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# Solutions for Space Waste: Biodegradation of Polyurethane by Pestalotiopsis Microspora in Microgravity

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**Abstract:** ***Reusing waste in space can significantly increase the longevity of missions in space. Traditional methods used aboard the International Space Station (ISS), such as returning waste to Earth or incinerating it in the atmosphere, are unsustainable for deep-space exploration. This experiment explores an innovative solution: leveraging the polyurethane-decomposing fungus Pestalotiopsis microspora for bioremediation in microgravity environments. With its unique ability to degrade polyurethane aerobically and anaerobically, P. microspora offers significant potential for recycling plastic waste into usable byproducts, aligning with NASA's sustainability objectives. This study involved the design of a custom-built experimental capsule to observe fungal degradation in microgravity conditions on the International Space Station. The capsule integrates a liquid pump system, a microspora chamber equipped with polyurethane samples, and a camera with LED lighting for real-time monitoring. Critical design considerations, such as microgravity-adapted bioreactors and contamination control, were addressed. Experimental methods included cultivating P. microspora on polyurethane foam and assessing decomposition efficiency through visual and biochemical analysis. Findings from this study could advance waste management strategies for long-term missions and contribute to closed-loop ecosystems essential for sustainable space colonization. By demonstrating the feasibility of cultivating P. microspora in space, this research lays the groundwork for integrating fungal bioremediation into future extraterrestrial habitats.***

**Keywords:** Bioremediation, polyurethane degradation, Pestalotiopsis microspora, microgravity research, sustainable space exploration

## 1. Introduction

In recent years, there have been efforts to promote sustainable living in space. Current means of waste disposal on the ISS require astronauts to manually process trash by collecting their waste in bags and storing them short term before either returning the trash to Earth or letting it burn in the atmosphere-- for missions beyond low-Earth orbit, this disposal method will not be sufficient. For this reason, NASA has started sustainability and recycling initiatives regarding spaceflight. (Lockhart 2018) In this context, it's clear that living sustainably in space is a challenge, but one that must be faced to ensure space travel runs smoothly.

Meanwhile, current research on Earth has paved the way for further advancements in this field. One breakthrough of note is the discovery of polyurethane decomposition by mushroom species[1]. Given that polyurethanes are one of the most used polymers on Earth,[2] having identified a novel way to biodegrade a common polymer has immense implications.

This experiment aims to understand the applicability and feasibility of cultivating polymer-decomposing fungi in microgravity environments. Specifically, the experiment will offer insights into how *Pestalotiopsis microspora* can decompose polyurethane sustainably in space, or microgravity. By looking at the results, further advancements in current biodegradation methods, particularly in polyurethane decomposition by fungi, could be achieved.

## 2. Literature Review

The accumulation of plastic waste is a critical environmental issue, both on Earth and in extraterrestrial environments, such

[1] Edwards, L. (2012). Amazon fungi found that eat polyurethane, even without oxygen. Retrieved from https://phys.org/news/2012-02-amazon-fungi-polyurethane-oxygen.html#:~:text=The%20authors%20suggest%20endophytic%20fungi,a%20process%20known%20as%20bioremediation.&text=Bioremediation%20is%20an%20important%20approach,down%20a%20variety%20of%20pollutants.

[2] Rey-Ting Guo, Xian Li, Yu Yang, Jian-Wen Huang, Panpan Shen, Rock Keey Liew, and Chun-Chi Chen, "Natural and Engineered Enzymes for Polyester Degradation: A Review," *Environmental Chemistry Letters*, vol. 22, no. 3 (2024): 1–22, https://doi.org/10.1007/s10311-024-01714-6.

as spacecraft or future space colonies. Recent research demonstrates the potential of bioremediation—a process that utilizes biological agents like fungi—to address this issue. Among the most promising organisms for plastic degradation is *Pestalotiopsis microspora*, a species capable of metabolizing polyurethane (PUR). This review synthesizes findings from several key studies to address why cultivating plastic-degrading *P. microspora* in space could play a vital role in reducing waste, ensuring environmental sustainability, and supporting long-term human survival in extraterrestrial environments.

