

Cultivation of *Pleurotus ostreatus* in Martian Regolith Simulant (MGS-1) for Dual-Purpose Food and Construction Applications



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Abstract

Establishing sustainable food production and construction material systems on Mars represents one of the most critical challenges for long-duration space exploration. This study investigated the feasibility of cultivating *Pleurotus ostreatus* (oyster mushrooms) across nine substrate formulations containing varying ratios of Martian regolith simulant (MGS-1), powdered straw or sawdust, wheat bran, and gypsum, with the dual goal of producing edible fruiting bodies and structural mycelium-based composites (myco-bricks). Substrates were inoculated with *P. ostreatus* spawn cultivated on Uncle Ben's rice bags and monitored over an 8-week period for colonization progression, fruiting body formation, and brick consolidation. Environmental conditions were tracked using Inkbird hygrometers and ranged from 18.0–22.5°C with relative humidity between 66–96%. Mycelial colonization was observed in several batches, particularly those with higher organic content, but growth stalled within 2–3 weeks in all substrates. No fruiting bodies or structurally consolidated bricks were produced. The control substrate (90% MGS-1) showed the weakest colonization. Contamination from a competing mold species, inconsistent watering, lack of HEPA filtration, and uncontrolled airflow in the atrium incubation environment are identified as the primary constraints on fungal development. These findings contribute to the growing body of literature on fungal cultivation in regolith-based systems and highlight the critical importance of environmental control for mycelium-based ISRU applications on Mars.

Introduction & Theory

Introduction

Long-duration space missions face strict mass, volume, and resource constraints, making in-situ resource utilization (ISRU) essential for sustainable extraterrestrial living. Every kilogram launched into space increases mission cost and complexity, and every additional item introduces new variables and potential points of failure. For future Martian habitats, systems that serve multiple functions—such as producing food while also generating construction materials—are especially valuable.

Fungi, particularly oyster mushrooms (*Pleurotus ostreatus*), present a promising avenue for multifunctional ISRU systems. Oyster mushrooms can convert low-value organic substrates into edible fruiting bodies while simultaneously producing mycelium, a dense fibrous network capable of forming lightweight, insulating, and biodegradable structural materials (Islam et al., 2017). Mycelium-based composites have been studied for their mechanical strength, adaptability, and potential as sustainable building materials.

Fresh food also plays a critical psychological and nutritional role in isolated environments. Studies show that access to fresh produce improves well-being and morale during spaceflight and military missions (Trovato et al., 2025). Mushrooms are particularly suitable for space agriculture due to their compact growth requirements, rapid life cycle, and ability to grow on recycled substrates (Cotter, 2014).

This experiment explores whether *P. ostreatus* can be cultivated in Martian regolith simulant (MGS-1) supplemented with organic materials to produce both edible mushrooms and structural mycelium bricks. Understanding fungal growth behavior in regolith-based substrates is a key step toward developing integrated food-and-material systems for future Martian settlements. Notably, a parallel study by Chavez et al. (2025) in the same Plant the Moon Challenge season also explored *P. ostreatus* in MGS-1, finding that sealed, high-humidity environments facilitated more robust mycelial colonization than open pot-based substrates—a finding that aligns closely with the constraints encountered in our own study.

