Team Fungi
Northern Arizona University & Hooper Undergraduate Research Award

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

CENE476: Capstone Prep

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Table of Contents

Table of Figures

Table of Equations

Table of Tables

Table of Abbreviations

ADEQ: Arizona Department of Environmental Quality
CENE: Civil and Environmental Engineering
CFU: colony forming units
E. Coli: Escherichia coli
EnE: Environmental Engineering
EPA: Environmental Protection Agency
L: liters
m: meters
mL: milliliters
mg: milligrams
NAU: Northern Arizona University
QA/QC: Quality Assurance and Quality Control
RWQA: Recreational Water Quality Act
SLF: Science Lab Facility

1.0 Project Understanding

1.1 Project Purpose

E. Coli is a common pathogen found in surface water. It can cause illness in the populous such as abdominal pain, diarrhea, nausea, fever, and dehydration. When high levels of *E. Coli* are found, facilities by law, must be shut down until levels are at regulation. An example is Slide Rock State Park, which closes regularly during Summer conditions due to a spike in *E. Coli* numbers in Oak Creek. If officials do not catch the *E. Coli* spike; recreational users may get sick [1]. *E. Coli* in fresh and marine water is controlled by Environmental Protection Agency (EPA) and Arizona Department of Environmental Quality (ADEQ) standards. The removal of *E. Coli* from local watersheds and larger surface water bodies is intensely important to the wellbeing of the community.

Fungi are adaptable organisms, which can be used to remediate a plethora of contaminants, nutrients or dyes in a water system for example. Fungi in a biofilter has already been found to reduce the amount of *E. Coli* in a water system [2, 3]. The purpose of this project is to complete a preliminary proof of concept study of Arizona fungi to determine whether any can remove or reduce *E. Coli* from a water system using a biofilter. Local fungi are different because in past studies, the fungi were from more humid, wet environment; as opposed to Arizona's arid environment.

1.2 Project Background

In contaminated water systems such as Oak Creek, *E. Coli* levels of 125 CFU/100 ml or higher have been found [1]. For reference, a stage of high risk is defined as 130 CFU/100 ml or higher [1]. According to the Recreational Water Quality Act (RWQA), the 30 day mean acceptable limit of *E. Coli* is 126 CFU/100 ml, and this is also the level where illness in the populous begins to occur [4, 5].

Currently there is very little peer-reviewed research around the topic of stormwater remediation using fungal biofiltration. There has been research on Pleurotus Ostreatus and Stropharia Rugosoannulata [2, 3].

Paul Stamets lead a study on the removal effectiveness of *E. Coli* using fungi in a mycofiltration column. Paul Stamets' research on storm water mycofiltration using Stropharia managed to yield a removal rate of more than 90% relative to the control media. 10 kg of biofiltration media was placed in an 18.6 L plastic bucket with drain holes drilled out. The media was 75% alder wood chips and 25% rice straw. *E. Coli* was added to previously *E. Coli* free water, then the water was passed through the biofiltration media at a rate of 0.3 L/min. [2]

In 2007 and 2008, a study was held on the Dungeness River in Washington for removing fecal coliforms. Contaminated water was held in 3 m by 9 m bioretention basin. Under 1.5 m of water was a permeable landscape fabric over 15 cm of gravel. In the gravel was a perforated drainpipe. The average outflow through the drainpipe was 11 Liters per minute. Over the fabric was roughly 75 m^3 of Alder mulch. The mulch was inoculated with Pleurotus and Stropharia.

For this study, the water was treated for 6 months and over that time, the basin was shown to remove an average of 90% of the fecal coliforms [3]. These studies are starting points to explore the mycoremediation capabilities of fungi native to arid climates like Arizona.

1.3 Technical Considerations

To conduct quality work, there are many necessary technical considerations to be made. For the proposed project, the following technical topics must be considerations: biofilters, contaminant removal mechanism, testing methods for *E. Coli*, data analysis methods, fungi and bacteria cultivation methods, and health and safety. Technical considerations include important measurement and design parameters and methods.

1.3.1 Biofilters

Biofilters apply microorganisms for the filtration and removal of contaminants in an air or water stream [6]. To design a biofilter, it is important to understand and know the basic elements and parameters of a biofilter. As seen below in Figure 1-1, a schematic is shown of a packed bed biological control system treating volatile compounds [7]. Biofilters contain microorganisms and filter media [6]. The microorganisms create a biofilm around each filter media particle, as seen below in Figure 1-1 [8]. The filter media is usually packed within a vertical column, containing one or multiple stratified layers. When flow with a contaminant is sent through the filter, the filter media and microorganisms capture the contaminant. The contaminant is converted, by a biological process performed by the microorganism, to less harmful products [8].

