Incorporating Edible Decomposers into Sustainable Bioregenerative Life Support Systems for a Martian Colony

Team Name: Florida Tech Fungi's II

Team Number: 9552

Challenge Category: Undergraduate Division

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I. Introduction

Establishing a bioregenerative life support (BRLS) system with supplemental food production is key to sustainable space colonization efforts at sites too remote for easy resupply from Earth. In-Situ Resource Utilization (ISRU) practices leveraging existing resources such as regolith to support BRLS systems, further reducing a future colony's dependency on Earth for vital goods. Decomposers are organisms capable of breaking down organic material making nutrients available for reuse by other organisms. We propose that involving edible decomposers into BRLS systems is an efficient method of recycling valuable organic and inorganic wastes for a colony's ecosystem, while also introducing a supplemental nutrient source for colonists. As decomposers with edible species, fungi are prime candidates for this study. They are resilient, lack dependency on water, and are adaptable in growth, whereas vegetative feedstock requires extensive care. Additionally, certain fungi have potential medicinal properties, such as lowering blood cholesterol and inhibiting tumor growth ("Oyster Mushroom", 2020). *Pleurotus ostreatus,* the Pearl Oyster mushroom, is a prime choice for regolith-based growth due to its ability to thrive in multiple environments.

II. Theory

Pleurotus ostreatus, or Pearl Oyster mushrooms, was chosen as the modeled fungi due to its resilience against environmental abiotic stressors such as desiccation, salinity, heat, and cold. Although they are primarily known to germinate on wood based substrate, they have earned a booning reputation for their life cycle having one of the shortest time periods amongst edible fungal types and their ability to proliferate in a wide range of substrate without strict requirements (Tesfaw et al., 1970). This will substantially decrease time committed in plant waste recycling with the addition of edible decomposers compared to traditional methods.

As part of their life cycle, fungal fruit bodies known as mushrooms release spores from their gills once they reach maturity. Once a substrate is inoculated, mycelium growth occurs in dendritic structures that promote decomposing organic chemical secretions. These secretions are known to have hydrolytic and/or oxidative enzymatic properties and also contain secondary metabolites as a vehicle in depositing nutrients to the substrate (Beeck, 2021). At this stage of mycelium growth, the action of decomposition can be taken advantage of in the proposed colonizing plant waste recycling system (CPWRS). Traditionally, plant waste decomposition

requires a relatively long period of time before proper use, whereas the decomposition process of mycelium growth not only contributes to a shorter time period but also provides benefits of additional nutritional value to the compost and an early source of food for colonizers.

Important studies have supported the possibility of remediation of regolith for crop growth and the benefits of the use of on-site resources (Eichler, 2021). Coupled with preliminary studies resulting in successful production of Oyster mushroom in regolith, the same can be applied to fungi cultivation in martian regolith. In conclusion, we hypothesize that the organic plant waste to martian regolith volume ratio has an influential trend on growth and the decomposition process of Pearl Oyster mushrooms.

III. Methodology

1. Planting Method

A substrate mixture was created with a ratio of 25:10:8:1 composed of medium to coarse saw-dust, medium-sized wood chips, plant waste, and calcium sulfate, respectively. The plant waste utilized was an unspecified mixture of plant matter gathered from our student garden and landscaping debris from around our campus. The substrate was blended using a *Waring Laboratory LB10 1 Liter Laboratory Blender* until homogenous and the consistency was similar to medium to coarse sawdust (Appendix C, Figure 3). Deionized water, in amounts approximately 60% of the total substrate weight, was then added into the mixture. Using the substrate mixture and MGS-1 Martian regolith simulant, six 10L polypropylene autoclavable bags were filled with a specific mixture of 5L of substrate and regolith, as seen in Table 1. The composition of the bags was determined by the volume percentage of martian regolith they were assigned to contain. The control bag, Bag 0, contained 0% regolith. Each of the other bags contained increasing amounts of regolith, growing in increments of 5% up to 25%.

Bag Number	Regolith %	Regolith Mass (g)	Substrate Mass (g)
0	0	0	1850
1	5	322.5	1757.5
2	10	645	1665
3	15	967.5	1572.5
4	20	1290	1480
5	25	1612.5	1387.5

Table 1: A summary of the regolith and substrate mixture masses used for all 6 bags used.

All 6 bags were autoclaved for 30 minutes at at least 121ºC and 15 lbs per square inch of pressure to sterilize the final substrate mixtures. Post sterilization, all 6 bags were inoculated with 85.9 g of *P. ostreatus* grain spawn once they were completely cooled. The amount of spores per bag was chosen due to limited grain spawn supply as a result of mold contamination. We experienced a large takeover of *Trichoderma* mold in our grain spawn supply which later showed up again in all of our bags.

2. *Growth Setup*

The six bags under study were placed in an environmental chamber (*Low Temperature Illuminated Incubator 818*). The daily high, low, and current values for both temperature and humidity of the chamber were recorded daily. During the mycelium spawn phase, the tops of each bag were folded and taped down to prevent early pinning in the extra space. For the duration of the spawn phase, the mycelium received limited contact to light. The bags were rotated counterclockwise by one spot each day to ensure equal exposure to both outside light and the humidity being generated from the bottom of the incubator. Once the mycelium reached its primordial phase, each bag was cut to be level with the top of the substrate to help encourage the fungi to fruit. A sheet of plastic wrap was then placed over each bag to trap in humidity. The substrate was misted with a spray bottle whenever it appeared dry, which usually occurred 2-3 times a day. During this phase, the lights on the inside of the door to the chamber were placed on a timer built into the incubator to be on for 6-8 hours a day. Parchment paper was placed over the lights to reduce direct exposure. At the start of this experiment we had 2 small humidifiers with fans that ran for 8 hours a day, these broke on day 35 and were then exchanged for open beakers of water set at the base of the incubator for humidity for the remainder of the competition.

