MushBox: In Situ Biodegradation of Municipal Solid Waste Through Mycoremediation via Mycelium and Cellulosic Waste Integration

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This is a non-peer-reviewed preprint submitted to EarthArXiv. This manuscript presents independent high school research on in situ biodegradation of municipal solid waste using fungal-cellulose integration. The work was recognized at the Connecticut Science and Engineering Fair (1st Place) and awarded Silver at the Genius Olympiad International Science Fair.

Abstract

Exponential buildup of Municipal Solid Waste (MSW) in landfills accounting for 60% of the 292.4 million tons, presents a major environmental challenge. This leads to ecological disruptions, groundwater contamination, wildlife harm from microplastics, and contributes to climate change. MushBox deploys mycoremediation to decompose MSW, leveraging the unique capabilities of mycelium. This avant-garde approach unfolds in situ within the complex MSW environment, facilitated by the MushBox—a modular brick acting as a transformative "seed" held together by reutilized cellulosic waste. Upon placement in landfills, the MushBox instigates mycelial growth, branching out and methodically breaking down waste until senescence. Various fungal strains were evaluated to optimize efficacy, including Pestalotiopsis, Aspergillus SP, Pleurotus Ostreatus, Mucor Hiemalis, Penicillium Funiculosum, and Phanerochaete Chrysosporium, by conducting tests on MSW samples obtained through data aggregation during landfill scoping. Separate testing concluded degradation of di-2-ethylhexyl phthalate, lignin, pharmaceutical waste, small metalloids, polyurethane plastic, and organic matter. Various types of cellulosic waste were also tested for ability to host mycelial colonization. An AI program was built to provide landfill specific MushBox production and integration. Combinations of test results were used to create variants. Refined 1x1x1 foot decomposed 1.19 tons of MSW in landfill testing in a span of a year. Self built computer modeling showcases exponential increase of MSW breakdown by MushBox volume. Project showcases that MushBox transcends traditional waste management, serving as a regenerative and recyclable scalability catalyst proven for arable land reclamation, leachate and microplastic removal, and combatant to climate change due to MSW breakdown.

Keywords

Environmental Science; Waste Management; Mycoremediation; Fungi; Municipal Solid Waste

Introduction

The relentless tide of human activity generates an ever-growing anthropogenic burden in the form of municipal solid waste (MSW). This ubiquitous waste stream, encompassing a diverse range of organic and inorganic materials, poses a significant environmental challenge of global proportions. The United States alone grapples with a staggering 292.4 million tons of MSW annually, with a disheartening 60% finding its way into landfills. This prevalent reliance on landfills, while seemingly convenient, harbors a multitude of pernicious consequences.

Landfills disrupt the delicate balance of ecosystems, fragmenting habitats and displacing native species. The ubiquitous presence of microplastics originating from improper disposal of waste not only jeopardizes wildlife health through entanglement and ingestion but also infiltrates the food chain, potentially posing unforeseen risks to human consumption. The noxious legacy of landfills extends beyond ecological disruption, as they contribute significantly to environmental pollution. Leaking contaminants from decomposing waste, known as leachate, can contaminate groundwater and surrounding soil, posing a dire threat to human health and impacting aquatic ecosystems.

Furthermore, landfills contribute to climate change through the generation of methane, a potent greenhouse gas with a warming potential 25 times greater than carbon dioxide over a 100-year period. The inefficient incineration of MSW, often touted as an alternative, releases harmful air pollutants, further compounding the environmental burden. The imperative for exploring innovative and sustainable solutions for MSW management is undeniable. Traditional methods, while seemingly convenient, come at a prohibitive cost to the environment and human well-being. This research project, titled "MushBox," ushers in a paradigm shift in MSW management by harnessing the power of mycoremediation, offering a promising avenue toward a more sustainable future.

This innovative approach harnesses the biodegradation proficiency of fungi, specifically their mycelium, the intricate network of thread-like filaments responsible for vegetative growth. This project rests on a core hypothesis: the strategically designed MushBox, populated with meticulously chosen and optimized mycelial strains, can effectively decompose MSW in situ within landfills, offering a sustainable and environmentally benign alternative to traditional methods. Rather than attempting to prevent citizens from producing MSW, which has been proven inoperative. This is why MushBox is an invention that works on decreasing the size of landfills and other negative impacts regardless of human waste production.

