

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

CENE 486C: Capstone Project Presentation

By: David Hammond, Chase McLeod, & Mishael Umlor

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1.0 Introduction

- **Objective:** Quantify the capability of Arizona native fungi to remediate *E. coli* from water using biotrickling filters
- **Client & Stakeholder:** Dr. Wilbert Odem & Hooper Undergraduate Research Award (HURA)
- **Location:** Research performed in Science Lab Facility (SLF), Mycology Lab



Figure 1: Team Fungi in the SLF's Laminar Flow Hood

1.1 Background

Past studies proved fungal mycelium, including *Stropharia rugosoannulata* and *Pleurotus ostreatus*, remediate *E. coli* from synthetic storm water in the Northwest [1,2]

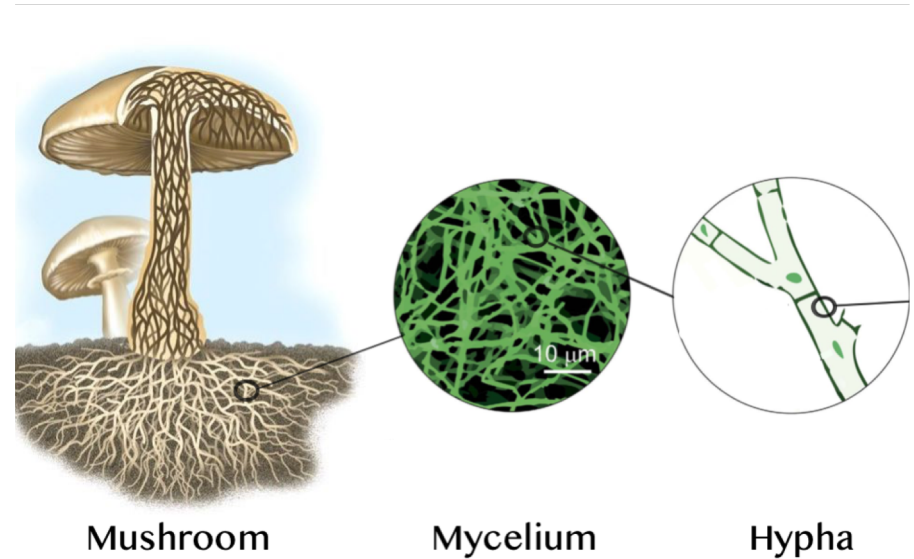


Figure 2: Schematic illustrating mycelium at different scales [3].

1.2 *Escherichia (E). coli* Overview

- *E. coli* is a “Rod Bacteria” found in the small intestine of warm blooded animals [4]
- Majority of *E. coli* are non-pathogenic
- Pathogenic strains may cause nausea, vomiting, diarrhea, and/or death [5]
- *E. coli* is a biological contaminant in surface waters, such as lakes, rivers and streams

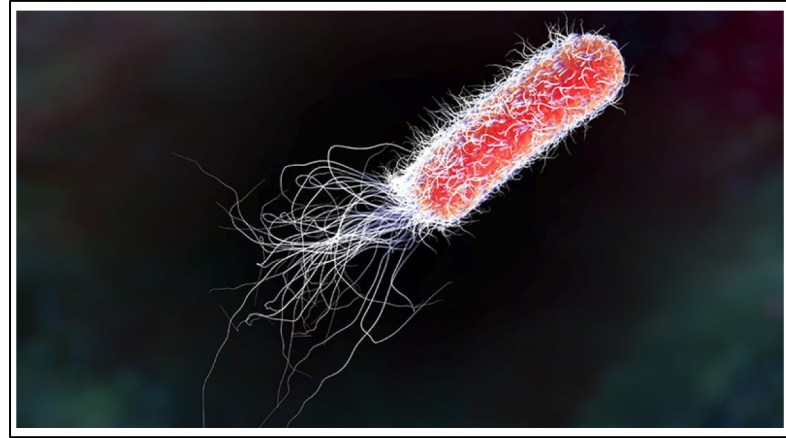


Figure 3: *E. coli* [4]

1.3 Constraints & Exclusions

Constraints

- **Time**
 - Fungi growth time
 - HURA cutoff: April 30th, 2020
- **Fungi**
 - Commercially available native fungi cultures
- **Budget**
 - Manpower
 - Equipment and laboratory use

Exclusions

- **Fieldwork**
 - Fungi was not field-tested



Figure 4: Past mycoremediation projects [6]

1.4 Project Scope Overview

- **Task 1:** Select Fungi
- **Task 2:** Cultivate Fungi
- **Task 3:** Design and Construction of Biofilters
- **Task 4:** Loading and Testing Biofilters
- **Task 5:** Data Analysis
- **Task 6:** Evaluate Project Impacts
- **Task 7:** CENE Deliverables, HURA Deliverables, & Publication
- **Task 8:** Project Management