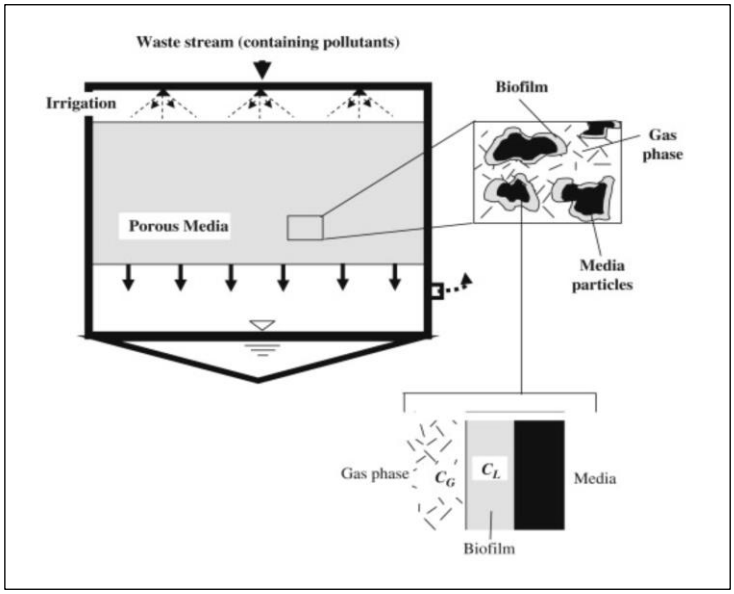

Figure 1-1 Schematic of biofilter [7].

Important parameters for a biofilter include residence-time, flow rate, and filter media size and distribution [9]. The most prevalent issue associated with biofilters include clogging of flow path, which reduces flow rate [10]. Over long-term use of a biofilter, biomass from the microorganisms build up. The biomass may block flow paths, which contributes to clogging,

channeling, and head loss within the system [10]. One suggested clogging control technique includes occasional mixing of the filter media to break up biomass [10].

An example of a biofilter experimental apparatus is shown below, where *E. Coli* contaminated water is delivered to the loading area of the biofilter via a peristaltic pump [11]. The pump ensures a steady delivery of flow, the filter material is sawdust, and a catchment basin is located directly below the vertical column, collecting the outflow. Gravity is the only force conveying the inflow solution through the filter [11].

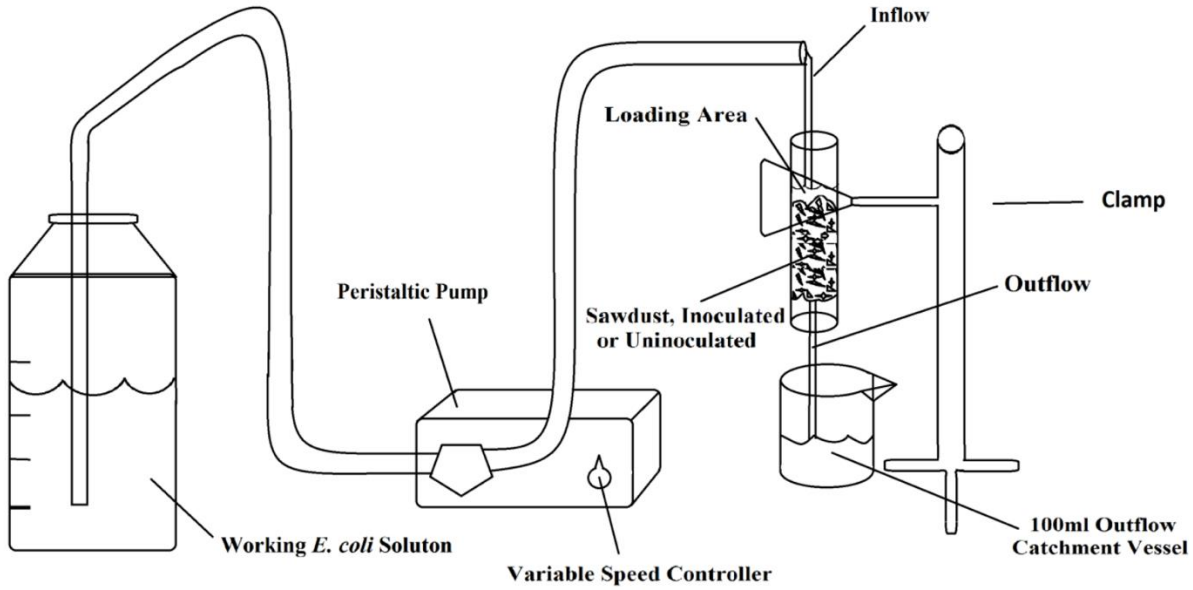

Figure 1-2 Experimental apparatus showing the path of the working solution [11].

1.3.2 Contaminant Removal Mechanism

Depending on the fungi, contaminants may be removed via filtration, biosorption, or chemical reaction. For filtration, the fungal mycelium simply catch the contaminant. Biosorption is defined generally as the ability for living organisms to uptake contaminants via adsorption, ion exchange, transformation, and stabilization [12]. For adsorption, isotherms provide a way of modeling the data. The most common isotherm models are Langmuir and Freundlich [13]. These isotherms use specific experimentally derived equations to fit data and establish a relationship between sorbent and contaminant. If the experimental data does not fit a known isotherm, the isotherm model must be developed from the obtained experimental data.

1.3.3 *E. Coli* Testing: Standard Method (9222)

When analyzing the ability of a designed process to remove a contaminant, the contaminant enumeration method is a crucial part of the experiment. The Standard Method, *9222: Membrane Filter Technique For Members Of The Coliform Group* allows for the enumeration of *E. Coli* [14].

A simple, effective, and affordable test kit, *Coliscan C MF method*, follows the United States EPA approved method, 9222 [2]. The *Coliscan C MF method* simultaneously tests for *E. Coli* and coliforms in water, measured in CFU, and is also an approved method for testing drinking water [15].

1.3.4 Data Analysis

The data generated for the project includes initial and final concentrations of contaminant, *E. Coli*, in water. The *E. Coli* concentration is analyzed in CFU/100mL. The change in concentration is calculated following equation 1 for percent removal, below. The initial and final concentrations denoted C_{in} and C_{out}, are measured using Standard Method 9222.