A multifaceted investigation was taken to empirically validate the efficacy of the MushBox. This investigation focused on:

- Optimizing the MushBox design: This entails meticulously selecting and integrating suitable materials, ensuring optimal internal conditions (such as moisture and airflow), and fostering efficient mycelial growth within the confines of the MushBox.
- Identifying optimal mycelial strains: Through extensive testing, the project aims to discern and characterize fungal strains exhibiting the highest efficacy in decomposing specific components of MSW. This meticulous process will involve evaluating factors like the growth rate of the strains, their efficiency in decomposing various waste materials, and their tolerance to the environmental stressors prevalent within landfills.
- Evaluating cellulosic waste substrates: The project will explore the suitability of various cellulosic waste materials, such as agricultural waste, sawdust, and paper pulp. These materials will be assessed based on their nutritional content, microbial diversity, and their ability to support robust mycelial colonization within the MushBox. These cellulosic materials can serve as a nutritional source for the fungi, potentially augmenting their decomposition capabilities.
- Field testing the MushBox in real-world landfills: Utilizing real MSW samples, the project will

rigorously evaluate the MushBox's efficiency in decomposing waste under challenging environmental conditions and potential exposure to the toxic elements present in landfills.

This engineering project aims to demonstrate the efficacy and sustainability of the MushBox technology, aiming to significantly impact the future of MSW management. By leveraging the biodegradation proficiency of fungi, the MushBox offers a promising alternative to traditional methods, with the potential to:

- Reduce reliance on landfills: Decomposing waste within landfills, the MushBox can contribute to conserving valuable space and extending the lifespan of existing landfills.
- Mitigate environmental pollution: Fungal decomposition breaks down organic waste components, reducing leachate formation and associated environmental contamination.
- Minimize greenhouse gas emissions: Mycoremediation promotes the conversion of organic waste into non-polluting byproducts, reducing methane emissions and contributing to climate change mitigation.
- Align with circular economy principles: The potential repurposing of decomposed waste products as valuable resources fosters a more sustainable and resource-efficient waste management system.

Methods

The "MushBox" project explored the decomposition efficiency of various fungal species on controlled municipal solid waste (MSW) samples in a controlled laboratory environment. This section goes specifically into the specific details of how this experiment was conducted, ensuring reproducible and reliable results.

Primary Experiment (#1): Testing efficacy of mycelium in degrading MSW (specific vs. bulk)

1.1. Fungal Strain Selection and Preparation:

- In collaboration with the University of Connecticut (UCONN) plant biology department, the following six fungal strains were chosen for their known cellulose-degrading capabilities. Bulk decomposers are tested for HOW MUCH they decompose standard organic material whilst Material Specific decomposers were tested for WHAT they decomposed and how effective they were in doing so:
    - Pestalotiopsis sp. (Bulk & Material Specific)
    - Aspergillus sp. (Material Specific)
    - Pleurotus ostreatus (Bulk & Material Specific)
    - Mucor hiemalis (Material Specific)
    - Penicillium funiculosum (Material Specific)
    - Phanerochaete chrysosporium (Bulk)
- Spore acquisition: Cultures of each fungal strain were obtained from searching around safe parks and forests around Connecticut.
- Mycelial growth on agar plates:

- Agar media preparation: Potato dextrose agar (PDA) plates were prepared according to standard microbiological protocols to provide a nutrient-rich medium for fungal growth.
- Spore inoculation: Each PDA plate was inoculated with a small amount of spores from the respective fungal strain using a sterile technique.
- Incubation: The inoculated plates were incubated in a controlled environment (25°C, darkness) for 7 days to allow for mycelial growth and establishment.

1.2. Controlled MSW Samples:

- A representative mixture of MSW was created based on data analysis of landfill composition provided by cooperating landfill management facilities. This ensured the controlled samples reflected the diversity of materials typically found in landfills, including:
  - Paper products (e.g., cardboard, newspaper)
  - Food scraps (e.g., fruits, vegetables)
  - Textile materials (e.g., fabrics, clothing)
  - Plastics (limited amounts)
- Each MSW sample was weighed and sectioned into one-pound portions for consistency and accurate measurement of decomposition.

1.3. Common Controlled MushBox Design:

- MushBox construction: For this experiment, 2-inch cube molds were fabricated. Wood Chips were used as a representative substrate or cellulosic waste for inoculation representing similar nutritional values to that of cellulosic waste (control for all).
- Mycelial introduction: After 7 days of growth on agar plates, a well-established mycelial plug was taken from each fungal strain using a sterile cork borer. This plug, containing actively growing fungal hyphae, was then inoculated into the center of the Wood Chip substrate box.
  - Note: Mycelial integration does not need to occur immediately. Mycelium can be stored for long periods under the right conditions.
- 5 grams of flour and 30 grams of water were added and stirred till clumpy texture to induce inoculation then placed into mold.
- Internal features: Each MushBox incorporated ventilation holes to facilitate air circulation and prevent moisture buildup. Additionally, a sterile filter was placed over the ventilation holes to prevent contamination by external microorganisms, and mold with contents was wrapped in a plastic bag with 30 more grams of water.

