was *E. coli OP50* which was provided by the Gehring Lab within the Science Lab Facility (SLF) at NAU. Creating an *E. coli* contaminated water supply for testing the biofilters included using viable *E. coli* colonies from a culture plate with Luria-Bertani (LB) broth to help the *E. coli* transition from plated colonies to liquid form. The *E. coli* culture plate was considered viable if it was cultured within the past two weeks. The LB broth was created from LB broth powder and reverse osmosis (RO) water from the lab, which was mixed and then autoclaved for a "Liquid 30-Minute" cycle to sterilize the solution. To inoculate the LB broth, one loop of the *E. coli* was aseptically transferred from the culture plate to a centrifuge tube with about 1 mL sterile water and mixed using the inoculating loop. 400 µL of the *E. coli* and water was transferred from the centrifuge tube to a sterile flask containing 15 mL of LB broth, and the solution was placed in a New Brunswick Scientific C24 Incubator Shaker at 29.5˚C and 105 revolutions per minute (RPM), as seen in Figure 3-8. After about two hours, the mixture turned from clear to slightly murky/cloudy, showing that the *E. coli* were growing throughout the broth. Based on concentration of the *E. coli* in LB broth, the mixture was diluted with RO water to get a desired concentration.

Figure 3-8: E. coli and LB Broth on Incubator Shaker Table with Other Lab Mixtures

3.4.2 Contaminated Water *E. coli* Concentration Standardization

Several trials were performed with different ratios of stock *E. coli* to RO water to determine the best mixture for achieving an influent concentration of approximately 1500 CFU/100mL. The dilution of the stock *E. coli* solution to RO water was determined to create the contaminated water supply based on Equation 3-1.

Equation 3-1: Stock Dilution [12]

$$C_1V_1 = C_2V_2$$

Where:
C_1: Concentration of stock solution
V_1: Volume of stock solution
C_2: Final concentration
V_2: Volume of dilution solution

Once the stock solution of *E. coli* was transferred to the dilution RO water, the mixture was mixed using a stir bar on a Fisher Scientific Stir Plate within a laminar flow hood, as seen in Figure 3-9. The mixture was stirred initially at a high speed to ensure distribution of *E. coli* in the RO water, and then set at a low speed to keep the solution well mixed until testing. The contaminated water supply, also referred to as synthetic wastewater (SWW) was made in two liter batches, where one batch made enough SWW to test three biofilters. The SWW was used for biofilter testing directly after the stock *E. coli* solution was mixed with the RO water. The process was repeated for each two liter batch of SWW.

Figure 3-9: SWW with Stir Bar on Stir Plate

The concentration of stock *E. coli* solution was determined using a spectrophotometer method, described in section 3.5.1. While the spectrophotometer was used to get a general idea about the dilution ratio, the method was verified using membrane filtration, explained in section 3.5.2. To verify that Equation 3-1 was yielding proper dilution ratios for stock *E. coli* to RO water, trial runs were performed prior to biofilter testing. The trial runs involved testing the SWW using membrane filtration to verify the applicability of Equation 3-1 and the spectrophotometer method.

3.5 Quantifying *E. coli*

The *E. coli* within the contaminated water supply was quantified using two methods – spectrophotometer and membrane filtration. The reason for this was that the membrane filtration required 18-24 hours of incubation time, whereas the spectrophotometer yielded instantaneous results. However, the spectrophotometer method was not an approved standard method, and therefore the accuracy of the method was verified using the membrane filtration method, following EPA approved Standard Method 9222 [12].

3.5.1 Spectrophotometer Method

The stock *E. coli* solution concentration was measured using a Shimadzu UVmini-1240 UV-Vis Spectrophotometer at a wavelength of 600 nanometers (nm). To

account for the yellow color of LB broth, the spectrophotometer was zeroed with a LB broth blank. To prepare the blank, the same ratio of LB broth to water (15 mL of LB: 400 µL water) was prepared to ensure the blank accounted for the small amount of water that was mixed with the *E. coli*. The spectrophotometer allowed for the quantification of the concentrated stock *E. coli* in LB broth, which was then diluted with RO water to create a supply of contaminated water, also referred to as SWW. The spectrophotometer method was not used for the quantification of *E. coli* in the SWW because the level of *E. coli* was below the detection limits of the machine.

3.5.2 Membrane Filtration Method

After obtaining a reading from the spectrophotometer, it was important to get an accurate reading from an EPA accepted testing method to enumerate to *E. coli* in the influent. To do this, the Coliscan® C Membrane Filter (MF) kit from Mycrology Laboratories was used [13]. This kit utilized a nutrient liquid formulation to detect glucuronidase which is produced only by *E. coli* strains. Two sample were taken from each biofilter, which included the three replicates per species and two controls. The reason for two samples per biofilter was because two dilutions were used to avoid getting a reading of too numerous to count (TNTC). The two dilutions used were 1:100 and a 1:10, which was the ratio of sample fluid to sterile dilution water. Two mL of nutrient broth were aseptically added to a pad-lined petri dish. After this, the 1:100 sample was taken first which included 99 mL of RO water and 1 mL of sample. The filter kit provided filtration equipment, which had a pump and catch basin for the filtered water, as seen in Figure 3-10. The two liquids were mixed and put into the reservoir lined with 0.45 µm grid filter paper, which was drawn through the filter using the vacuum pump, as seen in Figure 3-10. Due to the size of the filter paper, *E. coli* and any other potential microorganisms were left behind on the grid filter paper. The grid filter paper was then added to the petri dish with the nutrient broth, using forceps that were sterilized with ethanol and a flame between uses. The petri dish was then incubated at a temperature of 35 degrees Celsius for 18-20 hours. Once the incubation period was complete, the colonies were counted to enumerate the *E. coli* concentration in the influent. Readings of 30-300 are deemed appropriate for counting colonies [12]. Additionally, all Coliscan® testing was completed aseptically under a laminar flow hood with consistent disinfection after each petri dish was completed. Disinfection was performed according to the method, using 70 percent ethanol alcohol [13].

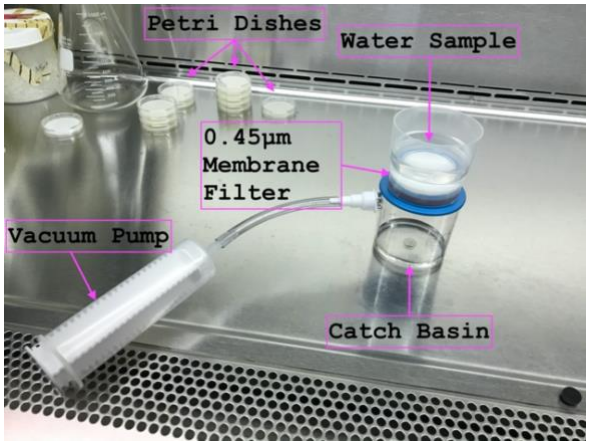

Figure 3-10: Coliscan C ® MF Kit in Use Within the Laminar Flow Hood

For *E. coli* concentrations that were TNTC, a different method was used to count colonies. Colonies from five squares were counted under a microscope, using 10 times magnification to get a representative number of colonies throughout the whole petri dish. To avoid human error, a laboratory counting device was utilized to tally the number of colonies observed. The five squares were then averaged and multiplied by the number of squares on the petri dish to get a reading. This procedure provided a standard way of counting TNTC concentrations for the MF *E. coli* quantification method.

3.6 Biofilter Testing

Figure 3-11 depicts the filter apparatus, consisting of burettes, ring stands, biofilters, a wooden stand, and catch basins. The dotted, solid, and dashed lines outline the burette holding the contaminated water, the biofilter itself, and the catchment basin, a beaker. Again, a computer rendering of this setup can be seen in Figure 3-5.

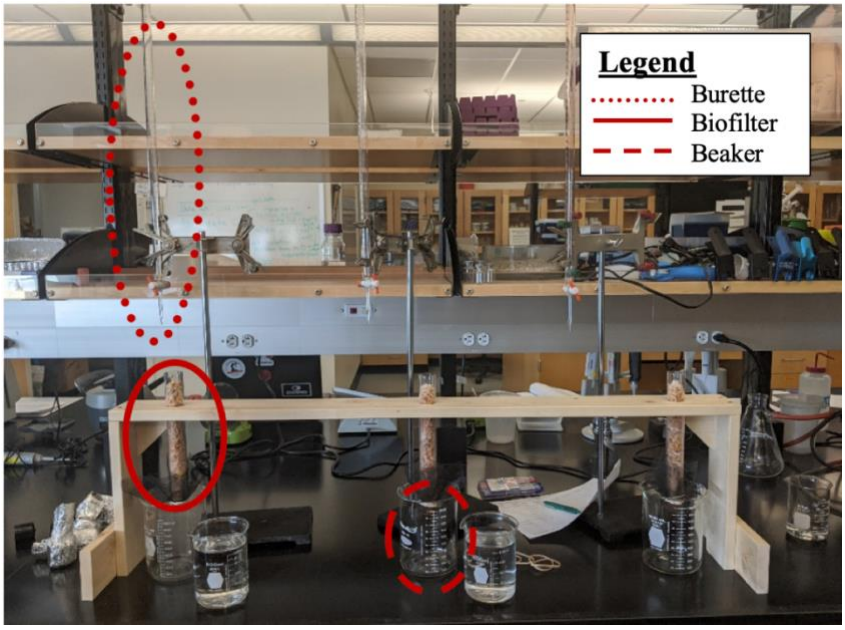

Figure 3-11: Filter Setup

The entire project, except biofilter testing, was performed aseptically to reduce contamination. The testing itself did not practice aseptic technique due to the limited size of the laminar flow hood. Thus, the biofilter testing was done in the open air of the laboratory.

The biofilter testing process started with a preliminary flush of 600 mL of RO water through the filter. 600 mL was chosen because it was roughly five times the volume of the packed media within the biofilter. The flush was meant to both dislodge any loose material in the filter and saturate the media with water to keep a constant flow rate of fluid throughout the testing process. To conserve time, the flushed water was applied directly into the filter, versus through the burette. As soon as water stopped coming out of the base of the filter, when the water flowed less than one drop every 10 to 15 seconds, the contaminated SWW was then sent through the respective biofilters.

The flowrate of each burette was standardized to one mL per second (mL/s). This was done by turning the stopcock, seen in Figure 3-11, to get the preferred flow. This was tested by filling water up to the top of the burette, then recording the time it took for the

burette to drain down to the 20 mL tick. The burettes were set to drain 20 mL in 20 seconds, plus or minus half a second.

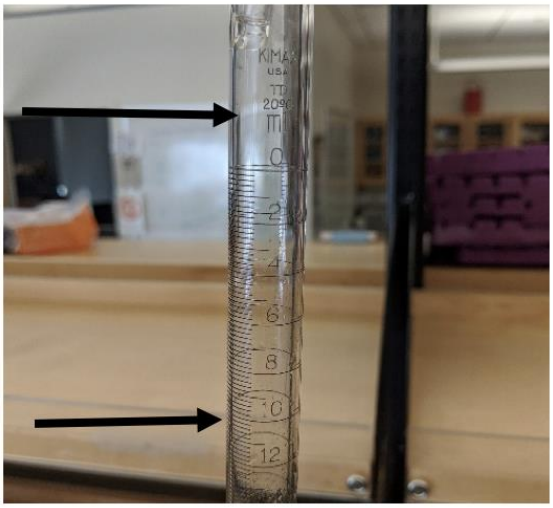

Figure 3-12: Burette Target Head

For a similar reason to the flushing volume, 600 mL of SWW was sent through each biofilter. Since the burettes were only 100 mL, they needed to be constantly filled. The tester applied the water at the top of the 100 mL burette, with a small funnel for support. To keep a constant head on the burette, the water level was kept between the zero and 10 mL tick on the top of the burette, as seen in Figure 3-12. This process was continued until all 600 mL had been passed through the filter. Again, the filter was run until less than one drop of water fell every 10 to 15 seconds. Figure 3-13 shows water dripping into the filter, flowing through, and water dripping out of the filter. It was observed that water flow within the biofilter was influenced differently for each fungal species. For example, residence time differed between the fungal species.

Figure 3-13: Water Flowing Into, Through, and Out of a Biofilter

3.7 Quantifying *E. coli* in Biofilter Effluent

After the biofilters were tested, the concentration of the effluent that was collected in the catch basin was determined in order to compute percent removal from the biofilters. The Coliscan® MF method for the influent concentration testing was also used to find the effluent concentration. Four samples were taken, which included the three replicate filters per species as well as one duplicate. With the four samples, seven petri dishes were prepared to handle two dilution ratios, 1:5 and 1:20. A 1:5 ratio was 20 mL of effluent and 80 mL of sterile RO water. Sterile water was created by autoclaving glass bottles of the RO water. A 1:20 ratio was 5 mL of effluent and 95 mL of sterile RO water. Each

person testing a biofilter, tested that biofilter's effluent. Overall, the person testing the second replicate biofilters took a duplicate sample to ensure quality assurance and quality control (QA/QC). The naming scheme used to label the petri dishes was as follows: the species abbreviation code, the replicate number representing what biofilter was tested, a D for duplicate, or no D if a duplicate was not appropriate, as well as the dilution ratio. For example, if replicate one of *Pleurotus ostreatus* was sampled and a 1:20 dilution ratio was used, the naming label would be PO-R1-1-1:20, which was written on the bench sheet and petri dish lid. See Appendix C for bench sheets and raw data. The same procedure highlighted in section 3.5.2 was used to enumerate the concentration of *E. coli* in the effluent. Figure 3-14 is an example of used, labeled petri dish samples that were labeled accordingly with the naming scheme.