Equation 1-1 Percent Removal [2]

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

Where:

C: concentration [CFU/100 mL]

1.3.5 Fungi and Bacteria Cultivation Methods

Microorganisms can be cultivated from pure cultures, which are cultures derived from a single organism [16]. Cultures may take many different forms – broth, agar slant, stab, or plate cultures [16]. Cultures are stored and incubated at appropriate temperatures, depending on the specific microorganism.

Air contains many microorganisms such as bacteria, therefore during inoculation or transfer of culture, it is imperative to practice aseptic techniques [16]. Aseptic techniques ensure that other microorganisms do not infect the pure culture. To accomplish aseptic techniques, tools and inoculation media must be sterile [16]. There are many sterilization methods; widely accepted methods include an autoclave and dry heat from Bunsen Burner [16]. After transfer of culture to growing media, the microorganisms shall be allowed to grow at appropriate temperatures and conditions according to the specific preferences of the microorganism.

1.3.6 Health and Safety

Human safety is about physical and mental safety, where project members are not hurt, maimed, or otherwise prevented from living their lives normally due to participating in the project. Maintaining project safety begins with training, staying informed, and following safety protocols. For the proposed project, researchers will be trained in laboratory safety. Project members can stay informed about their working environment, the Environmental Engineering laboratory, through the lab manager, Dr. Terry Baxter, lab assistants, and protocol binders (which contain SDS). Individuals can maintain safety by following proper safety and operating procedures in the lab. Safety is the responsibility of every team member, and it is each person's job to follow procedures to the best of their ability and say something when it is recognized that others are not performing duties safely.

1.4 Potential Challenges

In lab settings, challenges will arise and by having a preset plan for how to overcome each set of challenges, better results are assured. The first challenge the team can face is the fungi not maturing or dying. First, redundancy in project settings are to provide a safety net. Five fungi will be grown and the four healthier, more developed fungi will be used in experimentation. To ensure healthy fungal spawn, cultures will be obtained from reputable sources. Having samples grown at lab standards will mitigate risk of poor growth. Finally, to ensure the optimum growth, the team will carefully attend to the fungi.

Specific fungal substrates may affect the accuracy of final *E. Coli* concentration readings. Issues in prior research found that readings with straw substrate were higher than actual levels of *E. Coli* [2]. This was due to straw interrupting the testing method and skewing results. This challenge will be overcome by not incorporating straw into the biofilter design.

1.5 Stakeholders

There is a wide range of individuals who are affected by the project, therefore categorizing them as stakeholders. The primary stakeholders are the client, Dr. Wilbert Odem, and the investors, Hooper Undergraduate Research Award. Other stakeholders include the team members performing the project, and the people who the project will affect – which for this project is the general public in Arizona and other arid climates.

The client is a stakeholder because they acquisition and have a vested interest in the project outcomes. When funding is involved, there usually are a set of requirements and criteria defining necessary major outcomes of the project. The stakeholder is interested in seeing that the requirements and criteria are met. By meeting requirements and criteria, the client will generally be satisfied with the project.

The project team members are considered stakeholders because the outcomes of the project will affect member's experience and resume. Performing research at an undergraduate level is a resume builder for students. Therefore, each member of the team has a stake in the successful implementation of the project.

Finally, the project outcome – research contributing to the remediation of *E. Coli* contaminated water via biofilters with fungi – affects general public because it may have a future effect on alternative stormwater management in Arizona or other arid climates. By determining which types of fungi remediate *E. Coli*, future research may be performed in the design and application of fungal biofilters within Arizona watersheds. The goal of applying biofilters in watersheds would be to reduce *E. Coli* contamination in rivers, streams, and lakes, allowing the general public to recreate safely in those bodies of water.

2.0 Research Plan

The research plan does not involve field work, as the main objective of the research is to perform a proof of concept, executed within the laboratory. The project tasks are outlined in the following subsections.

2.1 Task 1: Select Fungi

The objective of selecting fungi is to select the appropriate fungi to be tested. A total of four types of fungi will be selected for testing. To do this a literature review, a meeting with a mycologist, and a decision matrix will be completed.

2.1.1 Task 1.1: Literature Review

The literature review involves researching different fungi and selecting species that could be used based on prior research. The objective of the literature review is to determine several species of fungi with potential capabilities for the remediation of *E. Coli*. in water.

2.1.2 Task 1.2: Conduct Interview with a Mycologist

This task involves contacting professional or NAU university mycologists, and requesting an informational interview. Interviewing with a mycologist will allow students to discuss the feasibility of growing and testing selected fungi from the literature review. Furthermore, the mycologist may have suggestions for other applicable fungi. The objective is to come away from the interview with a clearer understanding of the fungi to be chosen.

2.1.3 Task 1.3: Decision Matrix

A decision matrix will be made for specific criteria the team choses, based on overall project needs. The objective of this task is to select five fungi from those deemed eligible via the literature review and mycologist interview.

2.2 Task 2: Cultivate Fungi

The objective of cultivating fungi is to obtain, grow, and sustain selected fungi in preparation for biofilter analysis.

2.2.1 Task 2.1: Authorize Environmental Engineering Laboratory Use

To have a workspace for performing the project, access to the NAU Environmental Engineering (EnE) Lab will be necessary. This task involves obtaining necessary training certifications, compiling a protocol binder, and initiating the process with a request form. Additional laboratory access may include the Science Lab Facility (SLF).