Theory: The key consideration in this experimental design was the composition of the soil, which once colonized could hypothetically be concretized. Given previous experimental success with growing *Pleurotus ostreatus* to flush in Exo-Regolith simulant, by past Plant the Moon teams, the procedure was designed specifically to experiment with creating growth medium recipe conducive to creating a structurally sound myco-concrete. Definitionally, some concrete is the mixture of a cementing medium with a fine aggregate, (Neville, 2010) usually silicates which also comprises 46% of MGS-1 Global Version. (Long-Fox, 2023) Myco-concrete, lacking a traditional cementing medium, instead binds the aggregate matrix in a dense mycelium network. Through the colonization of lignocellulosic aggregate components, in this case saw dust or straw dust, *Pleurotus ostreatus* will in theory extend a network of hyphae between the aggregates, compacting the smaller substrate particulates. (Zhang, 2025) Saw dust and straw dust, both being common lignocellulosic substrates for *P. ostreatus* cultivation, exist as independent variable groupings. These groupings test efficiency in growth rate, as well as composite strength in the matrix, as each has a unique particulate size, mass, and density, which could affect the structural integrity of the concrete. They also may alter the fruiting group's rate of maturation. Once *Pleurotus ostreatus* fully colonizes its aggregate matrix, the fungus begins the production of its fruiting bodies, which results ultimately in considerable fungal die back. Such an event is not preferable and as such fruiting must be prevented, by way of heating and trying the newly formed 'composite myco-cement' mixture into a solid non-living structure. Fungal concrete using *P. ostreatus* is already common in engineering experimentation as a more sustainable alternative to semi-permanent building materials, however changing the aggregate base from a terrestrial silicate mixture to one of Martian origin is still a new and widely unexplored venture. Consideration should be made regarding the biological interactions between components found in Martian regolith that *P. ostreatus* would find unfamiliar in its usual life cycle, as they may lead to variation in growth compared to terrestrial myco-concrete recipes.

The importance in testing both the growth of these fungi into edible fruit as well as into a structural composite, returns to the core principle of ISRU, which is limiting payload weight on rockets. In this case, should the fungus be capable of both fruiting and composing a structure it may then be possible to reuse once or twice spent mycelium post fruiting as the basis for growing concrete.

Hypothesis

Research Question: Can *Pleurotus ostreatus* be successfully cultivated in substrate formulations containing Martian regolith simulant (MGS-1) and lignocellulosic organic matter to produce both edible fruiting bodies and structurally consolidated mycelium-based composites (myco-bricks) suitable for in-situ resource utilization (ISRU) on Mars?

Hypothesis: When cultivated in optimized substrates composed of powdered straw or sawdust, nutritional supplements, and Martian regolith simulant, *Pleurotus ostreatus* will exhibit enhanced mycelial development and produce fruiting bodies suitable for consumption. The resulting mycelium will bind the substrate into a solid composite capable of functioning as ISRU myco-concrete.

The scientific basis for this hypothesis rests on four key areas of research:

1. **Fungal Growth Requirements:** Oyster mushrooms require a carbon-rich substrate (straw, sawdust), nitrogen supplementation (wheat bran), minerals such as calcium sulfate (gypsum), and adequate moisture and oxygen. Previous studies show that substrate composition significantly affects colonization speed and yield (Z.A.S. et al., 2004; Moonmoon et al., 2010).
2. **Role of Gypsum and Supplements:** Gypsum has been shown to improve fungal growth and fruiting by enhancing substrate structure and mineral availability (Li et al., 2022).
3. **Mycelium as a Structural Material:** Mycelium forms a dense, interwoven network of hyphae that can bind particulate materials into lightweight composites with measurable compressive strength (Islam et al., 2017). When grown in molds, this network can create "myco-bricks" suitable for construction.
4. **Relevance to Martian ISRU:** Using Martian regolith as a bulk substrate reduces the need to transport building materials from Earth. If fungi can grow in regolith-based mixtures, they could simultaneously support food production, waste recycling, and construction of habitat components.

Experimental Variables

Independent Variables

Independent variables are the factors deliberately manipulated across experimental batches to evaluate their effect on fungal growth and composite formation.

- 1. Type of lignocellulosic substrate:** Powdered strawdust versus fine sawdust was used as the primary carbon source across the eight experimental batches (Batches 1–4 used strawdust; Batches 5–8 used sawdust). These two materials differ in particulate size, density, and lignocellulosic composition, potentially affecting both colonization rate and myco-brick structural integrity.
- 2. Proportion of MGS-1 Martian regolith simulant:** The percentage of MGS-1 in each batch was varied across four levels: 50%, 55%, 60%, and 65% (with the balance made up by the lignocellulosic source). The control batch contained 90% MGS-1 with no lignocellulosic component. This gradient was designed to determine the maximum regolith concentration at which *P. ostreatus* can still colonize and potentially form a structural composite.