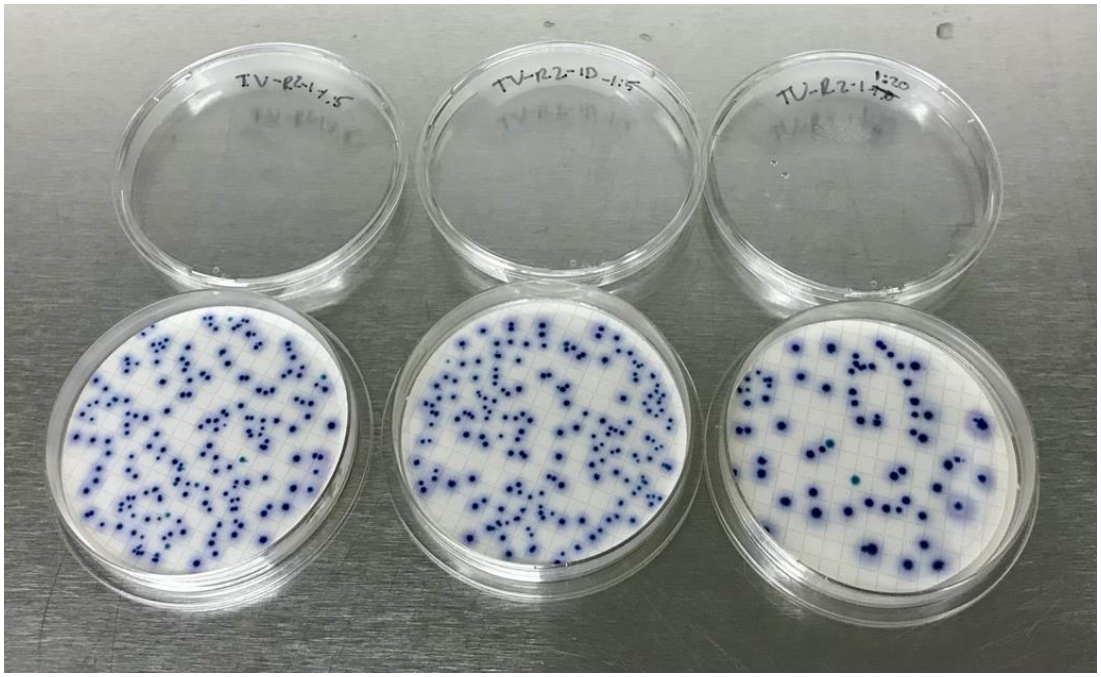

Figure 3-14: Naming Scheme Example for Samples

3.8 Analytical Methods

The analytical methods for the analysis of raw and processed data are explained in the following subsections.

3.8.1 Percent Removal

The percent removal was computed based on influent (Cin) and effluent (C_{out}) SWW *E. coli* concentrations, as seen in Equation 3-2.

Equation 3-2: Percent Removal [4]

$$\frac{C_{in} - C_{out}}{C_{in}} \times 100\%$$

Where:

C_{in}: Influent *E. coli* concentration

C_{out}: Effluent *E. coli* concentration

3.8.2 Statistical Analysis

Equation 3-3 was used to find the mean of a data set, which is an average of all data points taken. It was used based on the sum of all percent removal values for a species, divided by the number of data points.

Equation 3-3: Average

$$\bar{X} = \frac{\Sigma x}{n}$$

Where:
Σx: sum of data point values
n: number of data points
$\bar{X}$: mean of data

Equation 3-4 was used to analyze the standard deviation amongst biofilter replicates and their respective percent removal values.

Equation 3-4: Standard Deviation [14]

$$SD = \sqrt{\frac{\Sigma|x - \bar{X}|^2}{n}}$$

Where:
SD: is the standard deviation

Using the T-Distribution Table, the t-value was the range above and below the mean that statistically counted as quality data [14]. The T-test was performed on all data sets. A T-test was preformed to evaluate the likelihood that the data set was from the same population as the null hypothesis. To perform a T-test, a t-value was calculated based on Equation 3-5. Any data outside that range increased the probability (p), meaning the data could have been random numbers instead of a correlation, which was read from a T-table. This was done by applying a null hypothesis (H_0), which in this case the null hypothesis was no percent removal.

Equation 3-5: t-value [14]

$$t = \frac{|\bar{X} - H_0|}{\frac{SD}{\sqrt{n}}}$$

Where:
t: t-value
H_0: null hypothesis

The t-value was placed into a T-table with its respective degree of freedom to find the p-value. Equation 3-6 was used to compute the degrees of freedom (df). The T-table can be seen in Appendix D. The p-value was compared against a predetermined acceptable level of Type 1 error, α. Type 1 error, also known as a false positive, occurs when a researcher incorrectly rejects the null hypothesis. If the p-value was less than type 1 error, then the null hypothesis was rejected. If the p-value was not less than type 1 error, then the null hypothesis was not rejected, meaning that the resulting data may have just been random numbers.

Equation 3-6: Degrees of Freedom [14]

$$df = n - 1$$

Where:
df: degrees of freedom

4.0 Results

The four subsections below detail the pertinent project results.

4.1 Test times

During the testing process, each species had a slightly different test time. The test time is the time it took for 600 mL of SWW to completely pass through a biofilter. The test times were recorded, as seen in Table 4-1, which was used to compute the actual flowrate (Q_{actual}), velocity (v), and residence time (RT).

Table 4-1: Recorded Test Times for Each Species and Computed Residence Times

Fungi (Code)	**Test Time** (minutes)	**Actual Flowrate (Q_{actual})** (cfs)	**Velocity (v)** (fps)	**Residence Time (RT)** (minutes)
PO R1	12	2.94E-05	4.28E-03	3.24
PO R2	18	1.96E-05	2.85E-03	4.87
PO R3	13	2.72E-05	3.95E-03	3.52
SR R1	12	2.94E-05	4.28E-03	3.24
SR R2	13	2.72E-05	3.95E-03	3.52
SR R3	14	2.52E-05	3.67E-03	3.79
TV R1	79	4.47E-06	6.50E-04	21.36
TV R2	30	1.18E-05	1.71E-03	8.11
TV R3	120	2.94E-06	4.28E-04	32.45
TAs R1	19	1.86E-05	2.70E-03	5.14
TAs R2	19	1.86E-05	2.70E-03	5.14
TAs R3	37	9.54E-06	1.39E-03	10.00
C (-) R1	8	4.41E-05	6.42E-03	2.16
C (-) R2	8	4.41E-05	6.42E-03	2.16
C (-) R3	8	4.41E-05	6.42E-03	2.16
C (+) R1	13	2.72E-05	3.95E-03	3.52
C (+) R2	13	2.72E-05	3.95E-03	3.52
C (+) R3	11	3.21E-05	4.67E-03	2.97

4.2 Percent Removal

For the tested species, *Pleurotus Ostreatus* (PO), *Stropharia Rugosoannulata* (SR), *Trametes versicolor* (TV), *Trichoderma asperellum* (TAs), and controls negative (C(-)) and positive (C(+)), the percent removal was quantified, as seen in Table 4-2. The percent removal was calculated following Equation 3-2. The raw data used to calculate the percent removal was retrieved from in-lab bench sheets, as seen in Appendix C. The influent and effluent concentrations were quantified following the methods previously described.

Table 4-2 Percent Removal for Tested Fungal Species

Species	**Filter Replicate**	**Influent Concentration**	**Effluent Concentration**	**Percent Removal**
(code)	(replicate number)	(CFU/100mL)	(CFU/100mL)	(%)
11.5	R1	670,950	173124	74
PO	R2	670,950	150199	78
PO	R3	670,950	177660	74
SR	R1	0	0	0
SR	R2	10	0	100
SR	R3	0	0	0
TV	R1	1173	0	100
TV	R2	1173	932	21
TV	R3	1173	0	100
TAs	R1	0	0	0
TAs	R2	0	0	0
TAs	R3	0	0	0
C (-)	R1	325	4636	-1326
C (-)	R2	325	4283	-1218
C (-)	R3	325	3891	-1097
C (+)	R1	0	0	0
C (+)	R2	0	8	-100
C (+)	R3	0	0	0

Figure 4-1 represents the percent removal data gained from biofilter testing. Each filter type was placed next to each other and labeled with their species code abbreviations. If a filter had a negative percent removal, this represented an increase of *E. coli*. TAs did not

have a value because there was no change in concentration of *E. coli*. Both controls, C(+) and C(-) had a negative removal. The bar chart was truncated to negative 25 percent.

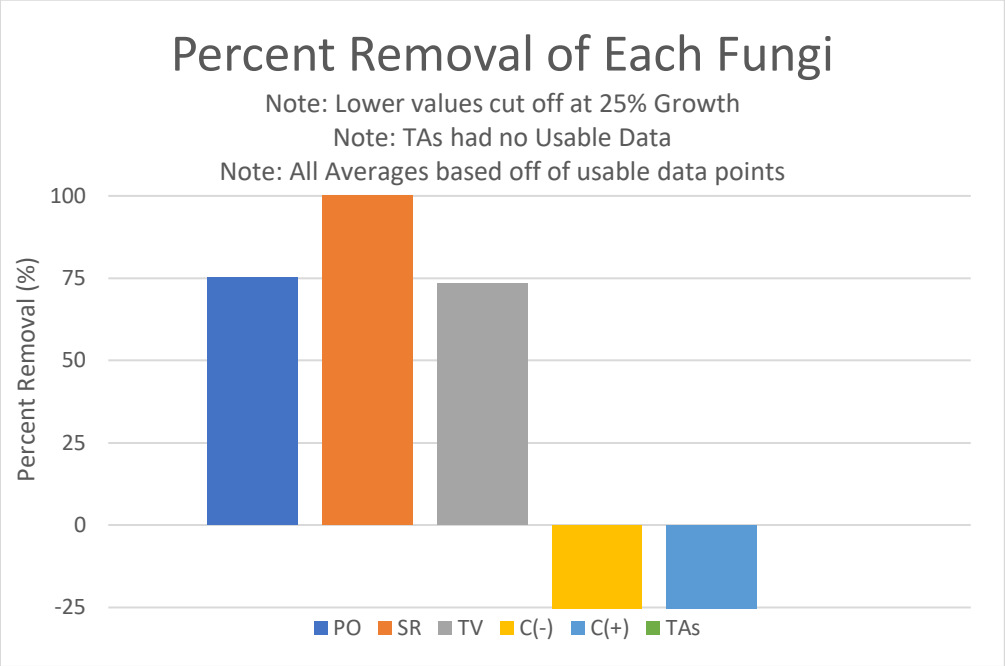

Figure 4-1: Percent Removal of Each Fungal Species

4.3 Statistical Analysis

Table 4-3 summarizes statistical analysis for each biofilter type. As seen below, average percent removal, standard deviation of data, null hypothesis, t-values, p-values, and whether each data set can reject the null hypothesis is shown for each species. For each filter type, the null hypothesis was zero percent removal. If data has a "N/A", it could not be analyzed for accuracy. Each filter type was compared to a type 1 error of 0.05.

Table 4-3: T-Test Summary

Species	**Average Removal**	**Standard Deviation**	**H_0**	**t**	**p**	**Reject? (α =0.05 df =2)**
(code)	(%)	(unitless)	(%)	(unitless)	(unitless)	(yes, no)
PO	75	2.19	0	59.295	0.0005	yes
SR	100	0	0	N/A	N/A	N/A
TV	74	45.88	0	2.775	0.057	no
TAs	0	N/A	N/A	N/A	N/A	N/A
C (-)	-1214	114.67	0	-18.335	0.0023	yes
C (+)	-100	N/A	N/A	N/A	N/A	N/A

4.4 Standardization of Synthetic Wastewater

Trials were performed to standardize the concentration of *E. coli* in the influent SWW. As seen in Appendix E, multiple ratios of stock *E. coli* to RO water were tested, with varying dilution methods. The table of data in Appendix E has columns for data such as the date the data was collected, a short description of the dilution and ratio of stock *E. coli* to RO water, the name of the sample (which coincided with the name written on the petri dish), colonies counted 18-24 hours after performing the MF testing, concentration which was calculated based on the colonies counted and the dilution used for the *E. coli* MF quantification method.

