



Student Spaceflight Experiments Program  
Fall 2022

***Galleria mellonella's Polyethyleneases – Pioneers of biodegrading plastic to establish a sustainable space environment***

It can take up to 500 years to decompose plastic waste in landfills (Cho, 2012). In 2021, Americans discarded 51 million tons of plastic, and approximately 95% of it ended up in landfills, oceans, or scattered in the atmosphere (Lakhani, 2022). Dr. Federica Bertocchini discovered wax worms' (*Galleria mellonella*) ability to biodegrade plastic through two enzymes produced in their saliva (Bombelli et al., 2017; Sanluis-Valverdes et al., 2022). As space travel becomes more prominent, it is important to sustain a clean space environment for future exploration. Such concerns raise the question of whether or not *Galleria mellonella* eggs can hatch and if their saliva enzyme, re-named PEases, can sustain the harsh changes experienced during the transition from a gravity to microgravity environment. Plastic pollution is spiraling out of control, and it is only a matter of time before society tries to use space as its next indispensable trash dump. The use of a natural resource, such as PEases, to biodegrade plastic in a microgravity environment can make revolutionary advances in stunting the exponential growth of plastic pollution on earth as well as the space commons. If proven that the enzymes can sustain themselves under such conditions, further research would evaluate the large-scale production of the enzymes to biodegrade more plastic simultaneously. The future is space and someday humans will inhabit other planets. It is critical to take advantage of the resources on earth and mimic it in a microgravity environment to help keep it pollutant free.

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## **I. Student Team Members and Professional Advisors**

### **Co-Principal Investigators**

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### **Collaborators**

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NASA SPOCS Team

Contribution to Team: Provided wax worm expertise from their mission to the ISS (2022) via Zoom

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### **Professional Advisors**

Name: Dr. Federica Bertocchini

Organization: Madrid's Margarita Salas Center for Biological Studies (CIB)

Spanish Research Council (CSIC), Madrid, Spain

Plastic Entropy

Contribution to Team: Provided wax worm expertise via Zoom

Name: Nicolas Dubaut

Organization: Plasticentropy

Contribution to Team: provided wax worm expertise via Zoom

Name: Shea Harris

Organization: Arkansas State University, Biosciences Institute Outreach Director

Contribution to Team: Provided wax worm expertise from their mission to the ISS (2022) via Zoom

Name: Dr. Katie Johnson

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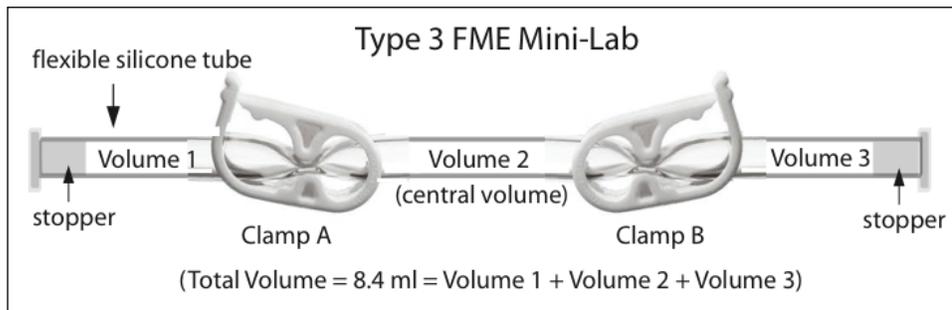
Contribution to Team: Biology and Scientific inquiry expertise

## II. Experiment Materials and Handling Requirements

### 1. Fluids Mixing Enclosure (FME) Mini-laboratory Proposed to be Used (check one):

- Type 1 FME Mini-lab (1 experiment volume: no clamps used)
- Type 2 FME Mini-lab (2 experiment volumes: one clamp used)
- Type 3 FME Mini-lab (3 experiment volumes: two clamps used)

### 2. List of Proposed Experiment Samples (Fluids and Solids to be Used)



#### ***Volume 1***

*List each fluid/solid to be used and the amount of each sample:*

- 2.5 mL ground oatmeal

#### ***Volume 2***

*List each fluid/solid to be used and the amount of each sample:*

- 20 *Galleria mellonella* eggs

#### ***Volume 3***

*List each fluid/solid to be used and the amount of each sample:*

- 5x5 cm grocery bag piece (polyethylene plastic) or 2.5 mL powder polyethylene

### **IMPORTANT: Are any of the proposed samples human in origin? (check one):**

- Yes
- No

**3. Special Handling Requirements During Transportation**

**Table 1: Requesting Thermal Control for Your Experiment**

		<b>Refrigeration</b>	<b>Ambient Conditions</b>
PRE-FLIGHT	Shipping from your Community to NanoRacks in Houston	X	
	At NanoRacks until Handover to NASA	X	
FLIGHT	Handover to NASA Until Arrival at ISS	X	
	Onboard ISS		<b>X (required)</b>
POST-FLIGHT	From ISS until Arrival at NanoRacks		<b>X (required)</b>
	At NanoRacks through Return Shipping to Community		X

