Reduction of *Escherichia coli* Using Mycofiltration in Freshwater Streams

<u>Project Team</u>:

Christopher Vacher
Principal Mycologist

Robert Whitescarver
Watershed Restoration Consultant

Victoria Vacher
Mycologist/Horticulturist

Cory Egger
Research Assistant/Mycologist

Enchanted Nature
1496 Hankey Mountain Highway
Churchville, VA 24421

July 2026

Abstract

This study evaluates the effectiveness of fungal mycelium (mycofiltration) using Oyster mushroom fungi (*Pleurotus ostreatus)* colonized wood-based media for reducing *Escherichia coli* (*E. coli*) concentrations in freshwater systems. *Pleurotus ostreatus* is an edible mushroom producing fungi native to the region. It is classified as a Biosafety Level 1 (BSL-1) agent, signifying that it poses no known hazard to healthy human adults, healthy animals, or the environment, making it safe to handle without specialized containment.

Controlled laboratory column experiments demonstrated that treatment performance improves substantially with increased media depth, with reductions of E. coli more than 30% at 15 cm and up to 99% at 23 cm media depths within a 10 cm diameter column. These findings establish mycofiltration as a viable, low-cost, and low-impact strategy for improving water quality in small, low-flow freshwater stream systems, while also providing a practical foundation for future field-scale implementation and performance validation.

Hypothesis

Mycofiltration using *Pleurotus ostreatus* colonized wood-based media will significantly reduce *Escherichia coli* concentrations in freshwater under low-flow conditions, with treatment effectiveness increasing as media depth increases.

Introduction

Project Background

Non-point-source pollution remains a significant challenge in small headwater streams and ephemeral waterways across rural, suburban, and urban landscapes. Pollution sources such as agricultural runoff, failing septic systems, stormwater runoff, leaking sewer infrastructure, pet waste, and wildlife contribute to elevated levels of biological contaminants, including *Escherichia coli (E. coli)*. In suburban and urban environments, impervious surfaces such as roads, parking lots, and rooftops accelerate runoff and transport pollutants directly into waterways, often without adequate filtration. These combined inputs impair water quality and pose risks to downstream ecosystems and human health. While a range of best management practices (BMPs) and stormwater control measures (SCMs) exist to address water quality concerns, these approaches are often difficult to implement in small, low-flow channels due to cost, access limitations, or the potential for ecological disturbance.

The site for this project is located within the Potomac-Shenandoah basin in an unnamed tributary of the Middle River watershed, classified as an intermittent stream by the Virginia Department of Environmental Quality.

Mycofiltration is an emerging, low-cost water quality enhancement approach that utilizes fungal mycelium integrated into organic substrates such as wood chips, sawdust, and straw. These mycelial networks form complex, porous structures that increase surface area, retain moisture, and support diverse microbial communities. Within these systems, contaminants may be reduced through a combination of physical straining, adsorption, and biological interactions. While some fungi are known to produce enzymes and secondary metabolites with antimicrobial properties, current evidence suggests that in flowing water systems, the primary mechanisms of *E. coli* attenuation are driven by physical capture, biofilm interaction, and microbial competition rather than rapid enzymatic inactivation. A biofilm is a complex, sticky community of microorganisms (like bacteria or fungi) that attach to a surface and encase themselves in a protective matrix of proteins and sugars. In an oyster mushroom mycofilter, the fungus creates a thick, living, web-like biofilm that safely traps, neutralizes, and digests waterborne bacteria like *E. coli.*

Due to its relative low cost, use of biodegradable materials, and compatibility with natural systems, mycofiltration has the potential to complement existing BMPs by enhancing biological processing within permeable, low-impact structures. However, despite growing interest, there remains limited experimental data on system performance, design parameters, and scalability, particularly in the context of small stream environments. This study seeks to address these gaps by evaluating the effectiveness of oyster mushroom colonized wood media for *E. coli* reduction by development of field-deployable system designs.

