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

Team Name: Florida Tech Fungi's

Team Number: 8628

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 leverage 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* is a prime choice for Regolith-based growth due to its ability to thrive in multiple environments.

II. Theory

Under the original hypothesis, the mycelium would no longer be supported by the substrate regolith mixture around the 400-600 gram range of added regolith, primarily due to the regolith's majority mass. *Pleurotus ostreatus*, the primary specimen in the experiment, is a rather resilient mushroom. These mushrooms are easy to grow and do not require strict environmental conditions. Additionally, it has a shorter growth time than other mushroom types, which is beneficial for this experiment (Tesfaw et al., 1970). As resilient as it may be, though, the biology of the mushroom is not suited for the harsh and unforgiving substrate that it was exposed to. In a separate experiment determined to examine the effects of *Pleurotus ostreatus* production with various substrates (wheat straw, leaves, saw-dust), sawdust produced the highest yield of mushrooms compared to all other substrates and is "recommended as [the] best substrate for Oyster mushroom cultivation" (Shah et al., 2004). The optimal temperature for growth of both mycelium and *Pleurotus ostreatus* is 28 ℃ (Hoa et al., 2015), while the optimal humidity is a range of 80-90% (Cikarge et al., 2018). It has been shown previously that a range of crops can be grown in simulated regolith with added organic matter, producing fruit and seeds (Wamelink et al., 2019).

III. Measurement Methods

1. *Planting Method*

Substrate was mixed to homogeneity in a ratio of 25:10:8:1 and was composed of medium to coarse saw-dust, medium-sized wood chips, hay straw, and calcium sulfate, respectively. Water was then mixed into the substrate up to approximately 60% of the substrate weight. The substrate mixture was then divided into 10 identical bags. A specific amount of Martian Regolith was added to each of the 10 bags, with the exception of the control bag. Each bag was then inoculated with the fungi spores.

2. *Growth Setup*

Bags were placed in two separate environmental chambers (*Low Temperature Illuminated Incubator 818*). Each chamber stored five bags. The temperature and humidity of each chamber were recorded daily. During the mycelium spawn phase, the chamber temperature was set to 24°C for the duration of the spawn growth with limited contact to light. Once the mycelium reached its primordial phase, the chamber temperature was then set to 13°C until the end of the growth period.

3. *pH Measurement*

pH levels were initially taken with litmus paper strips in the first three measurements. A pH probe was used for the remaining six measurements. Approximately 5 grams of the substrate was extracted from each bag, ground via mortar and pestle, and combined with 10 mL of distilled water in an Erlenmeyer flask. All samples were shaken simultaneously for 1 minute four times with 5-minute rests in between shakes. The samples then sat for 30 minutes and were tested for pH.

4. *Photo Recording*

Photos of every bag in their respective incubator placement were taken daily. For every bag, a simple photo with standard lighting was taken, as well as a photo with a red light shined over the bag. Additionally, any growth or points of interest on the bags were also photographed. This method was combined with the previously described data notebook to maximize the efficiency of data collection.

IV. Analysis & Results

1. *pH Trend*

In the initial three testing series, pH measurements were acquired by using litmus strips. By using these paper strips, pH was determined by color association between the color change as a result of the liquid sample coming into contact with the paper and a color coded pH chart. Through this method, the overall trend across all bags displays a curved slope-like decline as seen in *Graphs 5-14*. After week 3, a pH probe was used (*Vernier LabQuest Data Collection Handheld* and *Vernier pH Sensor pH-bta*) which gave a clearer trend. From this, we can see a gradual decline in pH levels ranging from the 200-800 gram bags, however, the control, 100 gram, and 900 gram have opposite behaviors displaying an increase in their pH trend.

2. *Temperature Trend vs Mycelium Growth*

For nearly the first 50 days, chamber temperatures were kept at approximately 22°C. During this period, the mycelium went through underwhelming growth as seen in *Appendix B* in the day 1 and day 23 figures for all bags. On day 49, after decreasing temperatures to approximately 13°C, the mycelium grew at a faster rate than the previous temperature setting and began to produce fruiting bodies around day 51.

