Mission Dine In Extraterrestrial Terrain (D.I.E.T.): Effects of the Near-Space Environment on *Chlorella vulgaris*

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Abstract
One of the biggest challenges we will face to send manned missions to explore Mars is to keep the human well-nourished so she can accomplish all the goals set for the mission. This report describes the objective and results of Mission D.I.E.T., which will evaluate the survivability of Chlorella vulgaris when sent out to near space on a balloon-sat flight to simulate Mars-like conditions. We will do this by recording and analyzing environmental conditions such as UV radiation, and changes in temperature that C. vulgaris will be exposed to during flight, and its ability to grow after flight. C. vulgaris is a photosynthesizing algae that is a great and renewable nutritional source (60 percent protein content, 19 different amino acids, rich in unsaturated fatty acids, more than twenty bio-available vitamins, and detox properties) that could possibly survive on actual Martian terrain. If C. vulgaris continues to grow normally after flight, the next step is to analyze its morphology to see if its shape and nutritional value changes at all. Also, we suggest evaluating the survivability of other types of edible algae in the near space environment that have similar nutritional values.

1.1. Mission Purpose

The purpose of Mission D.I.E.T is to research ways to provide a nutritional food source for space explorers that requires minimal maintenance and can survive on different terrains such as the surface of Mars. For this mission we chose edible algae because it is a perfect candidate to supplement our daily diet and get almost all the nutrients that we need, especially protein. Out of the all the edible algae, we chose to culture Chlorella vulgaris because of its significant nutritional value to us humans. C. vulgaris, is approximately 60% protein, it contains 19 amino acids including all eight amino acids considered to be essential for us humans, rich in unsaturated fatty acids, and more than 20 bio-available vitamins. Moreover, it is estimated that more than 7 million people in Japan consume C. vulgaris daily and proves it to be a highly potent nutritional "all-in-one-formula" whole food (Dr. Frank Liebke, Klinghardt Academy). Assuming that we get to Mars, we would want a food source that is small and renewable to reduce transportation costs, that also contains all the essential nutrients for human survival. If C. vulgaris survives its exposure to space-like environment we might have found a possible candidate that will provide most of the nutrients needed by our space explorers.

1.2. Primary Mission

We plan to discover the possible effects that extreme temperatures and UV radiation found on the near-space environment will have on C. vulgaris. It is crucial for us to look for changes that might rule out its effectiveness to us once it has been exposed to a Mars-like environment. One of the most important parts of this mission is the analysis of the algae before and after flight, this way we will be able to tell any significant differences between the pre-flight and post-flight algae. Multiple studies had been done about Algae’s adaptation and survival procedure. These studies were done either by artificially exposing the algae to space-like weather or just by observing the changes that happens to them here on earth because of the breaking of the ozone layers (reduction of the stratospheric ozone due to CFCs). An article published on December 30th, 1999 by Jose
Aguilera and the team at Marine Ecology Progress Series showed that UV radiation of wavelengths varied from 300-695nm influenced microalgae’s growth rate and photosynthesis efficiency, in almost all species of algae the photosynthetic production of oxygen decreased after only 2 hours of incubation in the lab. (Jose Aguilera, Marine Ecology Progress Series). This said, we want to see what happens to C. vulgaris when it is exposed to UV rays of shorter wavelengths while also being exposed to the stratospheric pressure (~1kPa), we expect the algae to survive the exposure and continue growing, and if the algae are damaged during flight, we will be able to determine if they can repair themselves.

1.3. Experiment Overview

Mission D.I.E.T will be completed via a three-stage plan. The first stage consists of exposure and familiarity with the methods needed to properly care for and grow C. vulgaris, this stage includes the recording of all matter placed in the algae’s environment, the rate of growth of the colony which will be measured by volume it will occupy during its different growth stages, and cell morphology under a microscope.