Project Goals

- Quantify *E. coli* reduction across varying media conditions, including uninoculated and mycelium-colonized substrates
- Evaluate the relationship between media depth and treatment performance
- Identify the relative contribution of biological versus physical filtration mechanisms
- Develop design considerations for field-scale, flow-through mycofiltration systems

Methods

This study employed controlled laboratory column experiments to evaluate the effectiveness of mycofiltration for reducing *Escherichia coli (E. coli)* concentrations in water. The experimental design compared uninoculated wood-based media to media colonized with oyster mushroom fungi across varying substrate depths.

Three column configurations were evaluated:

Uninoculated and unpasteurized coarse wood chips and sawdust (control)
15 cm depth wood media inoculated with *Pleurotus ostreatus*
23 cm depth wood media inoculated with *Pleurotus ostreatus*

Each configuration was used to process water samples collected from the study site, and resulting *E. coli* concentrations were compared to unfiltered influent samples.

Site Description and Sample Collection

Water samples were collected from site SH1, located at approximately 38° 9'59.59"N, 79° 2'56.08"W, within a small headwater stream system characterized by low flow and intermittent hydrology.

Sampling conditions were recorded as follows:

Water temperature: approximately 22°C
Air temperature: approximately 27°C
Weather: clear conditions with no recent rainfall

Water appearance: clear with no noticeable odor

Samples were collected and immediately placed on ice to preserve integrity prior to laboratory analysis.

Column Construction and Media Preparation

Columns were constructed using 10 cm diameter vertical housings designed to allow gravity-driven flow through the filtration media.

The filtration media consisted of a mixture of coarse wood chips and sawdust. The filtration media used for fungal inoculation was sterilized at 15psi for 1 hour. *Pleurotus ostreatus* spawn was introduced to the filtration media and allowed to fully colonize prior to experimental use. The control column contained non-pasteurized, uninoculated wood media.

Media depths for each configuration were as follows:

Non-sterile wood media: 23 cm depth (uninoculated media)
Treatment 1: 15 cm depth colonized media
Treatment 2: 23 cm depth colonized media

All columns were prepared to maintain consistent packing density and flow conditions.

Experimental Procedure
For each trial, a fixed volume of 1.5 liters of water was introduced into the column system under gravity flow conditions. Flow characteristics were observed.

Effluent samples were collected following complete passage through each column and prepared for microbial analysis. All trials were conducted under consistent conditions to allow for direct comparison between column configurations.

To validate initial findings, the experiment was repeated using identical methods and sampling location. E. coli colony-forming units (CFUs) were quantified for control and processed samples (15 cm and 23 cm media depths) using the Coliscan Easygel method. Results were consistent with initial trials, confirming reproducibility.

Microbial Analysis

Enumeration of *E. coli* was conducted using the Coliscan Easygel method. For each sample, a 3 mL sample of water was added to prepared media plates and incubated at approximately 35°C for 24 hours.

Following incubation, colonies exhibiting dark blue or purple coloration were identified and counted as E. coli. Non-target colonies (e.g., pink, blue-green, or white) were excluded from analysis.

Colony counts were converted to colony-forming units per 100 mL (CFU/100 mL) using standard conversion methods based on sample volume. All samples, including untreated influent controls and filtered effluent, were processed under identical conditions.

Data Analysis

Treatment performance was evaluated by comparing E. coli colony-forming unit (CFU) counts between unfiltered influent samples and processed effluent for each column configuration. Percent reduction values are reported as averages or ranges where variability between trials was observed.

Results

Flow Characteristics

All column configurations operated under gravity-driven flow conditions. For each trial, 1.5 liters of water were introduced into the column system. Initial breakthrough typically occurred within approximately 2 minutes, followed by complete drainage within 3-5 minutes.

Flow behavior varied slightly between configurations, with colonized media exhibiting slower and more uniform flow compared to uninoculated media. This effect is attributed to increased structural complexity and water retention within the mycelium-colonized substrate.

