Mycoremediation: Waste to Fuel to Food

Maddy Bridge, Ella Buchanan, Ben Davenport, Oliver Kendall, Sage McGinley-Smith, Tia Peterson

Advisors: Alex Cook, Dorlan Curtis Jr.

Key Terms

Fungi: multi-cellular, eukaryotic, heterotrophic organism that depend on extracellular enzymes to break down organic substrates for nutrition
Mushroom: the fruiting, reproductive structure of a fungus
Mycelium: the thread-like root system that makes up the majority of a fungi and produces enzymes that break down complex organic materials
Inoculation: the introduction of fungi onto a sterile growth medium to stimulate the propagation of new mycelium
Spawn: any organic substance that has been inoculated with mycelium, and used to transfer mycelium onto any substrate for mushroom growth
Substrate: the medium that a fungus grows upon
Pinning/fruiting: the mushroom growth cycle of a mature fungus. Pinning is the development of very small immature mushrooms, and fruiting is their growth to full size.
Mycoremediation: form of bioremediation that employs the enzymatic properties of fungi to detoxify contaminated environments.
Ex-situ mycoremediation: contaminating soil samples in a controlled environment and colonizing them with fungi to observe the unaffected remediation
Potato Dextrose Agar (PDA): a nutritional substrate used to grow pure fungal cultures (on a petri dish)
Minimal Salts Medium (MSM): a culture medium for fungi that contains only inorganic salts and nitrogen, and is supplemented with a carbon source

Research Objectives

Mycoremediation
- Screen and isolate oil-degrading fungi to be used for remediation
- Measure the efficacy of using Phoenix Oyster Mushroom (PHX) spawn for mycoremediation of soil contaminants

Edible Mushroom Cultivation
- Investigate the viability of a sustainable mushroom cultivation system at The Island School

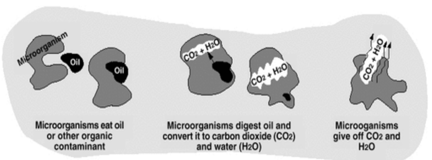
Figure 1: Edible Summer White Oyster mushrooms (SWOY) cultivated at CSD

Figure 2: Edible Grey Oyster mushrooms (GROY) cultivated at CSD

Introduction

Mycoremediation: Mycelium is the key to mycoremediation because it produces extracellular enzymes which can break down hazardous substances (such as petroleum hydrocarbons, pesticides, municipal waste) into less toxic form or into nutrients that fungi can use as a food source. Mycoremediation is typically associated with wood-rotting fungi species because many contaminants have similar molecular structures to those found in wood. At Center for Sustainable Development (CSD), approximately 1,000 gallons of crude glycerol are generated annually as a byproduct of campus' biodiesel production. In the absence of a usage and sustainable waste management system for oil-residues, crude glycerol creates a spatial challenge for storage and poses a high risk for soil and water contamination. Thus the first component of this study investigates, through the use of ex-situ experiments simulating oil spills, the potential of mycoremediation to provide a low tech, cost-effective solution to this problem. In this part of the study, the fungi used were Phoenix Oyster mushrooms (*Pleurotus pulmonarius*).

Figure 3: Mechanism of soil remediation by microorganisms



Edible Mushroom Cultivation
A significant issue faced by Small Island Developing States (SIDS) is food security, largely due to the lack of resources and arable land. As a result, SIDS accumulate a multimillion food import bill, and negatively impacts their carbon footprint. At the CEIS dining hall, 39% of the non-meat protein sources are imported soy-based products. This study investigates the on-campus cultivation of SWOY as a potential replacement for soy-based proteins. This species produces efficient yields, fruits quickly, and is adaptable to hot weather and many substrates.

Methods

Ex-situ Mycoremediation:
Soil was collected and sifted to pass a ¼" mesh screen, then dosed with known soil contaminants at varied concentrations to simulate soil contamination on-campus. The artificially contaminated soil was then inoculated with PHX agar cultures and observed for qualitative fungal growth. Soil samples were collected weekly to monitor the change in soil physicochemical properties (pH and NPK) due to fungal inoculation.

