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Environmental Engineering | Pollution Control

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Controlling Coliform Contaminated Water through Mycofiltration

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Abstract

Waterborne illnesses guarantee numerous deaths every year. One of the main causes is the absence of safe drinking water. The World Health Organization reports that over 3.4 million people die annually from water-related diseases. The greatest dangers from unclean water are sicknesses caused by microbes, protozoa, or parasites. The purpose of this study was to create an inexpensive and efficient water filter that has the ability to provide clean water to individuals living in the impoverished regions of the world. Mycofiltration uses fungal species in a substrate matrix to filter out pollutants (chemical and/or biological) from water. In this study, mycelia from *Stropharia rugosoannulata* and *Pleurotos ostreatus* to was to use remove *Escherichia coli* (K12 strain). Mycofilters were prepared by inoculating autoclave sterilized grain and perlite with *S. rugosoannulata* or *P. ostreatus* in sterile 50 mL conical tubes with a hole drilled in the bottom. The mycofilters were then placed on a ring stand, 10 mL of *E. coli* K12 suspension was aseptically pipetted at the top of the tube, and the water was collected at the bottom in sterile collection tubes. Samples were serially diluted, then plated to nutrient agar for colony counts. The mycofilters of *Stropharia rugosoannulata* and *Pleurotos ostreatus* significantly reduced the concentration of the *E. coli.*

1. Introduction

1.1. Mycofiltration

Mycofiltration is the process of using networks of fungal mycelium to facilitate improved water quality (Stamets, 2005). Paul Stamets discovered the technique of runoff management using mycofiltration while performing several treatment studies that documented bacteria removal from agriculture runoff. Stamets installed outdoor woodchip beds of *Storpharia rugosoannulata* species mycelium and other mushroom species in an area about 50 ft wide and 200 ft long. A year after planting the garden of these mushrooms, analysis of Stamets outflowing water showed "a hundred-fold drop in coliform levels despite the fact that I had more than doubled my population of farm animals" (Stamets, 2005). It should be noted that mycofiltration has very limited field experiments.

Thomas (2009) used mycoremediation treatment for two field sites; one field site was a control biofilter without fungi and the other was a biofilter with fungi. Thomas (2009) looked at fecal coliform and nutrient concentrations in source water and two outflow pipes from the two field sites. This study saw a 66% reduction in fecal coliform in the control biofilter and a 90% reduction in their biofilter containing fungi. Thomas concluded that the benefits of mycoremediation treatment application to a bioretention cell or other type of site were many and included: reducing fecal coliform and nutrients when properly designed, applicability to a variety of other contaminants, minimal handling, and low maintenance.

At Evergreen State College, Rogers (2012), completed a similar lab experiment that used *Pleurotus Ostreatus* mycelium inoculated sawdust in a column test. Rogers (2012) loaded the inoculated sawdust and non-inoculated sawdust with an *E. coli* solution and observed that the effects of mycofiltration significantly reduced the *E. coli* in solution. This study supported the evidence that Thomas (2009) and Stamets (2005) had found in that mycofiltration did reduce *E. coli* in a lab and field study.

1.2. Mycelium Species

Pleurotus ostreatus has been researched in a few different studies and was found to be effective in bacterial removal. *P. ostreatus* mushrooms are easy to grow and very adaptable to their environment. This species is edible and grown worldwide. The *P. ostreatus* mycelium was found to "attack and destroy bacterial colonies, which then serve as a nutrient source for the fungus" (Barron, 1987). Barron (1987) noticed fungal secretions from the mycelium stopped the colonies from growing and seemed to use the bacteria as an intermediate nutrient source to reach a higher nutrient content food source. The *P. ostreatus* produces a nematoxin that is contained in the fungal secretions. When the bacteria comes in contact with the secretion, the bacteria were immobilized, and the cell walls are destroyed and serves as a nutrient source for the fungus.

The wine cap is a species of mushroom, scientifically known as *Stropharia rugosoannulata*, that may hold a key to filtering harmful pollutants from stormwater runoff. Its mycelium is a microscopic, cobwebby, fungal thread that, when mixed with woody debris, decomposes bacteria. *S. rugosoannulata* mushrooms are easy to grow and very adaptable to their environment. They are also edible This species is grown worldwide. The research done the Environmental Protection Agency, Fungi Perfecti partnered with the Civil and Environmental Engineering Department at Washington State University in 2012 sought to determine which mushroom is most effective at filtering bacteria in urban environments. After testing a variety of species under various conditions, the research reaffirmed Stamets'

1984 findings: The wine cap mushroom is the most efficient species for removing *E. coli* bacteria.

1.3. Project Objectives

The objectives of this study were to investigate the treatment effectiveness of mycofiltration to reduce the concentration of *E. coli*. The objectives were met by simulating mycofilter in a laboratory where each filter contained either *P. ostreatus*, *S. rugosoannulata* or neither. *E. coli* was chosen for this study because it is a pathogen that causes harm to the natural environment and because the underdeveloped countries' water samples often exceed the standard concentration levels of *E. coli*. The overarching goals of this study were to determine treatment effectiveness of mycofiltration to reduce the concentration of *E. coli* after filtration.