Stage two comprises the construction of a balloon satellite box that will safely store and transport three 10-mL samples of C. vulgaris to the stratosphere; the balloon-sat will also have two on-board UV sensors (Sparkfun’s ML8511), a temperature sensor, a heating circuit, an Arduino UNO with SD-card shield and SD-card to record flight data, a digital camera equipped to take pictures of two of our algae samples every 30 seconds, three 9V batteries to power our heating circuit and Arduino, and switches to turn on Arduino and heating circuit. The third and final stage of this mission will be the cultivation of our exposed algae post flight at the lab, we will record daily changes in color and/or appearance which will be used to compare it to pre-flight algae; data will be collected in the same manner as in stage one.

Lastly, a cost per human analysis will be made alongside a calorimetry test to help determine how much algae is required to feed a team of four astronauts for prolonged times and whether the algae would be affordable during long term expeditions to high UV exposed environments such as Mars.

2.1. Algae Growth Medium

C. vulgaris will be grown in a purified water medium that contains dissolved Sodium Nitrate (NaNO₃) and Sodium Phosphate (NaPO₄) salts to provide the nitrate and phosphate sources that the algae require to grow. This medium will be added to the colony as it grows a few milliliters at a time to not overwhelm and/or stall the growth of our algae colony.
2.2. Distribution of Algae Samples Inside Balloon-Sat

To best understand the different effects of a Mars like environment on our specimen of C. vulgaris there will be three different scenarios. The first scenario focuses on a 10-mL specimen exposed to low UV radiation in a vacuum sealed container. This specimen’s major focus is to determine the effects of low light and no pressure change on the cell morphology.

The second specimen will have a similar focus with the difference of increased UV exposure using a plastic acrylic window pane. The final vial will be of the same volume (10-mL), in a vial susceptible to both pressure change and increased UV radiation. This is the key specimen that will help to give insight into the effects of both high UV and low pressure on cell morphology. All three vials will be kept at the same temperature which will be monitored via a temperature sensor.

2.3. On-Board Temperature and UV sensors

The temperature sensor will be accompanied by two UV-sensors in order to evaluate the amount of UV exposure to both the light exposed vials and the light protected vial. These are important to help analyze how much UV the specimens have been exposed to during their trip into Earth’s stratosphere. The temperature sensor will help to record temperature changes throughout its ascent and help to evaluate whether the specimen freezes, when it occurs and if so at what temperatures are we likely to notice said change.

3.1. Initial Algae Culture and Growth

Our specimen samples will be grown in four stages, doubling in size at each stage in the cycle. Our initial specimen consisted of two 50-mL vials filled with approximately 1-mL of algae and 49-mL of its mineral medium. This stage, along with all the other stages lasted for two weeks. Upon the end of the initial two weeks the specimens were transferred to two separate 500-mL sanitized jars. Each received an additional 50-mL of growth medium bringing each jar’s total sample size to 100-mL. At the end of this second two week period the jars were given an additional 100-mL of growth medium raising the total to 200-mL each. Lastly, during the final two weeks the specimens received a final 200-mL of growth medium bringing each jar to a total of 400-mL, a grand total of 800-mL for specimen trials.
3.2. Balloon-Sat Designs

The satellite that will contain our experiment has gone through three iterations. The first conceptual design was a 30 x 25 x 15 cm box. This box proved to be durable and contain all the space required for our experiment but consumed more of the weight mass budget (217 of 850 grams) than we had anticipated.

This led to our second iteration which looked to reduce the size by approximately 20% which gave us the minimum height required to store our vials before using sockets within the insulation as holders. This second box came out to be 24 x 20 x 12 cm. This box was much more ideal but during cold tests proved to be difficult to keep warm.

This led to our current and final iteration which is a 19 x 13 x 11 cm box which has slots cut into the top and bottom of the insulation. This was done to help reduce the height by another cm and to help hold the vials in place during flight. This box proved to be able to hold all the components needed, was able to keep a nominal temperature for the algae, and reduced the overall weight and footprint.
Final design. Passed all pre-flight tests and was the best design by far.

4.0. Test Results

We conducted multiple tests as the project proceeded to make sure our payload is durable, functional and extreme weather resistance.

4.1. Drop Test

After we had made our box, drop test was the first one to occur to test our box’s durability in case of rough landing and colliding with other payloads after balloon burst. We dropped the box from a second story building on a cement pavement. The interior was of the box was unaffected and the exterior was without any major damage, only a fatter corner on one side. But, it didn’t burst the box open or anything similar.