Earthworm Testing:
Red wriggler earthworms (*Eisenia fetida*) were utilized as an indicator species for soil health and viability to support the life of macro organisms after fungal inoculation. Adult earthworms were screened and allowed to clear their digestive tract for 24 hours, washed and weighed, and placed in a petri dish with 2 grams of the contaminated soil. After 48 hours of exposure to soil, specimens were weighed and observed for behavioral changes in response to light and touch sensitivity.

Figure 4: Qualitative scale used to interpret EW behavior and response to stimuli (light and touch) post-exposure to contaminated soil

In-vitro Fungal Adaptation:
To facilitate continued inoculation and maintain a consistent "in-house" supply of spawn, pure fungal cultures were created by cloning and propagating SWOY/PHX mycelium on PDA plates. These cultures were used to produce mycelium for edible mushrooms, in-vitro fungal adaptation process and mycoremediation purposes.
For in-vitro fungal adaptation, PHX agar cultures were transplanted to MSM plates on which the only carbon source was a dosage of the contaminant. If the mycelium showed signs of growth on the contaminant, a piece of that strain was then isolated on a PDA plate to grow. This process was repeated, increasing the dosage of contaminant each time until a strain was isolated that is adapted to consume the contaminant and can facilitate more effective remediation.

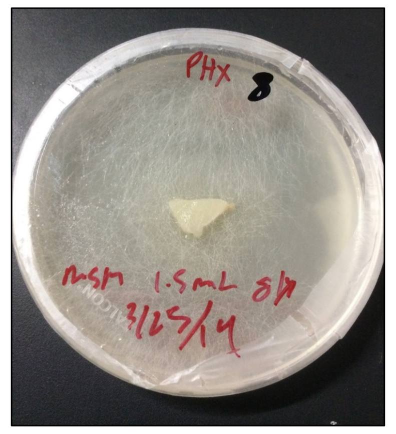


Figure 5: A pure culture of PHX growing on MSM dosed with 1% glycerol.

Edible Mushroom Cultivation:
PHX bulk spawn was used to inoculate sterile coffee grounds, incubated in the dark at 26° C and allowed to colonize for a period of 40-55 days. Once fully colonized, the substrate bags were transferred to a fruiting chamber where they were exposed to light, higher oxygen, and 90% relative humidity to incite the fruiting process. The mushroom bodies were then harvested to determine yield and size of mushroom caps.

Results & Analysis

Mycoremediation
The pH testing was unable to produce significant results, however the general trend shows that the soils became less alkaline over time (Fig. 6). This is a good sign because it may mean that the mycelium is remediating the soils and restoring them to a more normal pH.

Earthworm Toxicity
After the 48-hour period of exposure to contaminated soil, the worms were weighed and tested for light and touch sensitivity, and observed mortality. The mortality data shows that motor oil is the most toxic contaminant (Fig. 10) to macro-organism health. This conclusion is also supported by the unhealthy responses to light and touch observed in worms exposed to soil contaminated with motor oil. Analysis of the weight change (Fig. 7) shows that the earthworms may be able to consume and digest glycerol and vegetable oil; thus, it may be possible to utilize earthworms as an auxiliary bioremediator to fungi.