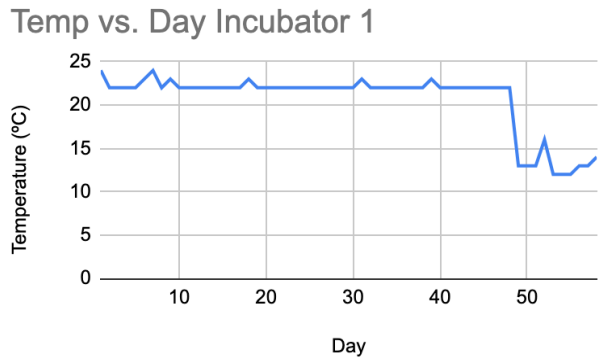
3. *Humidity Trend vs Mycelium Growth*

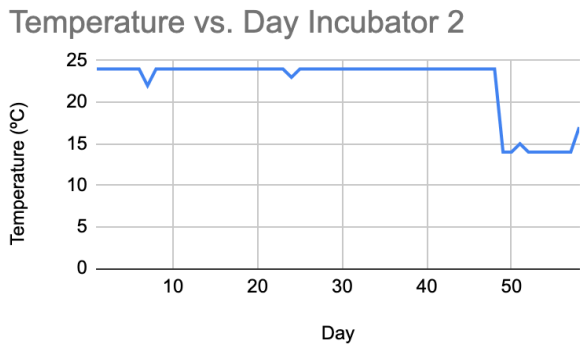
Humidity was maintained by placing two 500 mL beakers, unsealed, in each incubator as a source. From the initial day to around day 16, both incubators display an average trend humidity of about 33% as seen in *Graph 3* and *Graph 4* which seem to gain a dramatic oscillating trend up to day 49. Afterwards, in correlation to temperature change, the average trend seemed to stabilize at around 38% when fruiting bodies were observed.

4. *MGS-1 Simulant Addition vs Mycelium Growth*

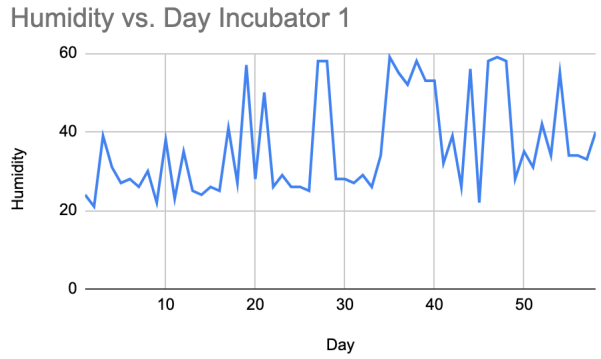
Growth bags that were able to produce a fruiting body were ones that had 500 grams and 700 grams of added MGS-1 simulant (*Appendix B*) with the 700 gram bag producing the highest amount of fruiting bodies.

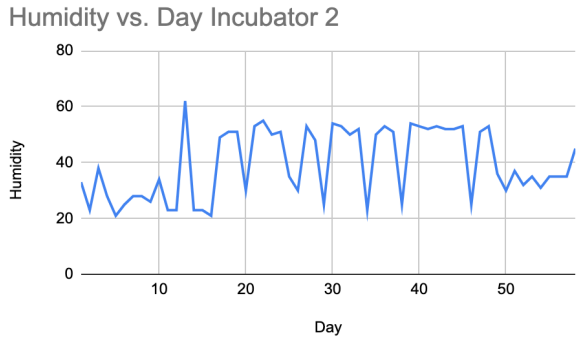
Figures #1& 2: Temperature vs. Day Incubator 1 & 2



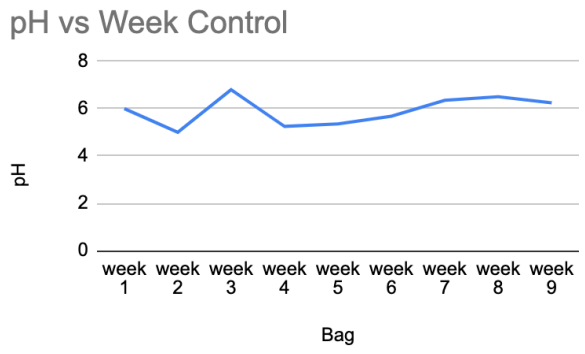


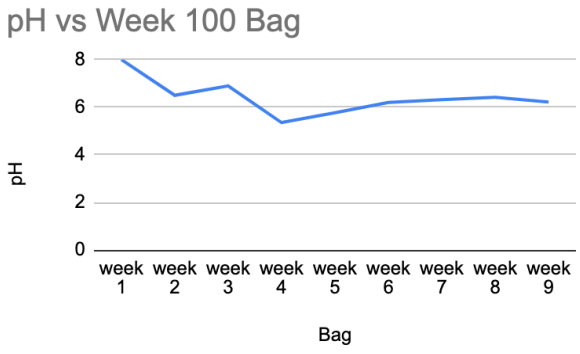
Figures #3 & 4: Humidity vs. Day Incubator 1 & 2