5.0 Discussion

The discussion section is composed of the following subsections.

5.1 Biofilter Testing

During testing, different biofilters had different residence times, as seen in Table 4-1. Typically, each species had similar residence times, with the exception of TV and TAs. Furthermore, replicates one and three of TV had the longest residence times, at 21 and 32 minutes. Observing the different species of fungi, PO appeared to have the thickest hyphae growing throughout the biofilter, but TV produced the longest residence time. According to the team's lab advisor, Ron Deckert, TV tends to produce hydrophobic barriers, therefore increasing the retention time. However, the residence time of replicate two of TV was much lower than the other replicates, which also showed a lower percent removal, seen in Table 4-2. It was suspected that the flow of SWW through replicate two of TV began channeling, meaning that the SWW had less contact with the fungal hyphae. This was considered as a potential reason for the vast difference in data for species TV. Additionally, during the five week fungi growth period, each biofilter was observed for contamination. TV biofilters were observed to have another type fungus growing within the filters. The other fungus was visible to the naked eye, due to its dark greyish color. The type of fungus within the TV filers was not determined, but was suspected to be a common type of airborne fungi which could have been introduced during the filter creation process. Filter replicate two had more contamination than the other two replicates. However, with time the contamination reduced, and the TV fungus was observed to take over each filter completely prior to testing. This contamination may have caused the TV to be less dense in filter replicate two than other replicates. Another species which exhibited variance amongst test times was TAs, as seen in Table 4-1. Replicate three of TAs had a residence time that was double the other replicates. This most likely did not have anything to do with packing, as all the filters were packed the same. The cause of variance in test times for Tas is unknown. Overall, aside from TV and TAs, there was high precision amongst each species testing, although there was variance between each different species, seen in Table 4-1. Considering each biofilter was created identically with respect to initial given nutrients, fungal culture, and Aspen woodchip media amounts, the data demonstrates the differences amongst species.

5.2 Quantifying *E. coli*

The spectrophotometer method for quantifying *E. coli* concentration, as expected, was not highly accurate. However, the spectrophotometer did provide an instant estimate for concentration. An issue encountered was that the spectrophotometer did not differentiate between dead and live *E. coli* cells, thus adding more error to the method. The MF method provided more accurate concentration readings; however, the data showed some anomalies that suggested error. The MF method required dilutions of 1:5 and 1:20 for effluent sample quantification, as explained in Section 3.7. Occasionally the different dilutions would yield highly variable concentration results for the same sample. For example, refer to the bench sheet in Appendix C for "Filter Replicate Number: 2"; petri dishes for TV-R2-1-1:5 and TV-R2-1-1:20 yielded concentrations of 825 and 1200 CFU/100 mL respectively. These dilutions ratios were performed for the same biofilter effluent sample, suggesting that the MF method yielded significantly varying results. Due to the lack of precision in the results between dilution ratios, the accuracy of the method may be questionable. This issue was also exemplified between 1:10 and 1:100 dilutions for the TV influent SWW, as seen in Appendix C.

Another issue arose from using ethanol alcohol for cleaning equipment between MF samples, such as the basin area that holds the water sample seen in Figure 3-10. If excess alcohol remained on the apparatus, it had the potential to kill the *E. coli* in the sample. This issue became noticeable early in the project and appeared as if the filter paper ink were blurred, as seen in Figure 5-1. Additionally, the *E. coli* growth on the sample plate was distinctly inhibited, seen by empty space on the plate where no colonies grew. When this issue was recognized, lab technicians were trained to take more care to completely dry equipment between sterilizations with ethanol alcohol.

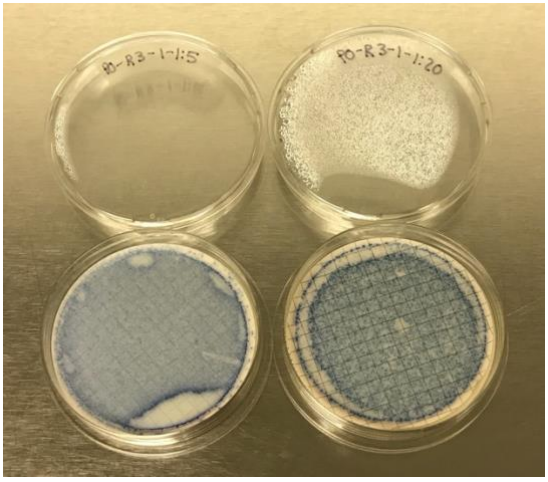

Figure 5-1: Ethanol Alcohol Contaminated MF Sample

5.3 Results

Several filter types had promising results. Figure 3-13 depicted a negative removal of *E. coli* for both control filters. This suggests there was little removal due to sorption by either the media the fungi was grown on or the fungal hyphae. However, the data for the fungal hyphae in C (+) was not as strong. This needs further testing to gain a better understanding of the impact of fungal hyphae on *E. coli* removal.

The same can be said for TAs. There was not enough viable data to accept the findings from this fungal biofilter. Additionally, the same can be said for SR, even though there was 100 percent removal, it was only for one influent concentration data point, as seen in Table 4-2. The percent removal for SR was also only a decrease of 10 CFU/100 mL. This

means that the SR data could not be treated as accurate because the influent concentration was too low.

The data for C (-) did show a negative growth and proved to have viable data. As seen in table 4-4, the null hypothesis could be rejected. This means that according to the data above, the results did not randomly occur. Thus, the filter media has no significant impact on helping removal of *E. coli.* Altogether, removal of *E. coli* was due to fungal biomass itself.

Species PO showed an average removal of 75 percent. This data can be backed up by table 4-4, showing that PO rejected the null hypothesis. The project's results for PO coincide with past research, as PO has been known to remove *E. coli* from surface water [4, 5]. This data also is an indicator that the *Coliscan*® test method provided accurate results.

Species TV had an average removal rate of 74 percent and was very close to the type 1 error margin of 0.05. This is most likely because of how vastly different the data was. Two of the three tests showed a 100 percent removal, whereas the second replicate only had a 21 percent removal. As explained in section 5.1, the severe difference may be due to the channelization of water through the second filter, causing a lower residence time. The data could not statistically reject the null hypothesis, but it was very close to being able to.

Based on the results, PO was the best filter fungi. TV was very close behind PO, but more tests will be needed to determine whether TV is an effective fungal species. The results also show the impact of retention time, and that all *E. coli* removal came essentially from the fungal biomass alone.

5.4 Challenges

Several challenges arose over the course of the project. The first came from cultivating *E. coli* and creating a standardized concentration of SWW. Initial verification of the dilution ratio performed on February 5, 2020, as seen in Appendix D, showed that the *E. coli* was easily cultivated, and that the dilution ratio was too high. Therefore, the results showed that the amount of stock *E. coli* used for making SWW needed to be reduced. Due to time constraints, the team made an educated guess on the ratio of stock *E. coli* to RO water, and performed biofilter testing for species PO and SR without further verification of the *E. coli* SWW procedures. Unfortunately, the testing resulted in differing concentrations for the influent SWW. The SWW concentrations for PO and SR were higher and lower than the preferred level, even though the method for making the SWW was performed identically. This may have come from the age of the *E. coli* used to create the SWW, which was why it became part of the protocol to use *E. coli* that was cultured within the last two weeks. However, later in the project, the issue of very little *E. coli* in the influent SWW arose again.

To mitigate the errors which occurred with species PO and SR, more time was dedicated to standardizing the influent SWW. As seen in Appendix E, more data was collected on

March 8, 2020, where different ratios of stock *E. coli* to RO water were tested. From this data, the conclusion was that approximately one to two µL of stock *E. coli* to two L of RO water would provide the desired SWW concentration of about 1500 CFU/100mL. This ratio was then adopted to the procedure method for creating SWW for the next species. The method worked for C (-) and TV; however, when following the procedure again the next day for C (+) and TAs, the *E. coli* was zero again. Thus, cultivating a standard amount of *E. coli* was the largest challenge of the project.

As time availed, additional work was performed to standardize the *E. coli* influent SWW concentration. Error may have been easily introduced when pipetting a volume of two µL of stock *E. coli* due to the small amount. As seen in Appendix E, a serial dilution approach was tested later. The serial dilution methods also showed variability in concentration results.

An additional and unexpected challenge arose from the COVID-19 pandemic. At a point, the research lab was closed for non-essential use. Furthermore, time necessary for ordering supplies may have also been impacted. For example, shipping and handling took more time during the pandemic than orders made previously. Additionally, more time had been planned for retesting each biofilter species because poor data was acquired from the first round of testing, but COVID-19 response prevented further use of the lab.

5.5 Project Constraints

The primary project constraints were manpower, time, and equipment availability. The team size of three people constrained how much work got done in one day. For time, the timing of some tasks was underestimated, resulting in a deficit for time. For example, ordering supplies took more time than estimated, due to the procedures for approvals and ordering. Additionally, Task 2.5: Microphotography Initial Proof of Concept, seen in Appendix A, got pushed to later in the schedule. Then, when the lab was closed, the task was not able to be completed [due to COVID-19]. Furthermore, the project was scheduled for about ten months, which was a relatively short amount of time for the project scope. Fortunately, equipment availability had a low impact on the project, as the Gehring Lab in the SLF was equipped with most necessary tools. A couple tools that were not available in the Gehring Lab included burettes and ring stands; however, these items were easily obtained from the EnE Lab with the lab manager's approval.

6.0 Example Field Scale Conceptual Design

The conceptual field scale design is presented and explained in the following two subsections.

6.1 Proposed Field Scale Design

The field scale design was configured based on the project results, past research, and engineering judgement. Appendix B shows results from an extended literature review, which focused on how fungi have been applied in pilot and field scale designs for treating contaminants. The last study shown in the table was most relevant, where fungi was applied in bioretention retention cells along the Dungeness River [5]. However, treating an entire river of water is not considered feasible. Thus, the design is meant for

application at a point source of pollution, such as a culvert or storm drain. The proposed field scale design is shown in Figure 6-1 on the next page and is like a detention basin. Water flows into the basin from a point source and can exit the basin through filter at the opposite end of the basin. The basin was designed as a trapezoid, to minimize the necessity of armoring the side walls, where side slopes adhered to specifications for Coconino County [15]. Additionally, the design provided one foot of freeboard to account for a safety factor. The design does not account for flow lost to infiltration. See Appendix F for all design parameters and methodology regarding trapezoidal cross-section design. Conceptually, the field scale filter could be built from welded steel gridded wire, an additional chicken wire liner (to keep media from washing away), and hardwood chip media. The woodchip media would need to be inoculated with adequate amounts of PO culture, prior to use for treating polluted water.

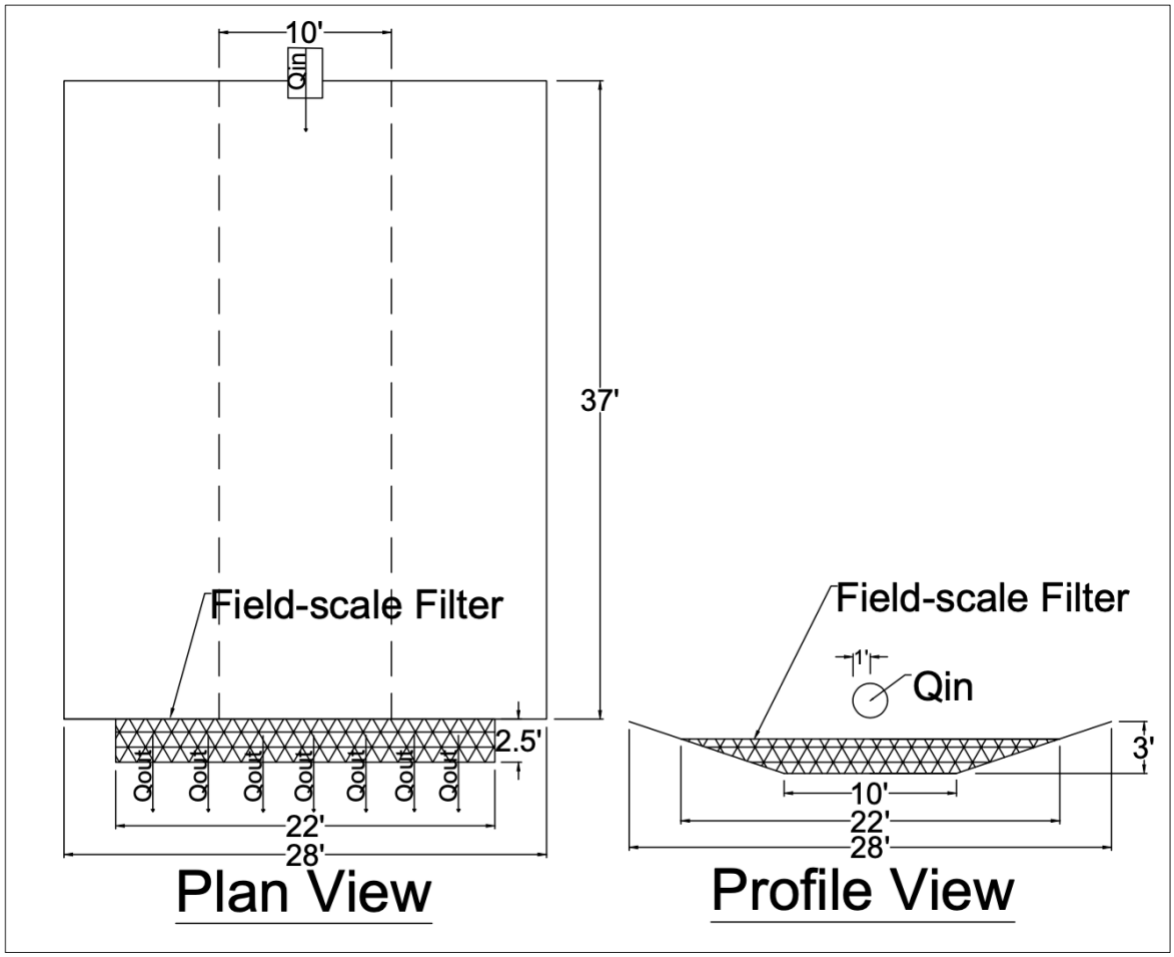

Figure 6-1: Field-scale Design Drawing

Scaling up the experiment based on results of the biofilters started with calculations based off the basin's outlet, known as the "Field-scale Filter" in Figure 6-1, where the media would be inoculated. The basin cross sectional area was then calculated, as seen in Appendix F. Given the biofilter cross sectional area and the flowrate for PO that was observed during testing, the flowrate (Q_{out}) for the field-scale filter was calculated. After the flow rate scale up, the residence time for the field-scale filter was determined to be