2.2.2 Task 2.2: Obtain Fungal Spawn

The NAU Mycology department and City of Flagstaff (COF) Arboretum will provide fungal spawn.

2.2.3 Task 2.3: Fungal Growth

The objective of fungal growth is to generate a standalone biomass for each fungal species. A fungi will generally take two to four weeks to fully mature.

2.2.3.1 Task 2.3.1: Sterilization

Before any media is inoculated, the media must be sterilized in an autoclave. The SLF and the EnE labs both contain a usable autoclave.

2.2.3.2 Task 2.3.2: Inoculation

The fungal spawn will be inoculated onto the chosen media in the within a laminar flow hood. The Fungi will be stored at the Science Lab Facility building until the media has been fully colonized by each fungus. The Media will then be transported to the NAU EnE Lab and maintained until lab testing in February.

2.2.4 Task 2.4: Sustain Fungi Until Testing Phase

The fungi will be maintained in a container with proper ventilation. Here the fungi will be held at room temperature and a constant humidity to prevent dehydration of the fungi. After two to four weeks, each fungi will produce fungal fruits, which will be removed and disposed to prevent the spread of fungal spores in the lab. The mushrooms will be sterilized and disposed as municipal solid waste. The mushrooms are sterilized to prevent cross contamination from other experiments in the lab. This is necessary because fungal spores are invasive and can spread into ventilation, causing uncontrolled fungal growth in lab vents.

2.2.5 Task 2.5: Microphotography Initial Proof of Concept

The goal of this task is to provide an initial proof of concept that the selected fungi may be used to remediate fungi. This will be completed by taking a sample of each fungi, placing it in a petri dish with a sample of *E. Coli*, then monitoring the two over time. This will identify if, on a microscopic scale, the fungi will consume the *E. Coli*.

2.3 Task 3: Design and Construction of Biofilters

Before testing, the biofilter must be designed and constructed. To achieve the goal of this task, the biofilter apparatus will be designed then constructed to fit the desired expectations.

2.3.1 Task 3.1: Fabricate Biofilter Apparatus

The main objective of fabricating biofilters is to have apparatuses for the testing of fungi. With four types of fungi being tested, and three replicates for each species, there will be a total of 12 biofilter apparatuses will be built. An additional three apparatuses will be built to serve as experimental controls. For example, one of the control biofilters will contain media without fungal biomass, therefore, representing any changes the media have on the filtration of *E. Coli* from the contaminated water stream. The biofilters will be built with purchased supplies, based on the determined biofilter design.

Fabrication of the biofilters will take place outside of the laboratory, and sterilized as necessary prior to the integration of the biological component. It is expected that no specialized equipment or skills will be necessary to fabricate the biofilters beyond those already in possession of Team Fungi.

2.3.1.1 Task 3.1.1 Biofilter Design

Biofilter design includes determining the design parameters, dimensions, shape, and materials. Necessary dimensions to be determined include the volume of biological filter media, desired hydraulic head, desired hydraulic retention time, and catchment basin capable of containing desired volume of flow for each biofilter test. The hydraulic head is the elevation of contaminated water above the filter media, which will cause pressure head driving the liquid through the filter. The necessary parameter to determine is the volumetric flow rate, which will be based on the pore space within biological media and the desired volume of contaminated water per test. Necessary materials will be determined for the biofilter outer structure, and other necessary components to make the filter work. For example, a porous support will be necessary for the filter media to rest upon. A computer aided draft of the biofilter design will be provided as part of the biofilter design task. By completing the biofilter design, a comprehensive plan for necessary dimensions and supplies for the fabrication of the biofilter will be accomplished.

2.3.1.2 Task 3.1.2: Purchase Supplies

Supplies will be purchased using the Hooper Undergraduate Research Award grant, based on the determination of the biofilter design. By purchasing and obtaining all necessary supplies, the biofilter apparatuses may be fabricated.

2.3.2 Task 3.2: Integrate Fungal Biomass into Biofilter Apparatuses

Post fabrication, the biological component, the fungi growing on their respective media, will be placed within the four biofilter apparatuses. This event will take place within the laboratory, following any necessary standard operating procedures. For the control biofilter, sterile filter media will be placed within the fifth apparatus. Media will be representative of the type that the fungi are growing on, and will be sterilized following aseptic techniques. Aseptic technique includes sterilization via autoclaving and performing transfers within a laminar flow hood. An additional control will include a column with fungal biomass that has been killed via sterilization. The purpose of the additional control with fungal biomass is to account for any *E. Coli* removal due to the biomass itself, such as adsorption.

Once the biological component is applied, the apparatuses will be ready for biofilter testing. Until testing, the biofilters will be stored within the lab in a designated, safe location. Safe location means that appropriate storage will be implemented where the biofilters will not cause health and safety hazards, nor will they be problematic for other lab activities in the lab.

2.4 Task 4: Loading and Testing Biofilters

The main objective of biofilter analysis is to quantify the effectiveness of each fungi in removing *E. Coli* from a contaminated water source. To meet this objective, each type of fungi will be tested within its own biofilter apparatus, where the biological media is the specific type of fungi growing on its preferred substrate. The *E. Coli* contaminated water will be run through each filter with a gravity driving force. Data will be acquired for the percent removal of *E. Coli*. Biofilter analysis includes many tasks and sub-tasks, including design, fabrication, purchasing supplies, integrating the biological component (fungi), creating a reliable source of *E. Coli* contaminated

water, cultivating *E. Coli*, and finally testing each biofilter. Only four of the five grown fungi will be tested. The extra fungi provides redundancy in the case of one species lack of growth.