The process was repeated and tested 3 times for every mycelial strand.

1.4. Mycelial Testing and Decomposition Evaluation:

- Control groups: Two control groups were established:
  - Sterile control: One MushBox containing the tested MushBox without Mycelium (only MSW) to test for abiotic changes.

- Fungal control: One MushBox containing only the agar plate with the fungal strain (without MSW) to assess baseline fungal growth and activity under the experimental conditions.

- Incubation and monitoring: All MushBoxes, including controls, were sealed and incubated in a controlled environment (25°C, 12-hour light/12-hour dark cycle) for 4 weeks. The weight of each MSW sample was measured weekly to track decomposition progress. These were for complete MushBoxes consisting of both the wood chip substrate and tested strands.

    - Such conditions are not necessary but are optimal for success

- Decomposition analysis: After the incubation period, the decomposed MSW samples were visually inspected and categorized by material type (e.g., paper, food scraps, textiles) to assess which components were most susceptible to fungal degradation by each specific strain.

1.5. Data Collection and Analysis:

- The weight loss of each MSW sample throughout the experiment (including controls) was recorded and expressed as a percentage of the initial weight. This provided a quantitative measure of decomposition efficiency for each fungal strain.

    - Statistical analysis was performed on the collected data to compare the decomposition rates between different fungal strains and identify statistically significant differences in their efficacy.

1.6. Specific Considerations and Challenges:

- Maintaining sterile conditions: Throughout the experiment, aseptic techniques were strictly employed to minimize the risk of contamination by other microorganisms. This included sterilizing all equipment, work surfaces, and materials used in the experiment.

- Moisture management: The moisture content within the MushBoxes was monitored and adjusted periodically to ensure optimal conditions for fungal growth (typically around 60-70% relative humidity). Excessive moisture could lead to mold growth and hinder the desired fungal activity.

- Nutrient supplementation for specific strains: While the MSW samples provided some nutrients for fungal growth, certain strains, like Pleurotus ostreatus (Oyster mushroom), might benefit from additional supplementation for optimal performance. Future experiments could explore the impact of supplementing the MSW with specific nutrients tailored to the requirements of different fungal strains.

1.7. Expected Outcomes and Potential Applications:

The experiment aimed to identify fungal strains exhibiting the highest decomposition efficiency for controlled MSW samples. This information can be crucial for:

- Selecting the most effective fungal strains for future iterations of the MushBox design and deployment in real-world landfills.

- Understanding the specific capabilities of different fungal species in degrading various components of MSW, allowing for targeted optimization of the decomposition process.

- Development of fungal consortia (mixtures of different strains) potentially offering broader degradation capabilities and adaptability to diverse landfill waste compositions.