3. *pH Measurement*

Data on the substrates' pH from each of the 6 bags was collected once a week for the duration of the project's grow period. For each bag, 5 grams of the substrate was

extracted and ground as fine as possible via mortar and pestle. The samples were then combined with 10 mL of distilled water each in an Erlenmeyer flask and shaken simultaneously for 1 minute, set to rest for 5-minutes, and repeated again four times. After agitation, samples were left to rest for 30 minutes before taking the pH measurements using a Vernier LabQuest Data Collection Handheld and Vernier pH Sensor pH-bta. The procedure followed was provided by the challenge but with the modification that we halved the amounts due to the lightweight material of our substrate.

4. *Photo Recording*

Photographs of all 6 bags were taken daily throughout the grow period in order to monitor the mycelium and decomposition. These images were taken on an iPhone and stored on Google Drive. Supplemental images were taken periodically with a DSLR camera in addition to the daily data collection.

IV. Analysis & Results

1. *pH Trend*

For all pH measurements, a Vernier pH probe was used to collect the data. Over the course of the grow period, a correlation between the amount of regolith in the soil compared to the pH was found. The lower regolith concentration consistently had the lowest pH recording, and as the pH measurements went from Bag 0 to Bag 5, the pH had a steady increase.

The first week of data collection yielded more basic results in the 8.3 to the 9.3 range, but as the growth period progressed, the pH began to decrease and settle around a neutral pH of 7. The overall trend of the pH in all bags over the course of the grow period can be seen in Figure 1 in Appendix A. We are unable to determine if the pH levels were affected by the presence of contamination, the green mold, *Trichoderma.*

2. *Analysis of Mushroom Dimension and Mass*

A common trend could be observed in the dimensions of the fruiting bodies in the bags containing a higher regolith to organic concentration. In comparison with the Control, which produced healthy and proportioned mushrooms, the bags containing

regolith produced fewer fruiting bodies, with much longer stems and with smaller and deformed caps. We believe this is due to the fungi attempting to create its next generation as fast as possible due to the stress from the regolith, and thus spending more energy on getting away from the substrate and sporing rather than generating a larger biomass for its fruit.

The fungi grown in regolith showed greater signs of stress, resulting in a decrease in its ability to decompose and reliably produce viable fruit bodies. We observed mushrooms from all 6 bags creating spores, but the higher regolith concentrations weren't always able to produce fruiting bodies capable of sporing.

3. *Growth Rate of Fruiting Bodies*

The full growth period for Oyster mushrooms typically lasts around 30 days. In this experiment, the first mushrooms were harvested from the control, bag 2, and bag 3 on day 48. From the data collected it can be observed that when in conditions of higher regolith concentration, the fruiting bodies require more time to develop. This result was most likely due to the hostility and lack in nutrients of a regolith-substrate, however, this can also be traced back to the longer mycelium spawn phase the bags sustained at the beginning of the experiment when the mycelium was attempting to colonize the substrate mixtures. We observed that the bags containing higher regolith concentration had mushrooms that matured faster once they began growing than the mushrooms from the control. We believe this is a result of the stress the regolith causes on the fungi and the fungi attempting to complete its life cycle as fast as possible.

4. *MGS-1 Simulant to Organic Plant Waste Volume Ratio vs Mycelium Growth*

All of the grow bags produced fruiting bodies and a trend was seen in mycelium growth versus the descending volume amount in organic plant waste. The higher regolith concentration resulted in the mushrooms growing longer stems rather than focusing on growing large caps and generating biomass. This is most likely due to the mushrooms pushing outwards away from the regolith in attempts to give future generations of mushrooms better growing conditions. Among the bags containing regolith, bag 2, which

contained 10 percent regolith simulant, provided the best growth. Overall, the control bag resulted in the most growth and mushroom biomass.

V. Discussion and Conclusion

1. *Feasibility of P. ostreatus Growth in Mars Regolith Simulant*

We were able to produce viable mushrooms in each of the 6 bags demonstrating that *P. ostreatus* can be grown in regolith-substrate mixtures with varying success rates. When compared to the control, the bags with a higher regolith concentration had less successful growth as well as a decrease in ability to decompose. The bags containing 5, 10, and 15 percent regolith did better than the bags containing 20 and 25 percent regolith by volume. Though the bags with an increased regolith to substrate ratio underperformed during the grow period, they still successfully provided viable offspring, further proving that *P. ostreatus* can be grown in regolith-substrate mixtures.

2. *pH Levels vs Mycelium Growth*

Throughout the duration of the grow period, the pH values of all 6 bags followed similar trends. It can be recognized that the bags that produced more successful yields showed slightly acidic pH readings, while the bags that struggled to produce viable offspring remained at pH closer to neutral. The bag containing the highest percentage of regolith struggled to decompose, remaining at a neutral or just below neutral pH after week 3. During week 3 of the competition, as the mycelium decomposition started to increase, the acidity of the pH started to decrease for all 6 bags. This trend was seen until the 6 week mark where the acidity of the bags started to increase again as the fungi began creating mushrooms, with the exception of bag 5 as mentioned. Based on our recorded pH measurements and growth data, it appears that the bags remain at a 6-7 pH range during the decomposition phase, which then raises to a 6.5-7.5 range when producing mushrooms. We are unable to determine if the pH levels were affected by the presence of contamination from green mold.

3. *Obstacles and Errors*

There were a number of obstacles that took place over the course of the challenge. At the start, we had to finely grind up the materials for our substrate as we were unable to obtain any pre ground varieties. We spent numerous hours ripping material up by hand

and using blenders to get the consistency desired. Additionally, we discovered that some of our grain spawn was contaminated with mold, specifically *Trichoderma*, which is very commonly found with oyster mushrooms. This mold was then found in the bags but was outcompeted by the mushrooms with the exception of the control bag and bag 4 which both had significant mold at the end of the grow period. Later in the competition, the humidifiers were no longer functional, therefore we adapted to using open beakers of water to provide humidity. When we reached the second phase, the lights within the incubator were turned on and covered with large sheets of parchment paper to reduce the direct light the bags were exposed to. The incubator itself was programmed to automatically turn the lights on and off at set times, however that system was not working properly and the lights had to be manually turned on and off each day. Lastly, samples were taken weekly from the bags in order to perform the pH measurements, which could have potentially disturbed the growth of the mushrooms, despite best efforts not to. The pH probe that was used was found to not be accurate; it was off by an average of +1.1 over the course of the challenge and the data was corrected accordingly.