2.4.1 Task 4.1: Create *E. Coli* Contaminated Water Supply

This task involves using an already cultured *E. Coli* sample and contaminating a known volume of water with a known amount of *E. Coli*. The purpose is to create a contaminated water supply to test the biofilters with the fungi.

2.4.1.1 Task 4.1.1: Cultivate *E. Coli*

This Task involves cultivating a sample of *E. Coli* in an agar broth and multiplying the *E. Coli* to get enough to contaminate a known volume of water. The purpose of this task is to cultivate *E. Coli* to test on the biofilter.

2.4.1.2 Task 4.1.2: *E. Coli* Concentration Testing

The objective of *E. Coli* testing is to determine the amount of *E. Coli* in the contaminated water supply, in CFU/100mL. Testing will follow guidelines within Standard Method 9222. The necessary supplies will be purchased from Micrology Laboratories, which are sold in a kit: EPA approved Coliscan® C MF [15].

2.4.2 Task 4.2: Test Biofilters

Testing biofilters will fulfill the objective of obtaining data for the percent removal of *E. Coli* from the contaminated water, for each type of fungi being tested. Furthermore, data will also be acquired for the control biofilter. Again, the control filter will determine any filtration effect of the media alone.

Each biofilter will be tested a series of three times, where each test will involve allowing a set volume of contaminated water to trickle through the filter. Data acquired include the initial and final concentrations (C_i and C_f) of *E. Coli* in units of CFU/100mL of water. The initial concentration will have already been established by task 2.3. In addition, as explained in task 2.3, the determination of the final *E. Coli* concentration will follow Standard Method 9222. By testing each of the biofilters three times each, sufficient data for the percent removal of *E. Coli* will have been obtained, meeting the overall objective of Task 2: Biofilter Analysis.

2.5 Task 5: Data Analysis

Data analysis for this project will include a percent removal equation, equation 1-1, to model the amount of *E. Coli* removed from synthetic wastewater by each type of mycelium. The control biofilter will attest to the amount of *E. Coli* removed by the biomass by quantifying any effects the filter media have on *E. Coli* removal. Task 5 also includes allnk, necessary engineering analysis for the project.

2.6 Task 6: Evaluate Project Impacts

The objective of project impacts are to understand and determine broader impacts the research has on regulations, public health, environment, economy, and society. The fungi selected will be local to Arizona, with potential application for other arid-type climates and riparian locations. Therefore, the broader impacts apply only to Arizona and similar environments. These potential impacts are dependent on the research results. As the research results are determined, an assessment of broader project impacts will be performed and thereby determined.

2.6.1 Task 6.1: Regulations

Stormwater is a typical carrier of *E. Coli*, spreading contamination to other bodies of water [1]. Fungal biomass implemented as biofilters may be a cost-effective biotechnology, impacting the regulation of stormwater.

2.6.2 Task 6.2: Public Health

Due to the undesirable health effects of *E. Coli* poisoning, the reduction of *E. Coli* in public recreational bodies of water may better public health.

2.6.3 Task 6.3: Environment

The potential for reducing *E. Coli* in watersheds would impact the total amount of environmental contamination. This may have an effect on the ecosystem.

2.6.4 Task 6.4: Socioeconomic

Fungi are low cost and durable microorganisms. Implementing the fungi as biotechnology would be cost-effective, therefore costing the community less money to implement than traditional stormwater management.

2.7 Task 7: Project Deliverables

The objective of project deliverables is to provide products to the client. Deliverable tasks are included for CENE 486, HURA, and publication.

2.7.1 Task 7.1: CENE 486 Project Deliverables

These deliverables will be the submittals for CENE 486 including the 30 %, 60% and 90% milestones; the final deliverable; and a website.

2.7.1.1 Task 7.1.1: 30% Report and Presentation

For this deliverable 30% of the final report and presentation will be the objectives. This report will include the plan for the lab analysis as well as fully designed and built biofilters. This task involves completing some of the work for the report. For the presentation, the same info in the 30 % report will be in the presentation.

2.7.1.2 Task 7.1.2: 60% Report and Presentation

This deliverable includes just over half of the work needed for a final report and presentation. Included in the 60 % report and presentation will be lab results and some of the data analysis. The objective of this task is to complete the 60 % report with the lab results and some of the data analysis. The objective of the 60 % presentation is to also have the information included in the report.

2.7.1.3 Task 7.1.3: 90% Report, Presentation, and Website

This task includes 90 % completion of the final report with edits completed from the previous report. This report will also include completion of the lab work and data analysis. Also included for the 90 % report will be the information included from the report into the presentation. A website will also need to be provided and 90 % complete.

2.7.1.4 Task 7.1.4: Final Report and UGRADS Presentation

This Task will be the final presentation (UGRADS) and report deliverable for the work completed for the entire semester. It will include everything completed. The purpose of this task is to show not only results, but the entire process of what took place.

2.7.1.5 Task 7.1.5: Website

This task will involve finalizing the website to highlight the fungi project. The objective of this task will be to update the website; which includes the project deliverables completed, methods, and results.

2.7.2 Task 7.2: HURA Deliverables

The objective of this task is to provide deliverables to the major stakeholder, HURA. These deliverables are outlined in the sub-sections below.

