The Highest Common Factor: Impacts of the High-Altitude Environment on Cyanobacteria

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Abstract:
The adaptation of space explorers’ lives could be made easier by researching ways of producing food and more importantly, oxygen by photosynthetic microorganisms. Based on that, our research will be searching for indications of flourishing life in a Mars-like environment that will be determined by sending an experiment with terrestrial cyanobacteria to the stratospheric environment on a high altitude balloon reaching an elevation of 30km in Earth’s atmosphere. The species selected for this task — Nostoc — is capable of photosynthesis, of human consumption, and is a primary producer in both marine and freshwater ecosystems. Keeping that in mind, we will be exposing two samples with different environmental variables in the high altitude setting. Our project will measure the health and viability of the samples by measuring carbon dioxide depletion and recording data real-time.

1.1 Mission Purpose
The purpose of this experiment is to research ways to create life support systems that could guarantee a supply of water, oxygen and food for future space missioners through the use of resilient photosynthetic organisms that do not require an inordinate amount of accommodations. The primary purpose of this experiment is to produce oxygen in a Mars-like atmosphere from cyanobacteria to prove that, one-day, breathable oxygen and a viable food source could be produced to make life easier with the adaptation of humans outside our own world.

Cyanobacteria is a type of bacteria that was originally called “Blue-Green Algae”, in part, because it is capable of photosynthesis. cyanobacteria are known to be quite resilient, and also healthy; some cultures use cyanobacteria as food, and there are even pills claiming to provide needed vitamins and minerals to humans, which compose primarily of cyanobacteria. Clearly, this type of life has been providing more than just oxygen to humans, and could continue to be an excellent resource in the future.

Previous work on this subject includes a study by the German Aerospace Center (DLR) where researchers subjected a multitude of life to a simulated Mars environment. After a month of exposure had passed, the only two types of life still living were Lichen, and Cyanobacteria.1 This study had successfully identified the types of life that can survive specifically on Mars, but did not focus on the aspects of growing these life-forms for practical reasons. Another experiment in this field was performed on the International Space Station recently wherein samples of fungi were left outside, and some were exposed to a simulated Mars environment. After 18 months 60% of the fungi were still alive, and their DNA were still very stable.2 These results further demonstrate the survivability of organisms beyond our world, but again, do not study the production of useful resources that these organisms provide.

1.2 Primary Mission
The BalloonSat ENSURE (Environmental Survival Experiment) will be running experiments on two separate samples of cyanobacteria (in this case, Nostoc) to investigate if it would be supportable in extraterrestrial - more specifically, the stratospheric - environments. This experiment is focused on identifying and testing a difference between a single environmental factor between two samples of Cyanobacteria aboard a BalloonSat that will reach approximately 30km in altitude. Each sample will be exposed to a different environmental variable, which is a difference in light exposure. Ideally, one sample will be exposed to the high-altitude environment in-full, with the unavoidable exception of pressure and atmospheric composition (modulating the composition to match what might be found on a different planet is impracticable for this experiment) and the second sample will be with a reduced amount of light, which would, for Nostoc, be ideal around 540 Lux (lumens/m²) to 1080 Lux. Lower light levels will be achieved by simply a sheer curtain over the sample. Additionally, this study will be comparing the effects of the light levels and the fact that the samples have flown through the stratospheric environment to a separate sample kept in optimal conditions on the ground. In doing so, an identification will be made to which variable is has the greatest effect on the rate of photosynthesis and in growth of the sample; also, in doing so, data will be collected as to how this constant variable will have an effect on the samples as it ascends into an ever changing environment.

1.3 Experiment Overview
The BalloonSat as a whole consists of two openings, inset into the box, that two 50ml centrifugal tubes rest in, being fully exposed to the outside environment — with the exception of the second sample, which has 32 layers of white shear curtain over the opening. At the top of each tube, a carbon dioxide sensor is affixed through a hole in the
top of the tubes, which will make real-time measurements of the levels inside each tube. On the outside of the box, there are four sensors; an infrared/visible light sensor, an ultraviolet light intensity sensor, a temperature sensor, and a pressure sensor. All of these sensors will be controlled by an Arduino Uno Rev.3 and be recorded to an SD card as a .txt file. The experiment also contains an internal temperature sensor to turn the heater circuit on or off as needed. Additionally, a camera is affixed, looking outward of the box to take pictures and document the ascent and decent.