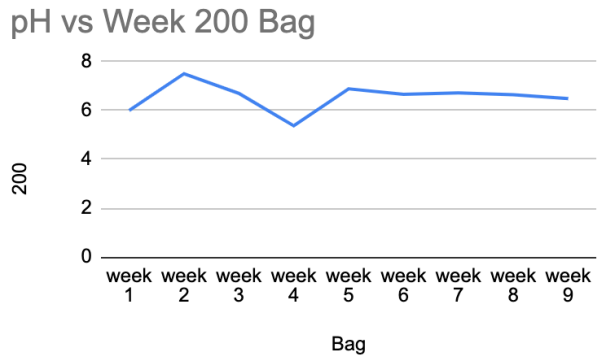


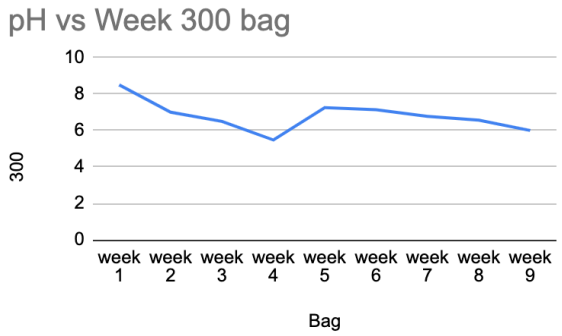
Figures #5 & 6: pH vs Week Control Bag & pH vs Week 100 Bag



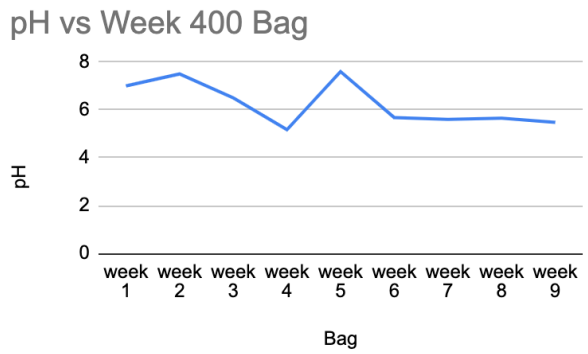


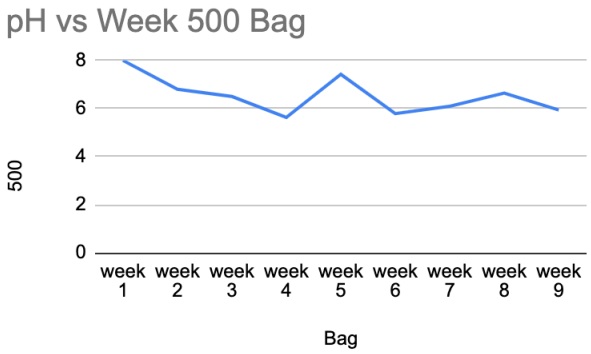
Figures #7 & 8: pH vs Week 200 Bag & pH vs Week 300 Bag



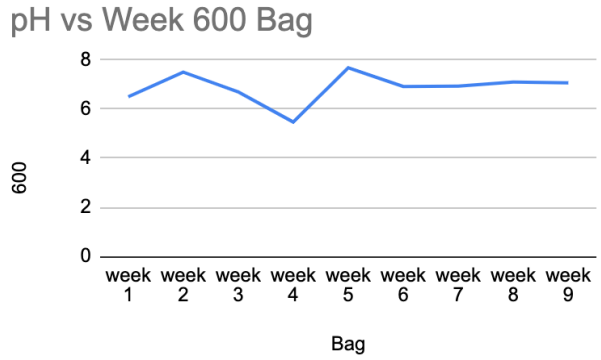


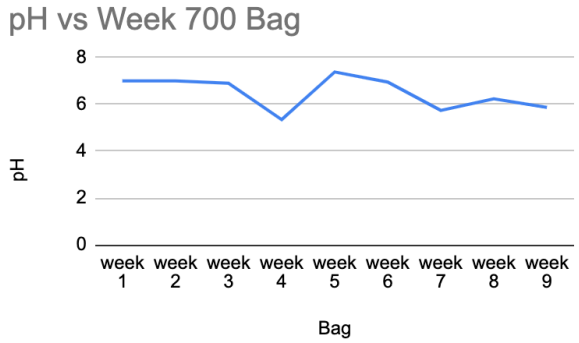
Figures #9 & 10: pH vs Week 400 Bag & pH vs Week 500 Bag