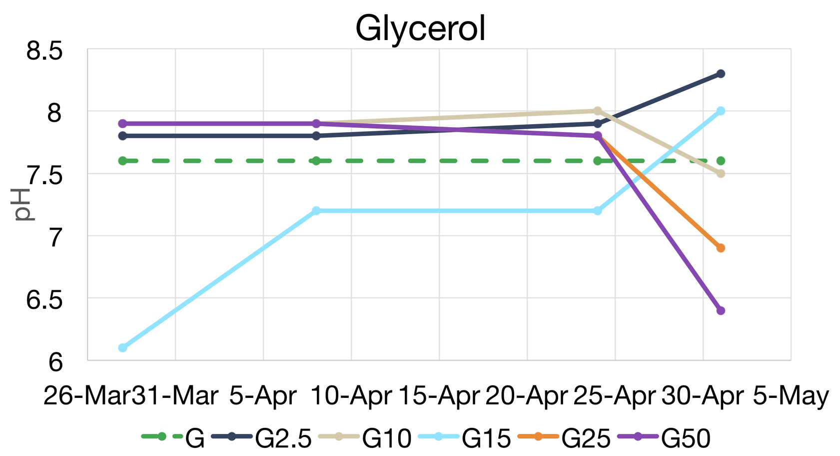


Figure 6: pH of glycerol-contaminated soil inoculated with PHX cultures

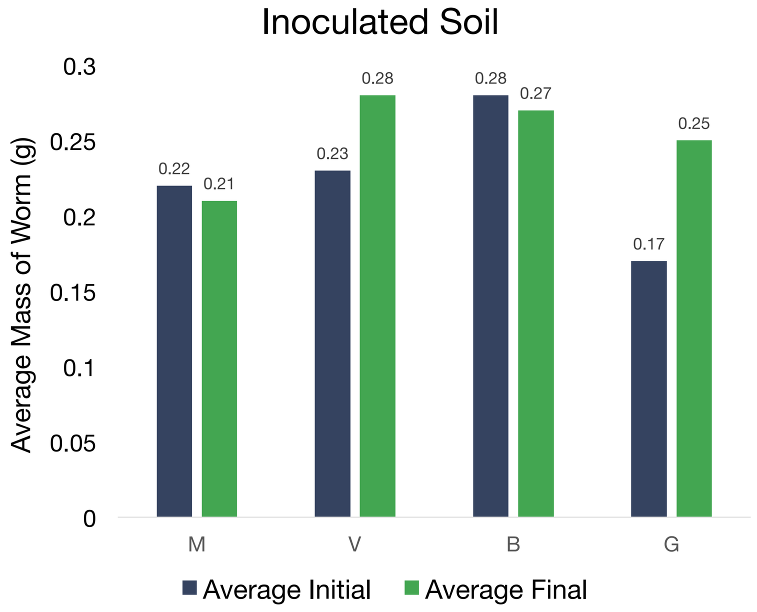


Figure 7: Average weight change of earthworms after 48 hour exposure to contaminated soil

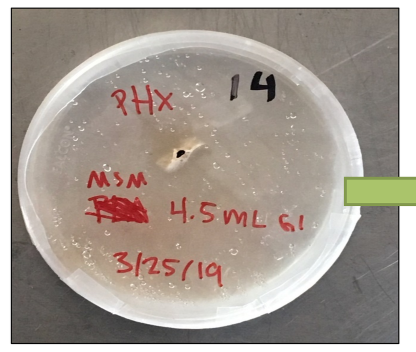


Figure 8: PHX strain screened on MSM dosed with 3% glycerol

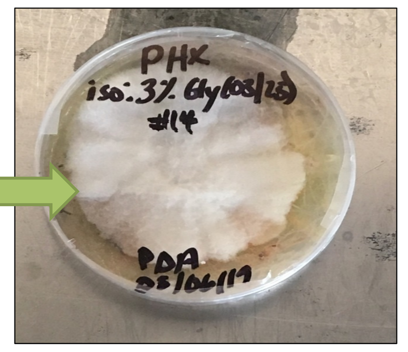


Figure 9: PHX strain transferred and isolated on PDA. This adapted culture has oil-degrading properties for mycoremediation purposes

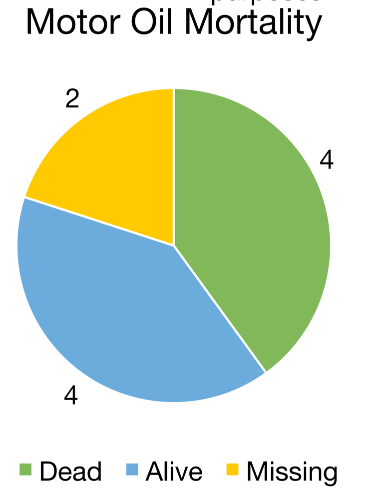


Figure 10: Mortality of earthworms after 48 hour exposure to motor oil-contaminated soil

In-vitro fungal adaptation
The Linear Growth Test measures the radial length of fungal cultures on PDA and MSM, using the mycelium on PDA as a baseline for ideal fungal growth. The similarities between the daily linear growth rates of fungi on PDA and MSM culture plates show that the fungi are able to consume the contaminants and utilize for growth (Fig. 8 and 9).