about 11 minutes, as seen in Appendix F. A comparison of the residence times of the lab-scale biofilters and the field-scale filter showed that the field-scale filter residence time was over three times greater than the lab-scale. Thus, the performance of fungi within the filter is expected to be comparable, if not better than the lab-scale performance. Thus, the field-scale design should remove at least 75 percent of *E. coli* from the water. Based on the dimensions of the design, the volume for the of fungi-inoculated filter media was calculated to be about 140 ft^3, as seen in Appendix F.

6.2 Field Scale Costs

For the field scale design, there were two categories of cost: maintenance and construction. Construction was based off cut/fill fees, metal grates (welded steel gridded wire), chicken wire and fungal growth. The cut/fill would depend on the company, but on average costs $15 per cubic foot. Grates cost roughly $250 per sheet; the detention basin would need roughly four. The chicken wire used to contain the filter would cost $5 per square-foot, needing roughly 28 square-feet of wire. The wood chips could be obtained from local tree-trimming companies for a low rate. Additionally, the PO fungi culture would require bulking, which can be performed by personnel for low the cost of nutritive media. These costs add up to a construction cost of $950 to $1200. These costs were based off the size of the of the basin and a cut and fill rate of $15 per cubic foot. The low cost comes from the basin being rather small, thus the overall cost is dependent on basin size [16, 17, 18].

The maintenance would be roughly $50 a year. This number is based off general retention basin maintenance costs and filter maintenance. The filter estimate is a rough estimate of an extra $30 for growing fungi. General basin costs are $0.15 per cubic foot for a dry detention basin, totaling $20 for the basin [19].

7.0 Project Impacts

Project impacts were evaluated for the topics outlined in the following subsections. The impacts are based on the results of the project.

7.1 Socioeconomic

A research project tends to have several socioeconomic impacts. A major example is the economic boon to create treatment plants, reactors, or swales for *E. coli* contaminated water. These treatment facilities create jobs, which then fuels the local economy. Two major areas that deal with *E. coli* are recreational water ways and farms. Generally, lettuce at farms get contaminated from the water used to water it. The canal which the irrigation water is pumped from is often contaminated. Therefore, if the amount of *E. coli* can be reduced or removed, millions of dollars in resources could be saved. An example of this is Monterey County, which lost $160 million in lettuce revenue. This was money that could not be spent in Monterey County's local economy. Additionally, this issue also can make people sick from consuming the contaminated lettuce. Arid sections of Arizona with lettuce farms, such as Yuma, deal with this problem as well [20].

This project may lead to economic benefits to an agricultural operation. On the flipside, these treatment plants may become expensive, between the cost of building an entire treatment facility or the manpower needed to create a swale or fill a swale with the filter technology.

Finally, as the data shows, even when several of these fungi do not completely remove *E. coli*, they can remove at least 75 percent. This brings down the amount of other treatments needed to disinfect a water system. For example, a wastewater treatment plant would only need to treat 25 percent of the water stream with expensive chemicals such as chlorine. This is an example of a positive impact.

7.2 Environmental

For this project several environmental impacts should be considered. The first being if the results and recommendations from this study were used in the field, it could ultimately improve stream water quality. Water quality in Oak Creek is a problem especially after heavy monsoon rains where Oak Creek *E. coli* levels spike. A negative impact however could be if the findings from this project were implemented in the field in a way in which the hydrology was altered, and the natural stream flow could be impeded.

If this project were to be tested in the field at different sources and tributaries within a watershed like Oak Creek, creating these basins could disrupt the natural environment and ecosystem. To add to that, introducing a fungus in bulk could have different affects. For example TAs releases numerous spores which could affect the ecosystem. However, all the fungi used are native to Arizona and if implemented in Arizona, the fungi are already part of the ecosystem.

In the lab testing phase of this project, starting with bulking fungi all the way to testing the biofilters, there has been ample amount of waste generated. Waste included plastic disposable pipets, gloves, packaging, Etc. By furthering this research, more waste would be produced, impacting the environment.

7.3 Public Health

E. coli is a bacterium which can cause serious health hazards [2]. Every year, 265,000 people are infected, and 100 people die from *E. coli* [21]. Most people who are infected in the US are infected from eating contaminated greens. If the project design is a success, it could reduce the number of people in the hospital infected with water born *E. coli.* The major public health impact meant to come from the project is to significantly reduce the amount of people who are contaminated at any level, from minor intestinal issues to death.

7.4 Regulations

A fungi that removes *E. coli* from water, such as PO, has impacts for regulations by providing a new technology for controlling pathogenic bacteria. The discharge of pollutants, including *E. coli*, into US waters is regulated by the Clean Water Act (CWA) under the National Pollutant Discharge Elimination System (NPDES) [22]. Under the

CWA, it is illegal to discharge from a point source into US waters without a NPDES permit [22]. With new technology, the limits for pollutant discharge may be affected. There are two types of effluent limits – technology-based effluent limits (TBELs) and water quality-based effluent limits (WQBELs) [22]. The limit set for an individual point source within the NPDES permit is derived from both TBELs and WQBELs. The TBELs are limited by the available technology, and therefore could change with the emergence of a new treatment technology. Quantifying the capacity of fungal species to remove *E. coli* from water demonstrates the potential for its use as a mainstream treatment technology, which could be considered when reviewing available treatment technologies.

Considering the percent removal results of the best fungi, PO, it was determined that the fungi may not treat *E. coli* to the level already set by other non-biological TBELs. TBELs set the treatment limit; however, any control technology may be employed to treat the water to the TBEL [22]. Using fungi is a cost-effective approach to treating biological contamination which may affect its decision for use. The removal rate of PO alone may not treat water to the TBEL; however, at common water reclamation plants, biotechnology is used for primary treatments. Microorganisms are used for the bulk of the water treatment, where other treatments are used later to finalize the process. While PO may not treat 100 percent of *E. coli*, it could serve as the primary step within a treatment process, thus impacting how TBELs and WQBELs are achieved for a NPDES permit. For example, implementing fungi in the form of a biofilter for stormwater quality could be adopted as a best management practice (BMP) for a state [23]. Real-world stormwater management involves multiple systems and takes into account pollutant control effectiveness and cost to obtain the most successful, holistic control strategy [24]. Therefore, the cost-effective nature and percent removal of PO poses a promising tactic for achieving NPDES water quality limits, and PO should be considered for a treatment control BMP.

8.0 Summary of Engineering Work

The engineering work is summarized based on the proposed versus actual hours and schedule. The following subsections provide the details regarding engineering work.

8.1 Personnel Hours

At the beginning of the proposal process, the scope of the project was created as well as the amount of hours the Senior Engineer (SE), Project Engineer (PE), Engineer in Training (EIT), Lab Technician (LT), and Administrative Assistant (AA) would spend on each task within the scope. Tables 8-1 and 8-2, from the original proposal, show the projected hours that would be spent on the project.

Table 8-1: Detailed Projected Project Hours Per Task Part 1 of 2

Tasks	SE	PE	EIT	LT	AA	Total Hours
Task 1: Select Fungi	4	8	19	0	0	31
Task 1.1: Literature Review	1	4	13	0	0	
Task 1.2: Conduct Interview with Mycologist	1	2	2	0	0	
Task 1.3: Decision matrix	2	2	4	0	0	
Task 2: Cultivate Fungi	6	14	36	54	0	110
Task 2.1: Authorize EnE Lab Use	1	8	35	0	0	
Task 2.2: Obtain Fungal Spawn	2	0	1	0	0	
Task 2.3: Fungal Growth	1	5	0	32	0	
Task 2.3.1: Sterilization	0	2	0	6	0	
Task 2.3.2: Inoculation	1	3	0	26	0	
Task 2.4: Sustain Fungi Until Testing Phase	0	0	0	6	0	
Task 2.5: Microphotography Initial Proof of Concept	2	0.5	0	16	0	
Task 3: Design and Construction of Biofilters	4	10	31	11	3	59
Task 3.1: Fabricate Biofilter Apparatus	3	8	27	0	3	
Task 3.1.1: Biofilter Design	3	7	20	0	0	
Task 3.1.2: Purchase Supplies	0	1	7	0	3	
Task 3.2: Integrate Fungal Biomass Into Biofilt. App.	1	2	4	11	0	
Task 4: Loading and Testing Biofilters	3	21	27	210	0	261
Task 4.1: Create *E. coli* Contaminated Water Supply	0	6	7	100	0	
Task 4.1.1: Cultivate *E. coli*	0	1	2	10	0	
Task 4.1.2: *E. coli* Concentration Testing	0	5	5	90	0	
Task 4.2: Test Biofilters	3	15	20	110	0	
Task 5: Data Analysis	6	20	30	6	0	62
Task 6: Evaluate Project Impacts	2	8	20	0	1	31
Task 6.1: Regulations	0.5	2	5	0	0.2	
Task 6.2: Public Health	0.5	2	5	0	0.25	
Task 6.3: Environment	0.5	2	5	0	0.25	
Task 6.4: Socioeconomic	0.5	2	5	0	0.25	
Task 7: Project Deliverables	15	39	85	0	17	156
Task 7.1: CENE 486 Deliverables	5.5	18	40	0	9	
Task 7.1.1: 30% Report and Presentation	1	4	10	0	2	
Task 7.1.2: 60% Report and Presentation	3	7	15	0	4	
Task 7.1.3: 90% Report, Presentation, and Website	1.5	7	15	0	3	

Table 8-2: Detailed Projected Project Hours Per Task Part 2 of 2

Task 7.1.4: Final	3	8	9	0	3	
Task 7.1.5: Website	2	4	8	0	2	
Task 7.2: HURA Deliverables	5	11	30	0	5	
Task 7.2.1: Interim Report for HURA	1		2	0	1	
Task 7.2.2: Final Report	1	5	12	0	2	
Task 7.2.3: HURA Poster Presentations	2	3	8	0	1	
Task 7.2.4: UGRADS Presentations	1	3	8	0	1	
Task 7.3: Publication	4	10	15	0	3	
Task 8: Project Management	19	49	38		36	142
Task 8.1: Resource Management	3	10	0	0	3	
Task 8.2: Client and TA meetings	3	6	10	0	7	
Task 8.3: GI Meetings	1.5	3	5	0	7	
Task 8.4: Team Meetings	6	15	20	0	14	
Task 8.5: Project Schedule Management	5	15	3	0	5	
Sum Of Hours Per Position	**58**	**169**	**286**	**281**	**57**	**851**

Again, Tables 8-1 and 8-2 show that 851 hours were projected to be spent to complete all tasks. Table 8-3 summarizes the actual hours spent on the project. Given that a grand total of 550 hours has been spent on the project, approximately 65 percent of the projected hours have been fulfilled.