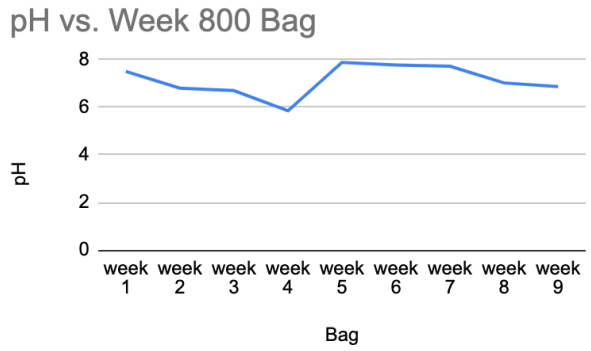


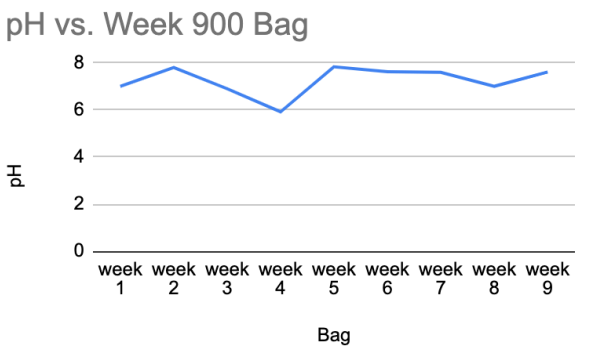
Figures #11 & 12: pH vs Week 600 Bag & pH vs Week 700 Bag





Figures #13 & 14: pH vs Week 800 Bag & pH vs Week 900 Bag





For a complete collection of daily photos not included in Appendix B, see the following link.
https://drive.google.com/drive/folders/1IyRz2zDcToweEWuyhDLkIzuLK6Z_jTyI?usp=sharing

For a complete collection of video updates of each bag, see the following link.
https://drive.google.com/drive/folders/1iYPtdPzx4Oy-bREBA74AnycI_SNQcgWP?usp=sharing

V. Discussion and Conclusion

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

This experiment showed that the mycelium is able to grow in the presence of up to 800 g of added regolith, or roughly 60% of content by mass. Our results nullified our previous hypothesis that the mycelium would no longer be supported by the substrate regolith mixture around the 400-600 gram range of added regolith. Our results suggest that the bags with 500 g and 700 g added regolith, or 51% content by mass and 57% content by mass respectively, were the optimal amounts of added regolith for the mycelium to still produce fruiting bodies. With the data collected from this growth period, we aim to find the optimal ratio of organic to inorganic waste that aids in the decomposition process for the next season of the competition.

2. *pH Levels vs Mycelium Growth*

A trend was noticed between pH levels and mycelium growth in the bags. The bags that were able to produce the beginning of fruiting bodies were the bags that became most acidic. The acidity of these bags indicate that the substrate is decomposing properly. This information will be useful in improving our next experiment as we can use the pH

levels to determine when the fungi have started decomposing and to time the temperature decrease.

3. *Obstacles and Errors*

Throughout the experiment, the growth of the specimen was slower than expected, resulting in a lack of visual data during the growth period. This blunder could be the consequence of a delay in temperature decrease due to waiting for a higher mycelium presence. With the concern of external obstacles, mold appeared in various bags, along with ice being discovered in the chambers (possibly as a result of the cooling system). There were also holes in the bags from the hay puncturing through that could have had many unknown effects. Lastly, samples were taken from the bags weekly in order to test the pH, which could have potentially disturbed the growth of the mushrooms, despite best efforts not to.