***Bioremediation and Metabolic Diversity***

Bioremediation offers an eco-friendly solution to reducing waste by exploiting the metabolic diversity of microorganisms. Screening for organisms capable of degrading plastics has revealed that *P. microspora* stands out for its unique ability to break down PUR, a synthetic polymer widely used in foams, coatings, and adhesives. Importantly, *P. microspora* not only degrades PUR under aerobic conditions but also exhibits anaerobic degradation—a rare and unprecedented trait among fungi. This capability suggests the potential for bioremediation in low-oxygen environments, such as sealed spacecraft or planetary habitats, making *P. microspora* a promising candidate for space applications.[3]

***The Role of Fungal Biodiversity***

A broad survey of fungal biodiversity highlights *P. microspora* as a particularly effective agent for degrading PUR. Studies have identified other fungi, such as *Pleurostoma richardsiae* and *Coniochaeta ligniaria*, with varying abilities to metabolize plastics. However, the exceptional efficiency of *P. microspora* in utilizing PUR as its sole carbon source underscores its unique suitability for space bioremediation systems. Additionally, the serine hydrolase enzyme involved in this degradation demonstrates a molecular mechanism that could be further optimized through genetic engineering to enhance plastic degradation rates in extraterrestrial conditions[4].

***Applications in Space Environments***

Space missions generate considerable plastic waste, including packaging materials, equipment components, and personal items. In closed environments like the International Space Station or future Mars habitats, managing waste is a logistical and environmental challenge. Traditional waste management systems rely on storage or incineration, both of which are resource-intensive and impractical for long-term missions. The ability of *P. microspora* to degrade PUR under anaerobic conditions offers an innovative alternative for waste recycling, reducing dependence on Earth resupply missions and mitigating waste accumulation.

***Environmental and Resource Sustainability***

Cultivating *P. microspora* in space could contribute to a closed-loop ecosystem by recycling plastic waste into usable biomass or other byproducts. This aligns with principles of circular economy and sustainability, which are critical for long-term extraterrestrial habitation. Moreover, the adaptability of *P. microspora* to different environmental conditions suggests that it could be integrated into bioreactors designed for microgravity or Martian gravity. These systems could transform waste plastics into materials that support life, such as carbon-based nutrients, creating a self-sustaining ecosystem for future colonists[5].

***Limitations and Future Research***

Despite its potential, the application of *P. microspora* in space requires further research. Studies are needed to understand its growth kinetics in microgravity, optimize its enzymatic pathways, and ensure its compatibility with bioreactors. Additionally, the potential for contamination or unintended ecological impacts must be carefully evaluated. Exploring the use of synthetic biology to enhance the metabolic efficiency of *P. microspora* could further improve its utility in space applications.

Farming and curating *Pestalotiopsis microspora* for use in space bioremediation systems addresses both the immediate challenge of plastic waste management and the long-term goal of sustainable extraterrestrial living. Its ability to degrade PUR under anaerobic conditions, coupled with its potential integration into closed-loop life-support systems, makes it a critical tool for future space exploration and colonization. By harnessing the unique properties of this fungus, humanity can take a significant step toward achieving sustainability beyond Earth.

## 3. Research and Experimentation Methods

The first step of the experiment involved consulting mycology professors from local universities, particularly the National University of Singapore. Dr. Choong, an expert in fungal degradation of waste and plastics, recommended using *Pestalotiopsis microspora*, a fungal species known for its ability to decompose polyurethane, a common polymer plastic. Following this advice, a syringe of *Pestalotiopsis microspora* hyphae was procured from Mycelium Emporium. To cultivate the hyphae into mycelium, potato dextrose agar (PDA) was

[3] Jonathan R. Russell, Jeffrey Huang, Pria Anand, Kaury Kucera, Amanda G. Sandoval, Kathleen W. Dantzler, and DaShawn Hickman, "Biodegradation of Polyester Polyurethane by Endophytic Fungi," *PubMed*, 15 July 2011, https://pubmed.ncbi.nlm.nih.gov/21764951/.