4.2. Whip Test

Then, we proceeded to the whip test. We penetrated the tube through the box and attached washers to prevent the box sliding of the tube. The arrangement was as it would be while attaching the payload to the balloon. We whipped the box with a string and gradually increased its swing. We swung the box as hard as it might interact during the flight and it held up perfectly.

4.3. Vacuum Test

One of the tests conducted for our experiment was a vacuum test. This was done by placing components susceptible to major change by pressure within a small pressure chamber. This mainly focused around the specimen samples and their respective containers. The vials proved to be able to withstand the change in pressure and helped to keep them from changing due to the change in the atmosphere around them. This however was not ideal for our third vial which requires a change in pressure. To help combat this we designed a top that utilizes insulation, paper towels, and tape. By placing the paper towels between insulation in the form of a sandwich and securing them to the top of the vial. Then by piercing the top with a thin blade, X-Acto knife preferable, we created a small channel that allows for pressure change. After running this through the pressure chamber we saw that the new lid allowed for a change of pressure within the vial. After we then simulated rough conditions by violently shaking the vial for approximately 30 minutes. Throughout this duration none of the liquid leaked all the way through as the minimal liquid that seeped was caught by the paper towels.

4.4. UV Sensor Test

We are using two UV sensors as a part of our experiment. One of the UV sensor is placed in between the two vials facing the window and the other is in the dark compartment with the other vial. The sensors were connected as the schematics show below, and calibrated using the data sheet
provided by Sparkfun on their website. To test their effectiveness and accuracy of data, we exposed them in different conditions such as: dark room, flash lights, halogen lights, sun lights in different angles and varies intensity. Both sensors were giving returning data and they were the same almost all the time. Sometimes we saw the voltage values being returned to be 0.01 or 0.02 apart for the same intensity.

4.5. Cold Test

For the cold test, we integrated our whole payload with all the experiment’s pieces where they are supposed to be. We didn’t glue the top as we still wanted to have to option of moving things around if needed for the mission simulation test, that’s why we just taped the top part. We placed the payload inside a freezer where the lowest recorded temperature was -25°C. After we let the system run for more than 90 minutes, we took it out and started gathering information about its effectiveness in cold weather. The camera along with the Arduino ran for the whole time collecting pictures and data, the lowest temperature inside the box was recorded 2.79°C.

4.6. Mission Simulation Test

To ensure payload’s functionality throughout the whole flight, we conducted a mission simulation test. It was two and half hours long and all parts and pieces were integrated into payload as a preparation for the actual launch. We let the system run and turned the camera on. We took the payload outside the building and walked with it around campus so we could catch any changes in temperature and UV intensity. Unfortunately, sun was not up that day so we did not see a huge spike of UV intensity as we had seen during the sub system test. But, the Arduino recorded data and the camera took pictures throughout the whole test without any unexpected interruption. Our simulation test collected data is as follows:

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<th></th>
<th>Output(Voltage)</th>
<th>Intensity(mW/cm²)</th>
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<tbody>
<tr>
<td>Inside class</td>
<td>196</td>
<td>0.03</td>
</tr>
<tr>
<td>Inside class(w/Flash)</td>
<td>196</td>
<td>0.11</td>
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<tr>
<td>Outside in Sun</td>
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<tr>
<td>Outside in Sun</td>
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<td></td>
<td>324</td>
<td>5.74</td>
</tr>
</tbody>
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5.0. Expected Flight Results

Since the actual flight has yet to happen we have no actual data or exposed algae to analyze, despite this, we can indicate a few expectations from our flight. Firstly, we expect our exposed algae to be alive and able to grow under the same conditions as they were maintained before the flight. Secondly, we expect that the algae sample that was in
the dark room of our balloon-sat will show growth rates like those shown by our original algae colony because it will be exposed to less UV light. Lastly, we expect that our on-board sensors and camera will return data that will help us figure out what kind of physical and environmental changes the algae went through during flight, and if these changes should or will affect the growth rate and morphology of the algae.

6.0. References