Table 8-3: Final Hours Log Per Major Task

Task Name	SE	PE	EIT	LT	AA	Total Hours
Task 1: Select Fungi	0	2	13	4	0	19
Task 2: Cultivate Fungi	0	2	12	50	0	64
Task 3: Design and Construction of Biofilters	0	2	13	3	1	18
Task 4: Loading and Testing Biofilters	0	0	18	106	0	124
Task 5: Data Analysis	2	10	12	0	0	24
Task 6: Evaluate Project Impacts	8	18	14	0	0	40
Task 7: Project Deliverables	25	44	126	0	7	201
Task 8: Project Management	12	12	25	0	12	60
Sum Of Hours Per Position	46	90	233	162	20	550

Again, Table 8-3 shows that between the various roles, 550 hours were actually spent on the project. Most of those hours came from the LT and EIT since the project was highly lab-work oriented and the EIT was the major contributor for outside of lab tasks. For example, tasks two and three, Cultivate Fungi and Design and Construction of Biofilter Apparatuses, required bulking up the fungi and transferring biomass into the biofilter

apparatuses by the LT, whereas the EIT prepared contributed most in Task 7: Project Deliverables. More detail for hours spent on subtasks is shown in Tables 8-4 and 8-5, which also further demonstrates the distribution of hours per role.

Table 8-4: Detailed Final Hours Log Per Task Part 1 of 2

Tasks	SE	PE	EIT	LT	AA	Total Hours
Task 1: Select Fungi	0	2	13	4	0	19
Task 1.1: Literature Review	0	0	13	0	0	
Task 1.2: Conduct Interview with Mycologist	0	2	0	4	0	
Task 1.3: Decision matrix	0	0	0	0	0	
Task 2: Cultivate Fungi	0	2	12	50	0	64
Task 2.1: Authorize Environmental Engineering Lab Use	0	2	10	0	0	
Task 2.2: Obtain Fungal Spawn	0	0		0	0	
Task 2.3: Fungal Growth	0	0	3	48	0	
Task 2.3.1: Sterilization	0	0	0	11	0	
Task 2.3.2: Inoculation	0	0	3	38	0	
Task 2.4: Sustain Fungi Until Testing Phase	0	0	0	0	0	
Task 2.5: Microphotography Initial Proof of Concept	0	0	0	2	0	
Task 3: Design and Construction of Biofilters	0	2	13	3	1	18
Task 3.1: Fabricate Biofilter Apparatus	0	2	13	3	1	
Task 3.1.1: Biofilter Design	0	2	6	3	0	
Task 3.1.2: Purchase Supplies	0	0	7	0	1	
Task 3.2: Integrate Fungal Biomass Into Biofilter Apparatuses	0	0	0	0	0	
Task 4: Loading and Testing Biofilters	0	0	18	106	0	124
Task 4.1: Create E.coli Contaminated Water Supply	0	0	13	79	0	
Task 4.1.1: Cultivate E.coli	0	0	0	21	0	
Task 4.1.2: E.coli Concentration Testing	0	0	13	58	0	
Task 4.2: Test Biofilters	0	0	5	27	0	
Task 5: Data Analysis	2	10	12	0	0	24
Task 6: Evaluate Project Impacts	8	18	14	0	0	40
Task 6.1: Regulations	2	4	5	0	0	

Table 8-5: Detailed Final Hours Log Per Task Part 2 of 2

Task 6.2: Public Health	1	3	4	0	0	
Task 6.3: Environment	1	6	2	0	0	
Task 6.4: Socioeconomic	5	5	3	0	0	
Task 7: Project Deliverables	25	44	126	0	7	201
Task 7.1: CENE 486 Deliverables	20	36	109	0	4	
Task 7.1.1: 30% Report and Presentation	2	5	18	0	2	
Task 7.1.2: 60% Report and Presentation	5	3	34	0	0	
Task 7.1.3: 90% Report, Presentation, and Website	7	16	23	0	2	
Task 7.1.4: Final	5	8	15	0	0	
Task 7.1.5: Website	1	5	20	0	0	
Task 7.2: HURA Deliverables	2	4	8	0	1	
Task 7.2.1: Interim Report for HURA	0	0	3	0	0	
Task 7.2.2: Final Report	1	2	4	0	1	
Task 7.2.3: HURA Poster Presentations	0	0	0	0	0	
Task 7.2.4: UGRADS Presentations	1	2	1	0	0	
Task 7.3: Publication	3	4	9	0	2	
Task 8: Project Management	12	12	25	0	12	60
Task 8.1: Resource Management	3	2	9	0	2	
Task 8.2: Client and TA meetings	1		3	0	2	
Task 8.3: GI Meetings	1	3	3	0	8	
Task 8.4: Team Meetings	4	2	3	0	0	
Task 8.5: Project Schedule Management	3	6	8	0	0	
Sum Of Hours Per Position	**46**	**90**	**233**	**162**	**20**	**550**

Tables 8-4 and 8-5 allowed for the comparison between hours proposed in Tables 8-1 and 8-2 and the hours spent on completed tasks. For example, Task 1 projected 31 hours, but only 19 hours were spent. The lightest shade of blue represents subtasks. When comparing the other completed tasks, less hours were necessary than projected for Tasks 2 and 3. Alternatively, the hours necessary to complete project deliverables was underestimated. It was originally proposed that Task 7: Project Deliverables would require 156 hours, but it actually took 201 hours. Lastly, Task 2.5 was not completed due to uncountable circumstances dealing with lab closure, which is another reason the hours spent on Task 2 were less than estimated.

8.2 Project Schedule

The proposed project schedule, as seen in Appendix A: Gantt Chart, guided the project throughout its duration; however, some minor changes to the timeframe of tasks were made. The changes are summarized in Table 8-6, showing tasks, including task number, initial deadline, and the actual completion date.

Table 8-6: Final Schedule Change Summary

Tasks	Initial Deadline	Actual Completion Date
Task 2.5: Microphotography Initial Proof of Concept	12/10/2019	Incomplete
Task 3.1.1: Biofilter Design	11/27/2019	1/20/2020
Task 3.1.2: Purchase Supplies	12/6/2019	1/24/2020
Task 3.2: Integrate Fungal Biomass Into Biofilter App.	1/23/2020	1/28/2020
Task 4.1: Create *E. coli* Contaminated Water Supply	3/23/2020	4/8/2020
Task 4.2: Test Biofilters	3/24/2020	3/15/2020
Task 6.1: Regulations	3/26/2020	4/15/2020
Task 6.2: Public Health	3/27/2020	4/16/2020
Task 6.3: Environment	3/28/2020	4/17/2020
Task 6.4: Socioeconomic	3/29/2020	4/18/2020

As seen in Table 8-6, Task 2.5 did not get completed. However, as seen in Appendix A, Task 2.5 was not a critical task, and therefore did not delay any other project tasks. The omission of Task 2.5 did not hinder the final design, although it would have enhanced the overall project results. Task 3.1.1 extended until the final purchase of supplies because the design of the apparatus was dependent upon supplies availability. Purchasing supplies was the primary delay of the project. This was due to an unrealistic proposed deadline and issues with purchasing supplies through the Human Resources (HR) department. Task 3.2 was delayed five days due to the delay of Task 3.1.2. Despite minor delays with the four tasks shown in Table 8-6, the overall project was not delayed. Based on the observed growth rates of the fungi during the inoculation and bulking up phase in Task 2.3.2, the fungi growth time within the apparatuses was projected to be no more than five weeks. Because of this, the biofilter testing was actually completed before the inital deadline. Task 4.1 according to the original schedule was supposed to be completed the last day of scheduled biofilter testing. This task was ongoing all the way to 4/8/2020 because more *E. coli* standardization was needed from the initial biofilter testing, as only two types of filter gave accurate results. Because of this, more time was spent on standardization of *E. coli* performed in the lab. The idea was to completely standardize the *E. coli* concentration methods, then test the biofilters one more time to gain more results. However, again the second round of lab testing was not performed due to lab closure for COVID-19 response. Tasks 6.1 through 6.4 were not completed on time, as

seen in the above table. The reason for this was because more results were desired to aid with the project impacts. Because a second round of testing was not performed, the project impacts were evaluated based on the first round of testing.

9.0 Summary of Engineering Costs

The team estimated that the project would cost roughly $80,000 overall. The breakdown of these estimates is shown in Table 9-1. These costs included payroll for employees, overhead, and supply costs.

Table 9-1: Proposed Project Cost

Project Cost Estimate				
1.0 Personnel	**Classification**	**Hours**	**Rate $/hr**	**Cost ($)**
	Senior Engineer	58	240	$13,920
	Project Engineer	169	120	$20,220
	Engineer in Training	286	100	$28,600
	Lab Technician	281	40	$11,240
	Admin. Assistant	57	20	$1,140
	Total Personnel Cost			$75,120
2.0 Travel		**Person(s)**	**Rate $/Person**	
	2.1 Roundtrip Flights	3	350	$1,050
		Days	**Rate $/Night/Rm.**	
	2.2 Hotel	4	94	$1,128
	2.3 Per Diem	5	55	$825
3.0 Supplies		**# of Items**	**Rate $/Item**	
	3.1 Fungal Spawn	5	25	$125
	3.2 Biofilter Materials			
	3.2.1 Apparatus Materials			$200
	3.2.2 Filter Media	1	5	$5
	3.3 Coliscan Kits	2	72	$144
4.0 Fees		**Days**	**$/Day**	
	4.1 Laboratory Use	15	75	$1,125
5.0 Total				**$79,722**

In table 9-2, a sum of the actual projects costs can be seen. The costs included employee wage, overhead, and materials costs. The project spent a grand total of $53,694. This is roughly 65 percent of the estimated amount. Therefore, the project was finished under-budget, saving an estimated 35 percent of project costs.

Table 9-2: Actual Project Cost

Actual Project Cost				
1.0 Personnel	**Classification**	**Hours**	**Rate $/hr**	**Cost**
	Senior Engineer	46	240	$ 11,040
	Project Engineer	90	120	$ 10,800
	Engineer in Training	233	100	$ 23,300
	Lab Tech	162	40	$ 6,480
	Admin. Assistant	20	20	$ 400
	Total Personnel			$ 52,020
2.0 Travel		**Person(s)**	**Rate $/Person**	
	2.1 Flights	0	350	$ -
		Days	**Rate $/Day**	
	2.2 Hotel	0	288	$ -
	2.3 Per Diem	0	55	$ -
3.0 Supplies		**# of Items**	**Rate $/Item**	
	3.1 1 1/4" OD x 1/18" ID 10" Clear Polycarbonate Tubing	2	$20	$ 41
	3.2 Tube cutting	1	$56	$ 56
	3.3. Aspen Chips	1	$10	$ 10
	3.4 1 1/4" Tube Caps	1	$21	$ 21
	3.5 1 1/8" Tube Caps	1	$18	$ 18
	3.6 2 ft 1 1/4" Tube	1	$7	$ 7
	3.7 Silicon	1	$5	$ 5
	3.8 Bleach	1	$2	$ 2
	3.9 Coli plates	8	$104	$ 832
	Total Supplies			$ 999
4.0 Fees		**Days**	**$/Day**	
	4.1 Lab Use	9	$75	$675
	Total Lab Use			$675
5.0 Total				**$53,694**

Personnel, travel, supplies and fee costs were detailed in Table 9-2, again showing that the project was completed under the estimated budget. The biggest cost difference was derived from less personnel hours spent than estimated, which drove down the personnel cost significantly. Again, the personnel hours were estimated at 851 but only 550 hours were spent. Considering the project was completed with a team-size of three individuals, the hours spent are significant. Additionally, the trip to Bozeman, MT for the National Conference on Undergraduate Research (NCUR) was canceled [Due to CVID-19]. Therefore, no travel costs were incurred. The supplies costs were slightly underestimated, as seen in Tables 9-1 and 9-2. This was due to needing more

Coliscan® MF kits than expected initially. It was estimated that four kits would be used, when the project actually required eight Coliscan® MF kits. Finally, the laboratory use fees were slightly overestimated. This was due to three LTs working in the lab together on lab rental days, instead of one LT working per rented lab day. This maximized productivity within the laboratory on days the lab was used.

10.0 Recommendations

The project recommendations are detailed in the following subsections.

10.1 Highest Performing Fungi

Based on the project results and the statistical analysis, seen in Tables 4-2 and 4-3, the highest performing fungi was species PO (*Pleurotus ostreatus*). Furthermore, species PO demonstrated a reasonable residence time, seen in Table 4-1, making it highly applicable for use. For example, species TV (*Trametes versicolor*) had a similar percent removal to PO, but its residence time was much higher, meaning that it would take longer to treat water. Therefore, it is recommended that PO was the best and highest performing fungi of the project.

10.2 Field Scale Implementation

The design proposed in section 6.0 is conceptual and requires site specific modifications. Furthermore, the design is based on laboratory scale results, meaning that the fungal performance is unknown for a larger scale. However, past research showed PO to remove over 90 percent of *E. coli* on a large scale [5]. Therefore, it is expected that the field-scale design would produce reliable results for *E. coli* removal.