[4] Saurabh Singh, Srikrishna Subramanian, Neha Gupta, Abhay Bajaj, Natesan Manickam,
Genomic insights on gene clusters and pathways for the biodegradation of plastic compounds: Unravelling the metabolic versatility in a Dietzia kunjamensis IITR165, Current Research in Biotechnology, Volume 8, 2024, 100258,ISSN 2590-2628, https://doi.org/10.1016/j.crbiot.2024.100258.(https://www.sciencedirect.com/science/article/pii/S2590262824000844)

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prepared as the growth medium. Each batch of PDA was autoclaved before inoculation to eliminate contamination risks. The hyphae were then gently applied to the sterilized agar using the syringe under aseptic conditions.

To ensure the fungi could survive the journey to the International Space Station (ISS) without prematurely degrading plastic or dying en route, we lyophilized the *Pestalotiopsis microspora*. Lyophilization (freeze-drying) involved removing water from the mycelium while maintaining its viability. This process kept the fungi dormant during transport, ensuring they only became active when rehydrated with water on the ISS. On Earth, we conducted extensive tests on reactivating lyophilized fungi by introducing water under controlled conditions. These tests confirmed that the rehydrated fungi retained their ability to break down polyurethane polymers, as evidenced by visible degradation of foam samples.

To prevent contamination during the growth process, samples were kept in a biosafety cabinet, and lab equipment was sterilized meticulously. Since pure polyurethane samples were unavailable, a foam mixture containing polyurethane was selected to replicate real-world waste conditions. Various inoculation methods were tested, such as placing agar pieces containing mycelium directly onto foam, which proved successful. This approach not only enabled fungal colonization but also demonstrated consistent polymer breakdown, validating the potential of *Pestalotiopsis microspora* for waste management applications in extreme environments like space.

These systematic steps allowed us to ensure the feasibility of the fungi's activation and decomposition capabilities, laying the groundwork for a novel approach to sustainability in extraterrestrial settings.

**Capsule and Electronics Setup and Design**

The capsule design consists of the following components: the outside container, the liquid pump system, the microspora chamber and carbon-dioxide sensor, the camera and LED lighting setup, and the electronic section panels.

The design for the outer shell of the capsule was provided to fit the given dimensions which we are permitted to use for our experiment. These dimensions were based on the precise fitting of the capsule shell into the McMek (the system which runs the experiment) and on the rocket and ISS. The shell is a rectangular-like prism with chamfered edges to produce an irregular octagon base. On each of these chamfered edges is also a screw hole to secure the lid to the shell. Furthermore, to ensure that the components are fixed securely, the shell has components stacked with no dead-space, and all components are near-perfect fits to the interior of the container. Additionally, foam layers were placed under the lid to compress and secure the components even more tightly. To meet strength requirements and ensure that future edits could be made while staying within a reasonable budget, the container was 3D printed with standard PLA filament.

The liquid pump system is a novel design introduced to activate the freeze dried microspora. A pump system was needed to introduce fluids to the microspora, activate it, and grow it. A solid option for a pump on the market is the Aquatech RP-Q1 Micro Ring Pump. It can easily fit within the capsule while providing enough torque to pump liquids more viscous than water. To secure the pump to the outer container, a 3D-printed cage was designed, and the pump was fitted in the middle. Multiple holes were also designed to allow for the pump's tubes to start from the liquid chamber, run through the cage and reach the microspora. The liquid chamber is made from medical-grade nitrile and secured by sliding a ring and epoxy.