Dependent Variables

Dependent variables are the measurable outcomes observed in response to the manipulated independent variables.

- 1. Mycelial colonization rate and extent:** The speed and completeness of white mycelial thread growth through each substrate batch, assessed qualitatively (none, light, moderate, heavy) through weekly visual inspection and photographic documentation.
- 2. Fruiting body formation:** The presence, number, and size of primordia and mature *Pleurotus ostreatus* fruiting bodies produced in the fruiting bag format across all nine substrate batches.
- 3. Myco-brick structural consolidation:** The degree to which the inoculated substrate in brick molds ($10 \times 1.25 \times 1.25$ inches) consolidated into a self-supporting solid composite, assessed by handling and visual inspection at the end of the 8-week period.

Controlled Variables

Controlled variables are factors held constant across all batches to ensure that observed differences in outcomes are attributable to the independent variables rather than extraneous influences.

1. **Fungal species:** *Pleurotus ostreatus* (oyster mushroom) was used as the sole inoculum species across all nine batches, ensuring that any differences in colonization are due to substrate composition rather than species variation.
2. **Spawn source and inoculation method:** All nine batches were inoculated from the same four Uncle Ben's ready-to-eat rice bag spawns, ensuring a consistent and genetically identical inoculum was distributed evenly across all substrates.
3. **Supplemental components (wheat bran and gypsum):** All experimental batches (excluding the control) contained a fixed 5% wheat bran (nitrogen supplement) and 5% food-grade gypsum (calcium sulfate), ensuring that nutritional supplementation was equivalent across all batches.
4. **Incubation environment and duration:** All batches were incubated in the same engineering atrium location using two identical covered containers monitored with Inkbird digital hygrometer/thermometers. The experimental period was fixed at 8 weeks for all batches, with environmental monitoring conducted from March 9–29, 2025.
5. **Substrate format (brick molds and fruiting bags):** Each of the nine substrate batches was simultaneously prepared in two formats: a $10 \times 1.25 \times 1.25$ -inch brick mold for structural composite assessment and a fruiting bag in a 3D-printed $3 \times 3 \times 3$ -inch box for mushroom production assessment. This dual format was kept consistent across all batches.
6. **MGS-1 source and preparation:** All batches used Martian regolith simulant sourced from the same Plant the Moon Challenge kit supply to ensure consistent mineral composition (MGS-1 Global Version) across the experiment.

Measurement Methods

Experimental Design Overview

The experiment tested nine substrate formulations containing varying ratios of Martian regolith simulant (MGS-1), gypsum, wheat bran, and either powdered straw or sawdust. Each substrate was inoculated with *P. ostreatus* mycelium and monitored for colonization, fruiting, and structural formation over an 8-week period (March 9 – March 29, 2025 for environmental monitoring; inoculation began approximately 6 weeks prior).

Substrate Formulations

A total of nine batches were prepared with the following compositions:

Batch	Regolith (%)	Gypsum (%)	Wheat Bran (%)	Lignocellulosic Source (%)
1	50	5	5	40% strawdust
2	55	5	5	35% strawdust
3	60	5	5	30% strawdust
4	65	5	5	25% strawdust
5	50	5	5	40% sawdust
6	55	5	5	35% sawdust
7	60	5	5	30% sawdust
8	65	5	5	25% sawdust
Control	90	5	5	0% (none)

Table 1. Substrate formulations tested across nine experimental batches. All components were mixed thoroughly to ensure even nutrient distribution.