2. Methodology

The design of the mycofilter came from Stamets (2005) where he suggested multiple filling options for the mycofilters depending on the mycelium species. For *P. Ostreatus* and *S. rugosoannulata,* boiled rye grains were determined to be the most effective.

The experimental design included nine mycofilters. The nine mycofilters were separated into three different groups: (1) three mycofilters without mycelium; (2) three mycofilters with *S. rugosoannulata* mycelium and (3) three mycofilters with *P. Ostreatus* mycelium.

To guarantee a rapid flow, 300 mL of perlite were uniformly mixed with 300 mL of sterile rye grains. Mycelium does not grow on perlite; therefore, it prevented the mycelium from overgrowing and allowed the liquid to filter through by creating air space. 35 mL of the mixture of perlite and rye grains were added to each of the 9 conical tubes. The liquid cultures of *P. Ostreatus* and *S. rugosoannaulata* were grown in Hansen's medium. The Hansen medium was prepared by dissolving 0,3 g KH2 SO4 , 0,2 g MgSO4, 5 g glucose in the distilled water as well as 1 g peptone which was first dissolved in 100 cm3 warmed water, and then added do the remaining components of medium.

Three conical tubes were inoculated with 4 mL liquid culture of *P. ostreatus.* Three conical tubes were inoculated with 4 mL liquid culture of *S. rugosoannulata.* Three conical tubes were not inoculated with anything. The mycofilters were then placed on a ring stand and let grown for 5 days. 10 mL of *E. coli* suspension was aseptically pipetted at the top of the tube, and the *E.coli* was collected at the bottom in sterile collection tubes. Samples were serially diluted, then plated on yeast malt nutrient agar for colony counts.

Data analysis involved counting the colonies that grown after incubation occurred. . Samples were serially diluted to a million, then plated on yeast malt nutrient agar using the plating and spreading technique for colony counts. The initial *E.coli* colonies (CFU/mL) were compared to the *E.coli* colonies (CFU/mL) counted after filtration.

Results

Overall the results of this study showed that the mycofilters lowered the *E. coli* concentration. All filters showed positive effects on the *E.coli* colonies present after filtration.

Average E. coli Colonies Reduced		
	Colonies reduced	Percent Reduced
Control	176	11.9%
S. rugosoannulata	476	32.2%
P. ostreatus	771	52.3%

Figure : Average number of *E. coli* colonies reduced after filtration

Figures 1,2 :There were 1496 *E. coli* colonies present initially. The controlled filter reduced the *E. coli* colonies from 1464 colonies to 1320, on average by 11.9 %. The filter inoculated with *S. rugosoannulata* on average reduced the *E. coli* colonies from 1469 to 1020, 32.2 %. *P. ostreatus* had the most dramatic effect on the *E. coli* colonies present.

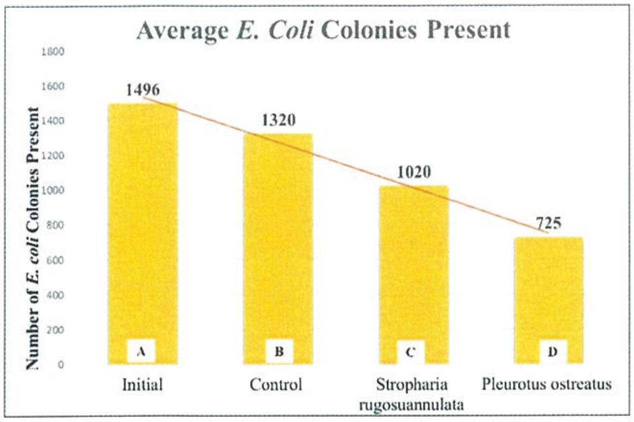

Figure 2: Number of *E. coli* colonies counted before and after filtration

It reduced the *E. coli* colonies on average from 1469 to 725 colonies. It was able to reduce the *E. coli* colonies by 52.3%.

Discussion

This study was done to create an inexpensive and efficient water filter. The study demonstrated that the *Pleurotos ostreatus* mycelium is the most effective at reducing *E. coli* colonies. The *Stropharia rugosoannulata* filter did not have a major effect on reducing the *E. coli* colonies because it only reduced the *E. coli* colonies by 32.2%. The *Pleurotos ostreatus* filter reduced the *E. coli* colonies by 52.3%. *P. ostreatus's* ability to attack and destroy harmful bacteria contributes to the creation of safe drinking water. Since mushrooms can be found everywhere, *P. ostreatus* is very inexpensive and accessible. *P. ostreatus* is also edible which assures that it will not have harmful effects on the water properties that are harmful to humans.

There were a few sources of errors in this experiment one of which includes the repeated use of the filters for different trails. Other factors include measurements error throughout the experiments and faults in the design/structure of the mycofilter.

In the future, different species of mushrooms will be tested to determine if it has any effects on the purity of the water. Various designs of the filter will also be tested to increase the filtration speed to reach the individuals necessities in daily life. Other factors that contribute to polluted water will also be filtered through, such as heavy meatal like lead, mercury and arsenic.

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