**4. Proposed Timeline of Crew Interactions – Your Proposed Crew Interaction Days and Crew Interactions Aboard ISS**

**Table 2: Proposed Timeline of Crew Interactions**

<b>Allowed Crew Interaction Day</b>	<b>Requested Interaction</b>
<b>A=0</b>	
<b>A+2</b>	Unclamp Clamp A, shake gently for 10 seconds Unclamp Clamp B, shake gently for 10 seconds
<b>U-14</b>	
<b>U-5</b>	
<b>U-2</b>	

### III. Question to be Addressed by the Experiment

Between 1950 and 2015, 8.3 billion metric tons of plastic have been produced (Dengler, 2017). The creation of this plastic comes at the price of leaving a carbon footprint that will last multiple generations. In an effort to reduce the carbon footprint created from plastic consumption, plastic recycling is proposed a solution. Incineration for energy production is an alternative but it does contribute to increase CO<sub>2</sub> emission (Cho, 2012). Overall, even though these methods of are available, or they have technical limitations, meaning they will not solve the problem at the large scale (recycling), or they can be toxic due to the products produced in the incineration chemical reactions. Americans discarded 51 million tons of plastic, of which almost 95% ended up in landfills, oceans, or scattered in the atmosphere in 2021 (Lakhani, 2022). At the current rate of plastic production, it is expected that the amount of waste will triple by 2060 with a meager increase in plastic recycling (2022). As such, it is imperative to put a hindrance on the exponential growth of plastic waste.

With the greater good of humanity in mind, the solution implemented needs to have great longevity so that future generations can reap the benefits of taking action now to create a more sustainable solution for plastic waste. Looking into the future brings awareness to the idea of becoming a space faring civilization that will inhabit another planet. Even though there is not an established community on another planet yet, the closest comparison is the International Space Station. Astronauts on the ISS utilize plastics every day. Before space becomes home to more than the select few onboard the ISS, it is necessary to implement a sustainable solution for plastic waste now. Establishing a way to safely dispose of plastic early on due to the importance of preserving the space commons would terminate any misconception similar to the idea of space being an indispensable resource and a place to dump trash.

Rather than allowing plastic to take its natural 500-year degradation path in a landfill, Dr. Federica Bertocchini, a molecular biologist at Madrid's Margarita Salas Center for Biological Studies (Spanish Research Council), discovered that polyethylene can be degraded from two enzymes in the saliva of the wax worm, the larvae of lepidopteran *Galleria mellonella* (Sanluis-Valverdes et al., 2022). Dr. Bertocchini and her business partner at Plasticentropy, Nicolas Dubaut, are investigating how to produce the enzymes in a way such that there will not be an infestation of *Galleria mellonella*, but rather create an established and sustainable method to harvest the enzyme to biodegrade plastic. Figure 1 depicts the ultimate goal of Dr. Bertocchini

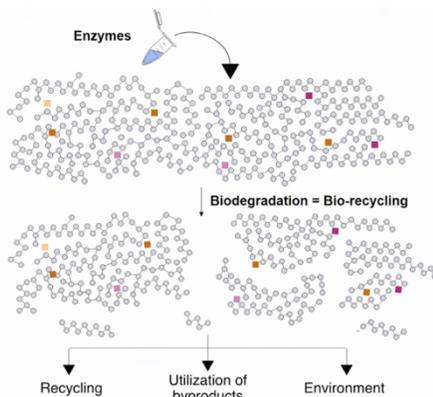


FIGURE 1. Enzyme and water solution used to biodegrade plastic (TEDxTalks, 2021, 0:08:39) and Dubaut's work. Thinking beyond earth's atmosphere and future generations, this experiment will identify if wax worms can produce PEases from the egg stage in a microgravity

environment. Upon the success or failure of *Galleria mellonella*'s ability to produce PEases in microgravity, further research can be conducted on the practicality of solely using the enzymes for the purpose of biodegrading plastics in microgravity.