VI. References

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VII. Appendix A: Data Tables and Graphs

Figure 1: Change in pH for Each Bag Throughout Grow Period

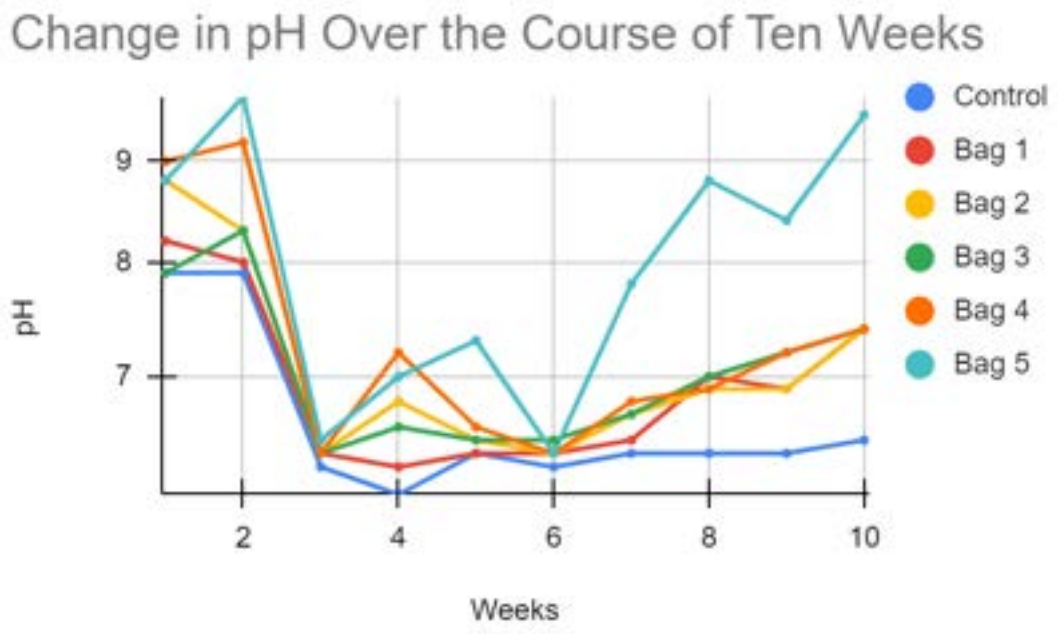

Figure 2: Daily Incubator Humidity Data Throughout Grow Period

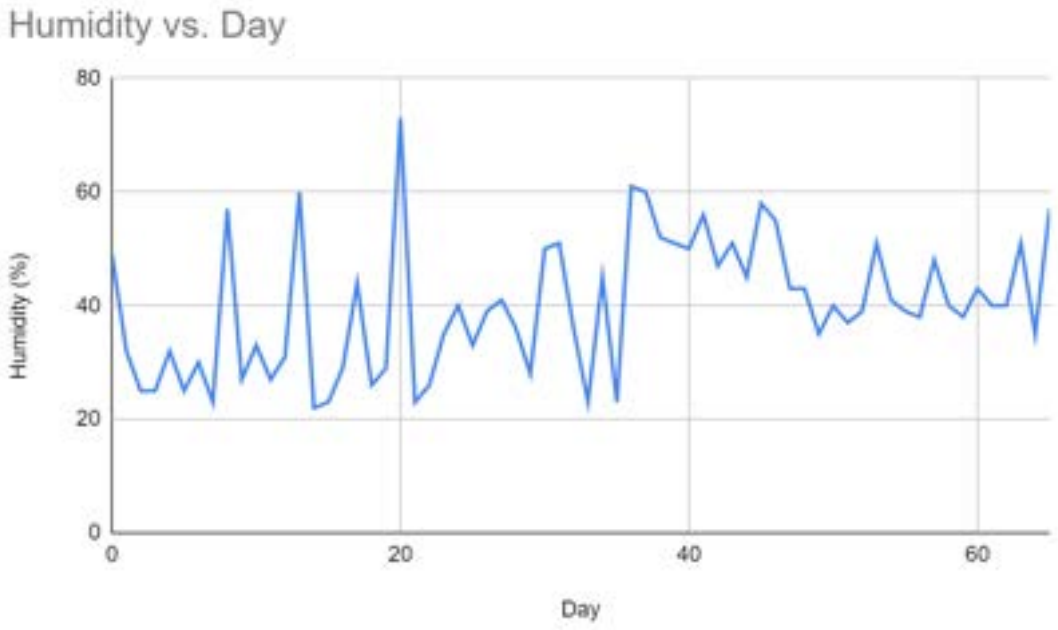

Figure 3: Analysis of Mushroom Dimensions and Mass Compared Across Different Volume Percentages of MRS to Organic Material