The microspora chamber is a half-acrylic, half-PLA design that contains the mycelium. The acrylic cover was laser-cut to exact specifications and fit exactly to the outer container's square-like face dimensions. Due to the transparency of acrylic, the cover allows for the camera setup to observe changes within the chamber. The 3D-printed PLA half was also fit to these specifications. These two pieces were secured together with 2 separate layers of epoxy resin. Additionally, a small hole is designed into the back of the chamber to fit a tube connected to the pump. Through this tube, water is directly pumped into the chamber. Inside of the chamber, two individual layers of polyurethane are placed. The base layer being a flat sheet to fit the chamber, and the second layer being the same but with two holes. One hole is suited to fit the microspora pellet, and the other for the tube to reach the pellet. This water was used to delyophilise the pellet so that we could reliably control when the experiment would begin. Furthermore, a small extension was attached to the back of the chamber to house the carbon-dioxide sensor. The sensor was covered by a tape specially designed to only allow gasses (such as CO2) to permeate it.

The camera and LED setup was designed around the microspora chamber. Due to the acrylic cutout being clear, the camera was placed directly facing the piece, allowing for it to capture any changes taking place inside the chamber. However, the camera had a 65° FOV, so spacing was important to ensure that the camera captured the entirety of the chamber. To help with this, 3D printed PLA standoffs were initially printed with M2 screw holes on each side so they could easily be attached to both the microspora chamber and the side of the camera insert. However, after testing, the PLA standoffs tended to be too flimsy and broke easily. Additionally, the nozzle size of the 3D printer used was too large to accurately print the screw holes, causing the standoffs to shift around when attached to the chamber and the camera insert. Therefore, wooden skewers cut to the specific length of the spacer were used as replacement. The design of the skewer inserts on the camera insert were also changed from screwholes to slots that fit the chopstick diameter. Finally, these chopsticks were secured to the slots through a layer of superglue. Furthermore, because of the limited amounts of light within the capsule, LED lights needed to be placed on either side of the camera insert to allow it to clearly capture the experiment.

The design underwent many iterations across versions. We first began with a design where the capsule was kept horizontal and

the camera faced down from the top panel of the capsule. Nevertheless, we moved on from this idea since this would greatly prohibit increasing the distance between the camera lens and the microspora chamber. Therefore, the camera looks "down the barrel" of the capsule instead. Furthermore, this design allows for an easier process of assembly since all of the components can be stacked in the capsule, instead of them being glued in specific places, which would prove very arduous considering the size of the capsule. In the end, we went with the most simplistic design which we could come up with to decrease possibilities of error with more complicated mechanical designs. That being said, the mechanical design which we employed achieves our goal of being able to effectively pump water into the microspora container, while also being able to reliably view it with the camera.

The electronic components of the capsules consisted of a pump, carbon dioxide sensor, LED lights, and a camera. To fit the electronic components onto the PCB we were given, we had to take into account the proposed mechanical design.

We used a SCD4x sensor, sourced from Digikey. This sensor measures carbon dioxide in Parts per Million, meaning that it measures the concentration of carbon dioxide in regards to one million (e.g. $CO_2$ levels of 1670 PPM would mean 1670 parts out of 1 million in the given environment are $CO_2$). The code was set up to read $CO_2$ levels every 15 minutes.

We used an electronic pump. This allowed us to activate the lyophilized mycelium pellet, by pumping in 10 milliliters of distilled water when the experiment started running. This would also create a moist environment for the mycelium to grow. The code was set up to ensure that the pellet activated when the command was to start the 30-day timer. The tubes were sterilized using ethanol and water. As the pump required more voltage than any of the other components, we had to solder a small connection to another IO pin to ensure enough voltage was going through. Two wires were soldered onto each small bump at the bottom (find the proper name) of the pump, which was then soldered to an appropriate place on the PCB which fit the mechanical design. Connections were then soldered to the IO pins.

**Software**

***Overview of Software Structure***

The software for the experiment is divided into two distinct sections: **Quest CLI** and **Quest Flight**. Both components are written in C for Arduino. Quest CLI serves to define functions and provides a simple methodology for testing individual components for functionality. Quest Flight, on the other hand, executes functions at specific intervals required throughout the experiment. These two sections work together to streamline troubleshooting and maintain well-structured code.