2.7.2.1 Task 7.2.1: Interim Report for HURA

This task involves providing a report for HURA in December to show progress of the research to HURA. This will include writing what has been completed in a report format. The objective of this task is to provide an interim report for HURA.

2.7.2.2 Task 7.2.2: Final Report

This is a report to show proof of concept and analyzed lab results to the client. The objective is to write a formal report showing the work completed. The purpose of this deliverable is to provide the client with a report, which outlines all the tasks and work completed.

2.7.2.3 Task 7.2.3: HURA Poster Presentations

This poster presentation will include making a poster of the research completed and providing it to HURA. Here others that attend the HURA poster presentations will have a chance to see the

research performed. The purpose of the HURA poster is to provide a way to show the audience at the HURA poster presentations the research performed.

2.7.2.4 Task 7.2.4: UGRADS Poster Presentation

This task will include making a poster to present at UGRADS. The objective will be to create a poster highlighting the research completed. The purpose is to present at UGRADS.

2.7.3 Task 7.3: Publication

A manuscript will be prepared and submitted to a journal related to environmental engineering or biotechnology.

2.8 Task 8: Project Management

A chosen engineer, who will oversee setting up meetings, will manage the project; lab times, and submit deliverables to the client. The project engineer will also oversee correspondence with the client instructor and technical advisor. The senior engineer will also set up any extraneous needed activities throughout the semester.

2.8.1 Task 8.1: Resource Management

The project manager will also coordinate and track project resources, such as the project grant funding for material, personnel, and travel expenditures. All expenditures will be recorded and accounted, ensuring the project does not go over budget.

2.8.2 Task 8.2: Client and TA Meetings

Meetings with Technical Advisor (TA) instructor and client will be scheduled and occur weekly, providing project updates. Any pertinent questions that arise will be addressed and discussed with the TA.

2.8.3 Task 8.3: GI Meetings

Meetings with Grading Instructor (GI) will be scheduled and occur bi-monthly. Meetings will serve as a schedule and project update and address any pertinent questions or issues.

2.8.4 Task 8.4: Team Meetings

Team meetings will be planned bi-weekly; this will keep the entire team up to speed, as well as make plans for any issues that may arise.

2.8.5 Task 8.5: Project Schedule management

The project engineer will oversee and update the project schedule as necessary. Any changes to the timeline will be tracked, and project-scheduling efforts will work toward minimizing schedule amendments.

2.9 Exclusions

The following tasks will be excluded from the research plan: field application of the tested fungi, and utilization of batch reactors for testing *E. Coli* removal capabilities of fungi. Field application of the tested fungi means performing any work in the field to apply the fungi to contaminated bodies of water or watersheds within Arizona, the American southwest, or anywhere else outside of the laboratory. Utilization of batch reactors for testing *E. Coli* removal capabilities would mean implementing the use of a batch reactor to determine *E. Coli* capabilities.

It is recognized that the excluded tasks would be included in a thorough research project; however, based on time and funding constraints, these tasks are outside the scope of work for the project.

3.0 Schedule Management

The project schedule was created in Microsoft Project. As seen in the Gantt chart, the time line of the project is approximately eight months. However, the work during December will be the part of fungi growth, in which no work from personnel will take place; instead, that time will be for the growth of the fungi. The project starts early September, with preliminary literature review of fungi and ends with the CENE 486 and HURA deliverables on May 7th, 2020. The deliverables for this project are shown in the schedule, which are the CENE 486 deliverables, which include 30%, 60%, 90% reports and presentations, final presentation and report, and a website. The last date for these deliverables is May seventh. The next set of deliverables are for HURA and are due at different times. These deliverables include an interim report due Nov 29th , HURA Poster presentations due April 24th , UGRADS Poster presentation due April 24th, and a manuscript for publication which is due May 7th. The major tasks include selecting fungi, cultivating fungi, designing and building the biofilters, loading and testing the biofilters, data analysis, evaluating project impacts, project deliverables, and project management.

The critical path is the shortest path to complete all necessary tasks that are linchpins to the rest of the project. The critical path starts with Task 1: Select Fungi. Nothing can be completed until background research and literature review on fungi is completed. After this, Task 2.1: Authorize Environmental Engineering Lab Use, Task 2.2: Obtain Fungal Spawn, Task 2.3: Fungal growth, and Task 2.4: Sustain Fungi Until Testing Phase are critical tasks. Obtaining lab access and selected fungi, and then growing chosen fungi are the next part of the critical path. After this, building the filter in Task 3: Fabricate Biofilter Apparatus is needed to continue lab testing. Once the filter is built, nothing can be tested until after a contaminated stormwater is produced in Task 4.1: Create *E. Coli* Contaminated Water Supply. Task 5: Data Analysis, is a critical task because deliverables, except for the 30% Report and Presentation, must include research data. In order to complete Task 5, Task 4.1.2: *E. Coli* Concentration Testing must be performed to quantify the concentrations. The critical path leads to completion of all milestones and Task 7.3: Publication, as seen in the Appendix: Gantt Chart Schedule. The publication task is the last critical task, which aligns with the project end date on May 7, 2020. If any of the critical tasks discussed above were not completed or delayed, it would put the rest of the project off-schedule, justifying these tasks as the critical path.