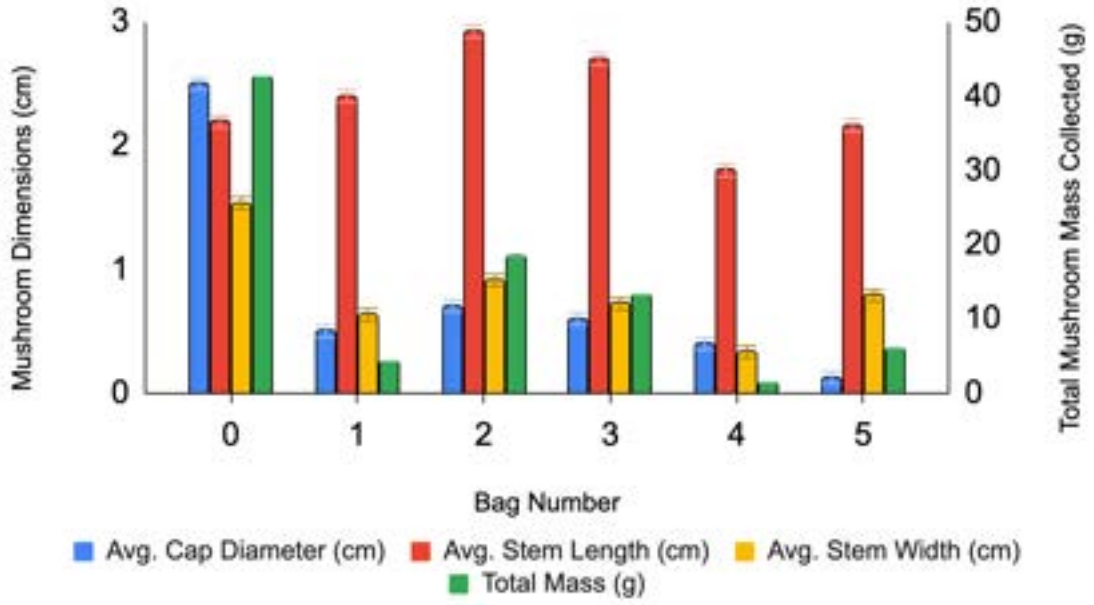

Table 1: Elements Present in Substrate Throughout Grow Period As Found Using EDAX Techniques Control Bag 100% Organic Material(OM) Substrate

Beginning (February 12th)				Middle (March 8th)				End (April 11)			
Element	Weight %	Atomic %	Error %	Element	Weight %	Atomic %	Error %	Element	Weight %	Atomic %	Error %
O	26.59	23.34	10.28	O	40.12	36.01	10.28	O	72.88	90.04	9.71
Ne	0.12	0.09	14.8	Ne	0.59	0.42	11.88	Ne	0	0	0
Na	0	0	0	Na	0.45	0.28	10.33	Na	0	0	0
Mg	0	0	0	Mg	0.29	0.17	8.45	Mg	1.19	0.96	9.19
Al	0	0	0	Al	0.17	0.09	7.51	Al	0.4	0.29	9.23
Si	0	0	0	Si	0.29	0.15	5.59	Si	1.46	1.03	6.33
K	0	0	0	K	0.28	0.1	5.41	K	1.07	0.54	5.38
Ca	5.4	1.89	2.35	Ca	2.71	0.97	2.2	Ca	8.21	4.05	2.9
Nb	2.06	0.31	3.48	Nb	0	0	0	Nb	0	0	0
Cu	0	0	0	Cu	0	0	0	Cu	0.29	0.09	18.11
Mo	2.57	0.38	3.25	Mo	3.91	0.58	2.95	Mo	14.51	2.99	3.65

Table 2: Elements Present in Substrate Throughout Grow Period As Found Using EDAX Techniques Bag 1 95% OM/5% MSG-1 Substrate Mixture

Beginning (February 12th)				Middle (March 8th)				End (April 11)			
Element	Weight %	Atomic %	Error %	Element	Weight %	Atomic %	Error %	Element	Weight %	Atomic %	Error %
O	29.41	27.94	10.18	O	22.97	19.09	10.55	O	52.15	73.13	9.9
Na	0	0	0	Na	0	0	0	Na	1.13	1.1	10.82
Mg	0.56	0.35	7.42	Mg	0.52	0.29	7.06	Mg	3.59	3.31	8.09
Al	0.34	0.19	7.04	Al	0.22	0.11	6.23	Al	3.79	3.15	7.06
Si	3.3	1.79	4.02	Si	0.57	0.27	4.38	Si	9.1	7.27	5.83
K	0.47	0.18	9.46	K	0.23	0.08	4.12	K	1.2	0.69	4.86
Ca	3.55	1.35	3.08	Ca	0.75	0.25	2.48	Ca	10.02	5.61	2.98
Fe	3.6	0.98	4.42	Fe	0.67	0.16	3.58	Fe	7.63	3.07	2.58
Nb	3.69	0.6	4.46	Nb	2.24	0.32	3.16	Nb	0	0	0
Mo	2.81	0.45	4.28	Mo	0	0	0	Mo	11.38	2.66	4.42

Table 3: Elements Present in Substrate Throughout Grow Period As Found Using EDAX Techniques Bag 2 90% OM/10% MSG-1 Substrate Mixture

Beginning (February 12th)				Middle (March 8th)				End (April 11)			
Element	Weight %	Atomic %	Error %	Element	Weight %	Atomic %	Error %	Element	Weight %	Atomic %	Error %
O	35.78	42.45	8.37	O	30.88	26.35	10.37	O	56.77	75.49	9.54
Na	0	0	0	Na	0.45	0.27	9.81	Na	1.91	1.76	10.06
Mg	14.71	11.49	4.72	Mg	0.82	0.46	7.24	Mg	4.65	4.07	7.92
Al	0.81	0.57	6.88	Al	1.38	0.7	5.61	Al	5.23	4.12	6.86
Si	11	7.43	4.07	Si	1.7	0.82	4.48	Si	9.31	7.05	5.87
K	0	0	0	K	0.13	0.05	8.53	K	0.97	0.53	5.15
Ca	0	0	0	Ca	0.83	0.28	2.62	Ca	4.65	2.47	3.15
Fe	2.17	0.74	9.95	Fe	1.64	0.4	2.8	Fe	5.71	2.17	2.52
Nb	13.69	2.8	4	Nb	0	0	0	Nb	0	0	0
Co	0	0	0	Co	0	0	0	Co	0.2	0.07	23.89
Mo	0	0	0	Mo	0	0	0	Mo	9.65	2.14	4.5
Ba	0	0	0	Ba	0	0	0	Ba	0.33	0.05	31.01
W	0	0	0	W	0	0	0	W	0.43	0.05	6.97