Spawn Preparation

Initial Attempt (Discarded): Ten wheat-bran bags were inoculated with *P. ostreatus* spores but developed mold contamination and were entirely discarded prior to substrate inoculation.

Successful Spawn: Four Uncle Ben's ready rice bags were subsequently inoculated with *P. ostreatus* spores and successfully colonized. These colonized rice bags served as the primary inoculation source for all nine substrate batches.

Inoculation Procedure

Rice spawn was broken apart and mixed evenly into each substrate batch. Substrates were then placed into two formats: nine brick molds ($10 \times 1.25 \times 1.25$ inches) intended for myco-brick formation, and nine fruiting bags inserted in a $3 \times 3 \times 3$ inches 3D printed boxes intended for mushroom production. All samples were distributed across two incubation containers and placed in an atrium environment.

Environmental Conditions and Monitoring

Environmental conditions were monitored using two Inkbird digital hygrometer/thermometers, one placed in each incubation container. Temperature and relative humidity readings were recorded approximately every 1–2 days from March 9 to March 29, 2025. Figures 2a and 2b below summarize the recorded environmental data:

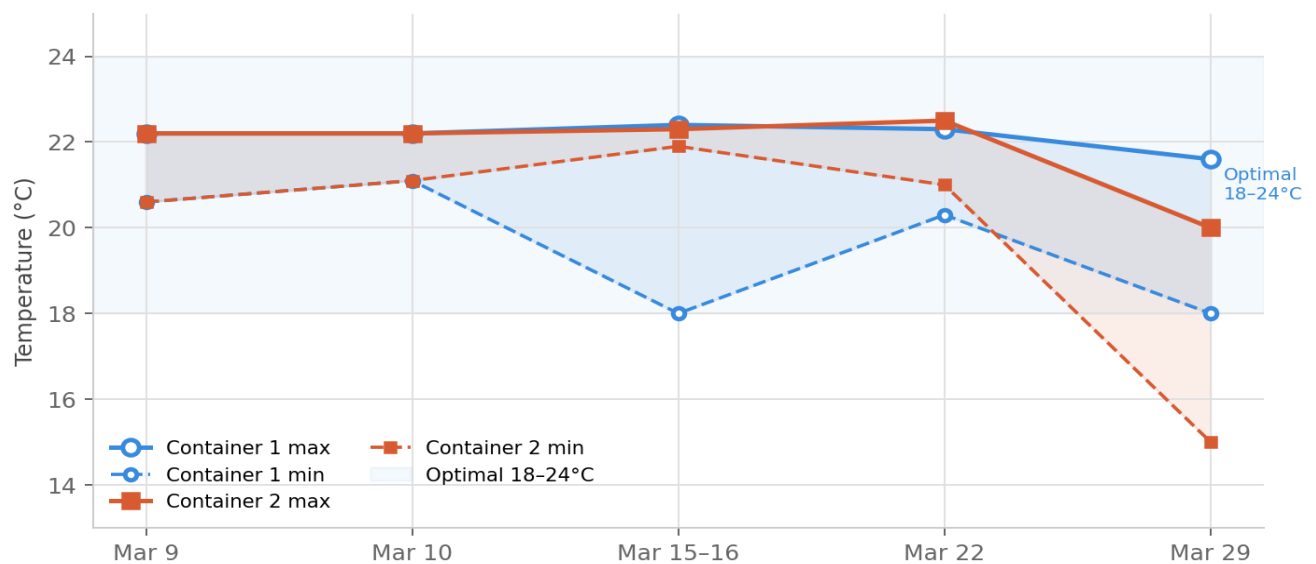


Figure 2a. Temperature recorded in both incubation containers (March 9–29, 2025). Solid lines = daily maximum; dashed lines = daily minimum. Shaded band = optimal growth range.