2.0 Selecting Fungi [7, 8, 9, 10]

Weighted Decision Matrix			Table 1: Fungal Species Decision Matrix			
Criteria Description	Arizona Native (Abundance) [1, 2]	Reasonable Growth Time [3]	Human/Environment Hazard [1]	Cost [4]	Supporting Research [5, 6]	Criteria Total
Weight	0.20	0.20	0.30	0.10	0.20	1.00
Fungi Options	Score	Score	Score	Score	Score	Weighted Score
<i>Trametes versicolor</i> (TV)	10	7	10	10	1	7.6
<i>Pleurotus ostreatus</i> (PO)	8	10	10	10	10	9.6
<i>Hericium erinaceus</i> (HE)	6	6	10	10	1	6.6
<i>Armillaria mellea</i> (AM)	2	6	1	10	1	3.1
<i>Inonotus arizonicus</i> (IA)	8	6	8	1	1	5.5
<i>Stropharia rugosoannulata</i> (SR)	1	8	10	10	10	7.8

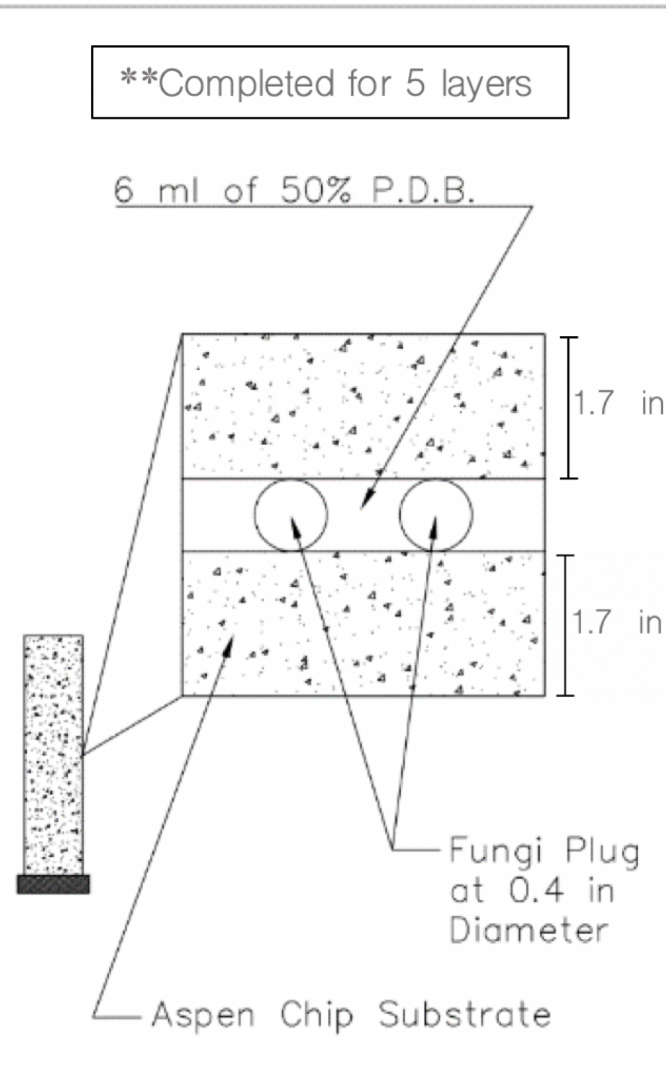
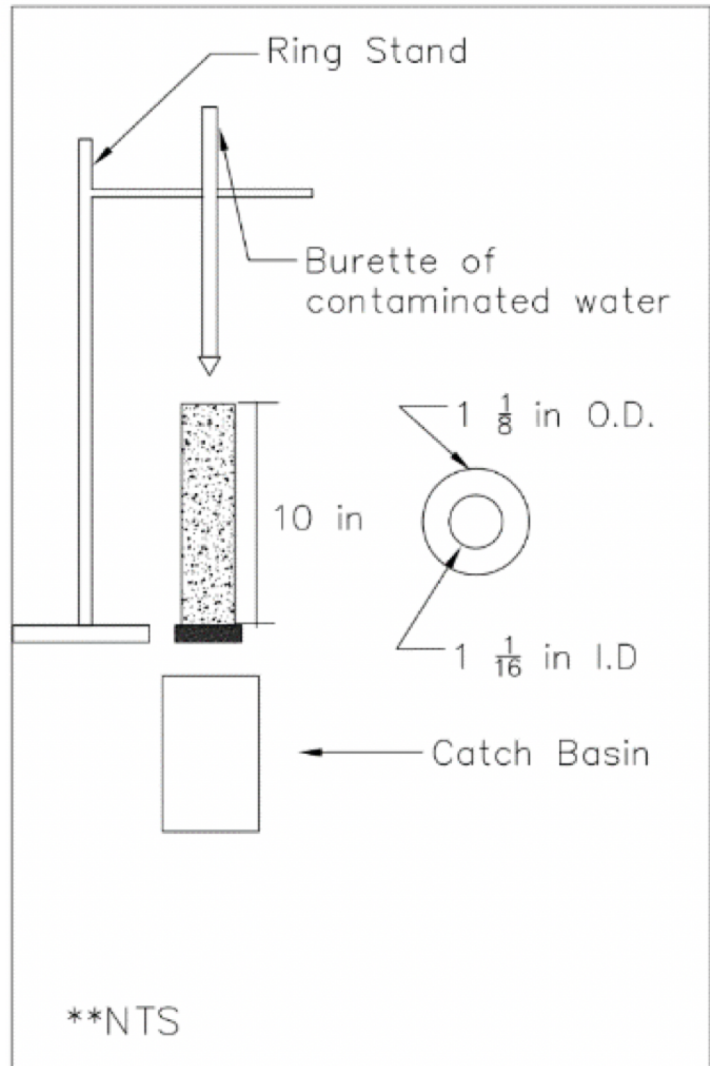
3.0 Bulking Fungi

