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

David Hammond, Chase McLeod & Mishael Umlor

Civil and Environmental Engineering & HURA Programs

NAU Northern Arizona University — HURA Programs

Team Fungi

Abstract

Many of Arizona's surface waters are contaminated to unacceptable levels with *Escherichia coli* (*E. coli*) bacteria. The most prevalent *E. coli* cases nearby Northern Arizona are Oak Creek and the Verde River [1]. Research shows that fungal species may be used to remediate many pollutants in water, including *E. Coli* [2, 3]. Minimal research has applied fungi to remediate surface waters in arid climates. This project focuses on quantifying the capacity of common Arizona fungi to remediate *E. coli* – the first step toward implementing fungal-based biotechnology for restoration of contaminated surface water in arid climates.

Research and testing of four fungal strains was performed to determine their individual capacities for removing *E. coli* from water. The tested fungi included: *Pleurotus ostreatus*, *Stropharia rugosoannulata*, *Trichoderma asperellum*, and *Trametes versicolor*. The biofilter design consisted of small-scaled identical vertical columns which were aseptically packed with Aspen wood chips, nutritive broth, and the respective fungal test strain per filter tube. After a five-week growth period, the filters were tested with water containing known amounts of *E. coli*. The flowrate for each fungal column was standardized, assuring each fungi was tested in like manner. A control column with no fungi accounted for any *E. coli* removal due to the media. An additional control contained dead fungi, accounting for any biosorption due to the fungal hyphae. The concentration of *E. coli* coming out of each column was quantified using EPA approved methods. Statistical analysis showed *Pleurotus ostreatus* was the highest performing fungi with a removal of 75 percent.

1.0 Introduction to the Problem: *Escherichia (E.) coli*

- ***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 [1]**

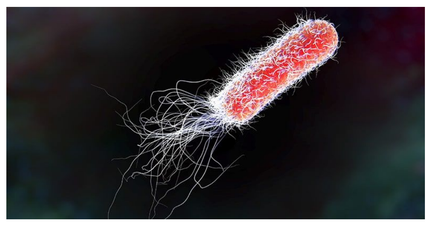


Figure 1: E. coli bacteria [4]

2.0 Methods

2.1 Selecting Fungi

The team chose 4 fungi to grow based on the following criteria:

- **Native abundance in Arizona [6]**
- **Growth time [7]**
- **Human & environmental hazard [8]**
- **Supporting research [2, 3]**

The selected species are shown below:

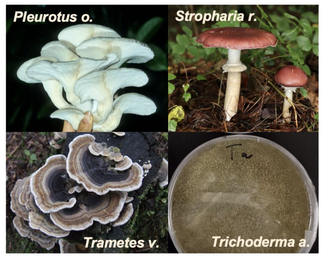


Figure 2: Fruiting bodies of Selected Species (except Trichoderma a.) [9]

2.2 Bulking Fungi

The selected fungi were bulked up, as seen below. Cultures were provided by the Gehring Lab.

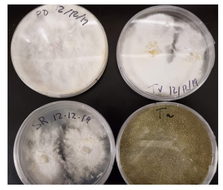


Figure 3: Fungal Species Growing on Potato Dextrose Agar Plates

Table 1: Species Code Names and Culture Replicates

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

2.3 Biofilter Assembly

A total of 18 biofilters were created, as seen below:

Table 2: Species Code Names and Biofilter Replicates

Biofilters (type)	Species (code)	Replicate Biofilters (number
Pleurotus o.	PO	3
Stropharia r.	SR	3
Trametes v.	TV	3
Trichoderma a.	TAs	3
Negative Control	C (-)	3
Positive Control	C (+)	3

Biofilter Controls

- Negative control: Accounted for mechanical filtration
- Positive control: Accounted for removal due to fungal hyphae.

The biofilters consisted of 1.1-in diameter clear tubes, Aspen wood chips (media), and pure fungal cultures, as seen below.

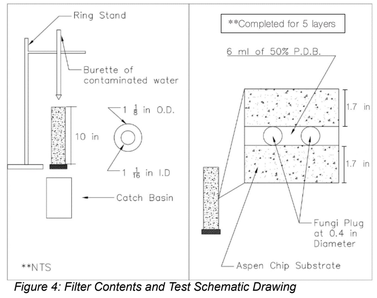


Figure 4: Filter Contents and Test Schematic Drawing

2.0 Methods Continued

Biofilters were created following aseptic techniques, where filters were prepared within a laminar flow hood.