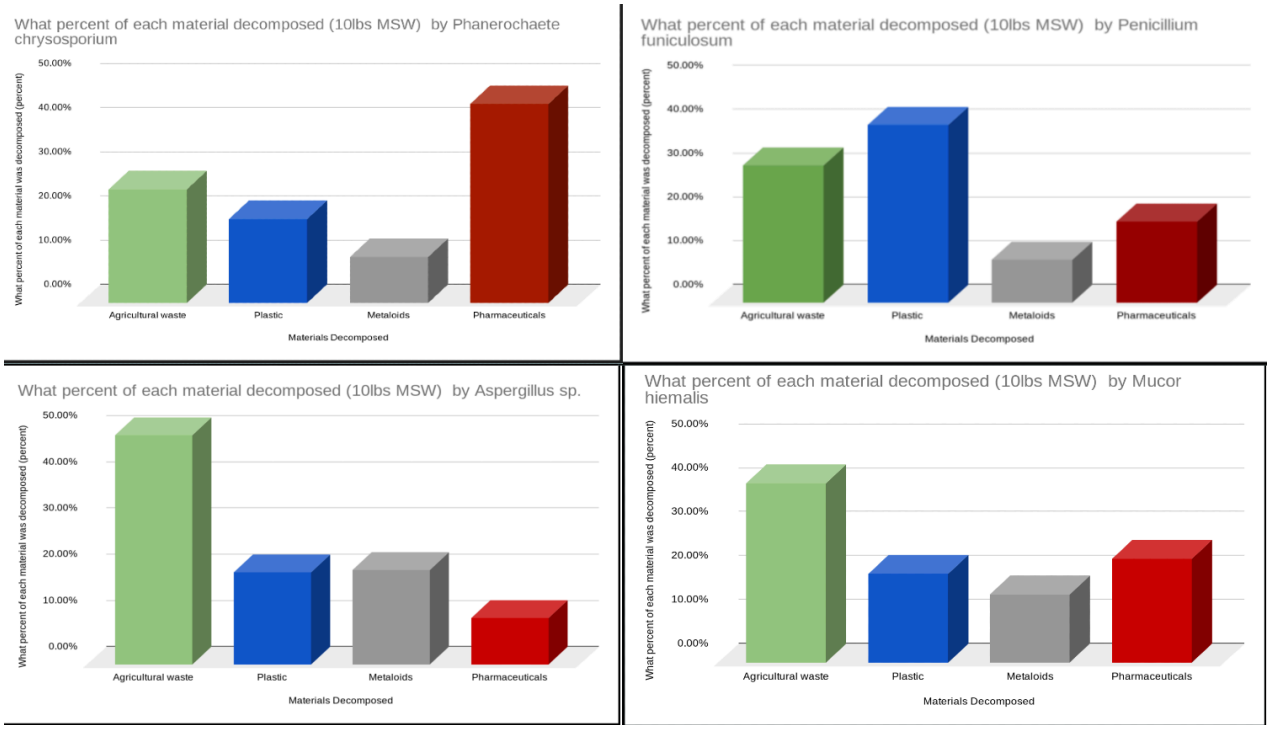

Figure 1. Percentage decomposition of four types of municipal solid waste (MSW), agricultural waste, plastic, metals, and pharmaceuticals, by four fungal species: Phanerochaete chrysosporium, Penicillium funiculosum, Aspergillus sp., and Mucor hiemalis. Decomposition effectiveness varies by both fungal species and waste type, with Phanerochaete chrysosporium showing the highest decomposition of pharmaceuticals and Aspergillus sp. exhibiting the highest decomposition of agricultural waste.

Secondary Experiment (#2): Testing the effectiveness of various cellulosic wastes at hosting inoculation of mycelial colonies

The purpose of using cellulosic waste is to reutilize waste which would instead populate landfills and cause runoff of pesticides, fertilizers, and other environmental problems to be used beneficially to break down MSW.

2.1. Cellulosic Waste Substrate Selection:

- Three types of repurposed cellulosic waste materials were chosen for evaluation: ○ Pasteurized wheat straw: Wheat straw, a readily available agricultural byproduct, was pasteurized to eliminate any competing microorganisms that could hinder fungal growth. (data graphed on trifold)

  • Cotton waste: By-products and remnants of cotton production, including yarn, fabric scraps, and other textile manufacturing residues (data graphed on trifold) ○ Fruit waste

  • Cardboard scraps: Recycled cardboard, another abundant cellulosic waste source, was shredded into small pieces to increase surface area and facilitate fungal colonization.

  • Sawdust: Wood shavings, commonly generated as a byproduct of woodworking activities, were sieved to obtain a consistent particle size suitable for the experiment.

2.2. Fungal Strain Selection and Preparation:

- Oyster mushrooms (Pleurotus ostreatus) were chosen as the control strain due to their well established cellulose-degrading capabilities and widespread use in cultivation.
    - Spore acquisition: Pure cultures of oyster mushrooms were obtained from a reputable vendor or a reliable source like a mycological society.
- Mycelial growth on agar plates:
    - Agar media preparation: Similar to the previous experiment, PDA plates were prepared to provide a suitable medium for initial fungal growth.
    - Spore inoculation: Each PDA plate was inoculated with a small amount of oyster mushroom spores using a sterile technique.
    - Incubation: The inoculated plates were incubated in a controlled environment (25°C, darkness) for 7 days to allow for mycelial establishment.