Table 2: Species Code Names and Culture Replicates

Fungi Type	Code	Replicates (number)
<i>Trametes versicolor</i>	TV	5
<i>Pleurotus ostreatus</i>	PO	5
<i>Stropharia rugosoannulata</i>	SR	5
<i>Trichoderma asperellum</i>	TAs	5



Figure 5: Fungal Species Growing on Potato Dextrose Agar Plates



*Figure 6:
Filter
Contents
and Test
Schematic
Drawing*

4.0 Constructing Biofilters

Filter Materials

- 1 1/16" base caps
- 1 1/8" Top caps
- Aspen Wood chips
- Filtered to 3/8" >Media> 0.08"
- 10" Polycarbonate tubing

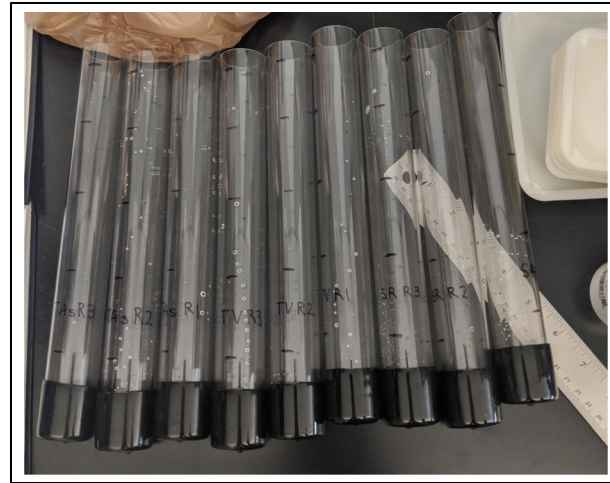


Figure 7: Polycar. Tubes w/ 1 1/16" caps



Figure 8: Aspen Wood Chip Filter Media

4.1 Aspen Media Sterilization Proof



Figure 9. Aspen wood chips non-sterile (left) vs. sterile (right) plated with PDA