To implement the proposed design, it is recommended that a full hydrologic and hydraulic analysis is performed for the site. These analyses would provide the actual incoming flowrate that needed to be treated. Based on that actual incoming flowrate, the basin size could be tailored to the needs of the project. Additionally, the proposed design does not account for infiltration. Depending on the ground water table, a non-permeable liner may be desirable. For example, if the ground water table is two feet below ground surface, an impermeable liner may be desirable to keep polluted water from infiltrating into the groundwater table. For places where groundwater is very far below ground surface, an earthen bottom may be more cost-effective and desirable. While the proposed design in section 6.2 proposes capital and maintenance costs, they are just ballpark estimates. The cost to implement is highly dependent on the site needs, and the overall size of the design. A professional engineer should be consulted before implementing a field scale design.

10.3 Future Research

In the conclusion of this project there is need for more experiments to be done to further this project's results. The biofilters that were tested were only done once. In reality, if this technology was implemented in the field, the fungi would have to be able to have multiple passes of water from different storms. It is recommended to have further research showing the exhaustion of the biofilters after multiple test to see how the fungi

behave. Additionally, the concentration leaving the biofilters were not tested on a timed basis. Therefore, a removal rate was not computable. Thus, additional testing should also focus on effluent concentration at different times in the filtration process. This will allow for the calculation of the filter removal rate, which will help with removal efficiency prediction over time.

While doing the testing, it was unclear the mechanism at which the fungi remove the *E. coli.* One guess at how the fungi remove the bacteria is the fungi looking for alternative nitrogen sources. However, there are many ways in which the fungi could remove the bacteria, which provides an excellent avenue for future research.

A new species of fungi used was *Trichoderma asperellum* (TAs). When testing this species, the testing did not produce results, showing error in the *E. coli* in the water. The same issue was happening for *Stropharia Rugosoannulata* (SR) and the positive control with dead PO fungi (C (+)). More research on *Trametes versicolor* (TV) is recommended because of the promising results from two of the three replicates.

Finally, it is recommended that additional research be performed for creating a standard concentration of *E. coli* in water. This was one of the project's largest problems, which means the issue should be addressed prior to further research dealing with *E. coli* concentrations in water. With a standardized influent concentration, the species will be able to be better compared to each other with statistical analysis.

11.0 Conclusion

The primary project objective was to quantify the capacity of four native Arizona fungi to remove *E. coli* from water within the laboratory. While all biofilters were tested, including two types of control biofilters, the data acquired had considerable error, making half of the results unusable. Therefore, the primary project objective were only half-fulfilled. The project objectives that were not met provide many avenues for future research. One native fungi did prove to be a viable option for implementation, meeting the objective to further research for arid climates such as Arizona. The socioeconomic, public health, regulation, and environmental impacts were evaluated based on the final project results. The project impacts are mainly beneficial. Finally, with the best performing fungi, a field-scale conceptual design was created and proposed. The design offered insight into the capital and operational costs of applying the best performing fungi. Additionally, the field-scale design demonstrated how the best performing fungi could be used in the future for the control of biological contaminants such as *E. coli*.

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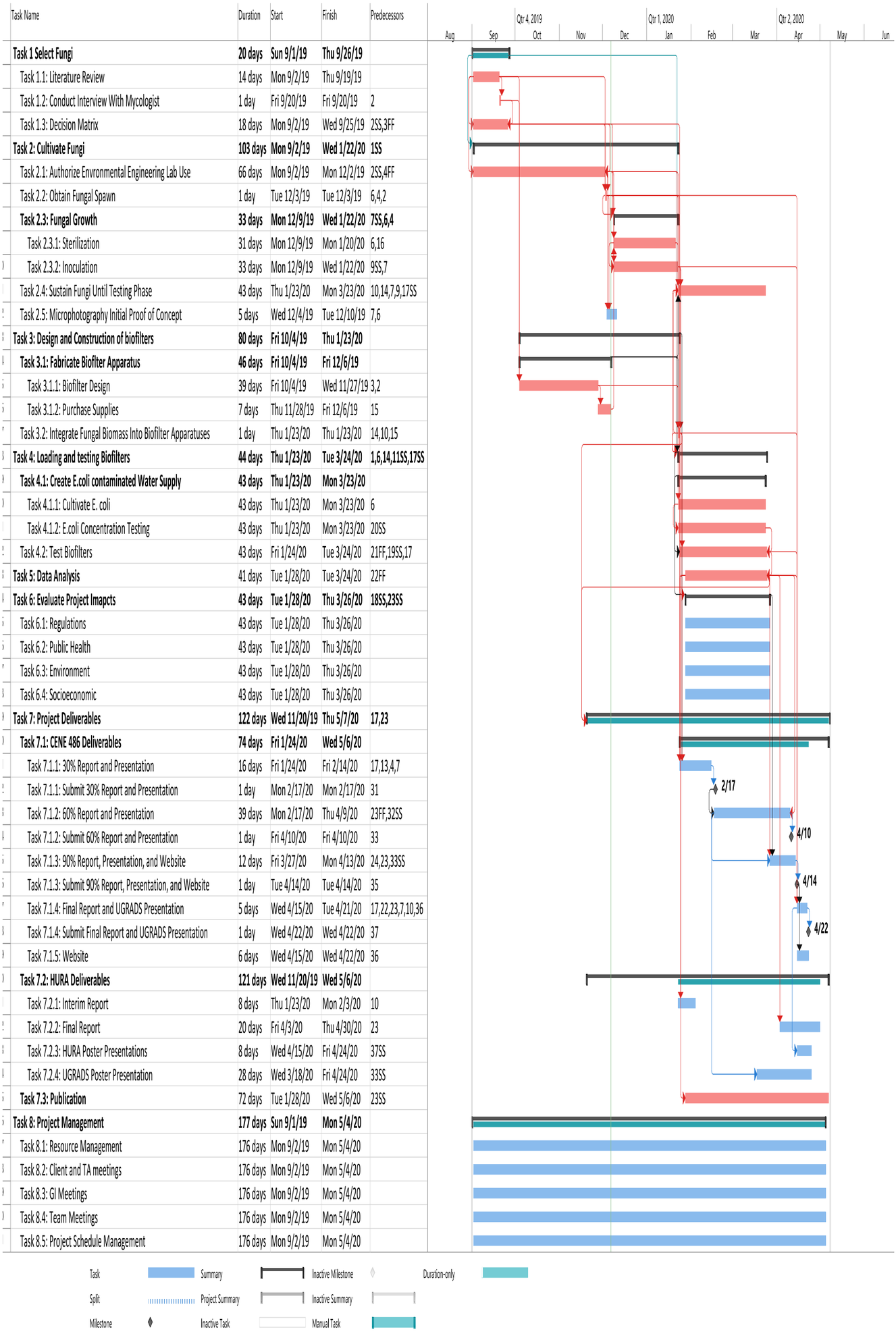
Appendices

Appendix A: Gantt Chart



Task Name	Duration	Start	Finish	Predecessors
Task 1 Select Fungi	**20 days**	**Sun 9/1/19**	**Thu 9/26/19**	
Task 1.1: Literature Review	14 days	Mon 9/2/19	Thu 9/19/19	
Task 1.2: Conduct Interview With Mycologist	1 day	Fri 9/20/19	Fri 9/20/19	2
Task 1.3: Decision Matrix	18 days	Mon 9/2/19	Wed 9/25/19	2SS,3FF
Task 2: Cultivate Fungi	**103 days**	**Mon 9/2/19**	**Wed 1/22/20**	**1SS**
Task 2.1: Authorize Envronmental Engineering Lab Use	66 days	Mon 9/2/19	Mon 12/2/19	2SS,4FF
Task 2.2: Obtain Fungal Spawn	1 day	Tue 12/3/19	Tue 12/3/19	6,4,2
Task 2.3: Fungal Growth	**33 days**	**Mon 12/9/19**	**Wed 1/22/20**	**7SS,6,4**
Task 2.3.1: Sterilization	31 days	Mon 12/9/19	Mon 1/20/20	6,16
Task 2.3.2: Inoculation	33 days	Mon 12/9/19	Wed 1/22/20	9SS,7
Task 2.4: Sustain Fungi Until Testing Phase	43 days	Thu 1/23/20	Mon 3/23/20	10,14,7,9,17SS
Task 2.5: Microphotography Initial Proof of Concept	5 days	Wed 12/4/19	Tue 12/10/19	7,6
Task 3: Design and Construction of biofilters	**80 days**	**Fri 10/4/19**	**Thu 1/23/20**	
Task 3.1: Fabricate Biofilter Apparatus	**46 days**	**Fri 10/4/19**	**Fri 12/6/19**	
Task 3.1.1: Biofilter Design	39 days	Fri 10/4/19	Wed 11/27/19	3,2
Task 3.1.2: Purchase Supplies	7 days	Thu 11/28/19	Fri 12/6/19	15
Task 3.2: Integrate Fungal Biomass Into Biofilter Apparatuses	1 day	Thu 1/23/20	Thu 1/23/20	14,10,15
Task 4: Loading and testing Biofilters	**44 days**	**Thu 1/23/20**	**Tue 3/24/20**	**1,6,14,11SS,17SS**
Task 4.1: Create E.coli contaminated Water Supply	**43 days**	**Thu 1/23/20**	**Mon 3/23/20**	
Task 4.1.1: Cultivate E. coli	43 days	Thu 1/23/20	Mon 3/23/20	6
Task 4.1.2: E.coli Concentration Testing	43 days	Thu 1/23/20	Mon 3/23/20	20SS
Task 4.2: Test Biofilters	43 days	Fri 1/24/20	Tue 3/24/20	21FF,19SS,17
Task 5: Data Analysis	41 days	Tue 1/28/20	Tue 3/24/20	22FF
Task 6: Evaluate Project Imapcts	**43 days**	**Tue 1/28/20**	**Thu 3/26/20**	**18SS,23SS**
Task 6.1: Regulations	43 days	Tue 1/28/20	Thu 3/26/20	
Task 6.2: Public Health	43 days	Tue 1/28/20	Thu 3/26/20	
Task 6.3: Environment	43 days	Tue 1/28/20	Thu 3/26/20	
Task 6.4: Socioeconomic	43 days	Tue 1/28/20	Thu 3/26/20	
Task 7: Project Deliverables	**122 days**	**Wed 11/20/19**	**Thu 5/7/20**	**17,23**
Task 7.1: CENE 486 Deliverables	**74 days**	**Fri 1/24/20**	**Wed 5/6/20**	
Task 7.1.1: 30% Report and Presentation	16 days	Fri 1/24/20	Fri 2/14/20	17,13,4,7
Task 7.1.1: Submit 30% Report and Presentation	1 day	Mon 2/17/20	Mon 2/17/20	31
Task 7.1.2: 60% Report and Presentation	39 days	Mon 2/17/20	Thu 4/9/20	23FF,32SS
Task 7.1.2: Submit 60% Report and Presentation	1 day	Fri 4/10/20	Fri 4/10/20	33
Task 7.1.3: 90% Report, Presentation, and Website	12 days	Fri 3/27/20	Mon 4/13/20	24,23,33SS
Task 7.1.3: Submit 90% Report, Presentation, and Website	1 day	Tue 4/14/20	Tue 4/14/20	35
Task 7.1.4: Final Report and UGRADS Presentation	5 days	Wed 4/15/20	Tue 4/21/20	17,22,23,7,10,36
Task 7.1.4: Submit Final Report and UGRADS Presentation	1 day	Wed 4/22/20	Wed 4/22/20	37
Task 7.1.5: Website	6 days	Wed 4/15/20	Wed 4/22/20	36
Task 7.2: HURA Deliverables	**121 days**	**Wed 11/20/19**	**Wed 5/6/20**	
Task 7.2.1: Interim Report	8 days	Thu 1/23/20	Mon 2/3/20	10
Task 7.2.2: Final Report	20 days	Fri 4/3/20	Thu 4/30/20	23
Task 7.2.3: HURA Poster Presentations	8 days	Wed 4/15/20	Fri 4/24/20	37SS
Task 7.2.4: UGRADS Poster Presentation	28 days	Wed 3/18/20	Fri 4/24/20	33SS
Task 7.3: Publication	72 days	Tue 1/28/20	Wed 5/6/20	23SS
Task 8: Project Management	**177 days**	**Sun 9/1/19**	**Mon 5/4/20**	
Task 8.1: Resource Management	176 days	Mon 9/2/19	Mon 5/4/20	
Task 8.2: Client and TA meetings	176 days	Mon 9/2/19	Mon 5/4/20	
Task 8.3: GI Meetings	176 days	Mon 9/2/19	Mon 5/4/20	
Task 8.4: Team Meetings	176 days	Mon 9/2/19	Mon 5/4/20	
Task 8.5: Project Schedule Management	176 days	Mon 9/2/19	Mon 5/4/20	