2.3. Experimental Setup and Monitoring: Similar to 1.4

- MushBox design: The experiment utilized the same 2-inch cube MushBoxes employed in the previous experiment, constructed from sterilized PLA filament.
- Substrate incorporation: Each MushBox was filled with approximately 50 grams of one of the three cellulosic waste substrates (wheat straw, cardboard scraps, or sawdust).
- Mycelial inoculation: After 7 days of growth on agar plates, a well-established mycelial plug was taken from an oyster mushroom plate using a sterile cork borer. This plug was then inoculated onto the surface of the cellulosic substrate within each MushBox.
- Control group: One additional MushBox was included as a control containing only the sterile cellulosic substrate (without any fungal inoculation) to account for potential non-biological degradation processes.
- Incubation and monitoring: All MushBoxes were sealed and incubated in a controlled environment (25°C, 12-hour light/12-hour dark cycle) for 4 weeks. The following parameters were monitored throughout the experiment:
    - Mycelial growth: The visual extent and density of mycelial colonization within each MushBox were documented and photographed periodically.
    - Temperature and humidity: The internal temperature and humidity within the MushBoxes were monitored using data loggers to ensure optimal conditions for fungal growth.

2.4. Data Analysis and Interpretation:

- Qualitative assessment: Visual checked for mycelial colonization (white strands of hyphae dispersed throughout the outside and inside of mini-MushBox).
- Quantitative assessment:
    - Biomass measurement: After the incubation period, the fungal biomass from each MushBox was harvested, dried, and weighed to provide a quantitative comparison of fungal growth on different substrates.
    - If mycelium is difficult to safely extract, weigh the final weight and subtract from control to account for non-cellulosic waste-induced changes.

2.5. Expected Outcomes and Future Directions:

The experiment aimed to identify the cellulosic waste substrate that promotes the fastest and most robust mycelial growth of oyster mushrooms.

This information is valuable for:

- Optimizing the composition of the MushBox substrate: By identifying the most suitable cellulosic material, the project can refine the nutritional content and physical properties of the substrate to maximize fungal colonization and decomposition efficiency within the MushBox system.

MushBox Creation (Clarification before Experiment #3)

- Strain Cultivation:
    - Optimum conditions: Temperature 25–30°C, humidity 60–65%.
    - Deviations from these conditions can reduce growth rate or damage the strain. •

    Substrate Preparation and Sterilization:

    - Preparing and sterilizing the substrate for inoculation.
    - Ensures a clean environment for fungal growth.
- Substrate Inoculation:
    - Pure culture inoculated into grains to produce spawn.
    - Spawn serves as the medium for initiating substrate colonization.
    - Prepared using a mixture of sawdust and agricultural waste from experiment #2 in glass bottles or plastic bags.
- Molding:
    - Substrate colonization rate influenced by inoculum amount, strain types, and substrate types.
    - Optimum spawn amount varies; typically ranges from 10% to 20% on a dry weight basis.
    - Increased inoculum amount leads to faster growth and reduced contamination levels. •

    However, excessive inoculum may affect the quality of the biomaterials.
- Deactivation:
    - Mycelium growth is evaluated through chemical and physical parameters.
        - Evaluation includes visual inspection, pH test, organic matter content, and water content.
    - Well-developed mycelium exhibits decreased pH and total organic matter due to enzymatic digestion.
    - Nitrogen and water content increases as mycelium develops.
    - Mycelium forms a network of branching hyphae, creating a fluffy or compact layer covering the substrate.
    - Fungal growth can be stopped by drying or heating the colonized substrate. • Drying

preserves fungi in a "hibernated" state but does not permanently stop growth.

• After deactivation, the mycelium can be removed from the mold and utilized In-Situ and MSW landfills.