Timing and duration of each task in the critical path will be maintained as planned via prioritizing those task items. Tasks along the critical path will be called critical tasks. Critical tasks will take priority over other tasks, ensuring the project is complete on the proposed deadline. For example, if a schedule change must occur, the non-critical tasks would be rescheduled instead of the critical tasks. By maintaining critical tasks so they do not get rescheduled to a later date, the total duration of the project will remain the same. The schedule float will be utilized to complete the non-critical tasks.

4.0 Staffing Plan

This section includes the staff positions and qualifications. The staffing plan provides a means for completing proposed tasks.

4.1 Staff Titles/Positions

The positions with their respective abbreviations for the project are listed below. For logging personnel hours, the position abbreviations will align with the work performed.

1. Senior Engineer: SE
2. Project Engineer: PE
3. Engineer In Training: EIT
4. Lab Technician: LT
5. Administrative Assistant: AA

4.2 Personnel Qualifications

The qualifications for all senior personnel are listed below. These qualifications will ensure the personnel are capable to complete the project on time meeting quality assurance and quality control (QA/QC) objectives.

1. Experiencing using the following software: Microsoft (MS) word, excel, and project, Autodesk AutoCAD
2. Microbiology lab experience
3. Experimental biological and chemical data analysis experience
4. Resource management
5. Project management experience
6. Research publication experience
7. Excellent interpersonal and technical commination skills
8. Leadership experience

Personnel will need to be experienced with the listed software to create computer aided drafting (CAD) designs, perform data and engineering analysis, and generate high quality reports. Software experience is applicable for all personnel, except the administrative assistant (AA). The AA will not need experience with AutoDesk AutoCAD. Microbiology lab experience is an important qualification that will allow for the collection of accurate data. For example, inoculating fungi requires good technique to ensure the culture is not contaminated by other airborne microorganisms during the transfer process. Microbiology lab experience is a necessary qualification for the lab technician (LT), which is the personnel who will be responsible for all lab work. Experimental biological and chemical data analysis experience is required for all personnel except the AA. It is expected that the Engineer in Training (EIT) will perform the bulk

of the raw data analysis, which includes determining the percent removal of *E. Coli*. The Project Engineer (PE) will be responsible for training the EIT on specific project data analyses, and the Senior Engineer (SE) will be consulted when errors or problems arise. The PE will review data and final results from data processing.

Resource management is an important skill which helps keep the project within its budgetary constraints. The SE and PE will have experience with resource management, and the EIT will receive training from the PE. All personnel will be responsible for submitting spending reports, and the PE will utilize those reports to track supply resources. For personnel resources, each personnel will also be required to log and track their hours. If the personnel log more than the allotted hours, the SE and PE will be responsible for making budget adjustments. Therefore, it is very important that all personnel have resource management skills.

The project management qualification applies to the SE and PE. These personnel will be in charge of managing the project. While the SE is the governing project manager, the PE will be in charge of managing field operations. For example, laboratory processes will be managed by the PE, whereas the SE will be in charge of critical project decisions. Similarly, the research publication experience applies to the PE and SE. With qualifying experience, the SE and PE will train the EIT on how to write a manuscript. While the EIT may spend more time writing the manuscript, the PE and SE will be heavily involved with ensuring the manuscript is prepared correctly based on their research publication qualification.

The final two qualifications apply to all personnel. Working on a team requires good communication skills and the ability to be a leader. For the preparation of deliverables such as reports, technical writing skills are paramount. Similarly, to work effectively and efficiently with a team, interpersonal skills are also important. For leadership, this will be mainly required for the SE and PE, but is also applicable for other personnel. The SE and PE will demonstrate their leadership skills when trouble shooting problems, and training the EIT.

4.3 Staffing Table

The following table organizes the amount of hours per position for each task of the project. The total number of hours estimated to complete the project is 851. The staffing table is broken between tables 4-1 and 4-2.

Table 4-1 Staffing Hours Part 1

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	13.5	36	54	0	110
Task 2.1: Authorize Environmental Engineering 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	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		1	7	0	3	
Task 3.2: Integrate Fungal Biomass Into Biofilter Apparatuses	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

Table 4-2 Staffing Hours Part 2

Task 6: Evaluate Project Impacts	2	8	20	0	1	31
Task 6.1: Regulations	0.5	2	5	0	0.25	
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	14.5	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	
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	18.5	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**	**168.5**	**286**	**281**	**57**	**851**

Below is a table of the sum of hours each position will complete throughout this project. The hours for each position are split amongst the positions, where the EIT and LT will log the majority of hours. These personnel have a lower pay rate, and possess the overall knowledge to complete their assigned tasks. The PE and SE will take on more leadership roles, which includes overseeing the project and managing the schedule and resources. Time wise, the SE, PE, EIT, LT, and AA will take up about 7%, 20%, 34%, 33%, and 7% of the total project hours, respectively. The percentages demonstrate the amount of work performed for each position, where the EIT and LT have the most work hours.

Table 4-3 Total Hours per Each Position

Task Name	SE	PE	EIT	LT	AA	Total Hours
Task 1: Select Fungi	4	8	19	0	0	0
Task 2: Cultivate Fungi	6	14	36	54	0	110
Task 3: Design and Construction Of Biofilters	4	10	31	11	3	59
Task 4: Loading and Testing Biofilters	3	21	27	210	0	261
Task 5: Data Analysis	6	20	30	6	0	62
Task 6: Evaluate Project Impacts	2	8	20	0	1	31
Task 7: Project Deliverables	15	39	85	0	17	156
Task 8: Project Management	19	49	38	0	36	142
Sum Of Hours Per Position	58	168.5	286	281	57	851

5.0 Cost Estimate of Engineering Services

Below is an estimate of the cost of engineering services, presented as Table 5-1. This table has five main categories into personnel, travel, and supplies costs, fees, and totals. Rates are taken from federal standards and online catalogs. The total cost to compete this project is estimated to be $79,722, as seen below in Table 5-1.