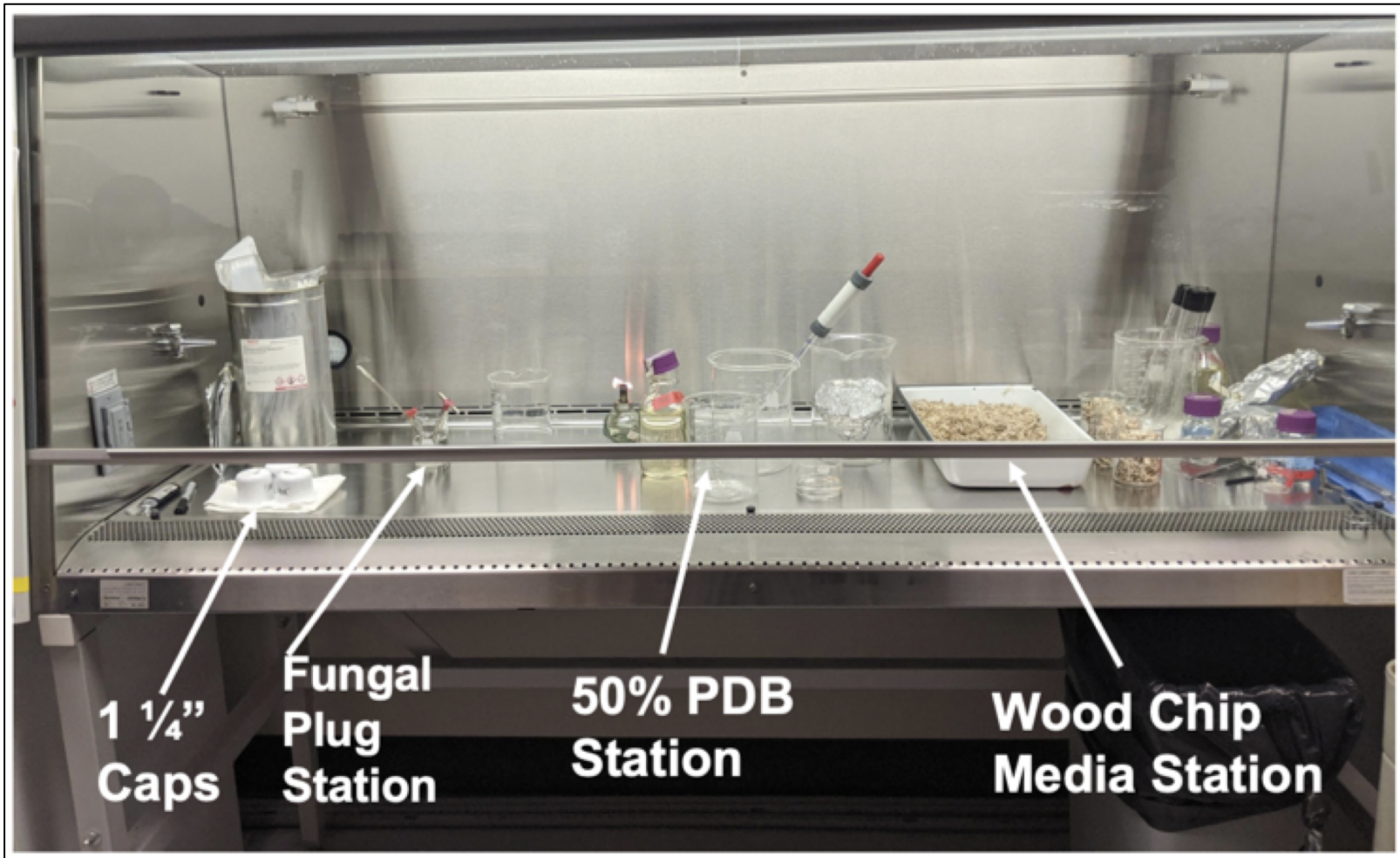


Figure 10: Biofilter Inoculation Set Up

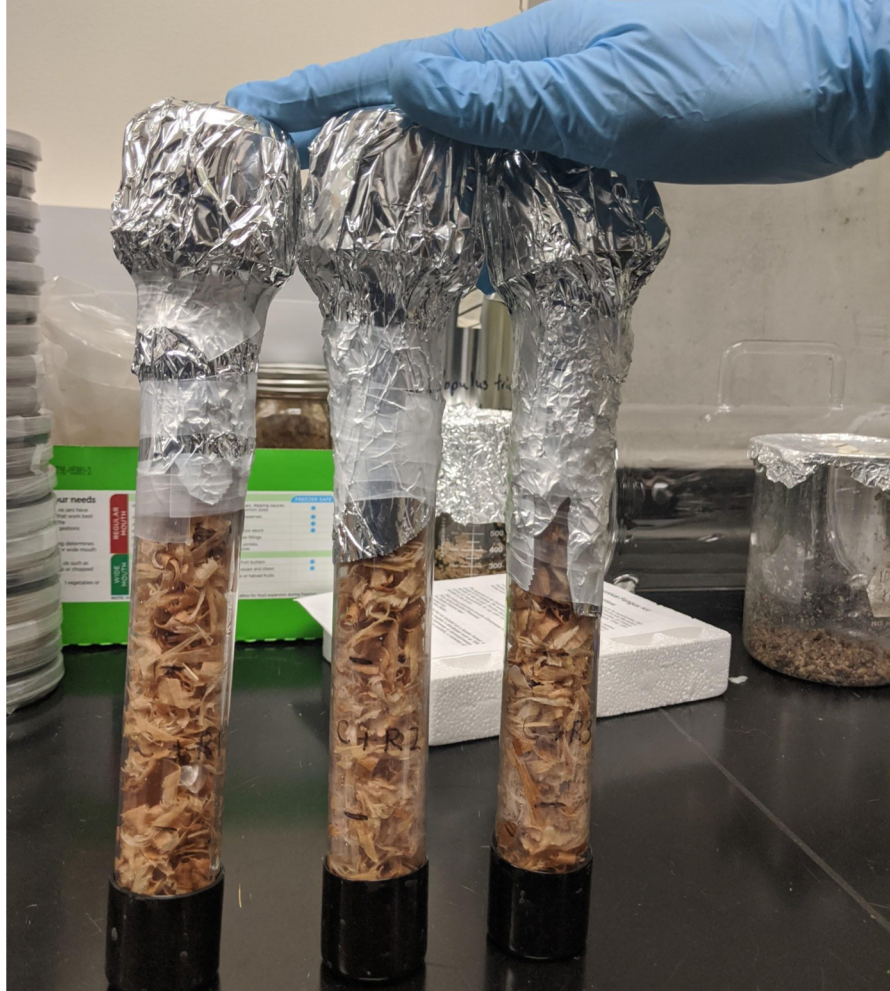


Figure 11: Completed Biofilters



Figure 12: Biofilters after 5 weeks

5.0 Create Contaminated Water Supply

Recipe:

- 1 Loop of *E. coli* inserted in 1 mL DI water
- 400 microliters transferred to Luria-bertani (LB) broth
- LB broth amount: 15 mL