VI. References

Cikarge, G. P., & Arifin, F. (2018). Oyster Mushrooms Humidity Control Based On Fuzzy Logic By Using Arduino ATMega238 Microcontroller. *Journal of Physics: Conference Series*. https://doi.org/10.1088/1742-6596/1140/1/012002

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Shah, Z. A., Ashraf, M., & Ishtiaq, M. (2004). Comparative study on cultivation and yield performance of Oyster Mushroom (pleurotus ostreatus) on different substrates (wheat straw, leaves, saw dust). *Pakistan Journal of Nutrition*, *3*(3), 158–160. https://doi.org/10.3923/pjn.2004.158.160

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Tesfaw, A., Tadesse, A., & Kiros, G. (1970). Optimization of oyster (Pleurotus ostreatus) mushroom cultivation using locally available substrates and materials in Debre Berhan, Ethiopia. Journal of Applied Biology and Biotechnology, 3(1), 015-020.

Wamelink, G., Frissel, J., Krijnen, W. & Verwoert, M. (2019). Crop growth and viability of seeds on Mars and Moon soil simulants. Open Agriculture, 4(1), 509-516. https://doi.org/10.1515/opag-2019-0051

VII. Appendix A: Data Tables

Table #1: pH Measurements by Week

Bag	week 1	week 2	week 3	week 4	week 5	week 6	week 7	week 8	week 9
Control	6	5	6.8	5.25	5.35	5.68	6.35	6.5	6.24
100	8	6.5	6.9	5.36	5.77	6.2	6.32	6.42	6.22
200	6	7.5	6.7	5.37	6.88	6.66	6.72	6.64	6.48
300	8.5	7	6.5	5.48	7.25	7.14	6.77	6.57	6
400	7	7.5	6.5	5.17	7.6	5.68	5.6	5.65	5.48
500	8	6.8	6.5	5.63	7.42	5.79	6.09	6.64	5.94
600	6.5	7.5	6.7	5.47	7.68	6.92	6.93	7.1	7.07
700	7	7	6.9	5.35	7.38	6.95	5.74	6.23	5.87
800	7.5	6.8	6.7	5.85	7.88	7.77	7.72	7.02	6.87
900	7	7.8	6.9	5.92	7.83	7.62	7.6	7	7.61

Table #2: Daily Temperature and Humidity Incubator 1 & 2

Incubator 1			Incubator 2		
Day	Temp (°C)	Humidity (%)	Day	Temp (°C)	Humidity (%)
1	24	24	1	24	33
2	22	21	2	24	23
3	22	39	3	24	38
4	22	31	4	24	28
5	22	27	5	24	21
6	23	28	6	24	25
7	24	26	7	22	28
8	22	30	8	24	28
9	23	22	9	24	26
10	22	38	10	24	34
11	22	23	11	24	23
12	22	35	12	24	23
13	22	25	13	24	62
14	22	24	14	24	23
15	22	26	15	24	23
16	22	25	16	24	21
17	22	41	17	24	49

18	23	27	18	24	51
19	22	57	19	24	51
20	22	28	20	24	30
21	22	50	21	24	53
22	22	26	22	24	55
23	22	29	23	24	50
24	22	26	24	23	51
25	22	26	25	24	35
26	22	25	26	24	30
27	22	58	27	24	53
28	22	58	28	24	48
29	22	28	29	24	25
30	22	28	30	24	54
31	23	27	31	24	53
32	22	29	32	24	50
33	22	26	33	24	52
34	22	34	34	24	23
35	22	59	35	24	50
36	22	55	36	24	53
37	22	52	37	24	51
38	22	58	38	24	25
39	23	53	39	24	54
40	22	53	40	24	53
41	22	32	41	24	52
42	22	39	42	24	53
43	22	26	43	24	52
44	22	56	44	24	52
45	22	22	45	24	53
46	22	58	46	24	25
47	22	59	47	24	51
48	22	58	48	24	53
49	13	28	49	14	36
50	13	35	50	14	30
51	13	31	51	15	37
52	16	42	52	14	32

53	12	34	53	14	35
54	12	55	54	14	31
55	12	34	55	14	35
56	13	34	56	14	35
57	13	33	57	14	35
58	14	40	58	17	45

Table #3: Water Added to Inside of Bags

Bag	**Water Used (mL)**
Control	435
100	495
200	555
300	625
400	675
500	745
600	805
700	855
800	925
900	975
Total water:	7090