In a recent zoom call with Dr. Bertocchini and Dubaut, they shared their knowledge of how wax worms degrade plastic. Bertocchini first recognized the wax worm's ability to biodegrade plastic when she pulled out her beehive from storage and found her stored honeycombs were infested with wax worms. She cleaned out the wax worms from the beehive and stored them in a plastic bag, and a few hours later she discovered there were holes in her bag similar to figure 2 (Bombelli et al., 2017). On earth, these enzymes can be applied to plastic in a

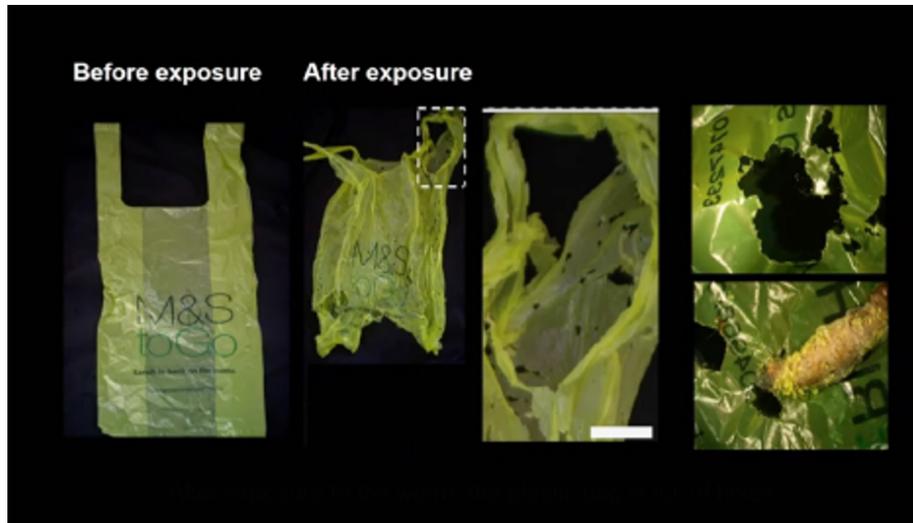


FIGURE 2. Polyethylene bag biodegraded by *Galleria mellonella* from Bombelli et al.

water solution at room temperature. The contact with polyethylene and enzyme solution causes the formation of degradation products such as ketones, among other compounds. Dubaut says, “This new discovery opens up highways of potential applications in the field of plastic bioremediation via bio-recycling” (Bertocchini & Dubaut). Dr. Bertocchini and her research team believes “a depository of degrading enzymes could revolutionize the cleanup of polluting waste”. Dubaut’s dream of living in a plastic waste-free environment may not be light years away (Bertocchini & Dubaut). This experiment hopes to contribute to the cause of creating a plastic free environment on earth’s surface as well as the atmosphere.

The idea of recycling in space is not new. In 2018, an article from the ISS National Laboratory, announced that a facility is being developed by in-orbit service provider, Redwire (formerly known as Made in Space), to implement a commercial polymer recycling system (CPRS). This system aims to repurpose trash into useful items for astronauts and demonstrate plastic recycling capabilities in microgravity (Elkavich, 2018). As the idea of recycling in space becomes more widely known, the use of wax worms could be crucial to the creation of and maintaining a sustainable space environment.

#### IV. Experiment Design

Carrying out this experiment will allow for a better understanding of the *Galleria mellonella*'s saliva enzymes', PEases, production and recycling capability in space. If *Galleria mellonella* can go through the phases of development from being an egg to a cocoon, then the biodegradation of plastic from the PEases enzyme will be evaluated. Once proven is capable of biodegrading polyethylene, then further research can evaluate the large-scale production of the enzymes to biodegrade more plastic simultaneously in a microgravity environment. When large amounts of plastic need to be recycled, the use the enzyme-water solution will allow for the biodegradation to be massified and applied at a much larger scale.

Materials needed for this experiment include *Galleria mellonella* eggs, oatmeal, and a small piece of polyethylene plastic. Further explanation of the materials is detailed in this section of the experiment proposal.