2.1 Growth Medium

The design of ENSURE is a rectangular box with both samples being exposed outwards on the same face, as to guarantee an equal amount of light exposure for both 50mL centrifugal tubes. Those tubes will contain both the samples, and the CO2 sensors; the Cyanobacteria will be cultured in a Soil-Water Medium (CaCO3, mixed with non-fertilized garden soil, and spring water, then steamed) and made semi-solid by mixing this medium with non-nutrient agar. The medium must be firmed up because in the top of each tube will be the detecting component of the CO2 sensor, which would not be able to operate if submersed in the liquid medium. Instead of creating our own medium, a Soil-Water Supernant has been used, essentially a concentrated medium, that is diluted with spring water — 1 liter of spring water per 50mL of supernant.

2.2 Photosynthesis Measurement Apparatus

The CO2 sensors are inside the tubes in order to keep the inside at the atmospheric composition of ground-level; this is easier than constructing an airtight container to affix over the tubes. The completed combination of the CO2 sensors and the centrifugal tubes compose the Photosynthesis Measurement Apparatus (PMA). The detector components are mounted in a 3D printed piece that fits over the cap of the tube, which has a hole drilled in it to allow air to flow to the sensor. The tubes themselves needed to be fitted with a rubber washer as a gasket, and possible hot-glued around the cap for good-measure. All three centrifugal tubes — two aboard the ENSURE and the one used for control kept on the ground — have been tested to be air-tight by placing them in a vacuum chamber. Additionally, the two completed Photosynthesis Measurement Apparatuses that are a part of the experiment have been tested inside the vacuum chamber as well, and proven to be capable of holding the atmosphere inside.

2.3 Light Distribution on Secondary Sensors

An additional feature of the experiment is the set of environmental sensors meant to record data about the light on the samples, the altitude at which it is at, and the temperature the samples are exposed to. This is needed in order to make conclusions on why the samples behave the way we see from the CO2 readings, and to help support why placing one sample behind a shear curtain proved to be better or worse for the health of the Nostoc in different setting. The sensors: temperature, ultraviolet power per cm2, visible light intensity, infrared light intensity, and a pressure/altitude sensor, have to be placed, not only on the same face as the samples, but also exposed to the same angle of light that the samples are exposed to as visualized in Figure 2. After the creation of the two larger inlets, the angle that was determined was 28.61°, and a mounting channel for the aforementioned sensors was 3D printed to ensure this angle of light exposure was achieved.
2.4 Carbon Dioxide Sensors
The CO2 sensors being used is the MG-8113, which relies on a chemical reaction inside the detecting components, allowing charge of a layer to be moved between different layers of the detector the more CO2 is a part of the reaction. The reaction follows the Nernst Equation, in terms of output voltage. Solving this equation provides:

\[ PPM = 10^{ \frac{(Voltage) - 4.38491}{-0.44}} \]

The two sensors are outputting about the same voltage just being left alone, with only a few hundredths of a volt difference.

In order to calibrate these sensors, there are a number of methods, but the only one that is achievable with what we have is “Calibration Using Fresh Air”. To use this method, it is known that the air outdoors is 390-400 PPM of CO2. To do this, we took both the sensors outside, away from any source of carbon dioxide, and recorded the readings for a few minutes. The results of this test found that one sensor, which will now be labeled “MG1,” needed to have the voltage multiplied by 1.40775039. The second sensor, or, “MG2,” needed to be multiplied by 0.8248695851 to reach the target PPM.

The readings of the sensors can be quite erratic because of its analog signal, and the high slope of the CO2 curve, so an average is added up and used for the voltage for any one reading. After some testing, it was found that more consistent results come from a lower number of readings spaced out for a longer amount of time. For the calibration, it was convenient to take 5 readings, each 200ms apart, wanting quality measurements over quantity.

3.1 Initial Culturing
The initial culture of Nostoc was received on 3/10/2016, and culturing began on 3/11/2016 under the supervisor of Professor Tom Dillon. All equipment was sterilized before use, and the Soil-Water Medium was prepared by diluting 50mL of powdered agar into 200mL of deionized water from the same filter as used before. Then, the 3% agar, the Soil-Water Medium, and test tubes were sterilized in an autoclave at 200º F at them into the flasks (i.e. pouring from the tube directly) could compromise the sterility of the samples, but single cells of Nostoc that were in the medium were transferred. Afterwards, the flasks were placed with the foil tops over them, were placed under the fluorescent light of a hood, with the blower of the hood turned off. The light produced by the hood was measured by an integrating light sensor (Model: TSL2561) attached to an Arduino Uno, and registered approximately 700lx at the floor of the hood. That reading is with the fluorescent room lights on, and the sun shining through the window of the lab, so, that should be considered the maximum light the sample could receive. Additionally, a temperature sensor (TMP36) also attached to the microcontroller was used and measured an average room temperature of 22ºC. The tube that had the initial Nostoc in it was placed in a dark drawer to preserve them for possible future use. The two samples were left under the hood in the recorded conditions.