Figure 2a. Temperature recorded in both incubation containers (March 9–29, 2025). Solid lines = daily maximum; dashed lines = daily minimum. Shaded band = optimal growth range (18–24°C). Optimal conditions for *P. ostreatus* mycelial growth are generally 18–24°C and 80–95% relative humidity (Cotter, 2014).

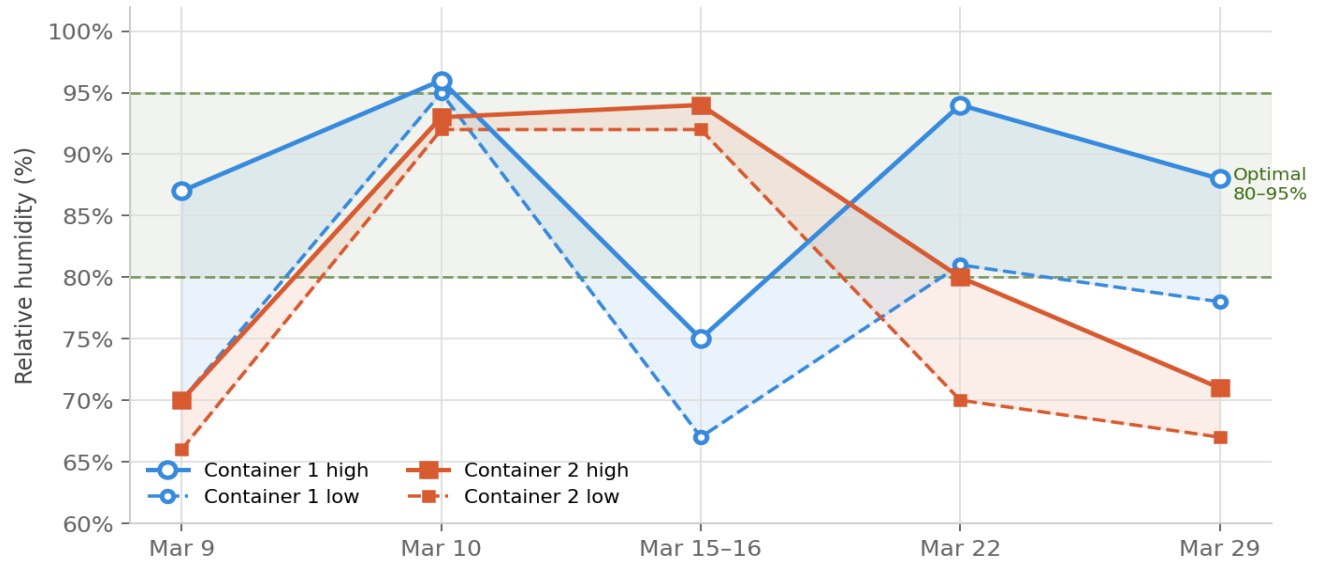


Figure 2b. Relative humidity in both incubation containers (March 9–29, 2025). Solid lines = daily high; dashed lines = daily low. Green band = optimal RH for *P. ostreatus* colonization.

Figure 2b. Relative humidity in both incubation containers (March 9–29, 2025). Solid lines = daily high; dashed lines = daily low. Green shaded band = optimal RH range for *P. ostreatus* colonization (80–95%, Cotter, 2014).

Constraints on environmental control were significant. The experiment was conducted in an engineering atrium with uncontrolled airflow. A still-air box was introduced partway through the experiment to reduce contamination risk but did not fully eliminate it. Temperature and humidity fluctuated considerably, particularly in Container 2 during Week 4 where minimums dropped to 15°C and 67% RH—conditions below the threshold for optimal mycelial activity. Watering was inconsistent throughout the experiment. No HEPA filtration or sterile laboratory equipment was available.