***Mission Sequence Execution***

The experiment begins with the mission clock being reset, and the current time and date recorded. Preliminary checks are conducted to verify the functionality of the abort function and confirm that the pump is turned off. Since no operations are required during the initial 24 hours, a 24-hour timer is started. After this period elapses, the pump is activated for 5 seconds to ensure that the lyophilization of the pellet is undone by pumping the necessary fluid.

***Hourly Photo Capture***

A loop is implemented to manage the photo capture process. Every hour, the LED is turned on, a photo is taken, and the LED is turned off to conserve power. This ensures that images are captured at regular intervals. Nested conditional statements are used to monitor the mission clock and trigger the sequence when the clock reaches the hour.

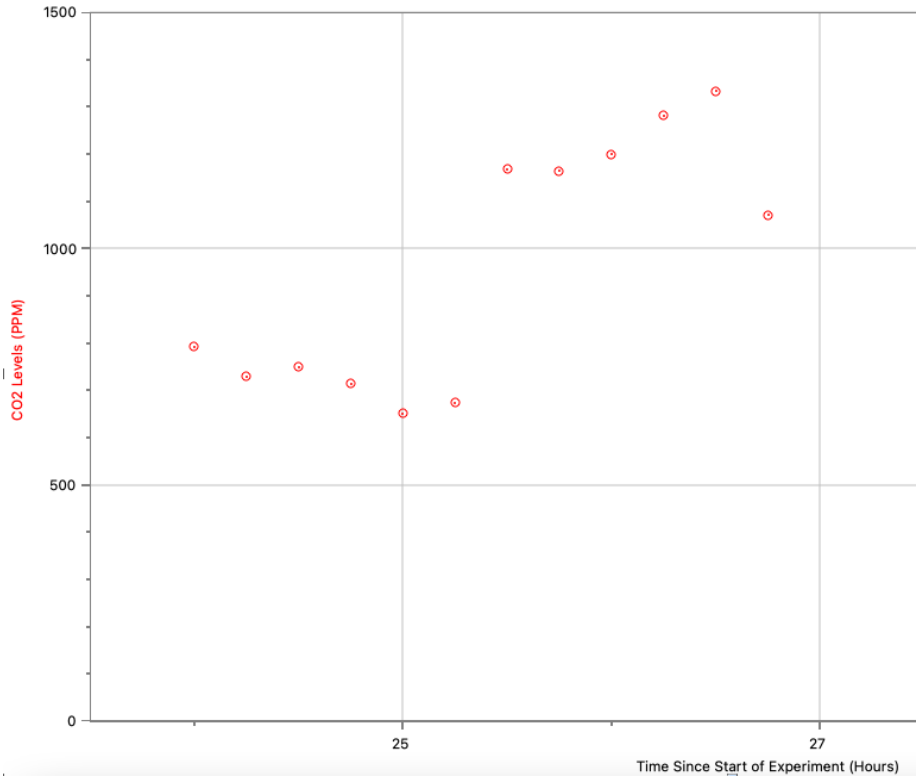
***Environmental Data Collection***

Every 15 minutes, data is collected from the temperature, humidity, and CO2 sensors. This process follows a similar structure to the photo-capture sequence, ensuring data recording is consistent. Error checks are integrated to return error messages in case of invalid or impossible inputs. Additionally, the system introduces a 5-second delay to avoid conflicts if the LED is active for photo capture during a data collection interval.