Table 4: Elements Present in Substrate Throughout Grow Period As Found Using EDAX Techniques Bag 3 85% OM/15% MSG-1 Substrate Mixture

Beginning (February 12th)				Middle (March 8th)				End (April 11)			
Element	Weight %	Atomic %	Error %	Element	Weight %	Atomic %	Error %	Element	Weight %	Atomic %	Error %
O	40.16	47.26	8.66	O	42.67	39.31	10.24	O	54.21	75.14	9.57
Na	0.92	0.76	7.99	Na	0.43	0.27	12.33	Na	0	0	0
Mg	0	0	0	Mg	2.06	1.25	7.58	Mg	5.11	4.66	8.11
Al	8.24	5.75	4.16	Al	0.89	0.49	6.76	Al	2.45	2.01	7.31
Si	10.33	6.92	3.91	Si	2.36	1.24	5	Si	9.98	7.88	5.86
S	0	0	0	S	0	0	99.99	S	0	0	0
K	0	0	0	K	0.19	0.07	11.14	K	0.84	0.48	5.41
Ca	5.57	2.62	4.5	Ca	0.78	0.29	3.9	Ca	4.74	2.62	3.17
Fe	0	0	0	Fe	1.32	0.35	4.39	Fe	11.2	4.45	2.36
Co	0	0	0	Co	0.02	0.01	64.76	Co	0.43	0.16	9.32
Cu	0	0	0	Cu	0.08	0.02	60.81	Cu	0.57	0.2	13.93
Nb	13.06	2.65	4.16	Nb	2.62	0.42	4.67	Nb	0	0	0
Mo	0	0	0	Mo	0.4	0.06	11.39	Mo	10.31	2.38	4.55

Table 5: Elements Present in Substrate Throughout Grow Period As Found Using EDAX Techniques Bag 4 80% OM/20% MSG-1 Substrate Mixture

Beginning (February 12th)				Middle (March 8th)				End (April 11)			
Element	Weight %	Atomic %	Error %	Element	Weight %	Atomic %	Error %	Element	Weigh t %	Atomic %	Error %
O	19.47	18.95	9.5	O	43.52	46.5	9.94	O	44.83	65.73	9.67
Na	0	0	0	Na	1.13	0.84	9.74	Na	0.75	0.77	11.34
Mg	1.09	0.7	5.1	Mg	2.57	1.8	7.52	Mg	3.57	3.45	8.09
Al	0	0	0	Al	4.91	3.11	6.2	Al	10.02	8.72	6.82
Si	2.33	1.29	3.73	Si	11.24	6.84	5.22	Si	11.68	9.75	6.17
K	0	0	0	K	0.61	0.27	4.94	K	1	0.6	5.21
Ca	0	0	0	Ca	4.31	1.84	2.52	Ca	4.74	2.77	3.24
Ti	0	0	0	Ti	0.43	0.15	5.05	Ti	0	0	0
Fe	0.81	0.23	12.01	Fe	5.28	1.62	2.37	Fe	12.56	5.28	2.33

Co	0	0	0	Co	0	0	0	Co	0	0	0
Cu	0	0	0	Cu	0	0	0	Cu	0	0	0
Nb	17.83	2.99	3.11	Nb	0	0	0	Nb	8.22	2.08	5.5
Mo	0	0	0	Mo	0	0	0	Mo	0	0	0

Table 6: Elements Present in Substrate Throughout Grow Period As Found Using EDAX Techniques Bag 5 75% OM/25% MSG-1 Substrate Mixture

Beginning (February 12th)				Middle (March 8th)				End (April 11)			
Element	Weight %	Atomic %	Error %	Element	Weight %	Atomic %	Error %	Element	Weight %	Atomic %	Error %
O	53.98	54.44	7.74	O	34.08	31.15	10.25	O	53.36	73.18	9.54
Mg	0	0	0	Mg	1.06	0.64	7.27	Mg	6.1	5.51	7.82
Al	5.72	3.42	5.01	Al	0.7	0.38	6.07	Al	3.95	3.21	6.95
Si	15.61	8.97	4.03	Si	3.58	1.86	4.56	Si	12	9.37	5.8
Cl	0	0	0	Cl	0	0	0	Cl	0.22	0.13	9.91
K	0	0	0	K	0.08	0.03	12.34	K	0.91	0.51	4.97
Ca	0	0	0	Ca	1.48	0.54	2.46	Ca	3.97	2.18	3.2
Fe	0	0	0	Fe	3.76	0.99	2.3	Fe	8.86	3.48	2.39
Co	0	0	0	Co	0	0	0	Co	0.25	0.09	13.45
Nb	0	0	0	Nb	2.61	0.41	3.9	Nb	8.02	1.89	5.13
Tc	0	0	0	Tc	0.1	0.01	18.84	Tc	0	0	0
Mo	0	0	0	Mo	0	0	0	Mo	1.54	0.35	6.67

Table 7: Total Water Used

Total Amount of Water Used (L)	4.011

VIII. Appendix B: Visual Data

Image 1a: Control Bag 100% Organic Material(OM) Substrate Initial Image

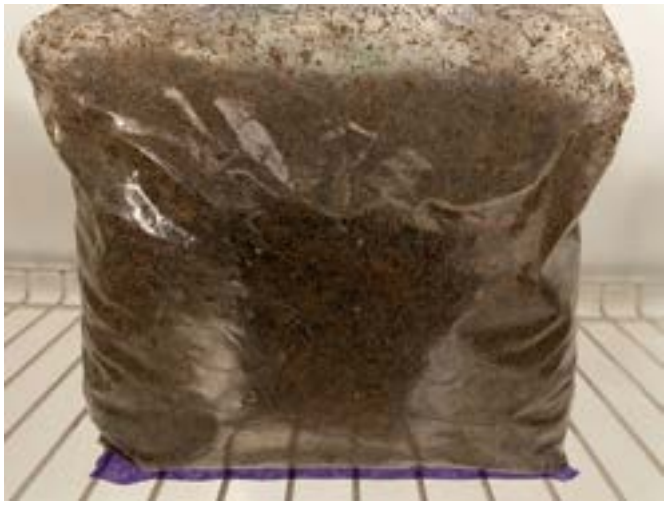

Image 1b: Control Bag 100% Organic Material(OM) Substrate Midway Image (33 days)