Edible Mushroom Cultivation
The study concluded that SWOY mushrooms would be suitable to cultivate for The Island School dining hall. This mushroom species has a quick spawn run, produces multiple flushes, is adaptable to the tropical climate, and can be cultivated on a variety of accessible substrates (coffee, woodchips, sawdust etc.). Based on the CEIS Spring 2019 food audit, campus cultivated mushrooms cost 40 cents less per pound in comparison to imported mushrooms and soy-based proteins (Fig. 11). This projected cost savings is more sustainable for local production and reduces the campus carbon footprint.

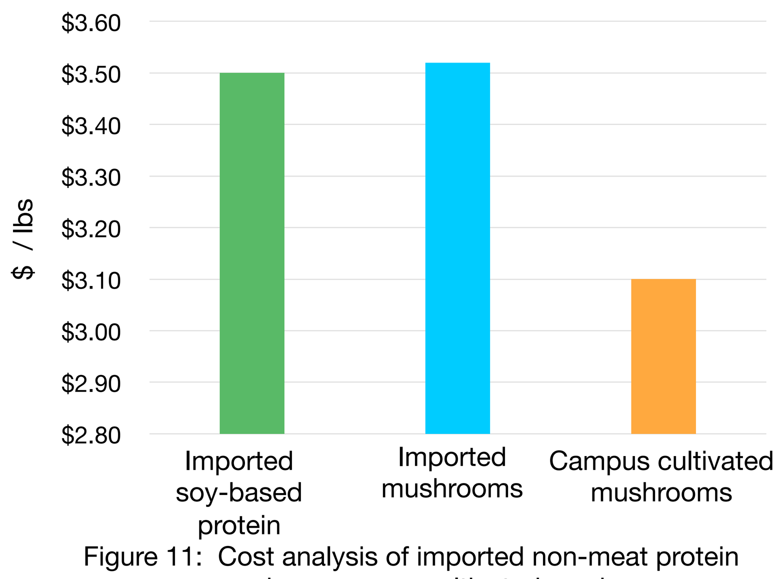


Figure 11: Cost analysis of imported non-meat protein sources and on-campus cultivated mushrooms.

Conclusion

In-vitro fungal adaptation: A viable strain of PHX mycelium was screened and isolated to survive on a contaminant dosage of 3%v/v of MSM which is indicative of its oil-degrading ability. In comparison to pure PHX cultures maintained on PDA which had a growth rate of 5.60±0.55 mm/day, the isolated oil-degrading strains on MSM displayed 6.11±0.33 mm/day linear growth rate. As a result, these oil-degrading fungal strains will be further adapted to a higher dosage of soil contaminants (10-15%), and propagated for in-situ mycoremediation purposes

Mycoremediation: Based on the qualitative observations of visible mushroom growth on WVO, BD and Gly in the ex-situ mycoremediation trials, it can be inferred that PHX spawn is suited for soil decontamination. Additionally, the change in soil pH reveals that fungi have an impact on the physicochemical property of soil. Furthermore, given the high mortality rate of EW as an indicator species, soil contaminated with motor oil and glycerol have been identified as the most toxic and should be top priority for decontamination.

Edible mushroom cultivation: The contribution to food security of the CEIS dining hall has been proven possible with the success of the edible mushrooms cultivated in this project. Nevertheless, it has been calculated that CSD will need to produce 50lbs/week of oyster mushrooms to offset the demand of imported soy-based proteins. The campus mushroom production would require approximately 12m^2 of cultivation space at a cost of $3.10/lb which is 40¢ less than soy-based meat substitutes.

Figures 12 and 13: Examples of indoor mushroom fruiting rooms that will need to be designed for mushroom cultivation at CSD

Project Future

Mycoremediation: Once a strain is successfully adapted to remediate a higher concentration of the contaminants, it will be grown in substrate bags. This will allow mycoremediation and monitoring of the on-campus soils. A research grant to fund production of oil-degrading fungal strains and analytical equipment for soil contamination and micronutrient levels has been applied for.

Edible mushroom cultivation: A shipping container is required to scale-up. This container provides enough area to produce the 50lbs of mushrooms needed per week. This environment would be climate controlled and sterile, which would increase the consistency of mushroom

Figure 14: Edible PHX cultivated on-campus

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