Measurement Techniques

Due to the absence of fruiting bodies and incomplete colonization, quantitative yield data could not be collected. The following qualitative and observational methods were employed:

- Qualitative colonization scoring via visual inspection (none, light, moderate, heavy)
- Photographic documentation of substrate, hygrometer readings, and environmental setup
- Daily and weekly observation logs of mycelial progression and contamination
- Structural integrity assessment of brick molds for early consolidation

Analysis & Results

Colonization Outcomes

Mycelial colonization was observed in several batches during the first 1–2 weeks of incubation, with the most visible growth appearing in substrates containing higher proportions of organic material (Batches 1 and 5, with 40% straw/sawdust respectively). However, growth stalled or reversed in all batches within 2–3 weeks. No primordia or fruiting bodies were observed in any substrate at any point during the 8-week experiment. No substrate achieved the degree of full colonization required for myco-brick consolidation⁴

Batch	Lignocellulosic Source	Initial Colonization	Final Colonization	Fruiting	Brick Formation
1	40% Strawdust	Moderate	Stalled	None	None
2	35% Strawdust	Light–moderate	Stalled	None	None
3	30% Strawdust	Light	Stalled	None	None
4	25% Strawdust	Light	Stalled	None	None
5	40% Sawdust	Moderate	Stalled	None	None
6	35% Sawdust	Light–moderate	Stalled	None	None
7	30% Sawdust	Light	Stalled	None	None
8	25% Sawdust	Light	Stalled	None	None
Control	None	Minimal	None	None	None

Table 3. Colonization outcomes across all nine substrate batches. Initial colonization reflects observations during Weeks 1–2; final colonization reflects the state at Week 8.

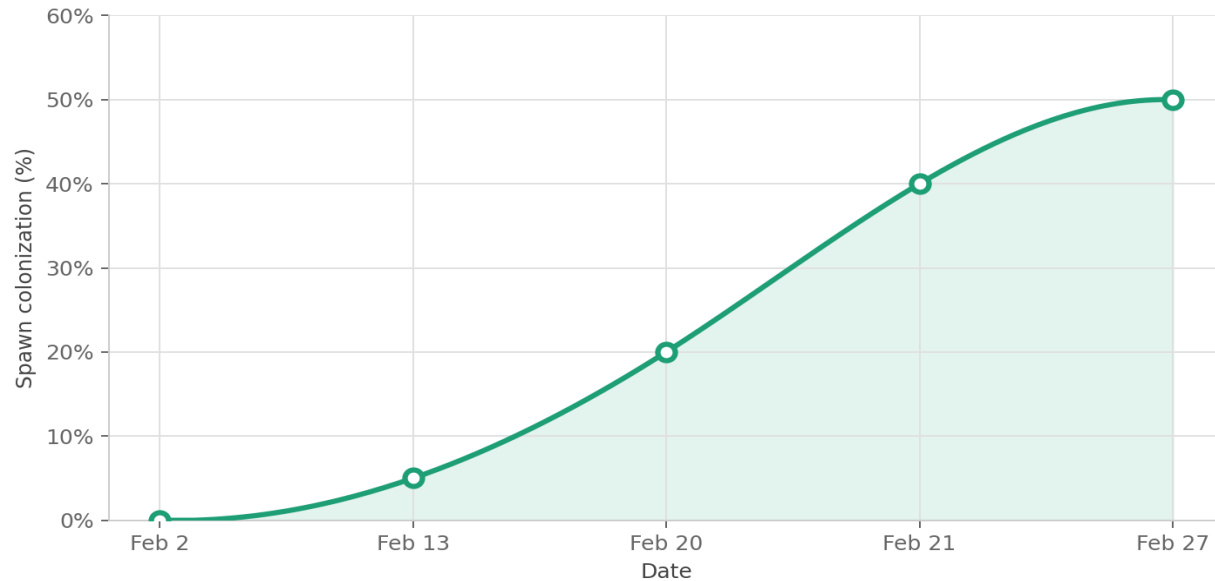


Figure 1. Spawn colonization increased slowly during the first two weeks after inoculation. Rapid expansion began around February 20 as the mycelium established itself within the substrate.