Image 1c: Control Bag 100% Organic Material(OM) Substrate Final Image (65 days)

Images 1d: Images of Mushrooms Growing in Control Bag 100% OM Substrate

Images 1e: Collected Mushroom Images Control Bag 100% OM Substrate

Image 1f: Collected Mushroom Cap to Test Viability Control Bag 100% OM Substrate

Image 1g: Scanning Electron Microscope Image Mycelium Control Bag 100% OM Substrate

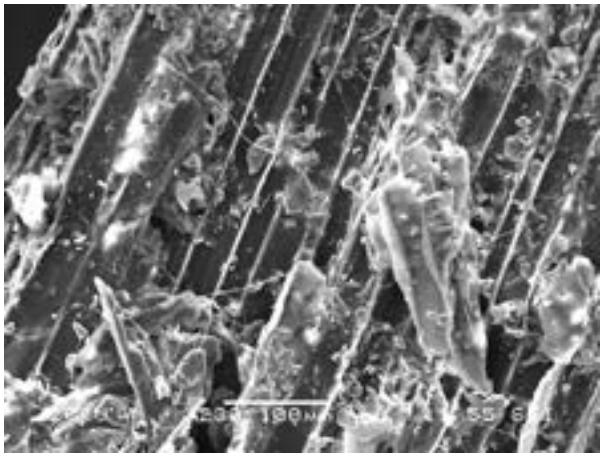

Image 2a: Bag 1 95% OM/5% MSG-1 Substrate Mixture Initial Image

Image 2b: Bag 1 95% OM/5% MSG-1 Substrate Mixture Midway Image (33 days)

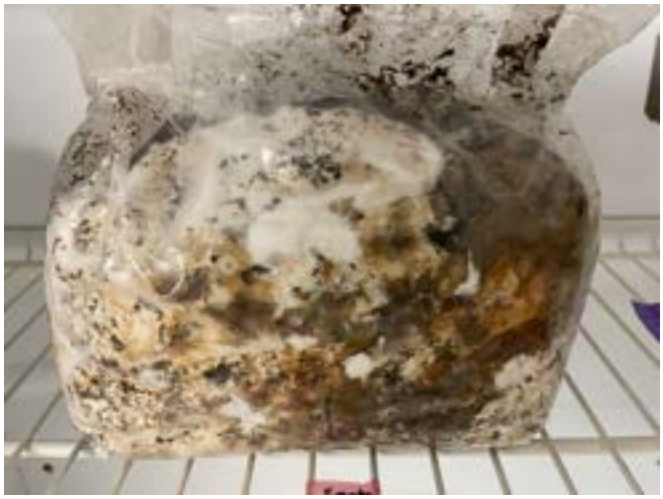

Image 2c: Bag 1 95% OM/5% MSG-1 Substrate Mixture Final Image (65 days)

Images 2d: Images of Mushrooms Growing in Bag 1 95% OM/5% MSG-1 Substrate Mixture

Images 2e: Collected Mushroom Images Bag 1 95% OM/5% MSG-1 Substrate Mixture

Image 2f: Scanning Electron Microscope Image Mycelium Bag 1 95% OM/5% MSG-1 Substrate Mixture

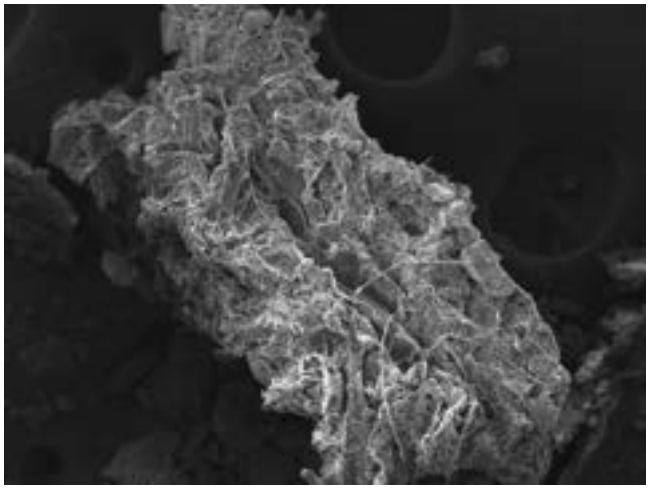

Image 3a: Bag 2 90% OM/10% MSG-1 Substrate Mixture Initial Image

Image 3b: Bag 2 90% OM/10% MSG-1 Substrate Mixture Midway Image (33 days)

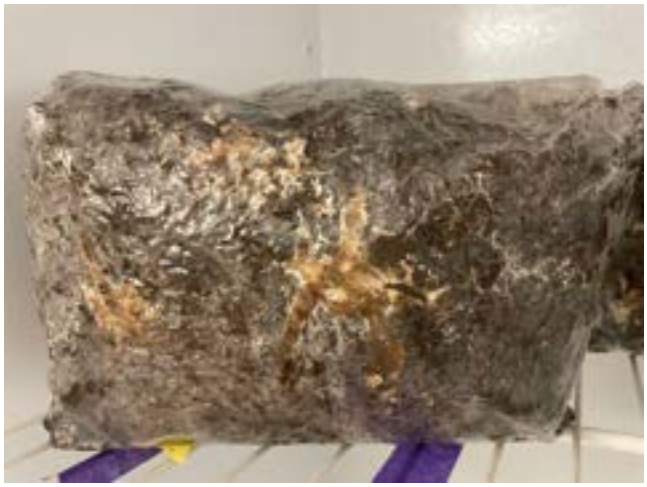

Image 3c: Bag 2 90% OM/10% MSG-1 Substrate Mixture Final Image (65 days)