Appendix B: Extended Background Research Findings

Location	Contaminant	Reactor Scheme	Removal Rate	Removal Mechanism	Design Parameters	Citation	Fungi Type
Bellingham, WA	Diesel and Heavy Oil in Soil	Mixed test mounds, pilot scale	1035 ppm removed in 17 weeks	Not stated	10 cu-yard test piles on top of impermeable tarp	S. Thomas, P. Becker, M. R. Pinza and J. Q. Word, "Mycoremediation of Aged Petroleum Hydrocarbon Contaminants In Soil," Washington State Department of Transportation, Olympia, 1998.	Not stated
Barcelona, Spain	carbamazepine (CBZ), pharmaceutical in water	Air pulsed fluidized bioreactor operated in batch and continuous mode, pilot scale	continuous mode: 11.9 μg CBZ g−1 dry weight d−1, batch mode: 96% removal in 2d	Aerobic degradation, adsorption	Continuous mode: HRT=3d, batch mode: t=2d, concentration	A. Jelic, C. Cruz-Morato, E. Macro-Urrea, M. Sarrà, S. Perez, T. Vicent, M. Petrovic and D. Barcelo, "Degradation of carbamazepine by Trametes versicolor in an air pulsed fluidized bed bioreactor and identification of intermediates," Water Research, vol. 46, no. 4, pp. 955-964, 2012.	*Trametes Versicolor*
Berlin, Germany	carbamazepine (CBZ), pharmaceutical in water	Non-sterile novel plate bioreactor operated in batch and continuous mode, pilot scale	9,9337,112 mg m−2 d−1, 60% removal with real STP effluent, 80% removal with synthetic effluent	Adsorption (fit Freundlich isotherm)	porosity (of foam plate) =10 PPI, T=34-37˚C, Volume=2L, Evaporation, concentration, Equilibrium reached in 4 hr, addition of nutrients necessary	Y. Zhang and S.-U. Geiben, "Elimination of carbamazepine in a non-sterile fungal bioreactor," Biosource Technology, vol. 112, pp. 221-227, 2012.	*Phanerochaete chrysosporium*
Santiago de Compostela, Spain	Diclofenac, ibuprofen, naproxen, carbamazepine, and diazepam, pharmaceuticals in water	Stirred tank reactors (STRs) and fixed-bed reactors (FBRs) consisting of glass jacketed column with an internal diameter of 4.5 cm and a height of 20 cm	60-90% removal	Adsorption	STR: Volume=2L, HRT=24h, feeding rate, DO, Ph, Temperature=30˚C. FBRs: HRT=24h, feeding rate, T=30˚C,	A. I. Rodarte-Morales, G. Feijoo, M. T. Moreira and J. M. Lema, "Operation of stirred tank reactors (STRs) and fixed-bed reactors (FBRs) with free and immobilized Phanerochaete chrysosporium for the continuous removal of pharmaceutical compounds," Biochemical Engineering , vol. 66, pp. 38-45, 2012.	*Phanerochaete chrysosporium*
South Central Mississippi	Cresote, polycyclic aromatic hydrocarbon in wastewater sludge from wood preserving facility	Testing mounds	Average decreases in 3- and 4-ring analytes of 91 and 45% after 45 days,	aerobic degradation	3x3 m test plots of soil	Davis, M.W., Glaser, J.A., Evans, J.W., and Lamar, R.T. "Field Evaluation of the Lignin-degrading Fungus Phanerochaete Sordida to Treat Creosote-contaminated Soil." Environmental Science and Technology 12 (1993): 2572-576. Web.	*Phaerichaete sordida*
Wood from District Kinnaur and Himachal Pradesh, India	Toxic Dyes: Congo Red, EBT.	Batch tests	92.4% of CR, 50% of EBT	Bioaccmulation, bioabsorbtion, then biodegradation.	25 ml of autoclaved aqueous sol'n with each dye was made, then incubated with the test fungi for 7 days at 24 C. Then the fungi were removed via filter paper. Then a percentage removal was calculated.	R. Kumar, S. Negi, P. Sharma, I. Prasher, S. Chaudhary, J. S. Dhau, and A. Umar, "Wastewater cleanup using Phlebia acerina fungi: An insight into mycoremediation," Journal of Environmental Management, vol. 228, pp. 130–139, 2018.	*Phlebia acerina*
Bratislava Croatia	selenium	Batch tests	70% removal efficiency for lowest concentration of selenium, 60 % removal for 2nd lowest conc, 40% removal efficiency for next, 20%, then 10% removal for 89.9 mg/L of selenium	Bioaccumulation, bio volatilization	Conducted in 250 mL Erlenmeyer flasks. 45 mL of sabourraud dextrose broth added. Conc of 4.2, 22.4, 44.1, 69.9, and 89 mg/L of selenium respectively	Urík, Martin, Katarína Boriová, Marek Bujdoš, and Peter Matúš. "Fungal Selenium (VI) Accumulation and Biotransformation—Filamentous Fungi in Selenate Contaminated Aqueous Media Remediation." CLEAN – Soil, Air, Water 44.6 (2016): 610-14. Web.	*A. clavatus*
Dungeness Watershed, WA	Fecal coliforms and nutrients in water	Bioretention cells with Fungi. Inflow to energy dissipator rocks, to fungi and plant zone, and exit through buried perforated pipe outlet.	nutrients: not viable, fecal coliforms: 97% removal after equilibrium (29 hr)	Biosorption	Flowrate, concentration	S. A. Thomas, L. M. Aston, D. L. Woodruff and V. I. Cullinan, "Field Demonstrations of Mycoremediation for Removal of Fecal Coliform Bacteria and Nutrients in the Dungeness Watershed, Washington," Battelle, Richland, 2009.	*Pleurotus ostreatus, Pleurotus ulmarius, Stropharia rugoso-annulata*

Team Fungi Test Data Bench Sheet

Data collected by: Team Fungi **Data Description:** Synthetic wastewater (SWW) creation data

Creation Date	Creation Time	Species Batch	E. coli in LB Conc.	SWW Conc. Target	SWW Conc. Target	E. coli in LB For SWW	RO water	Notes
(mm/dd/yy)	(hh:mm) (AM/PM)	(species code)	(cells/mL)	(cells/mL)	CFU/100mL	mL	(mL)	
3-6-20	11:15	~~C(-)~~ SR	1.4×10^6	150,000	1500	0.08 mL	2000	E. coli in LB made on 3/5 @ 3PM
3-7-20	Created: 12:20PM Tested: 2:20pM	PO	103×10^6	150,000	1500	0.5 mL	2000	
3-12-20 ~~1:00PM~~	1:00 PM	TV	291×10^6	150,000	1500	1 mL	2000	
3-12-20	10:00 AM	~~SF~~ C(-)	120×10^6	150,000	1500	2 mL	2000	
3/13/20	10:30 AM	C(+)	129×10^6	150,000	1500	3 mL	2000	
3/13/20	11:30AM	Tas	164×10^6	150,000	1500	2 mL	2000	

Note: Use spectrophotometer to quantify "E. coli in LB Conc.".

Additional Notes:

$15 \frac{\text{cells}}{\text{mL}} = 1500 \frac{\text{CFU}}{100\text{mL}}$

Team Fungi Test Data Bench Sheet

Data collected by: Team Fungi **Data Description:** Synthetic wastewater (SWW) *E. coli* enumeration raw data

Collection Date (mm/dd/yy)	Collection Time (hh:mm) (AM/PM)	Sample (name)	Name written on plate? (Name actually written)	Concentration CFU/100mL	Count Date (mm/dd/yy)	Count Time (hh:mm) (AM/PM)	Notes
3-12-20	10:30AM	Sample 1 C(-)-SWW-1:10	C(-)-SWW-1-1:10	570	3/13/20	2:15 PM	
			C(-)-SWW-2-1:10	80	3/13/20	2:15 PM	
3-12-20	10:30AM	Sample 1 C(-)-SWW-1:20	C(-)-SWW-1-1:100	Ø	3/13/20	2:15 PM	
			C(-)-SWW-2-1:100	Ø	3/13/20	2:15 PM	
3-7-20	4:00PM	Sample 1 PO-SWW-1:10 S2	PO-SWW-1-1:10	TNTC	3-8-20	12:30 PM	Appear as Ø on S.P.
			PO-SWW-2-1:10	TNTC	3-8-20	12:30 PM	
3-7-20	4:00PM	Sample 1 PO-SWW-1:~~20~~100 S2	PO-SWW-1-1:100	729,540	3-8-20	12:30 PM	Counted using AVG method
			PO-SWW-2-1:100	612,360	3-8-20	12:30 PM	
3-12-20	1:15PM	Sample 1 TV-SWW-1:10	TV-SWW-1-1:10	1680	3-8-20	2:05PM	
			~~TV-SWW-1:10~~ TV-SWW-2-1:10	540	3-13-20	1:47PM	
3-12-20	1:15PM	Sample 1 TV-SWW-1:20	TV-SWW-1-1:100	1300			
			~~TV-SWW-1:20~~ TV-SWW-2-1:100	0			
3-6-20	1:30PM	Sample 1 SR-SWW-1:10 S2	SR-SWW-1-1:10	0	3/7/20	5:14 PM	Performed in LFH
			SR-SWW-2-1:10	Ø 10	3/7/20	5:14 PM	
3-6-20	1:30PM	Sample 1 SR-SWW-1:~~20~~100 S2	SR-SWW-1-1:100	0	3/7/20	5:14PM	
			SR-SWW-2-1:100	6	3/7/20	5:14PM	
3-13-20	10:50AM	Sample 1 C(+)-SWW-1:10	C(+)-SWW-1-1:10	0	3/14/20	11:25 AM	E. coli conc. issue.
			C(+)-SWW-2-1:10	0	3/14/20	11:25 AM	"
3-13-20	10:50 AM	Sample 1 ~~1:100~~ C(+)-SWW-~~1:20~~	C(+)-SWW-1-1:100	0	3/14/20	11:25 AM	"
			C(+)-SWW-2-1:100	0	3/14/20	11:25 AM	"
3-13-20	12:18PM	Sample 1 TAs-SWW-1:10	TAs-SWW-1-1:10	0	3/14/20	11:25 AM	"
			TAs-SWW-2-1:10	0	3/14/20	11:25 AM	"
3-13-20	12:15PM	Sample 1 ~~1:100~~ TAs-SWW-~~1:20~~	TAs-SWW-1-1:100	0	3/14/20	11:25 AM	"
			TAs-SWW-2-1:100	0	3/14/20	11:25 AM	"

Note: Naming scheme: Species code-synthetic wastewater-Sample number-dilution ratio.

*LFH = Laminar Flow Hood

Team Fungi Test Data Bench Sheet

Data collected by: David Hammond **Filter Replicate Number:** 1

Collection Date (mm/dd/yy)	Collection Time (hh:mm) (AM/PM)	Sample (name)	Name written on plate? (Name actually written)	Concentration CFU/100mL	Count Date (mm/dd/yy)	Count Time (hh:mm) (AM/PM)	Notes
3-12-~~20~~	10:30AM	C(-)-R1-1-1:5	C(-)-R1-1-~~1:5~~ 1:10	TNTC ~9072	3/13/20	2:30PM	
3-12-20	10:30 AM	C(-)-R1-1-1:~~10~~ 20	C(-)-R1-1-1:100	200	3/13/20	2:30PM	
3-7-20	3:30PM	PO-R1-1-1:5	PO-R1-1-1:5	40,068	3-8-20	12:40PM	Appeared as 59 mainly 20, 68, 78 ons.P.
3-7-20	3:30PM	PO-R1-1-1:~~10~~ 20	PO-R1-1-1:20	306,180	3-8-20	12:40PM	
3-12-20	2:15PM	TV-R1-1-1:5	TV-R1-1-1:5	0	3-13-20	1:40PM	
3-12-20	2:15PM	TV-R1-1-1:~~10~~ 20	TV-R1-1-1:20	0	3.13.20	1:40PM	
03/6/2020	12:20 PM	SR-R1-1-1:5	SR-R1-1-1:5	0	3/7/2020	5:14 PM	R1 had a lower flow through, ?
03/6/2020	12:20 PM	SR-R1-1-1:~~1~~ 2 0	SR-R1-1-1:20	0	3/7/2020	5:14 PM	Spectrophot. conc = -0.0264x10^8 (zeroed w/ RO H2O)
3/13/20	11:00AM	C(+)-R1-1-1:5	C(+)-R1-1-1:5	0	3/14/20	11:20AM	E. coli issue.
3/13/20	11:00AM	C(+)-R1-1-~~1:10~~ 1:20	C(+)-R1-1-1:20	0	3/14/20	11:20AM	"
3/13/20	1:10 PM	TAs-R1-1-1:5	TAs-R1-1-1:5	∅	3/14/20	11:25AM	"
3/13/20	1:10 PM	TAs-R1-1-~~1:10~~ 1:20	TAs-R1-1-1:20	∅	3/14/20	11:25AM	"

Note: Naming system: Species code-replicate code-Sample #-Dilution Ratio. Naming Ex: C(-)-R1-1-1:5 means: Control negative-Replicate 1-Sample 1-Dilution Ratio of 1:5. Duplicate=D