3.2 Semi-Solid Medium Creation
After the initial culturing, on 3/17/2016, we met Professor Tom Dillon again to create different semi-solid mediums, all with a different concentrations of agar. We made 3% agar by mixing 6 grams of powdered agar into 200mL of deionized water from the same filter as used before. Then, we created a Soil-Water Medium that is five times as concentrated as the mixture we used for initial culturing by diluting 150mL of deionized water with 50mL of the Supernat to get a total of 200mL of medium. After that, the 3% agar, the Soil-Water Medium, and test tubes were sterilized in an autoclave at 200º F at.

![Figure 3: From the top, Nostoc growing in a beaker in the Soil-Water medium. Below, Nostoc in different concentrations of agar medium](image)
15 PSI for 15 minutes. After sterilization, we put all the remaining culturing cells of *Nostoc* into the Soil-Water we reserved before. Our goal was to make different concentrations of agar and to see how healthy the cyanobacteria can handle the variety of agar concentration; to determine which concentration we should use for the samples used for flight. We took 6 sterilized medium sized test tubes and made 7ml of agar + soil-water solutions. Deionized water was added to dilute the solution. Exact amounts as follows:

We labeled all the test tubes by their agar concentrations. Then we added 1ml *Nostoc* into each test tubes. We let the test tubes to cool down for 45 minutes. After 45 minutes, the 1.5% and 1% agar solutions were solidified. But 0.5% agar solution stayed liquid.

Now that we know 0.5% agar solution stays liquid, we decided to test 0.8% agar solution and 0.9% agar solution to see at what exact point the agar solution solidifies. 0.8% agar stayed semi liquid and 0.9% agar solution had the best growth of all samples. We then cultured 0.9% agar in four 50ml test tubes.

### 3.3 Creation of 0.9% Agar:

To make the soil-water medium with 0.9% agar, the proportions used to make the samples used for flight are: 5X soil water 5.6ml, 3% agar 8.4ml, deionized water 14.0 ml, Cyanobacteria 4ml, with the total coming out to 32 ml.

We chose the best two samples for the flight and kept the other two as control samples.

### 4.0 Test Results

To ensure that ENSURE can meet the structural requirements to withstand the landing and balloon burst of the flight, we subjected it to a number of different tests.

#### 4.1 Stair Test

The first structural test conducted on the box was the stair test. The box is rolled down a flight of stairs to simulate dragging and tumbling that would be experienced at balloon burst and when landing in windy conditions. The test was a success with no exterior damage aside from a little flatter corners.

#### 4.2 Whip Test

The second test was the whip test. The experiment was put onto a rope through the attachment point and spun by hand to simulate the maximum stress that the balloon’s cord could put on the box. The test was a success, with no damage to the attachment point and nothing falling out of place inside the box.

#### 4.3 Drop Test

The third structural test was the drop test. The experiment was dropped from a second story window (12-15ft) above the ground and let go. This is meant to simulate a very hard impact at either landing or at the balloon burst. The test was a success, with the only visible damage occurring to the corner on which it landed, but the box, and all its contents were still completely usable (figure 4).

#### 4.4 Thermostat Test

Another test done was to put the experiment in a refrigerator, although this isn’t the coldest conditions it will endure, it should be enough to turn on the heater, and, when the box is removed, it should turn off again at room temperature. The heater had turned on by itself, and when removed, it took a few minutes to turn off again. Since the box wasn’t actually sealed at the time of the test, it wouldn’t have held much heat, so the purpose of the test was just to see if the thermostat code had worked, and it had, making this a success.

### 5.0 Expected Flight Results

Since the flight has not happened yet, we do not have any data on the experiment. Despite this, we can specify a few expectations from the flight. First, since *Nostoc* grows optimally in 500-1000lx, we can expect a higher rate of photosynthesis from the second sample (the one with a shear curtain over it). Also, we expect that since it will be exposed to less of the intense ultraviolet light at higher altitudes it will be healthier than the other sample, although this will be hard to determine, especially because the flight is only 2 hours long, which does not give much time for much of an effect on the samples. After the flight, we will bring the two samples back to the lab and allow them to grow alongside the control sample, keeping a close eye on them over the coming days. One sample had previously died within a few days of being sealed in a tube, with the cells visibly missing from where colonies of Nostoc had been, so that indicates that cell
death can easily make a visible change inside the tubes. Additionally, we plan on seeing that as the light levels, particularly the ultraviolet intensity increased, the CO₂ production decreased, and the sample that was exposed to more ultraviolet will grow slower, or even die sooner than the one exposed to optimal light levels. What might also happen is that the second sample will do worse in comparison to the control sample, suggesting that other factors, light radiation and exposure to cosmic rays could have harmed that sample as well. If nothing else, we will determine if exposure to a near-space environment will effectively kill colonies of incredibly useful cyanobacteria.

6.0 References