Images 3d: Images of Mushrooms Growing in Bag 2 90% OM/10% MSG-1 Substrate Mixture


Images 3e: Collected Mushroom Images Bag 2 90% OM/10% MSG-1 Substrate Mixture


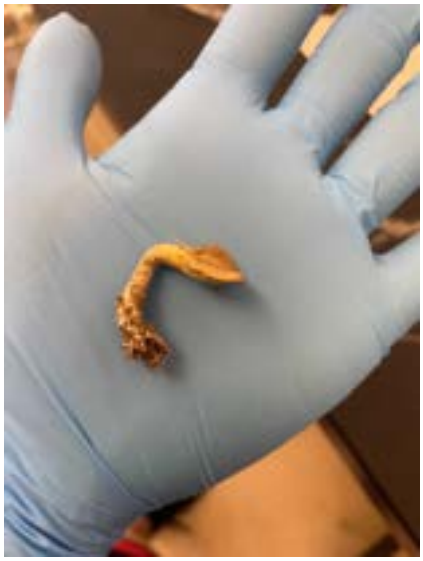

Image 3f: Scanning Electron Microscope Image Mycelium Bag 2 90% OM/10% MSG-1 Substrate Mixture

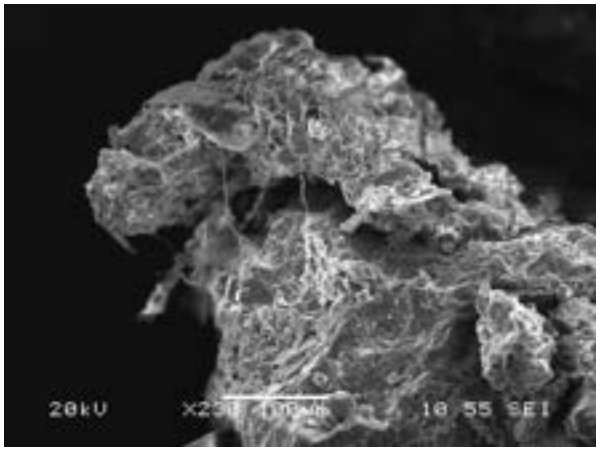

Image 4a: Bag 3 85% OM/15% MSG-1 Substrate Mixture Initial Image

Image 4b: Bag 3 85% OM/15% MSG-1 Substrate Mixture Midway Image (33 days)

Image 4c: Bag 3 85% OM/15% MSG-1 Substrate Mixture Final Image (65 days)

Images 4d: Images of Mushrooms Growing in Bag 3 85% OM/15% MSG-1 Substrate Mixture

Images 4e: Collected Mushroom Images Bag 3 85% OM/15% MSG-1 Substrate Mixture


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Image 4f: Scanning Electron Microscope Image Mycelium Bag 3 85% OM/15% MSG-1 Substrate Mixture Initial Image

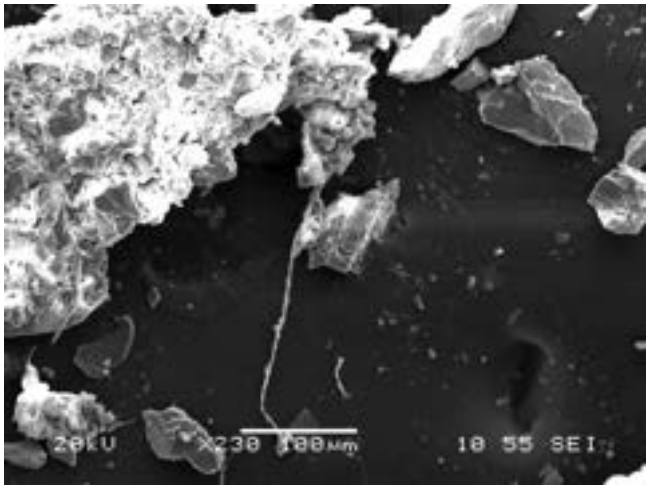
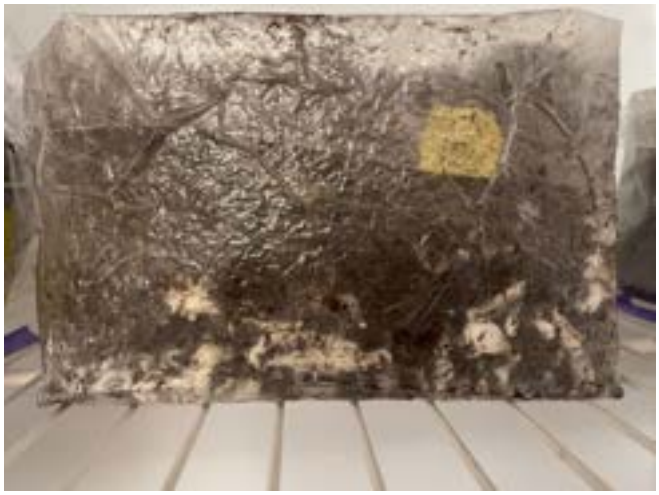


Image 5a: Bag 4 80% OM/20% MSG-1 Substrate Mixture Initial Image

Image 5b: Bag 4 80% OM/20% MSG-1 Substrate Mixture Midway Image (33 days)

Image 5c: Bag 4 80% OM/20% MSG-1 Substrate Mixture Final Image (65 days)

Images 5d: Images of Mushrooms Growing in Bag 4 80% OM/20% MSG-1 Substrate Mixture



Images 5e: Collected Mushroom Images Bag 4 80% OM/20% MSG-1 Substrate Mixture

Image 5f: Scanning Electron Microscope Image Mycelium Bag 4 80% OM/20% MSG-1 Substrate Mixture