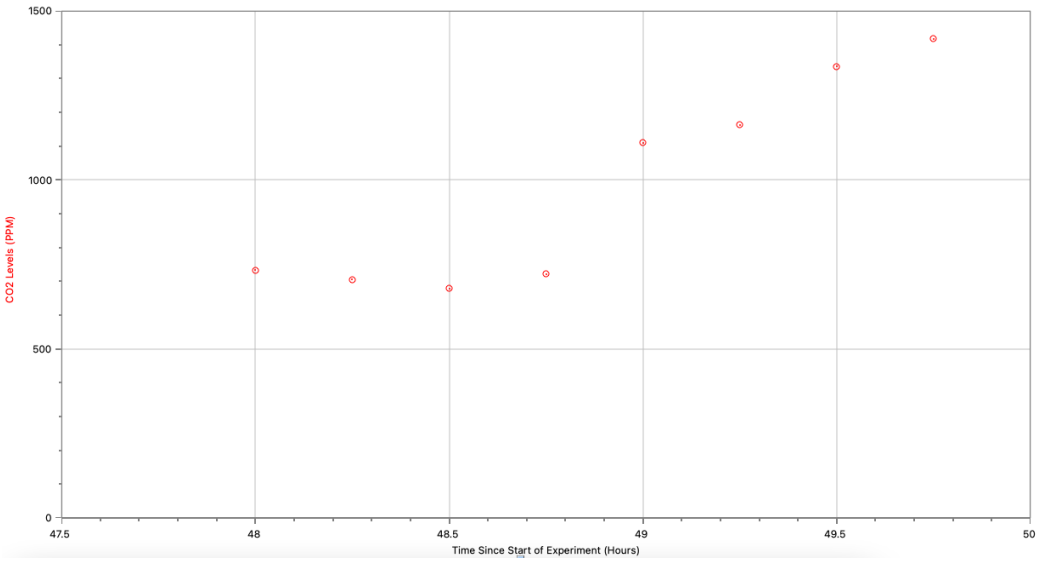
***Real-Time Mission Clock Monitoring***

To verify functionality and maintain an accurate sequence of events, the current mission clock is printed every second. This provides continuous monitoring and helps to identify any irregularities during the experiment. This structure ensures that all components function as intended, while incorporating robust error-checking mechanisms and logical workflows to support the mission's success.

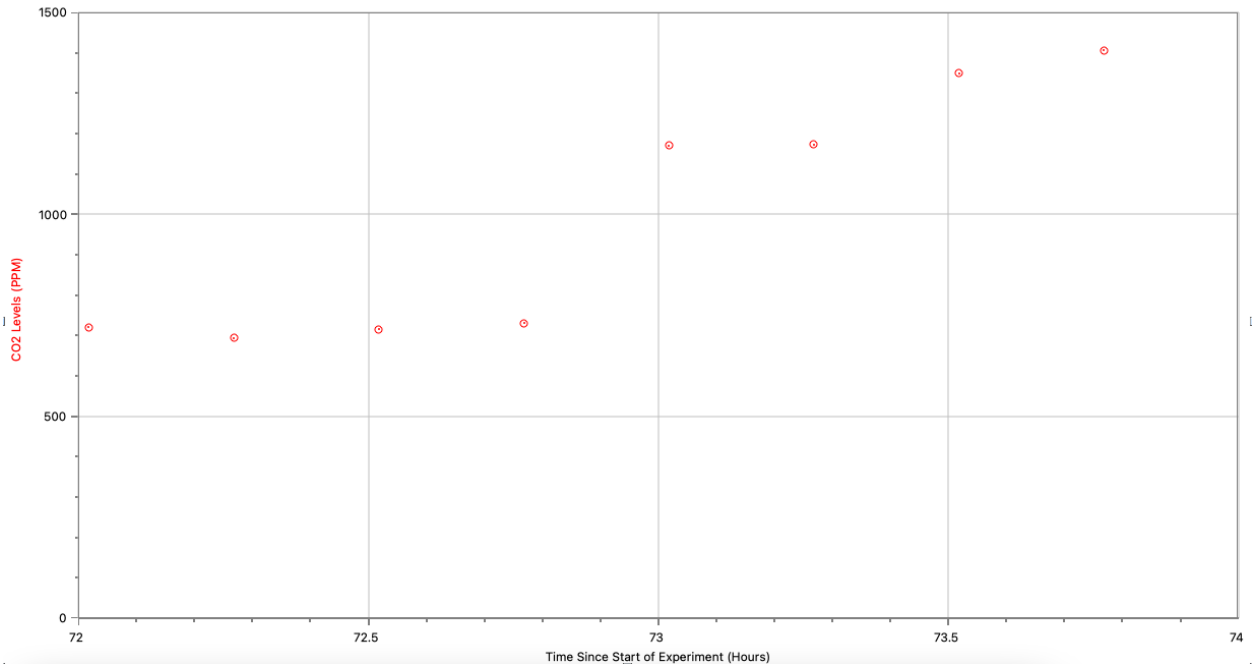
## 4. Data Presentation




Flight Unit Data (Time Stamp 1)