Team Fungi Test Data Bench Sheet

Data collected by: Chase McLeod **Filter Replicate Number:** 2

Collection Date (mm/dd/yy)	Collection Time (hh:mm) (AM/PM)	Sample (name)	Name written on plate? (Name actually written)	Concentration CFU/100mL	Count Date (mm/dd/yy)	Count Time (hh:mm) (AM/PM)	Notes
3-12-20	10:30AM	C(-)-R2-1-~~1:5~~ 1:10	C(-)-R2-1:10	~~1300~~ 1,030	3/13/20	2:00PM	
3-12-20	10:30AM	C(-)-R2-1-~~1:10~~ 1:100	C(-)-R2-1-1:100	10,800	3/13/20	2PM	
3-12-20	10:30AM	C(-)-R2-1D-~~1:5~~ 1:10	C(-)-R2-1D-1:10	750	3/13/20	2PM	
3-7-20	4:30PM	PO-R2-1-1:5	PO-R2-1-1:5	TNTC	3-8-20	12:15PM	Appeared as 29, 0.06C8
3-7-20	4:30PM	PO-R2-1-1:~~10~~ 20	PO-R2-1-1:20	150,199	3-8-20	12:15 PM	Technically TNTC but used microscope
3-7-20	4:30PM	PO-R2-1D-1:5	PO-R2-1D-1:5	TNTC	3-8-20	12:15PM	
3-12-20	1:30PM	TV-R2-1-1:5	TV-R2-1-1:5	825	3-8-20	2:01PM	
3-12-20	1:30 PM	TV-R2-1-~~1:10~~ 1:20	TV-R2-1-~~1:10~~ 1:20	1200	3-13-20	1:51PM	
3-12-20	1:30PM	TV-R2-1D-1:5	TV-R2-1D-1:5	~~825~~ 222	3-13-20	1:54PM	
03/06/20	12:20 PM	SR-R2-1-1:5	SR-R2-1-1:5	0	3/7/2020	5:14 PM	Spectrophotometer conc = ~0.0186x10^8 cells/mL (zeroed w/ RO H_2O)
03/06/20	12:20 PM	SR-R2-1-1:~~10~~ 20	SR-R2-1-1:20	0	3/7/2020	5:14PM	
—	—	SR-R2-1D-1:5	—	—	—	—	—
3-13-20	~~10:45~~ 11:10 AM	C(+)-R2-1-1:5	C(+)-R2-1-1:5	5	3/14/20	11:15 AM	E. coli conc. issue
3-13-20	~~10:45~~ 11:10 AM	C(+)-R2-1-~~1:10~~ 1:20	C(+)-R2-1-1:20	20	3/14/20	11:15 AM	"
3-13-20	~~10~~ 11:10 AM	C(+)-R2-1D-1:5	C(+)-R2-1D-1:5	0	3/14/20	11:15AM	"
3-13-20	12:45PM	TAs-R2-1-1:5	TAs-R2-1-1:5	0	3/14/20	11:15AM	"
3-13-20	12:45 PM	TAs-R2-1-~~1:10~~ 1:20	TAs-R2-1-1:20	0	3/14/20	11:15AM	"
3-13-20	12:45 PM	TAs-R2-1D-1:5	TAs-R2-1D-1:5	0	3/14/20	11:15AM	"

Note: Naming system: Species code-replicate code-Sample #-Dilution Ratio. Naming Ex: C(-)-R1-1-1:5 means: Control negative-Replicate 1-Sample 1-Dilution Ratio of 1:5. Duplicate=D

Team Fungi Test Data Bench Sheet

Data collected by: Mishael Umlor **Filter Replicate Number:** 3

3

Collection Date (mm/dd/yy)	Collection Time (hh:mm) (AM/PM)	Sample (name)	Name written on plate? (Name actually written)	Concentration CFU/100mL	Count Date (mm/dd/yy)	Count Time (hh:mm) (AM/PM)	Notes
3-12-20	10:30 AM	C(-)-R3-1-~~1:5~~ 1:10	C(-)-R3-1-1:10	~7182 TNTC	3/13/20	2:00 PM	
3-12-20	10:30 AM	C(-)-R3-1-~~1:10~~ 1:100	C(-)-R3-1-1:100	600	3/13/20	2:00 PM	
3-7-20	4:45 PM	PO-R3-1-1:5	PO-R3-1-1:5	TNTC	3-8-20	12:35 PM	Approx as 49, 59 on S.P.
3-7-20	4:45 PM	PO-R3-1-~~1:10~~ 1:20	PO-R3-1-1:20	177,660	3-8-20	12:35 PM	
3-12-20	3:00 PM	TV-R3-1-1:5	TV-R3-1-1:5	0	3-13-20	1:45 PM	
3-12-20	3:00 PM	TV-R3-1-~~1:10~~ 1:20	TV-R3-~~1:10~~ 1:20	0	3-13-20	1:45 PM	
3-6-20	12:20 PM	SR-R3-1-1:5	SR-R3-1-1:5	0	3/7/2020	5:14 PM	
3-6-20	12:20 PM	SR-R3-1-~~1:10~~ 1:20	SR-R3-1-1:20	0	3/7/2020	5:14 PM	
3/13/20	11:15 AM	C(+)-R3-1-1:5	C(+)-R3-1-1:5	0	3/14/20	11:22 AM	E. coli conc. issue
3-13-20	11:15 AM	C(+)-R3-1-~~1:10~~ 1:20	C(+)-R3-1-1:20	0	3/14/20	11:22 AM	"
3-13-20	1:00 PM	TAs-R3-1-1:5	TAs-R3-1-1:5	0	3/14/20	11:22 AM	"
3-13-20	1:00 PM	TAs-R3-1-~~1:10~~ 1:20	TAs-R3-1-1:20	0	3/14/20	11:22 AM	"

Note: Naming system: Species code-replicate code-Sample #-Dilution Ratio. Naming Ex: C(-)-R1-1-1:5 means: Control negative-Replicate 1-Sample 1-Dilution Ratio of 1:5. Duplicate=D

Appendix D: T Distribution Table [25]

T Table

Given below are two T-tables (also known as T-Distribution Tables or Student's T-Table). There are two T Tables provided below for you to use depending on whether you're dealing with an one-tailed T-distribution or a two-tailed T-distribution

T Table (One Tail)

DF	A = 0.1	0.05	0.025	0.01	0.005	0.001	0.0005
∞	ta = 1.282	1.645	1.96	2.326	2.576	3.091	3.291
1	3.078	6.314	12.706	31.821	63.656	318.289	636.578
2	1.886	2.92	4.303	6.965	9.925	22.328	31.6
3	1.638	2.353	3.182	4.541	5.841	10.214	12.924
4	1.533	2.132	2.776	3.747	4.604	7.173	8.61
5	1.476	2.015	2.571	3.365	4.032	5.894	6.869
6	1.44	1.943	2.447	3.143	3.707	5.208	5.959
7	1.415	1.895	2.365	2.998	3.499	4.785	5.408
8	1.397	1.86	2.306	2.896	3.355	4.501	5.041
9	1.383	1.833	2.262	2.821	3.25	4.297	4.781
10	1.372	1.812	2.228	2.764	3.169	4.144	4.587
11	1.363	1.796	2.201	2.718	3.106	4.025	4.437
12	1.356	1.782	2.179	2.681	3.055	3.93	4.318
13	1.35	1.771	2.16	2.65	3.012	3.852	4.221
14	1.345	1.761	2.145	2.624	2.977	3.787	4.14
15	1.341	1.753	2.131	2.602	2.947	3.733	4.073
16	1.337	1.746	2.12	2.583	2.921	3.686	4.015

Appendix E: *E. coli* Standardization Data

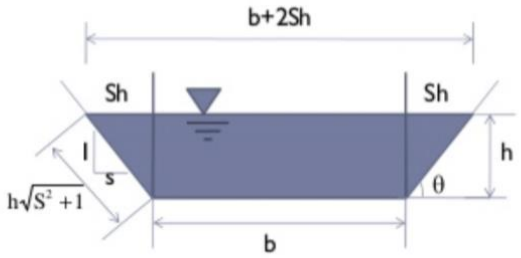
Date	Description	Sample Name	Colonies Counted	Concentration
(mm/dd/yy)	(text)	(code)	(colonies)	(CFU/100mL)
Non-serial Dilutions				
2/5/20	1.8 mL stock *E. coli*: 2L RO water	Trial1 1:10	50	500
2/5/20	1.8 mL stock *E. coli:* 2L RO water	Trial1 1:20	TNTC	TNTC
2/5/20	1.8 mL stock *E. coli*: 2L RO water	Trial1 1:50	TNTC	TNTC
2/5/20	2.16 mL stock *E. coli*: 2L RO water	Trial2 1:10 3/5	TNTC	TNTC
2/5/20	2.16 mL stock *E. coli*: 2L RO water	Trial2 1:20 3/5	33	660
2/5/20	2.16 mL stock *E. coli*: 2L RO water	Trial2 1:50 3/5	20	1000
3/8/20	1 µL stock *E. coli:* 2L RO water	1µL-1:10-3/8	103	1030
3/8/20	1 µL stock *E. coli*: 2L RO water	1µL-1:100-3/8	8	800
3/8/20	10 µL stock *E. coli*: 2L RO water	10µL-1:10-3/8	2600	26000
3/8/20	10 µL stock *E. coli*: 2L RO water	10µL-1:100-3/8	237	23700
3/8/20	100 µL stock *E. coli*:2L RO water	100µL-1:10-3/8	8957	89568
3/8/20	100 µL stock *E. coli*: 2L RO water	100µL-1:100-3/8	19	1900
Serial Dilutions				
4/6/20	2 1/100 serial dilutions, then diluted in 2L	T1-4/6-1:10	0	0
4/6/20	2 1/100 serial dilutions, then diluted in 2L RO water	T1-4/6-1:100	6	600
4/6/20	2 1/100 serial dilutions, then diluted in 2L RO water	T2-4/6-1:10	0	0
4/6/20	2 1/100 serial dilutions, then diluted in 2L RO water	T2-4/6-1:100	1	100
4/7/20	3 1/10 serial dilutions, then diluted in 2L RO water	T3-4/7-1:20	495	9900
4/7/20	3 1/10 serial dilutions, then diluted in 2L RO water	T4-4/7-1:20	0	0
4/7/20	1 1/100 dilution	T5-1/100-1:100	TNTC	TNTC
4/7/20	2 1/100 serial dilutions	T5-1/10000-1:100	TNTC	TNTC
4/7/20	2 1/100 serial dilutions, then diluted in 2L RO water	T5-2L-1:20	251	5020
4/7/20	2 1/100 serial dilutions, then diluted in 2L RO water	T6-2L-1:20	2150	43000

Appendix F: Field Scale Design

Variable	unit	value	Variable Key	Color Legend
Q_{in}	cfs	2	Point source inlet flowrate	Calculated
Q_{tube}	cfs	2.54E-05	Flowrate of lab-scale filter tube	Constant
A_{tube}	ft2	6.88E-03	Area of lab-scale filter tube	Input
L_{Tube}	Ft	0.83	Length of flow through lab-scale filter (thickness)	
V_{tube}	ft/s	3.70E-03	Velocity of flow through lab-scale filter	
RT_{Tube}	min	3.76	Residence time of lab-scale filter	
A_{basin}/A_{tube}	ratio	8145	Ratio of Area of basin to Area of lab-scale filter tube	
Q_{out}	cfs	0.21	Flowrate through fieldscale filter	
h	ft	2	height of fieldscale filter	
b	ft	10	Bottom basin width	
sh	ft	6	Top width of side slope area	
s	ratio	3	Basin side slopes (based on CCDDM, 3H:1V for unprotected sides) [15]	
A_{basin}	ft2	56	Cross-sectional area of fieldscale filter	
T_{drain}	hours	2	Time needed to completely drain detention basin	
V	ft3	1490	Volume of basin without freeboard	
T_{fill}	hr	0.21	Time for basin to fill, dependent on Inlet flowrate	
b+2sh	ft	22	Basin top width	
B_{filter}	ft	2.5	Field scale filter thickness	
V_{filter}	ft3	140	Total volume of filter, a.k.a. fungi volume needed	
L_{basin}	ft	27	Total length of basin	
V_{filter}	ft/s	0.0037	velocity of field scale filter	
RT_{filter}	min	11.28	Residence time of field scale filter	

Trapezoidal Section Equations [26]

- Let's consider a trapezoidal channel having bottom width, b, depth of flow, d, and side slope, S.



$$\text{Cross-sectional area of flow} = A = bh + Sh^2$$

$$\text{Wetted Perimeter} = P = b + 2\sqrt{Sh^2 + h^2} = (A/h - Sh) + 2\sqrt{Sh^2 + h^2}$$

$$A = bh + Sh^2 \qquad b = \frac{A}{h} - Sh$$