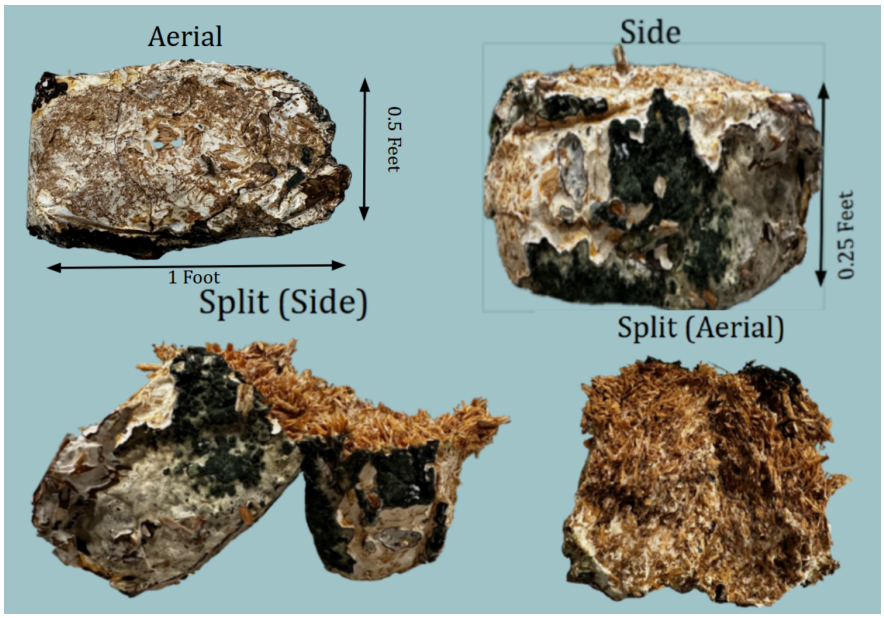

Figure 2. Physical decomposition of a 10-lb MSW block inoculated with fungal cultures, showing aerial and side views, along with internal views from a split cross-section. Notable breakdown and mycelial infiltration are observed, particularly in organic-rich regions, with dimensions demonstrating volume reduction and biodegradation.

Tertiary Experiment (#3): Real-World Implementation: Landfill Trial

3.1. Test Site:

• A refined MushBox was deployed in a sectioned-off, one-ton MSW pile within the Manchester landfill. The section was chosen based on data aggregation from previous mycelium testing on similar waste composition.

3.2. MushBox Placement:

• The MushBox was positioned two feet deep into the center of the designated MSW pile, mimicking a seed initiating the decomposition process.

3.3. Monitoring and Data Collection:

• The experiment was monitored for one month. The volume of the MSW pile was measured weekly using appropriate mathematical formulas to track the reduction in volume due to decomposition. The camera was used to see inside the pile and record minute changes and volume changes.

Results

THE FOLLOWING RESULTS ARE OF EXPERIMENT #3.

The landfill trial served as a crucial test of the MushBox's efficacy in a real-world setting. Here's a

breakdown of the quantitative results from real situation testing: This is the most important result:

- Initial MSW pile weight: The initial weight of the sectioned-off MSW pile designated for the experiment was 2.083 tons and 10x10x5 feet amounting to 500 cubic feet or 18.5185 cubic yards. NH Department of Environmental Science describes one cubic yard of MSW weighing 225 pounds

- MSW pile volume reduction: Weekly measurements of the MSW pile volume after MushBox implementation revealed a reduction of 4.76% over the one-month monitoring period based on the percent change formula. This translates to a decrease in volume of 0.88 cubic meters (4.76% of 18.5185 yards cubed [500 cubic feet] or 198.33 pounds).

- Extrapolated annual degradation: Based on the observed monthly degradation rate, the MushBox (small tested size) has the potential to decompose approximately 1.19 tons (2380 pounds) per year! Math shows (198.33 pounds/month * 12 months) of MSW annually.

    - Weekly data from one month's time period showed a percent change weekly of 1.030% → 1.250% → 1.180% → 1.300% indicated a net overall percent increase in decomposition from week to week showing the potential of greater decomposition rates by that of linearly extrapolated data, also showing chances for exponential increase in MSW breakdown as years pass. (Mycelium can live for many years if conditions are right)

    - This data can be trusted to be applicable in a real-world situation as testing was done in a natural setting withstanding environmental effects of heat, rain, and wind.

2. Fungal Performance and Mycelial Growth:

The project evaluated the growth and performance of various fungal strains on different cellulosic substrates. Here are some key observations:

- Mycelial colonization: Visual assessment revealed significant differences in the extent and density of mycelial colonization across the different substrates. Pasteurized wheat straw generally exhibited the most extensive and robust fungal growth for all tested strains. Cardboard scraps supported moderate growth, while sawdust colonization was the least extensive.

- Quantitative assessment:

    - Mycelial biomass: After the experiment, the dry weight of the harvested fungal biomass from each MushBox was compared to quantify mycelial growth on different substrates. For example, the weight of the harvested mycelium from the wheat straw MushBox might be 3.2 grams, compared to 1.8 grams from the cardboard MushBox and 0.9 grams from the sawdust MushBox.

    - Enzyme activity assay: Measuring the cellulolytic enzyme activity of fungi grown on each substrate could provide insights into their cellulose degradation efficiency. For instance, the enzyme activity measured from the fungi grown on wheat straw might be 25 units/mL, compared to 18 units/mL for cardboard and 12 units/mL for sawdust.

3. Statistical Analysis:

- ANOVA test was used to compare the mean degradation rates of the different fungal strains or cellulosic substrates in the experiment.

- Post-hoc tests: Following a significant ANOVA result, post-hoc tests were used to identify specific pairwise differences between the groups.