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Contamination

The first round of spawn preparation (wheat-bran bags) was lost entirely to mold contamination before any substrate inoculation occurred. Subsequent batches inoculated with the rice-bag spawn showed no macroscopically visible mold; however, the stalling and dieback of mycelium in all batches suggests the likely presence of microbial competition at a level not detectable by visual inspection alone.

Environmental Data Summary

Temperature across both containers remained within the acceptable range for *P. ostreatus* growth (18–24°C) for most of the experiment. However, relative humidity was highly inconsistent. Container 2 showed a marked decline in humidity during Week 4, reaching minimums of 15°C and 67% RH, which falls below the threshold for sustained mycelial activity. Container 1 maintained more consistent humidity (78–96%) but experienced its own periods of lower readings (67–75% in mid-experiment). The ideal humidity for *P. ostreatus* colonization is 80–95% (Cotter, 2014), which was achieved only intermittently in both containers.

Overall Result

The primary hypothesis—that *P. ostreatus* cultivated in MGS-1-based substrates supplemented with organic materials would produce fruiting bodies and consolidate into myco-bricks—could not be confirmed or rejected due to incomplete colonization and absence of fruiting. The experiment yielded important negative data regarding the constraints of non-sterile, non-climate-controlled environments on fungal cultivation in regolith-based substrates.

Discussion & Conclusions

The results of this study, while not achieving the desired outcome of fruiting body or myco-brick production, provide meaningful insights into the challenges of cultivating *P. ostreatus* in Martian regolith simulant under resource-limited conditions. The experiment serves as an important exploratory data point in a field where negative results are underreported but equally valuable.

The most significant limiting factor was the lack of environmental control. Oyster mushroom cultivation is highly sensitive to humidity fluctuations and airborne contamination. In professional mycology settings, HEPA-filtered clean rooms and humidity-controlled fruiting chambers are standard practice. Our atrium-based setup, while innovative in its use of two separate incubation containers and hygrometer monitoring, could not replicate these conditions. The recorded humidity data clearly shows periods where both containers fell below the 80% threshold critical for sustained mycelial growth—particularly Container 2 during the final week, where minimums reached 67% RH and 15°C.

The initial contamination of the wheat-bran spawn also set the experiment back significantly. The successful pivot to Uncle Ben's rice bags—a well-documented low-tech spawn cultivation method in the hobby mycology community—demonstrated adaptability, but the delay likely reduced the window for full substrate colonization within the 8-week period.

Inconsistent watering represents another critical variable. Fungal mycelium requires consistent substrate moisture (field capacity ~60–70%) to colonize effectively. Without automated or precision watering, moisture levels almost certainly fluctuated in ways not captured by surface hygrometer readings, contributing to mycelial dieback in later weeks.

These findings align closely with the parallel study by Chavez et al. (2025), who also cultivated *P. ostreatus* in MGS-1 during the same Plant the Moon Challenge season. That team similarly encountered mold contamination that outcompeted their target fungi, and found that only their sealed bag environment—where humidity was maintained by the mycelium's own respiration—produced robust colonization. Their open pot-based specimens, analogous to our setup, showed slower and more inconsistent progression. This convergence of results across two independent studies using similar organisms and substrates strongly suggests that environmental sealing and humidity control are non-negotiable prerequisites for successful fungal ISRU systems.

The control batch (90% MGS-1, no organic carbon source) performed worst, as expected. MGS-1 alone provides no bioavailable carbon or nitrogen for fungal metabolism, confirming that organic supplementation is essential—a finding consistent with the broader Plant the Moon Challenge literature (Chavez et al., 2025; Duri et al., 2020).