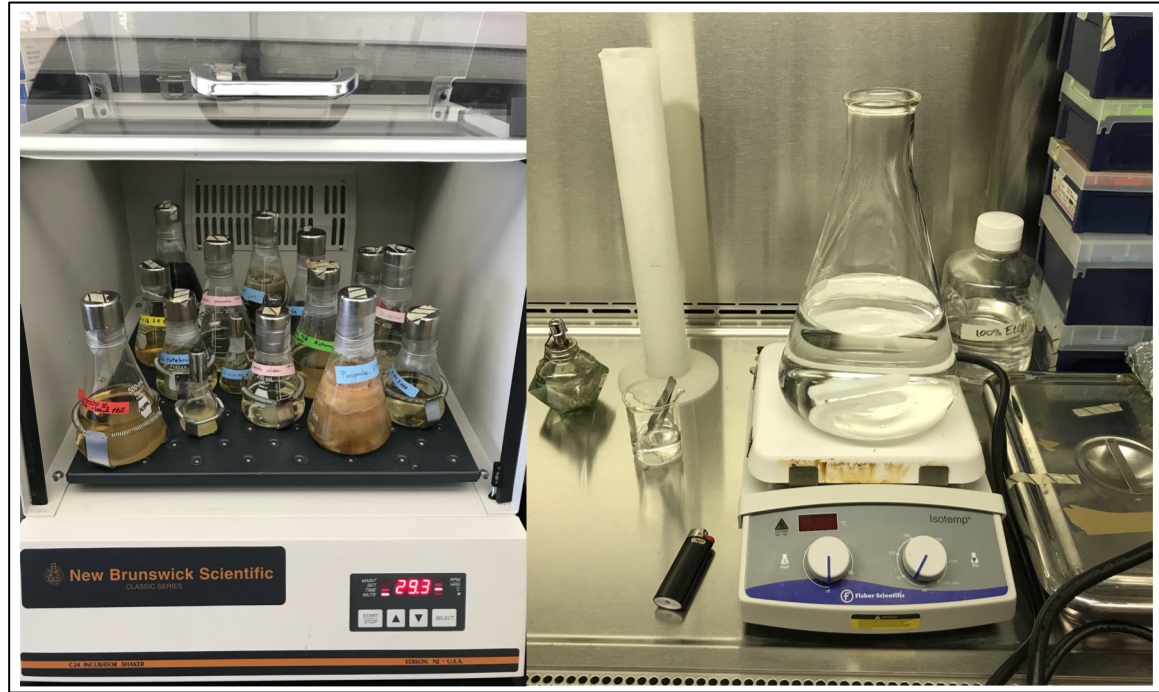


Figure 13: *E. coli* growing in incubator (Left) and Synthetic Wastewater (Right)

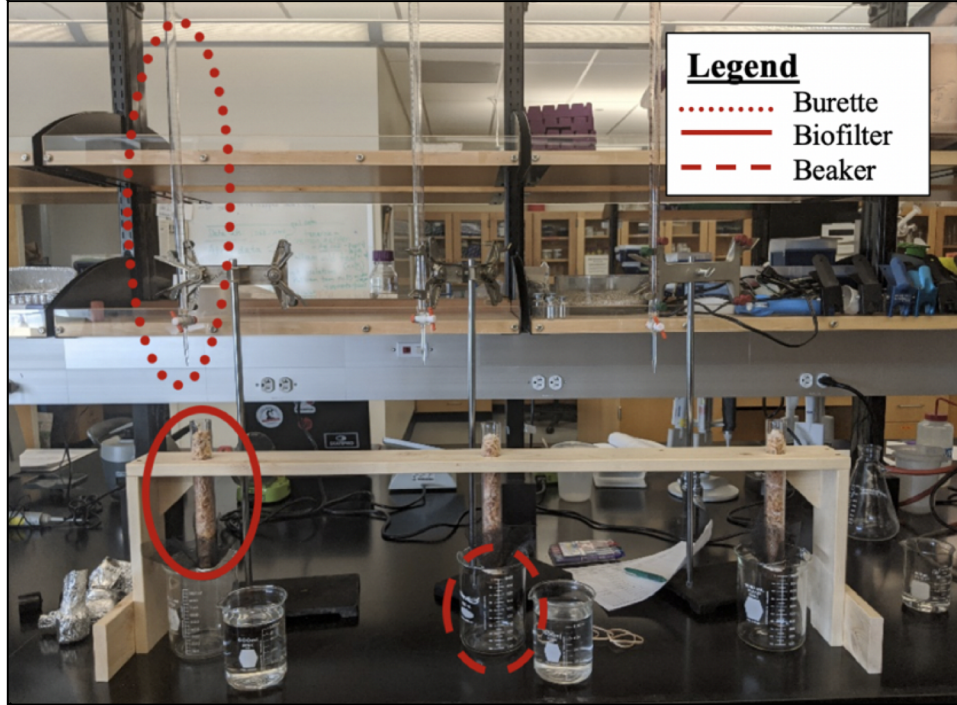
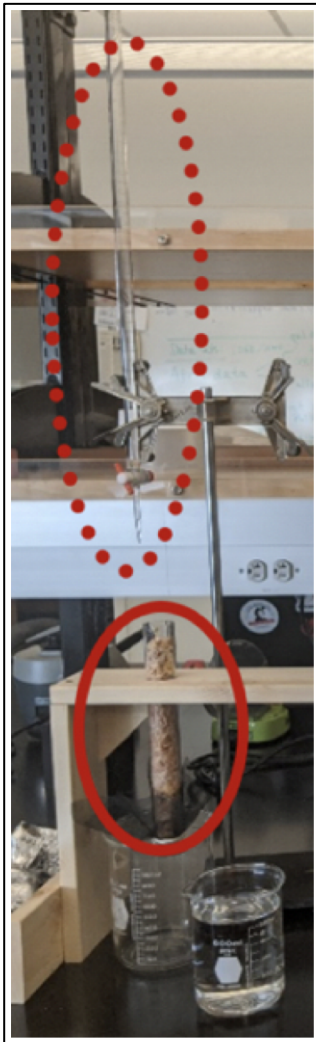