Table #4: Water Used for Humidity for Each Incubator

Incubator	**Water Used (mL)**
1	1615
2	1515
Total:	3130

Table #5: Total Water Used Duration Experiment

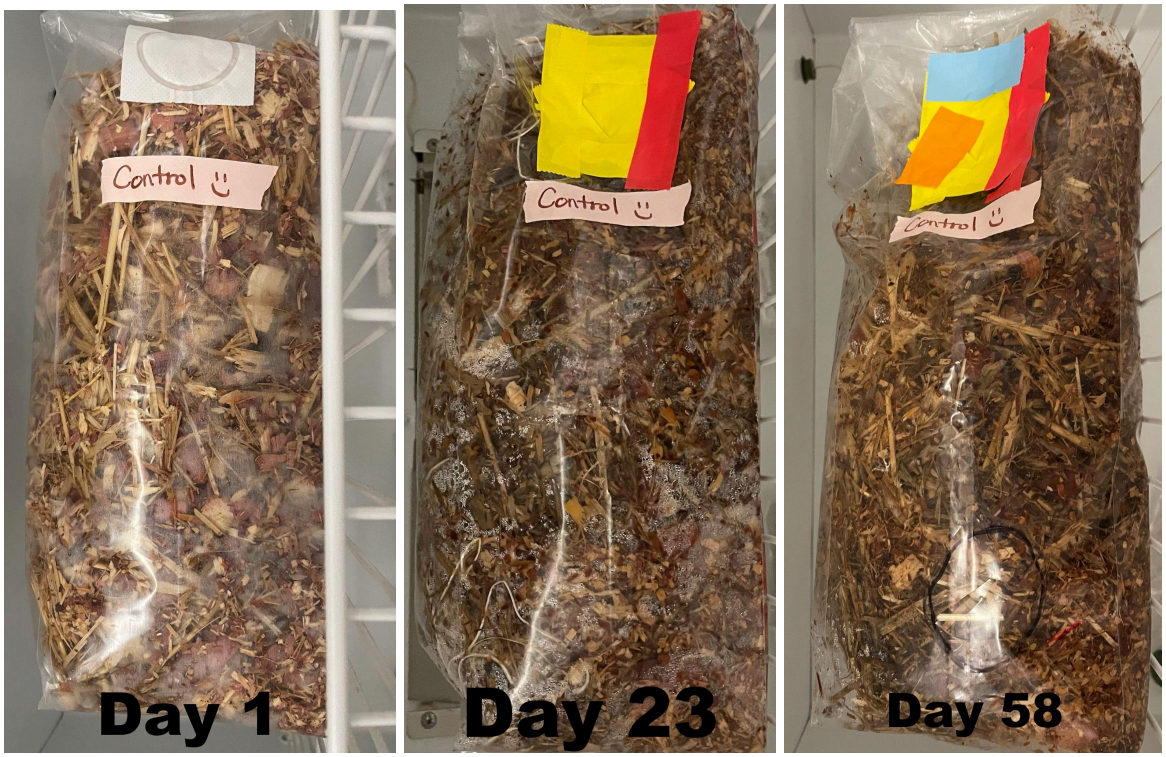
Total Water Used (mL):	10,220

Table #6: Total Substrate Material Masses Used

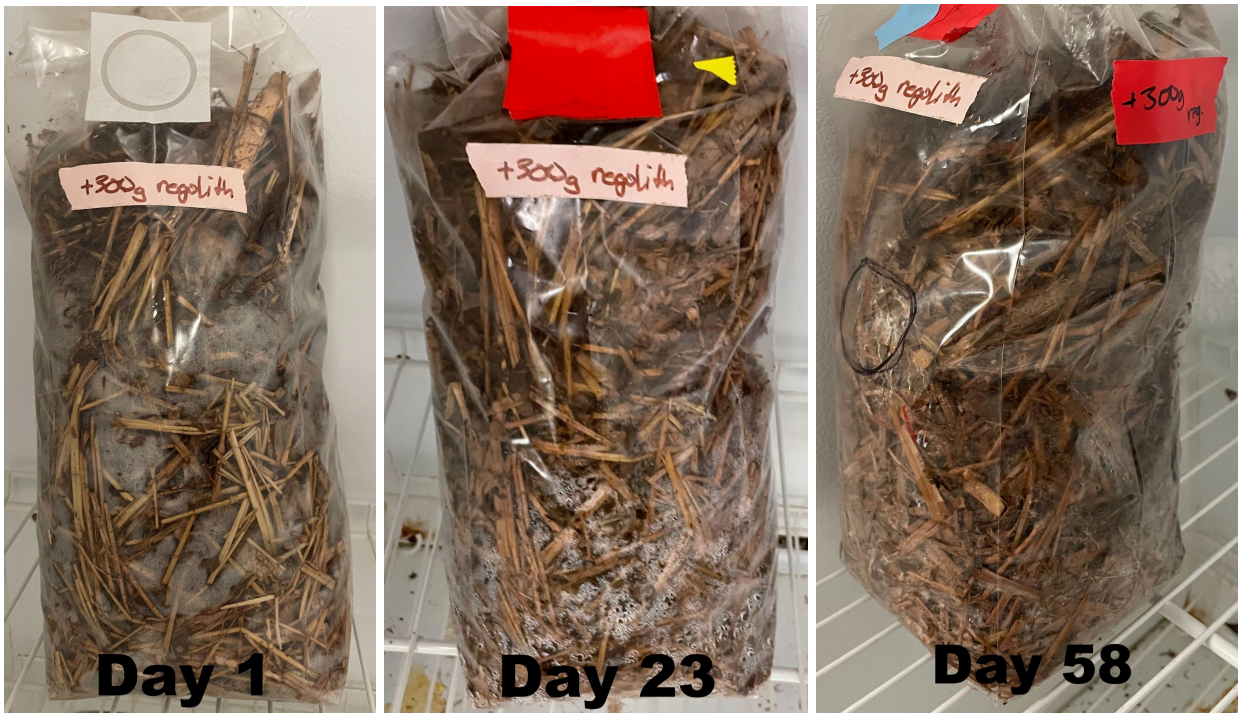
Material	**Amount (g)**
Sawdust	298.25
Woodchips	119.3
Straw	95.44
Gypsum	11.93