Future iterations of this experiment should prioritize: (1) a sealed, humidity-controlled fruiting chamber maintaining 85–95% RH consistently; (2) HEPA filtration or at minimum a laminar flow hood for all inoculation procedures; (3) automated or precision watering protocols; (4) extended colonization periods of 10–12 weeks before fruiting induction; and (5) the inclusion of *P. ostreatus* spawn specifically cultivated on straw rather than rice, as straw more closely resembles the carbon structure of the organic substrates used. Additionally, future studies should explore the potential of fully sealed bag cultures within the substrate molds themselves, combining the sealed humidity advantage observed by Chavez et al. (2025) with the brick-formation goal of our own study.

Despite the absence of fruiting bodies or myco-bricks, this experiment makes a genuine contribution to the literature on fungal ISRU for Mars. It documents a set of environmental conditions under which *P. ostreatus* cannot be reliably cultivated in MGS-1-based substrates, narrows the design space for future experiments, and provides the first multi-batch regolith ratio comparison (50–65% MGS-1 vs. organic content) in a non-sterile setting. As one of very few teams in the Plant the Moon Challenge to pursue a dual food-and-material system through a single organism.

Appendices

Appendix A: Environmental Monitoring Data

The following table presents all recorded temperature and relative humidity readings from both incubation containers over the monitoring period (approximately March 9–29, 2025).

Date	Container 1 Temp (°C)	Container 1 RH (%)	Container 2 Temp (°C)	Container 2 RH (%)
Mar 9 (start)	69.2°F / ~20.6°C	70% (max 87%)	69.2°F / ~20.6°C	66% (min)
Mar 10	71°F max / ~22.2°C	95–96%	71°F max / ~22.2°C	92–93%
Mar 15–16	max 22.4 / min 18.0	67–75%	max 22.3 / min 21.9	92–94%
Mar 22	max 22.3 / min 20.3	81–94%	max 22.5 / min 21.0	70–80%
Mar 29 (end)	max 21.6 / min 18.0	78–88%	max 20.0 / min 15.0	67–71%

Appendix B: Substrate Preparation Notes

All substrate components were weighed and mixed by hand. Mixing was performed thoroughly to ensure even distribution of nutrients. MGS-1 Martian regolith simulant was provided by the Plant the Moon Challenge kit. Wheat bran (nitrogen supplement) and food-grade gypsum (calcium sulfate) were sourced commercially. Powdered straw and fine sawdust were sourced locally and ground to a fine consistency before mixing. Substrate batches were prepared simultaneously and inoculated within 24 hours of preparation.

Appendix C: Spawn Preparation Notes

First spawn attempt: ten wheat-bran bags were inoculated with *P. ostreatus* spores under non-sterile conditions using a still-air box. All ten bags developed green/black mold contamination within seven days and were discarded.

Second spawn attempt: four Uncle Ben's ready-to-eat rice bags were inoculated with *P. ostreatus* spores. Rice bags were sanitized externally with isopropyl alcohol prior to inoculation. All four bags showed successful white mycelial colonization within 10–14 days and were used as the inoculation source for all nine substrate batches.

Appendix D: Observation Log Summary

Week	Observation
Week 1	Rice spawn and wheat bran bags inoculated. Light white mycelial threads visible in Batches few batches by end of week.
Week 2	Colonization progressing in higher-organic batches (1, 2, 5, 6). Humidity inconsistent.
Week 3	Growth slowing across all batches. First signs of dieback in a few batches. Some signs of contamination are visible in wheat bran bags.
Week 4	Significant slowdown. Rice bags maintain partial colonization. Ready to be transferred into substrates.
Weeks 5–8	No new growth. No fruiting bodies or brick consolidation in any batch. Experiment concluded.

Appendix E: Photos



Inoculated Uncle Bens rice



Inoculated Wheat Bran bags



Spawn growth of Rice bags



Spawn growth of Wheat Bran bags



Contamination of the Wheat Bran bags



Powdered straw and sawdust



Prepping substrate bags



Substrate mixture in sterile bags (batch 5)



Sterile watering of bricks with a syringe (30 mm)



Humidity and temperature tracking

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