Figure 14: Filter Test Setup, Full view (above), zoomed to one filter (left)

5.1 *E. coli* Concentration Testing: Spectrophotometer Method

- Equipment: Shimadzu UVmini-1240
- Analyte: *E. coli* + Luria-Bertani (LB) broth
- Wavelength: 600 nm
- Units: cells/mL
- Yields instant results



Figure 15. Stock *E. coli* and Spectrophotometer

5.2 *E. coli* Concentration Testing: Membrane Filtration Method

- Standard Method 9222: *Membrane Filter Technique For Members Of The Coliform Group* [1]
- EPA Approved Coliscan® C MF kits by *Mycrology Labs* [2]

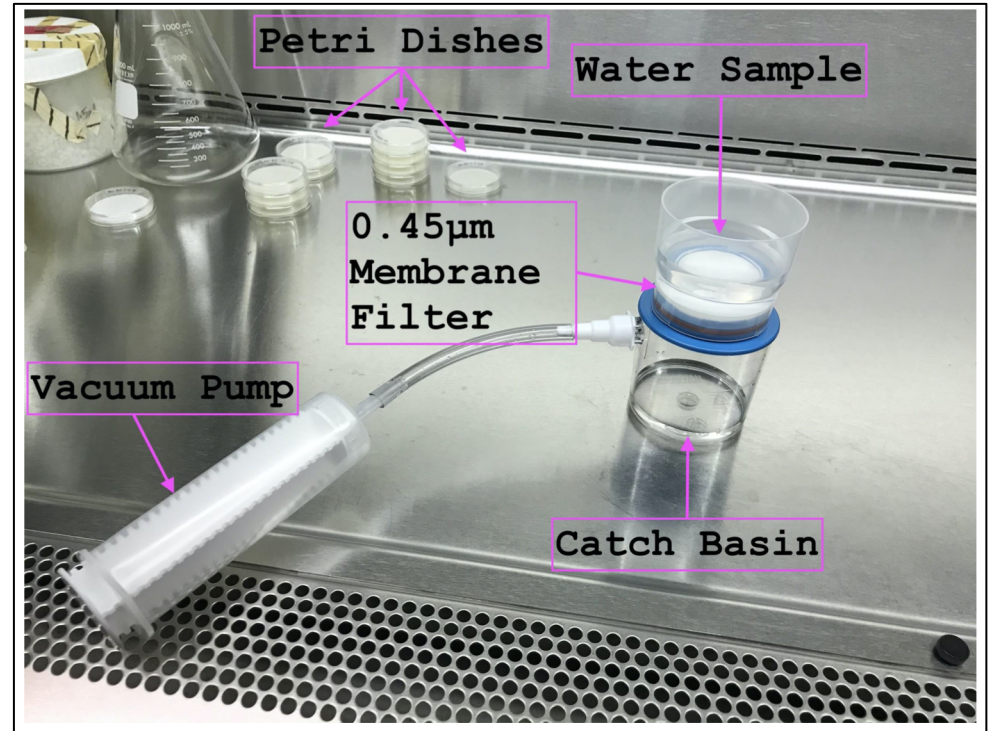


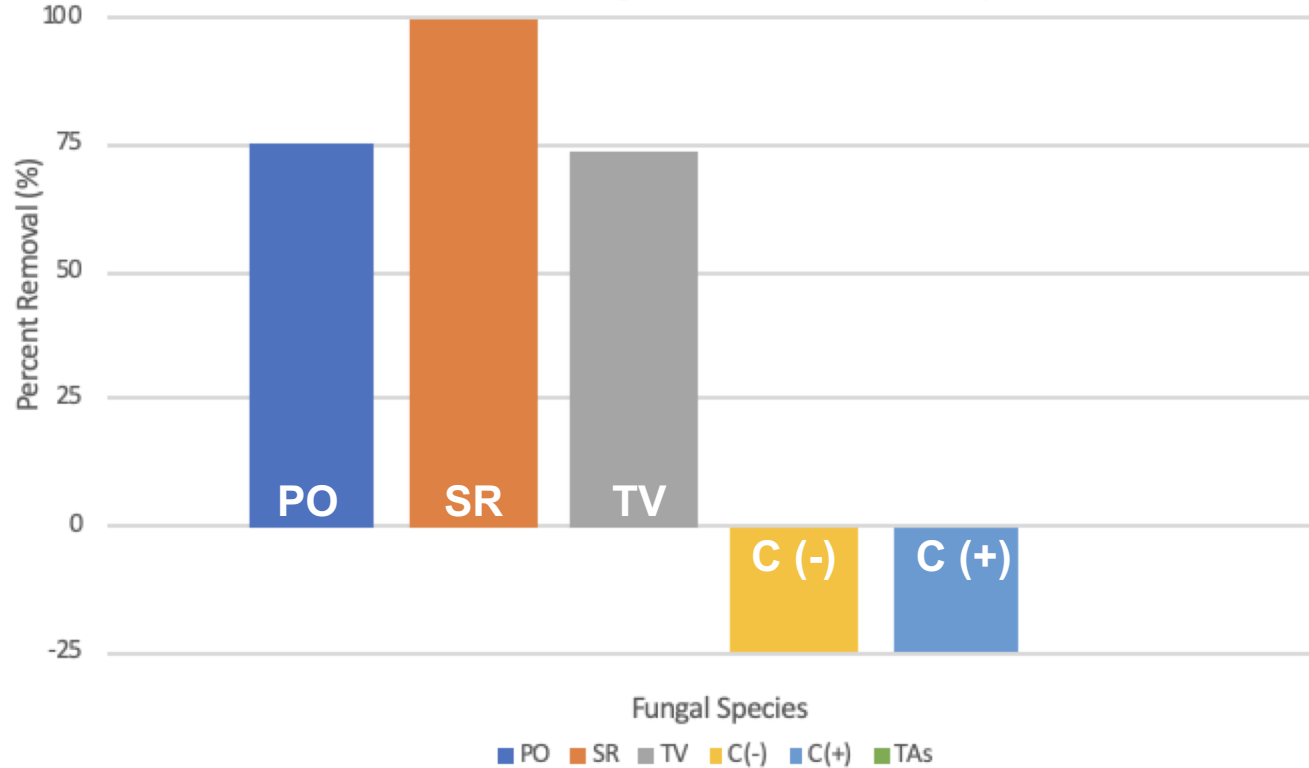
Figure 16. Coliscan Membrane Filter Kit Setup