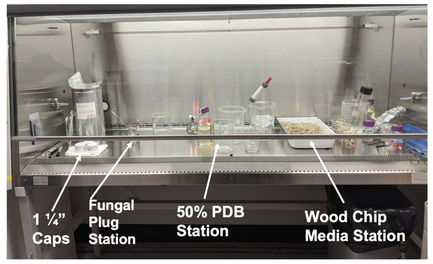


Figure 5: Laminar Flow Hood During Filter Creation and Inoculation

Fungi matured for five to six weeks before they were tested. As seen below, the fungi mycelium appeared white and stringy.

Figure 6: Completed Biofilters

Figure 7: Biofilters After 5 Weeks

2.4 Biofilter Testing

A supply of *E. coli* contaminated water was created for testing the capability of fungi to remediate the water. The contaminated water was made with *E. coli OP50*, provided by the Gehring Lab. The contaminated water was delivered to the filter at a constant flowrate, using burettes. The biofilters were tested in groups of three, seen below.

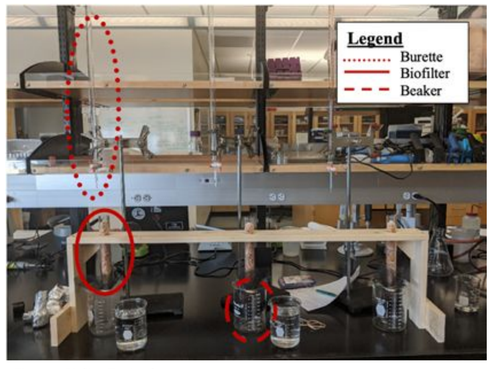


Figure 7: Biofilter Testing Setup

2.0 Methods Continued

2.5 Quantifying *E. coli* Concentration

Biofilter Influent and effluent concentrations were quantified using Standard Method 9222: *Membrane Filter Technique For Members Of The Coliform Group* [10].

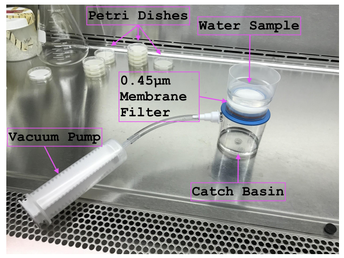
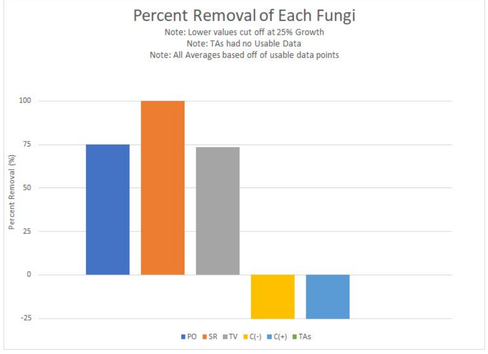


Figure 8: E. coli Concentration Testing Equipment Kit [11]

3.0 Results

3.1 Percent Removal

The percent removal was computed for all species and controls, shown below in a bar graph.



3.2 Statistical Analysis

The data was analyzed using the t-distribution, as seen below. The null hypothesis was no percent removal, and a type one error of 0.05 was used [12]. Species with "N/a" were not analyzed due to lack of data [due to COVID-19].

Table 3: Statistical Analysis Results Using A T-Table

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

4.0 Discussion

From the results, *Pleurotus o.* was found to be the best fungi for removing *E. coli*. *Trametes v.* was second best, however due to the variation in data, more testing is needed. The variation is believed to be caused by channelization of water in the filter, rather than flowing evenly through. *Trametes v.* deserves more lab testing because although the variation, two of the three showed 100 percent *E. coli* removal. The control negative results show that there is little to no filtration of *E. coli* from the Aspen wood chip media. All other results are inconclusive and more testing will be needed.

6.0 Recommendations for Future Research

If this technology was implemented in the field, the fungi would need to be able to handle multiple passes of water from different storms. It is recommended to further research the exhaustion of the biofilters by testing the same filters multiple times, observing how the fungi perform over time. Further testing should be performed with *Trametes v.* to verify its results. Finally, the removal mechanism for each species is unknown, which provides an additional avenue for research.

7.0 Conclusion

In conclusion, four fungi were chosen to try and remove *E. coli* from a synthetic wastewater. Of those, *Pluerotus o.* was found to be the best at removing *E. coli.* It was found that Aspen wood chips have no impact of removal. This data will be used to continue research on this topic.

8.0 References

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Acknowledgements

- **Gehring Lab Personnel:** Dr. Catherine Gehring, Dr. Ron Deckert, and laboratory members
- **CENE 486C Instructors & Technical Advisors:** Dr. B. Bero, Dr. J. Heiderscheidt, Professor Mark Lamer, Professor Gary Miller, & Dr. Wilbert Odem
- **Hooper Undergraduate Research Award (HURA)**
- **Office of Undergraduate Research & Creative Activity**