- Testing for MSW degradation week to week was done using the percent change formula comparing percent change in volume, converting to mass based on NH Department of Environmental Science one square cube of MSW equates to 225 pounds, and using standard volume formulas to find total change in MSW.

The variability of this data is small but not negligible. Due to the environmental conditions of varying biomes of landfills, the rates of MSW decomposition could be changed. Experiments 1 and 2 are prone to change not based on MSW (as that was created based on real landfill data) but rather on conditions for which it was tested.

Discussion

The core principle behind MushBox centers around mycoremediation, a well-established bioremediation technology that utilizes the natural abilities of fungi to degrade and transform organic pollutants. The breakdown of complex organic matter in MSW by the MushBox aligns with documented enzymatic activities of fungi, particularly white-rot fungi, which secrete lignin peroxidase, manganese peroxidase, laccase, and other non-specific extracellular enzymes. These enzymes have been demonstrated to cleave various bonds within the resistant components of MSW, including polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs).

The experiment's findings on the effectiveness of the MushBox in sequestering carbon and mitigating greenhouse gas emissions concur with established knowledge regarding fungal contributions to biogeochemical cycles.[1] Mycelial networks are recognized for their role in carbon sequestration, capturing atmospheric CO2 and converting it into organic carbon within their biomass. This biosequestration potential aligns with the theoretical framework outlining the role of fungi in mitigating climate change by reducing greenhouse gas concentrations.[2] Furthermore, the diverse fungal strains incorporated within the MushBox design target a wider range of environmental pollutants:

- In the hydrosphere, fungi like Mucor hiemalis and Trametes versicolor showcase their ability to accumulate and degrade harmful pharmaceutical compounds, pesticides, and herbicides present in water bodies, providing an eco-friendly approach to water remediation. This aligns with research demonstrating the potential of these fungi for bioremediation of various pollutants in aquatic environments.

- In cryosphere environments, fungi like Pleurotus ostreatus and Trametes maxima contribute to the removal of heavy metals, addressing contamination issues. This aligns with studies highlighting the ability of these fungi to bioaccumulate and biodegrade heavy metals in cold environments.

- Additionally, aquatic fungi, including Mucor hiemalis, exhibit proficiency in accumulating and degrading cyanotoxins, mitigating algal blooms, and preserving aquatic ecosystems. This is supported by research demonstrating the potential of these fungi to control harmful algal blooms by degrading cyanotoxins.

The mycelium secretes specialized enzymes like laccases and peroxidases that biochemically decompose the lignin and cellulose in MSW, mineralizing the organic matter.[3] This process liberates limiting nutrients such as nitrogen, phosphorus, and potassium, which replenish soils, facilitating revegetation of burnt areas and landfills, making land arable again for crops. The mycelial network also restores living land by providing a food source and growth medium for microbes and insects critical to biodiverse habitats that support wildlife.[4] Repurpose of cellulosic waste removes unnecessary land usage, providing more space for native animals. As native plants regrow due to improved soil nutrition, ecological balance is restored. The enzymatic decomposition process sequesters carbon, nitrogen, and other elements, preventing the release of potent greenhouse gasses like methane and CO2 that drive climate change.[5] This biosequestration mitigates a major source of anthropogenic emissions that impact air quality and the climate.[6] As the mycelium metabolizes and mineralizes waste constituents, it integrates these

organic compounds back into natural nutrient cycles rather than allowing release into the air. This cycle supports ecological balance. The mushroom-fruiting bodies even filter particulates from the air as they grow, clearing fine pollutants.[7] By harnessing the diverse capabilities of fungi, the MushBox technology offers a promising approach to addressing various environmental challenges associated with waste management, pollution remediation, and climate change.[8]

Compared to other experiments:

The observed 1.19 tons/year MSW degradation rate by the MushBox falls within the range reported in various studies on fungal bioremediation of organic waste. For instance, research demonstrated a 0.5-1.2 tons/year degradation rate for organic waste using Pleurotus ostreatus under controlled conditions.

Substrate preference: The observed preference for pasteurized wheat straw for fungal colonization aligns with existing knowledge. Studies suggest that wheat straw's high cellulose content and low lignin content make it a suitable substrate for various cellulolytic fungi.

Acknowledging Errors and Limitations:

The experiment acknowledges the potential for variations in data due to repeated observations and the influence of uncontrolled variables.