Total Mass:	524.92

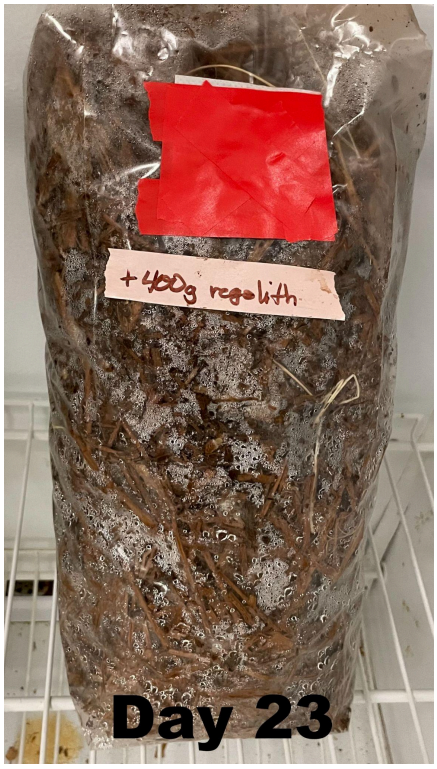
VIII. Appendix B: Visual Data




+200g regolith
Day 1
+200g regolith
Day 23
+200g reg
Day 58


+300g regolith
Day 1
+300g regolith
Day 23
+300g regolith
+300g reg
Day 58


+400g regolith
Day 1


+400g regolith
Day 23

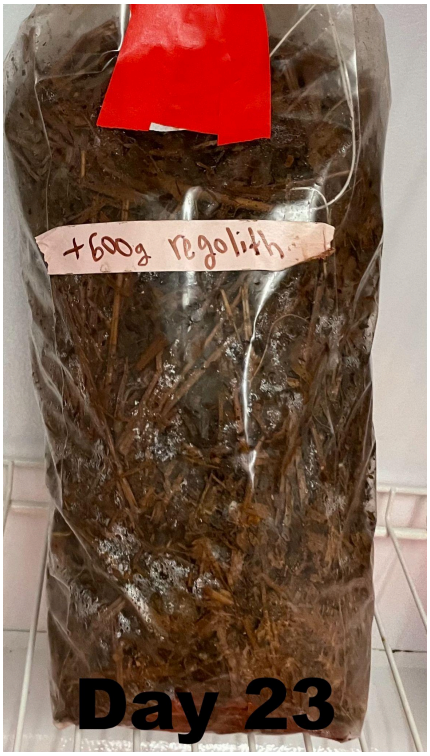

+400g reg
Day 58


+500g regolith