Flight Unit Data (Time Stamp 2)




Flight Unit Data (Time Stamp 3)

## 5. Discussion

During the experiment, periodic increases in $CO_2$ levels were observed, indicating metabolic activity by the *Pestalotiopsis microspora* fungus. As *P. microspora* is known for its ability to degrade polyurethane, the rise in $CO_2$ levels suggests that the fungus was actively breaking down the polymer and utilizing it as a carbon source for its growth and metabolism. The degradation process likely involved enzymatic breakdown of the polyurethane into smaller compounds, which were then metabolized, releasing $CO_2$ as a byproduct.

These fluctuations in $CO_2$ levels served as indirect evidence that *P. microspora* was effectively consuming the polyurethane material. By monitoring these changes alongside visual observations of mycelium growth on the foam, we were able to conclude that the fungus was successfully degrading the polymer.

Although upwards trends were seen between each time stamp, due to the lack of persistence in the sensor settings, by default, certain configurations (such as the automatic self-calibration feature, temperature offset, and sensor altitude), were not saved to the non-volatile memory. This means that every time the sensor restarted, it would revert back to its initial state. As a result, any calibrations or adjustments that happened during either operation or study were lost on every power cycle or reset. Such losses made the sensor readings inconsistent between time stamps.

Another related issue is about the automatic self-calibration mechanism itself. It is usually on unless it is specifically disabled. The self-calibration mechanism recalibrates the sensor to 400 ppm of $CO_2$ each week when it detects fresh air. If the sensor does not experience low $CO_2$ concentrations periodically, this recalibration can cause the baseline $CO_2$ readings to stabilize at values around 400-700 ppm upon each restart. Such behaviour can result in inaccurate readings until the sensor stabilises. Turning off the automatic self-calibration may potentially resolve this issue.

Hence, although the sensor readings did not show an upward trend across the entire scope of data collection, upward trends could be seen across each individual timestamp, indicating that carbon dioxide levels were indeed increasing over the course of the experiment.

**Experiment Limitations**

Our experiment faced several notable limitations. Firstly, we were only able to conduct the experiment in microgravity conditions on the International Space Station (ISS) once. This restricted our ability to replicate the experiment and verify the results obtained from our initial flight unit. Consequently, our conclusions are based solely on data from this single trial.

Secondly, the system controlling the experimental capsule experienced a shutdown midway through the study. As a result, we were only able to collect data for 15 days, rather than the originally planned 30-day duration. This reduction in data collection time limited the scope of our analysis and may have impacted the comprehensiveness of our findings.

Lastly, due to repeated restarts of the McMeck system, the carbon dioxide sensor reset its baseline measurement each day. This prevented us from recording absolute $CO_2$ levels over the duration of the experiment. Instead, we were only able to measure the rate at which $CO_2$ levels increased daily, which may have constrained the accuracy of our conclusions regarding fungal activity and polyurethane consumption.

## 6. Conclusion and Implications

In sum, this study demonstrates the potential of Pestalotiopsis microspora in microgravitational conditions, which can provide a sustainable solution for space waste management. Its results lay the groundwork for incorporating fungi bioremediation systems into space exploration initiatives to sustainably recycle waste in space. Despite our promising findings, more research is nonetheless required in more controlled environments, as well as research on how to best optimize the capabilities of Pestalotiopsis microspora in such a field. Yet, by harnessing innovative solutions like fungal bioremediation, this experiment was successful in taking a step forward to create sustainability initiatives beyond Earth, paving the way for more efficient future space exploration.

