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Research Plan

Cat - Environ. Eng.

Controlling Coliform Contaminated Water through Mycofiltration

A. Rational

Waterborne illnesses guarantee numerous deaths every year. One of the 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 technology, known as "mycofiltration" refers to the intentional and judicious use of cultivated networks of fungal mycelium to facilitate water quality improvements in engineered ecosystems. 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 used to remove *Escherichia coli* (K12 strain) from water.

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 come 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.

B. Hypothesis

The project is to develop and, test a mycofilter based on the mycelium driven by gravity that could be used to filter pathogens- free drinking water inexpensively in developing countries. I will test 2 different types of mushroom mycelia: *Stropharia rugosoannulata* and *Pleurotos ostreatus*. It is hypothesized that the mycofilter consisting of *Stropharia rugosoannulata* will be the most effective at reducing *E. coli* colonies.

C. Procedures

Conical tubes, 50mL (9x), Boiled Rye Grains (300 mL), *Pleurotus ostreatus* (Oyster mushroom) grow kit (Back to the Roots, Inc.) Mushroom Kit, *Stropharia rugosoannulata* Mycelia (Wine Cap Mushroom) on rice grains (from Garden Pleasure B07G2K7MPC), Perlite (300 mL), *Escherichia coli* K12 (Carolina Supply Co. #155065).

All filter materials were sterilized in an autoclave oven. To assemble the filter 300 mL of perlite and 300 mL of grains were mixed. 9 -50 mL conical tubes with a 1 cm hole on the bottom were filled with 35 mL of the mixture. 3 conical tubes were inoculated with 4 mL liquid culture

of *P. ostreatus* (Figure 1,5). 3 conical tubes were inoculated with 4 mL liquid culture of *S. rugosoannulata* (Figures 2,4). 3 conical tubes were not inoculated with anything and used as the control group (Figure 3). 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 nutrient agar for colony counts.

All experiments will be performed at the microbiology lab bench within the science research laboratory at the high school. It is a BSL-1 Lab. I will be trained in microbiology techniques and safety procedures. I will wear protective goggles, lab coat, and disposable gloves. all experiments will be directly supervised by the teacher. Surface decontamination will be done by flame sterilization or use of 10% bleach. All used materials will be given to the research teacher, put into autoclave bags, and then autoclaved by the teacher. Edible mushrooms: *Pleurotus ostreatus* (Oyster mushroom) and *Stropharia rugosoannulata* (Wine Cap Mushroom) are exempt from pre-approval and pose no safety hazard.

D. Bibliography

1) Stamets, P., Beutel, M. and Taylor, A. (2013). Comprehensive Assessment of Mycofiltration Biotechnology to Remove Pathogens from Urban Stormwater. [ebook] Fungi Perfecti,
2) Barron, G. & Thorn, R Greg. (2011). Destruction of nematodes by species of Pleurotus. Canadian Journal of Botany. 65. 774-778. 10.1139/b87-103.
3) Barron GL. 1988. —Microcolonies of Bacteria as a Nutrient Source for Lignicolous and Other Fungi.‖ Can. J. Bot. 66:2505-2510.
4) Davis AP, M Shokouhian, H Sharma, and C Minami. 2006. —Water Quality Improvement through Bioretention Media: Nitrogen and Phosphorus Removal.‖ Water Environ. Res. 78(3):284-293.
5) SA Thomas, LM Aston, DL Woodruff, & VI Cullinan. (2009). Field Demonstrations of Mycoremediation for Removal of Fecal Coliform Bacteria and Nutrients in the Dungeness Watershed, Washington [Ebook]. Richland, Washington: Pacific Northwest National Laboratory.

6) Rogers, T. (2012). Experimental Evaluation of Mycoremediation of Escherichia coli Bacteria in Solution using Pleurotus ostreatus [Ebook]. The Evergreen State College. Retrieved from Gapiński, Mariusz & Woźniak, Wanda & Ziombra, Mirosława & Murawska, Joanna. (2007). Oyster mycelium on the liquid medium. Acta Mycologica. 42. 125-128. 10.5586/am.2007.013