Percent Removal of Each Fungi

Note: Lower values cut off at -25% Growth

Note: TAs had no Usable Data

Note: All Averages based off of usable data points



6.0 Filter Results

*Figure 17:
Percent
Removal of
each Filter Type*

6.1 Statistical Analysis

Table 3: Statistical Analysis Results

Species Code	Average Percent Removal	Standard Deviation	P-value	Reject Null Hypothesis? (P<0.05)
PO	75%	2.2	0.0005	Yes
SR	100%	N/a	N/a	No
TV	74%	45.9	0.057	No
TAs	0%	N/a	N/a	No
C(+)	-100%	N/a	N/a	No
C(-)	-1214%	144.7	0.0023	Yes

*Type 1 Error: 0.05

*Null Hyp: 0% Removal

7.0 *E. coli* Concentration Problems

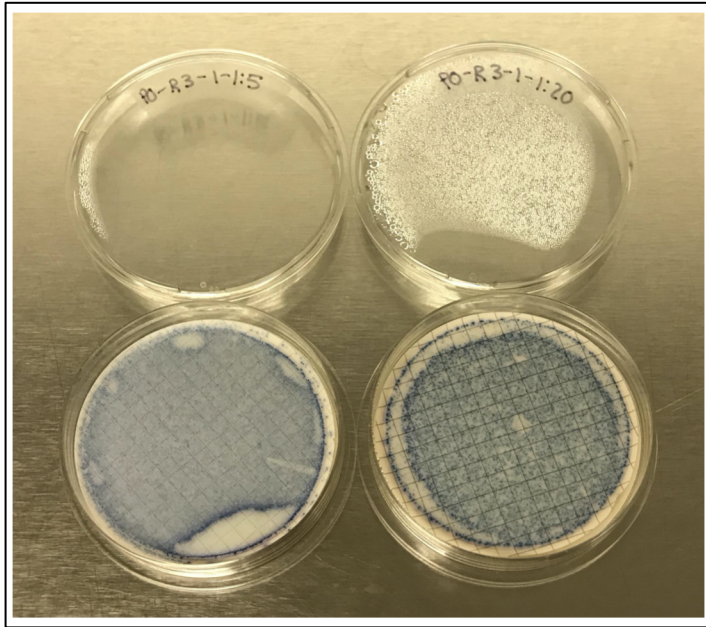


Figure 18: Ethanol Contamination on *Pleurotus o.* Petri Dishes

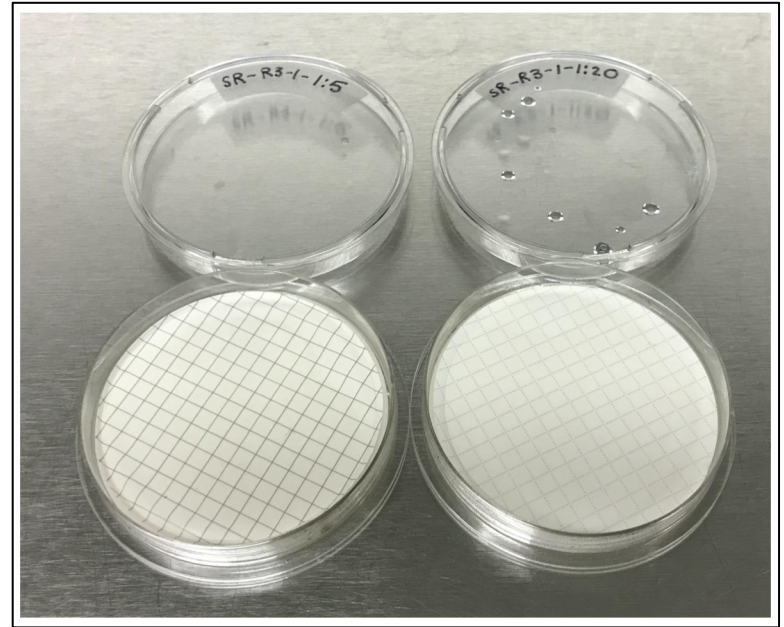