Day 1

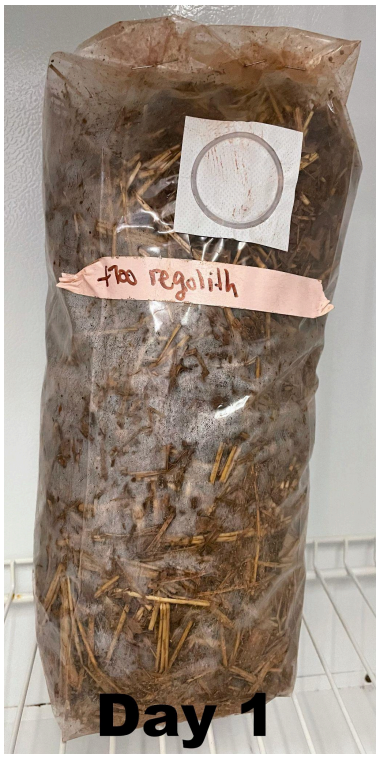

+500g regolith
Day 23


+500g reg
Day 58


+600g regolith
Day 1


+600g regolith
Day 23

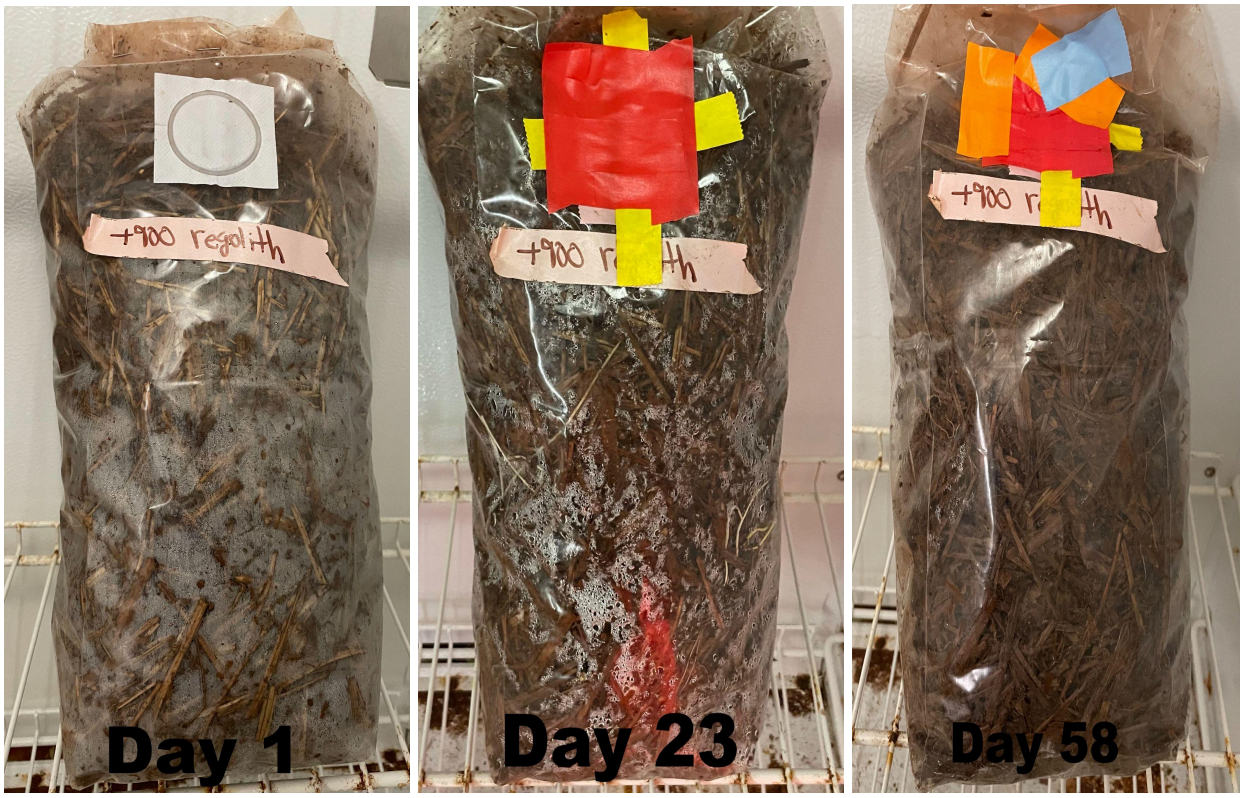

+600g regolith
Day 58


+700 regolith
Day 1


+700 regolith
Day 23


+700 regolith
Day 58


+800 regolith
Day 1
+800 regolith
Day 23
Day 58


+900 regolith
Day 1
Day 23
Day 58