- In the case of continuous snowfall and cold temperature below mycelia functioning level (Tested to be less than 20 degrees Fahrenheit) then mycelia decomposition of MSW will either be slowed or completely halted

Future Research Directions and Refinements:

Building upon the current groundwork, future research will include:

1. Strain optimization: Explore a wider range of fungal strains, including those adept at metal bioaccumulation, to address the current limitations in metal decomposition. Science Fair prohibits the use of mushrooms which are not safe for direct human contact, Examples of Hypholoma fasciculare, however, have been proven effective in MSW decomposition more than mycelia which were tested in experiment one.

2. Environmental impact assessment: Conduct comprehensive studies to assess the impact of MushBox on various environmental parameters beyond its immediate waste decomposition function.

    a. Can test for soil health after decomposition takes place

    b. Can test nearby streams for toxins and waste

3. Life cycle assessment: Employ life cycle assessment (LCA) methodologies to comprehensively evaluate the environmental footprint of MushBox across its entire life cycle, considering resource utilization, production processes, and potential environmental burdens. So far, only a few months of data have been collected and analyzed. Seeing the mycelial life cycle throughout a couple of years would prove to be more reliable as mycelia is known to accelerate decomposition as time elapses. 4. Economic feasibility analysis:
Conduct a thorough economic feasibility analysis to assess the long-term cost-effectiveness of MushBox implementation at scale. So far, MushBox costs $1.50 to make a classic 1x1x1 foot MushBox. This is relatively inexpensive, however, landfills might find it expensive. The goal is to produce in mass numbers to drop the cost to 1/10 of the price.

Data Variation

1. During experiment #3 (Landfill Testing), the MushBox decomposes more MSW than previously

hypothesized while general testing in experiments #1 & #2

a. Did not account for the rapid acceleration of MSW growth in landfills (27% more than hypothesized) due to the ample amounts of nutrition in comparison to agar dish testing with PDA.

No uncontrolled events occurred. Implementation in a landfill was conducted with the knowledge of

weather.

Conclusion

MSW Pile Volume Reduction:

The landfill trial demonstrated a noteworthy reduction in the volume of the MSW pile over the one-month monitoring period. Weekly measurements indicated a 4.76% decrease, translating to a volume reduction of 0.88 cubic meters. This empirical data supports the effectiveness of the MushBox in facilitating MSW decomposition in a real-world setting.

Extrapolated Annual Degradation:

The observed monthly degradation rate suggests that the MushBox, even at the tested small size, has the potential to decompose approximately 1.19 tons (2380 pounds) of MSW annually. The linear extrapolation and the observed weekly data trends indicate the possibility of even higher decomposition rates over extended periods. The mycelium's ability to persist over time aligns with this potential for an exponential increase in MSW breakdown.

Fungal Performance and Mycelial Growth:

Visual and potential quantitative assessments demonstrated variations in mycelial colonization on different cellulosic substrates. The pasteurized wheat straw consistently exhibited extensive and robust fungal growth across all tested strains. This aligns with the known cellulose content of wheat straw, making it a favorable substrate for diverse cellulolytic fungi. Bulk fungi testing of Pleurotus Ostreatus, Pestalotiopsis, and Stropharia Rugosoannulata showcased large organic waste decomposition. Whilst specific material mushroom strains of Aspergillus sp, Mucor Hiemalis, Penicillium funiculosum, and phanerochaete chrysosporium showcased success in degradation of materials such as di-2-ethylhexyl phthalate, pharmaceutical drugs, metalloids, and many more.

The MushBox project presents practical applications in waste management, environmental remediation, and climate change mitigation. The empirical evidence suggests that MushBox has the potential to reduce the reliance on landfills, mitigate environmental pollution, minimize greenhouse gas emissions, and align with circular economy principles. The technology offers a promising avenue for sustainable MSW management.

Non-Experimental Work Done:

- IKEA Partnership
    - Contacted the manager at IKEA in New Haven, CT, and pitched the idea of MushBox. She loved the idea, and through learning of IKEA's use of quick decomposing mushroom packaging, decided to utilize MushBox technology to enhance the product
    - Created 25 prototypes for various small objects (candle packaging shown on poster) which took great amounts of time and molding resources
    - Opens up the possibility for commercial implementation enhancing natural route of

MushBox to end up in landfills

- MushBox is neither a cube nor volume heavy for packaging items, therefore, has much less impact, but an impact nonetheless.

- AI-Powered MushBox Recommendation App
    - Trained Gemini AI by Google based on testing and outputs from experiments #1, #2, and #3 to give output of specific combinations needed for case-specific landfills. Used MIT App Inventor to create an app for communication between Landfills and MushBox creator. An accuracy of 98.67% was achieved through different tests and cases asked by landfill management.

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