Since the question being addressed specifically pertains to the enzyme produced by the *Galleria mellonella*, there are no other egg types that would be included. The fluids mixing enclosure (FME) will have 20 *Galleria mellonella* eggs in volume 2. Since the eggs themselves cannot be purchased, moths will need to develop to produce the eggs. To do so, the wax worm larvae will be purchased from Carolina Biological Supply Company. These larvae will then develop into a cocoon and then moths to produce the eggs. The moths are capable of laying 300 eggs at once, so this will ensure there will be plenty to select from in the case of a scrubbed launch or unhealthy eggs. In optimal conditions, the wax worms can hatch within 3-5 days of egg delivery (Topflight Dubia). However, to keep the eggs in a dormant state, they will need to be stored between 8 °C and 15 °C. If stored at 4°C, a normal refrigeration temperature, the eggs might not hatch. his dormant state can extend their hatching period to about 35 days. Considering the time for transportation of the experiment from Frisco, Texas to Houston, TX, to Cape Canaveral, FL, and finally to the ISS, refrigeration will allow the life cycle of the eggs to start once onboard the ISS. Another consideration when picking the number of eggs within volume 2 of the FME is that during the transportation, it is expected that some of the eggs will die. Having more within the tube will ensure that at least some of eggs will mature and become PEases-producing larvae. In the case that all 20 eggs survive transportation, overcrowding within the tube is not a concern. During a conversation on Zoom with Dr. Bertocchini and Dubaut, they emphasized the fact that the eggs are naturally laid in the crevices of beehives. If anything, the crevices of beehives is more confined than the FME.

Once the eggs hatch, the larvae need to be sustained with some form of nutrients. In this experiment, 2.5 mL of ground oatmeal will keep the larvae happy and healthy. Through a Zoom conversation with the Arkansas State University NASA SPOCS team that also sent *Galleria mellonella* larvae to the international space station, they explained that their choice of nutrients was oatmeal due to its accessibility and small size. The oatmeal will be purchased in the full-grain state at a local grocery store, but then ground up using a food processor. It is acceptable if all of the oatmeal is consumed by the larvae. These larvae are cannibalistic organisms and will eat each other if no other source of nutrients is available. In addition, if there are no more larvae available for consumption, they will still consume the plastic. During the Zoom conversation, Dr. Bertocchini stated that her past experiments have shown that the larvae will consume both the polyethylene and the oatmeal regardless of the amount left in the test section.

The main premise of the experimental question is based on whether or not the *Galleria mellonella* will survive and if their saliva enzymes, PEases, are capable of degrading plastic in microgravity after being exposed to harsh environmental transitions. As such, a 5x5 cm piece of

polyethylene from a plastic bag needs to be included within the FME. The decision to use polyethylene plastic was based on previous experimentation discussed between the ASU NASA SPOCS team as well as Dr. Bertocchini. Since it is already known that PEases are capable of biodegrading plastic on earth, it is only right to use a material that already has expected outcomes.

Before the experiment is sent to the ISS, it is necessary to experiment with the balance of plastic and oatmeal as a source of nutrients for the larvae. There should not be too much oatmeal such that it would be the only thing the larvae eats. Another experiment that would be beneficial



FIGURE 3: Polyethylene powder from Kompass.com

is a comparison between forms of polyethylene—powder or solid sheet (film). Polyethylene powder can be purchased online and is shown in figure 3. If the larvae consume the powder, then a more accurate ratio of ground oatmeal to ground plastic could be put into the FME.

To prepare the FME before departure from Frisco, Texas, the samples of each volume need to be premeasured by mass and volume. A scoopula and graduated cylinder will be used to measure the volume of ground oatmeal and, if selected, powder polyethylene. A nanogram-precision scale will measure the mass of the polyethylene for comparison to results from being subject to microgravity. If not using the powder polyethylene, a Vernier caliper will be used to measure the size of the plastic sheet that will go into FME. The solid end cap will be inserted into the tube and zip-tied off. Once the zip tie does not come apart, the first chamber will be assembled by using a funnel to insert the premeasured ground oatmeal. This is the first chamber that will be opened by the astronauts and will be denoted as such with a green mark. To prepare the second chamber for the *Galleria mellonella*'s eggs, compressed air and a long cotton swab will be used to ensure no remnants of the oatmeal are present. The second chamber will then be filled with the eggs using the same funnel as chamber one after it has been cleaned. The second chamber will be closed off and cleaned the same way as chamber one. The clamp between the second and third chamber will be marked blue to indicate it is the second chamber activated. Finally, the third chamber will contain the plastic. Depending on the form of polyethylene used either forceps or the same clean funnel will be used to insert the contents. The FME will then be prepared for shipment to Nanoracks via FedEx.

When the experiment is sent to the ISS, an identical experiment will be conducted on the ground as the control. The difference between the two experiments would be the gravity imparted on the test tube. To analyze the experiment results, the plastic's mass will be evaluated using a scale that has nanogram-precision. The nanogram-precision scale is available for use at the Collin College laboratory. The masses of the two pieces of plastic will serve as the

characteristic used to determine how much plastic was biodegraded in the different environments. The polyethylene plastic is the only material that is going to be assessed, so it will not affect the results if the larvae die during transportation.

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