Figure 19: Zero growth of *E. coli* for *Stropharia r.* Petri Dishes

8.0 Field Scale Design

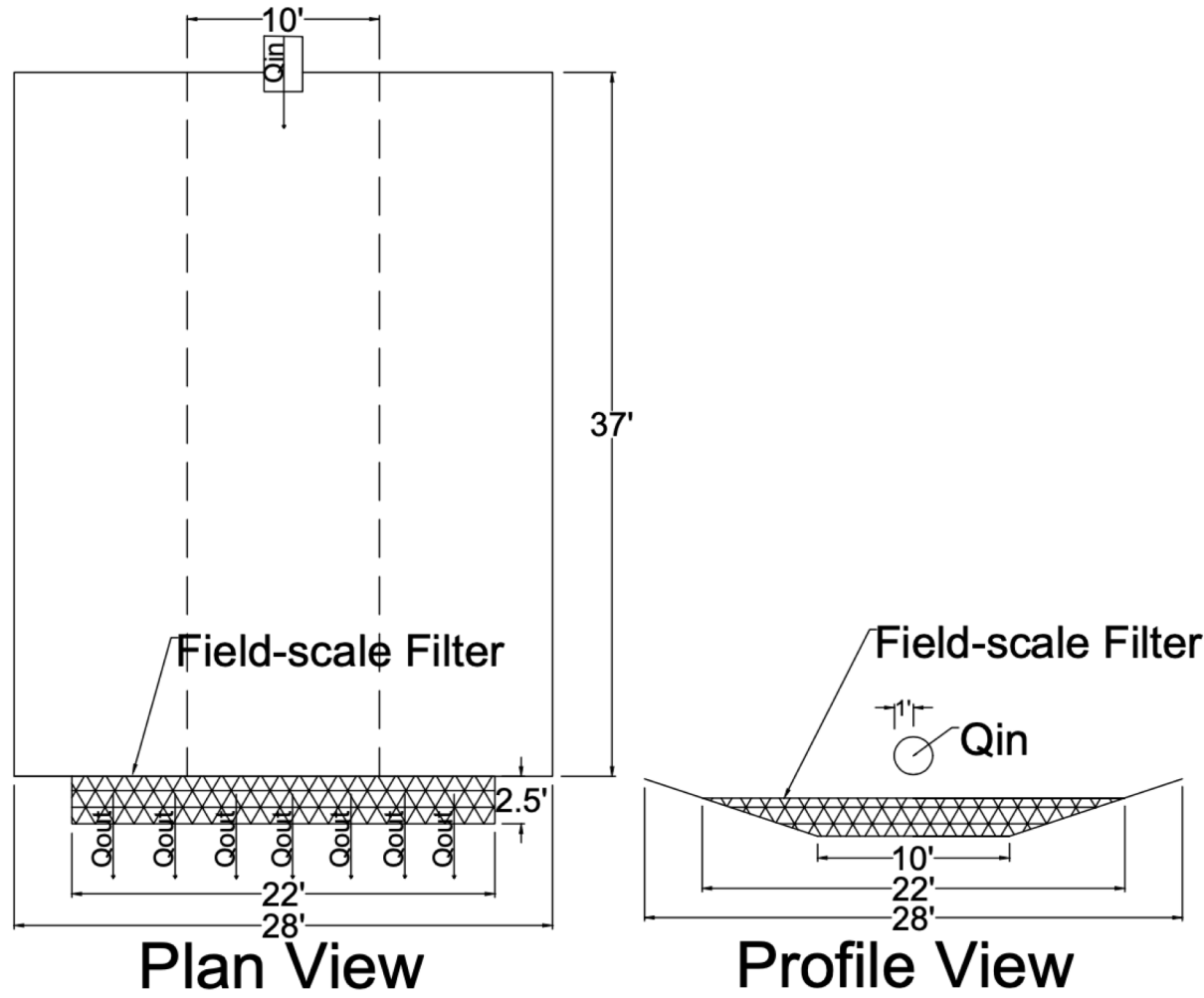


Figure 20. Field-scale Implementation Design Drawing

9.0 Project Impacts

- **Regulations** [12, 13]
- **Public Health** [14]
- **Environment**
- **Socioeconomic** [15]



Figure 21. Pristine Oak Creek [11]

10.0 Recommendations for Future Research

- **More Testing needed for each fungi used**
 - **Standardize *E. coli* concentration**
 - **Confirm TV and PO results**
 - **Retest TAs, SR and C (+)**
- **Determine removal rate (performance with respect to time)**
- **Reusability of filters**
- **Removal mechanism**

11.0 References

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