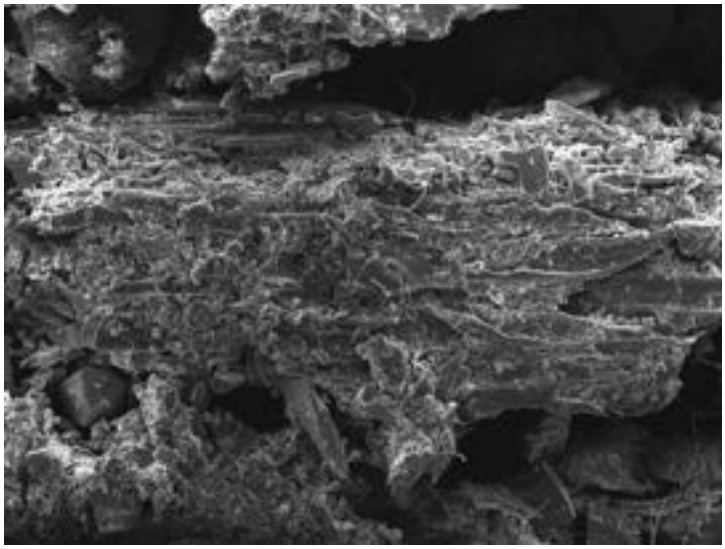


Image 6a: Bag 5 75% OM/25% MSG-1 Substrate Mixture Initial Image

Image 6b: Bag 5 75% OM/25% MSG-1 Substrate Mixture Midway Image (33 days)

Image 6c: Bag 5 75% OM/25% MSG-1 Substrate Mixture Final Image (65 days)

Images 6d: Images of Mushrooms Growing in Bag 5 75% OM/25% MSG-1 Substrate Mixture

Images 6e: Collected Mushroom Images Bag 5 75% OM/25% MSG-1 Substrate Mixture

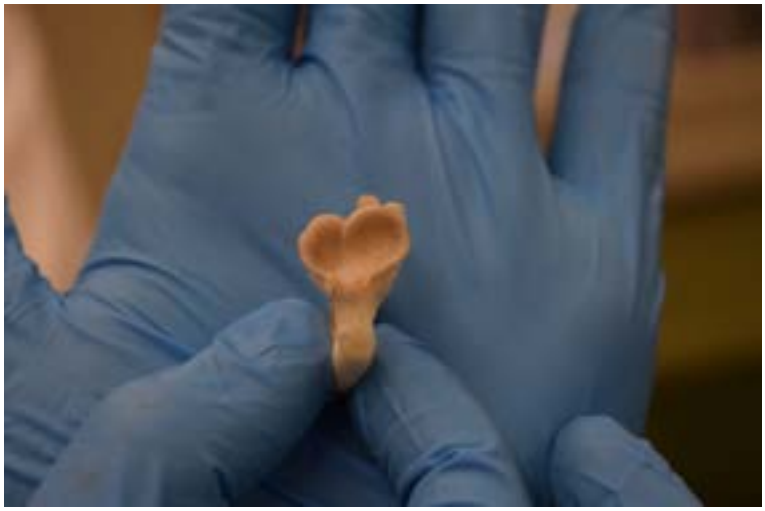


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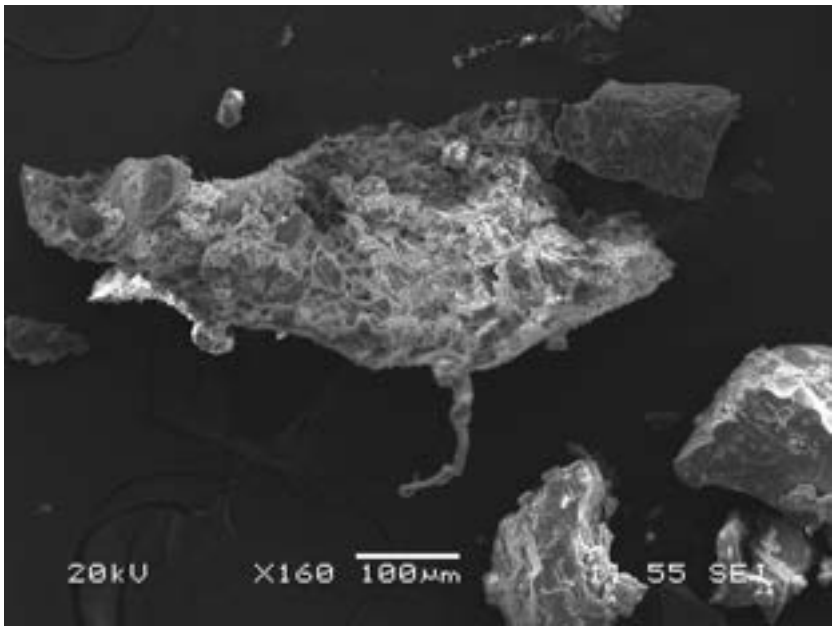
Image 6f: Scanning Electron Microscope Image Possible Mycelium Bag 5 75% OM/25% MSG-1 Substrate Mixture



VIII. Appendix C: Additional Visual Data

Figure 1: Incubator Set Up

Figure 2: Components of Organic Substrate

Figure 3: Uniform Blended Organic Substrate

Figure 4: Grain Spawn Used

Figure 5: Method of Collecting Daily Temperature and Humidity Data

Figure 6: Part of the team getting ready to shake the samples during pH data collection.

Figure 7: A member of the team grinding up a sample for pH data collection.

Figure 8: A member of the team transferring ground up samples for pH data collection.

Figure 9: A member of the team recording data.

Figure 10: Dimension measurement of mushroom grown in regolith/organic material mixture.

Figure 11: Image of Substrate and DI Water for pH Collection

Figure 12: Mold(*Trichoderma)* Growing in the Control

Figure 13: Mold(*Trichoderma)* Growing in Bag 4