Table 5-1 Cost Estimate of Engineering Services Table

Cost Estimate				
1.0 Personnel	**Classification**	**Hours**	**Rate $/hr**	**Cost**
	Senior Engineer	58	240	$ 13,920
	Project Engineer	168.5	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			$ 5
	3.3 Coliscan MF Kit	2	72	$ 144
4.0 Fees		**Days**	**$/Day**	
	4.1 Laboratory Use	15	75	$ 1,125
5.0 Total				**$ 79,722**

The highest cost comes from personnel, totaling at $75,120. The reason this is the highest cost is the lab and engineering analysis this project requires. The travel cost comes in at $3,003, which accounts for flights to Bozeman Montana for the 2020 National Conference on Undergraduate Research (NCUR) as well as per diem rates. Per Diem is allotted for daily food and hotel costs. For the Supplies cost, it totals to $474. This section is the lowest cost because only five fungi spawns are being purchased which have a low cost. Built into the supplies also are the Coliscan MF kits, which quantify the concentration of *E. Coli* [15]. Fees accounts for the money required for renting laboratory space. Based on 8-hour workdays, it is projected that a total of 15 days will be spent in the lab.

6.0 References

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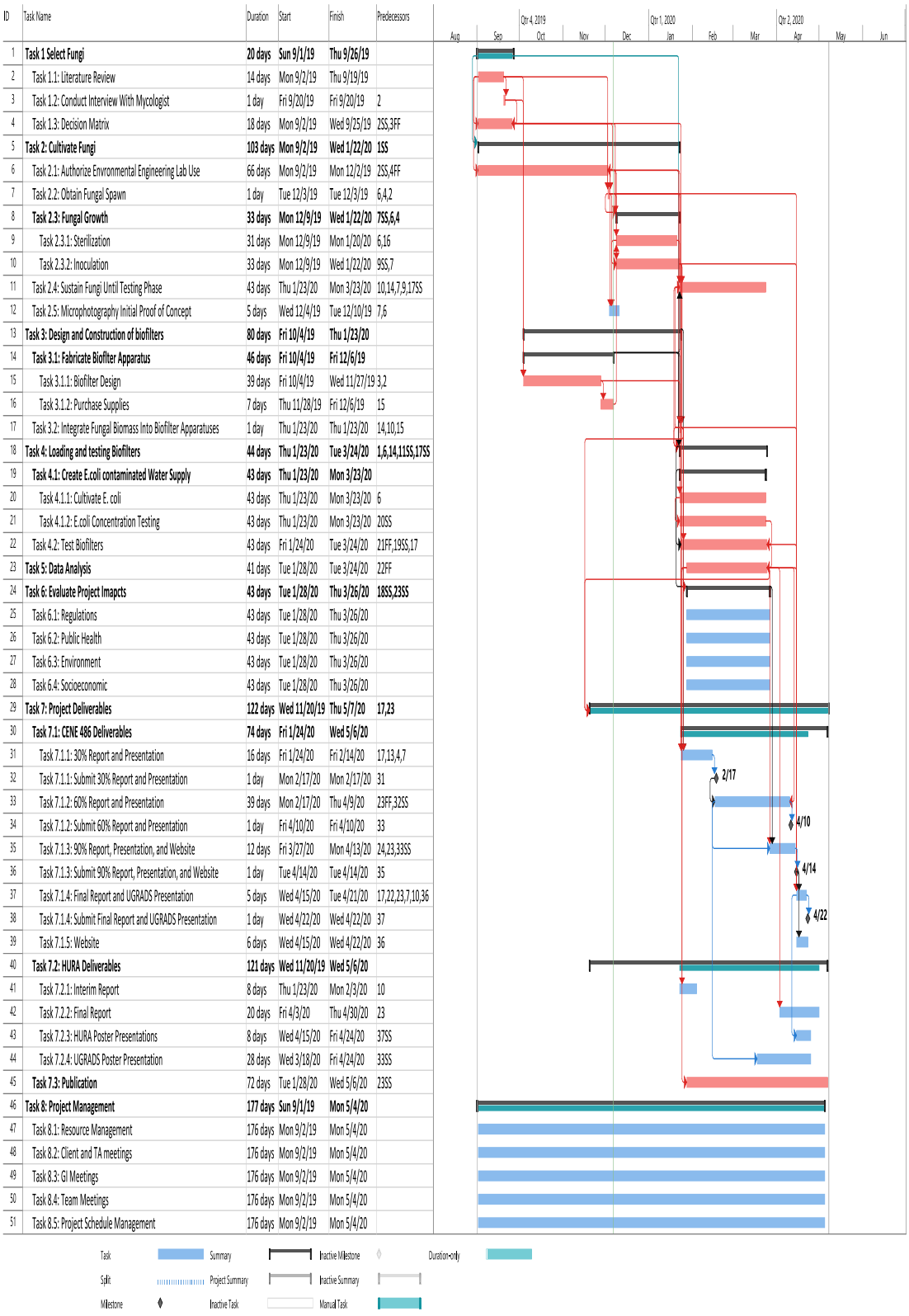
7.0 Appendix: Gantt Chart Schedule



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