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*Captains:* Aarna Agarwal, Ian A. Wilson

*Subteam Leaders*: Matthew Rahul Cortez, Sohrab Mehra, Joseph Oh

*Team Members*: Andrea Liang, Anthony Ding, Emilie Holiday, Jiayi Han, Peiming Xu

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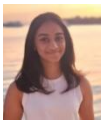
## Author Profile



**Aarna Agarwal** is one of the co-captains for Singapore American School's SpaceLab team. She's been interested in the STEM field since middle school, and has since been exploring her interests through clubs like Geek Girls and robotics. Being in Geek Girls, she advocates for more female participation in STEM classes and activities at school. In SpaceLab, she mainly worked in the electrical and experiment department. She is also an accomplished athlete, training at the Archery Academy of Singapore, and has competed at the national and international level. She was the head author of this article.

**Sohrab Mehra** is the lead of the mechanical engineering department of Singapore American School's SpaceLab team. His interest in engineering began long before he made his way into 3D printing and laser cutting, sparked by his love for Lego and transformers in his early childhood. Sohrab continued to pursue his passion for "building" by spearheading the team's innovative design and construction of the capsule, ensuring it was fully equipped and ready for space. He was the vice head author for this article.

**Anthony Ding** is a member of Singapore American School's SpaceLab team, responsible for assisting in the process of software development. His interest in engineering, programming, and scientific experimentation culminated in his participation in SpaceLab. Anthony hopes to continue pursuing his passion for innovation and problem-solving in college and beyond, with projects like SpaceLab paving the way.

**Ian Wilson** is one of the co-captains on Singapore American School's 2024 SpaceLab team. He's long been interested in theoretical physics, mechatronics, mycology, and olympiad math problems. Ian's SpaceLab contributions were mostly in the Software, experiment ideation, and biological departments.

**Joseph Oh** is the lead of the outreach and experimental team of SpaceLab 2023–24. His main contributions include NASA documentations, research, and bio experimentation with microspora. He is a passionate researcher with high interest in STEM and social sciences. He is grateful for this opportunity of designing an experiment to the International Space Station and is excited to be contributing to the field of Space Exploration. Outside of Spacelab, he is involved in Student Government, Quest, Public Policy and Civic Action Club, and drama.

**Andrea Liang** is part of the mechanical development team in Spacelab. With experience in MATE robotics, she uses her mechanical and design knowledge to contribute to the team. She has extensive experience with Fusion 360 to do Computer-Aided Design, which she uses to both model existing parts and create new ones. As for the other branches of STEM, she's actively involved in the science and math communities at SAS.

**Peiming Xu** is part of the SpaceLab mechanical team. With plenty of experience in robotics competitions such as FRC, and MATE, he brings creative designs and analytical problem-solving to the team. His expertise in CAD and fabrication allows him to work efficiently in the design and mechanical department. Deeply involved in the STEM community at Singapore American School, Peiming's passion for science and technology started at a young age with math and grew to include chemistry and physics. When he is not doing something STEM-related, he can be found playing basketball and listening to music.

**Jiayi Han** works in the outreach and communication department of SpaceLab team 2023–24. She also helps in various aspects such as documentation, research, and experimentation. She was incharge of NASA documentation and material ordering. She is interested in STEM-related projects and chose to join the SpaceLab team to discover more topics in science. Apart from STEM, she also likes crafting and arts.

**Emilie Holiday** is in the mechanical department of SpaceLab. Her interest in astronomy and engineering stemmed from her childhood, where she spent a good chunk of her time watching Mark Rober and alien documentaries. SpaceLab has taught her about the ins and outs of computer-aided design and simultaneously furthered her interest in space technology. Outside of SpaceLab, she enjoys making art, reading, and curating Spotify playlists.

**Vivaan Khushani** is an engineering enthusiast and technology advisor for the Singapore American School's SpaceLab team. As the president of the Code for All service club, Vivaan promotes accessible technology education and mentors students in technology skills. His leadership across STEM initiatives reflects his commitment to fostering innovation and collaboration. Beyond his work in STEM, Vivaan is an avid cricketer and a passionate teacher.