E. coli Reduction Performance

Experimental results demonstrated a clear relationship between substrate composition, media depth, and *E. coli* reduction.

The control column, consisting of non-pasteurized coarse wood chips and sawdust without fungal inoculation, exhibited an increase in E. coli concentrations relative to influent samples. This suggests either the introduction of bacteria from the substrate or conditions favorable to bacterial persistence within the media.

In contrast, columns containing wood-based media inoculated with *Pleurotus ostreatus* showed measurable and progressively greater reductions in E. coli concentrations.

The 15 cm colonized media depth column achieved an average reduction of approximately 31.3%, indicating moderate treatment effectiveness under limited 15 cm media depth conditions.

The 23 cm colonized media depth column demonstrated substantially improved performance, achieving reductions ranging from 89% to 99%, representing near-complete attenuation in trials.

Effect of Media Depth

A strong positive relationship was observed between media depth and treatment performance. Increasing media depth within the 10 cm diameter columns from 15 cm to 23 cm resulted in a disproportionate increase in *E. coli* reduction, suggesting a nonlinear response.

This improvement is attributed to:

Increased surface area for microbial interaction
Greater opportunity for physical straining and adsorption
Enhanced development of biologically active zones

These findings indicate that treatment effectiveness is driven by cumulative interactions within the media rather than a single-pass filtration effect.

Role of Biological Activity

The contrast between uninoculated and colonized media highlights the importance of biological activity in *E. coli* attenuation. The increase in *E. coli* observed in the control column demonstrates that untreated organic media alone may not provide effective treatment and can, under certain conditions, contribute to bacterial persistence.

In contrast, *Pleurotus ostreatus* colonization appears to enhance treatment performance by:

Increasing structural complexity of the media
Supporting biofilm development
Promoting competitive microbial interactions

These results suggest that biological processes play a critical role in system performance, supplementing physical filtration mechanisms.

Summary of Findings

Overall, the results demonstrate that:

- Uninoculated wood media is ineffective and may increase E. coli levels
- Mycelium-colonized media provides measurable reduction
- Treatment performance improves significantly with increased media depth
- Effective attenuation is achieved through cumulative, short-duration interactions within biologically active media

Discussion

Interpretation of Results

The results of this study demonstrate that mycofiltration using *Pleurotus ostreatus* colonized wood media can effectively reduce *Escherichia coli (E. coli)* concentrations under controlled conditions. A clear performance gradient was observed across treatment configurations, with increasing media depth resulting in substantially improved *E. coli* attenuation.

The 15 cm media depth column achieved a moderate reduction (31.3%), while the 23 cm media depth column achieved high reductions ranging from 89% to 99%. This disproportionate improvement indicates a nonlinear relationship between media depth and treatment performance. These findings suggest that effective attenuation is driven by cumulative interactions within the media rather than a single-pass filtration effect.

Mechanisms of *E. coli* Reduction

The observed reduction in *E. coli* is likely the result of multiple interacting mechanisms. While some fungi are known to produce antimicrobial compounds, the time scales observed in this study (<3 minutes of contact) suggest that rapid enzymatic inactivation is not the primary driver of reduction.

Instead, the following mechanisms are likely dominant:

Physical straining and retention within the porous wood matrix
Adsorption to organic surfaces and biofilms
Microbial competition within biologically active substrate

The presence of *Pleurotus ostreatus* appears to enhance these processes by increasing structural complexity, improving moisture retention, and supporting the development of biologically active zones within the media.

Role of Biological Activity

The increase in *E. coli* observed in the uninoculated control column highlights the importance of biological activity in system performance.

Untreated organic media may provide conditions that allow bacterial persistence or even growth. In contrast, colonized media demonstrated consistent reductions, indicating that fungal activity plays a critical role in transforming the media into an effective treatment system.

This finding reinforces that mycofiltration is not simply a physical filtration process, but a biologically mediated system in which microbial interactions are essential to performance.

Implications for Field Design

The results of this study have direct implications for the design of field-deployable mycofiltration systems. Most notably, the findings suggest that extended hydraulic retention time is not required to achieve meaningful *E. coli* reduction. Instead, treatment effectiveness can be achieved through repeated, short-duration interactions between flowing water and biologically active media.

This supports a distributed design approach in which:

Flow is slowed but not impounded
Water is directed through multiple contact zones
Media depth and surface complexity are maximized

Such an approach is well-suited for small headwater streams, where maintaining natural flow conditions and avoiding channel alteration are critical design constraints.

Limitations

While the results are promising, several limitations must be acknowledged. Laboratory conditions provide a controlled environment that cannot fully replicate the variability of natural systems. Factors such as fluctuating flow rates, sediment load, temperature variation, and biological disturbance may influence performance in the field.

Additionally, the experimental setup represents a simplified system with uniform flow conditions, whereas natural streams exhibit complex hydraulics and heterogeneous substrates. As such, the results should be interpreted as proof-of-concept rather than direct prediction of field performance.

Future Research Needs

Further research is required to evaluate mycofiltration performance under real-world conditions. Key areas for future investigation include:

Field-scale pilot installation
Long-term performance and durability of media
Seasonal and temperature-related variability
Optimization of media composition and configuration
Hydraulic performance under varying flow regimes

These efforts will be essential to validate laboratory findings and refine design parameters for practical implementation.

Conclusions

This study demonstrates that mycofiltration using *Pleurotus ostreatus* colonized wood media is an effective method for reducing *E. coli* concentrations under controlled conditions. Experimental results showed that treatment performance improves significantly with increased media depth, with reductions ranging from 31.3% at 15 cm media depth to as high as 99% at 23 cm media depth.

The findings indicate that treatment effectiveness is driven by cumulative interactions within biologically active media rather than reliance on extended hydraulic retention time. Additionally, the increase in *E. coli* observed in the uninoculated control highlights the importance of fungal colonization and biological activity in achieving meaningful reductions.

Overall, this study establishes a functional foundation for the use of mycofiltration as a low-cost, low-impact water quality improvement strategy in small stream systems.

Recommendations

The next phase of this work should focus on the design and implementation of field-scale pilot systems. Laboratory results cannot fully replicate real-world conditions, and in-situ testing is necessary to evaluate performance under variable flow, environmental, and ecological conditions.

Future designs should prioritize:

Increased media depth and surface complexity
Distributed treatment zones to maximize interaction frequency
Permeable structures that maintain natural stream flow
Integration with existing stream features such as riffles and channel morphology
Monitoring and Data Collection

Field deployments should include robust monitoring protocols to quantify performance over time, including:

Untreated water and treated water *E. coli* concentrations
Flow conditions and hydraulic behavior
Seasonal variability

Funding and Project Development

This study was self-funded. Advancement to field-scale implementation, including pilot installations and long-term monitoring, will require external funding and support. Potential funding sources may include environmental grants, watershed restoration programs, and partnerships with academic or governmental institutions.

Regulatory Integration

Given its low-impact and biodegradable nature, mycofiltration may be well-suited for integration into regulatory frameworks such as stream restoration and aquatic resource enhancement projects. Further engagement with regulatory agencies will be necessary to align design approaches with permitting requirements.

Final Statement

Mycofiltration represents a promising, process-based approach to improving water quality in small freshwater stream systems. By leveraging natural biological processes within a low-cost and adaptable framework, this method offers a practical alternative to conventional infrastructure in environments where traditional solutions are not feasible. Continued research and field validation will be essential to fully realize its potential as a scalable water quality management strategy.

Acknowledgments

The contributions of Fred Blanton, Cory Egger, Sarah Egger, Jeanne Hoffman, Victoria Vacher, and Robert Whitescarver are gratefully acknowledged. Their expertise, technical assistance, and support were instrumental in the successful completion of this research.