

Extremophile Collection and Identification

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April 09, 2011

Abstract

A satellite was built to endure the high altitude and any possible problems that it would experience in flight. We put the finished satellite through a series of tests. The satellite was attached to a balloon which had been released to reach a height 100,000 ft. Inside of the satellite there was a 200 m plastic tube which is similar to Dr. Pieter Tan's Aircore® concept. It was in our attempt to see if we could collect a sample of bacteria surviving in extreme environment. The problem that we faced was coating the inside of the tube with agar, sterilizing the tube and agar and maintaining a constant temperature inside the satellite to prevent the plastic tube from freezing at high altitude. We used a heater for the inside of the satellite to maintain a constant temperature. We flushed the inside of the tube with sand to make the agar bond to the inside of the plastic tube and we washed the inside of the tube with chlorine bleach. We also ran flowing sterile air through the tube. After our sterilizing technique we clamped the ends of the tube and it was lift off. We found bacteria in the tube, however we are not convinced that they are extremophiles, in part due to sterile field problems.

1. Introduction

With the launch of TSJC Balloon Satellite 2010, the opportunity to build, and test a device to collect microbes in an extreme environment was possible. In accordance with Pieter Tan's Aircore® invention which is an instrument that can record trace gases in air samples at atmospheric pressure (Figure 1) a design was initiated to collect microbes at extremely high altitudes. Extremophiles are microbes that can exist in the most extreme of environments.

A sterilized plastic tube with interior nutrient agar coating was used to collect air

samples from the atmosphere. Any collected microbes will be allowed to grow in the tube *in situ*. After 7 days of incubation, these will be transferred to petri dishes and further incubated and evaluated. The goal is to collect and identify high-altitude extremophiles.

The collection mechanism has no moving parts, only a 200 foot long, 0.19 inch inner diameter nylon tube coated internally with agar. Both ends are sealed, filled with air at atmospheric pressure and sterilized prior to launch. Just before launch, one end of the tube is cut open so that as the balloon-satellite rises into a lower pressure environment, the tubing gas moves outward, maintaining the sterile environment in the tubing. At maximum altitude, the residual sterile air is filling the tube, and then upon descent, compresses into the closed end of the tube as atmospheric air and particles migrate into the tube through the open end. The gas nearest the closed end represents the re-compressed ground-level gas and then gas samples from the highest altitude, then the next highest and so on until the gas at the open end represents the gas just above the landing site. All of this presupposes that no diffusion along the length of the tube occurs. This diffusion is minimal for the few hour flight. The basic gas motion is essentially the same at Dr. Tans' Aircore® experiment; our contribution is to coat the tubes inside with nutrient agar and incubate any collected microbes *in situ*.

On the ascent of the balloon satellite, the air in the tube will evacuate the tube, as the satellite descends the tube will begin filling up with air until it reaches landing. Upon arrival to the landing site, we shall seal the tube with clamps at 2m intervals. At TSJC we will then culture the Agar samples from the sectioned tubing in three petri dishes of regular nutrient agar and anaerobic and aerobic blood agar. We will perform a gram stain with the incubated samples. If large enough colonies are present, they will be identified microscopically and photographed.



Figure 1: Image of Petier Tan's Aircore®. It is the basis of our design concept.

2.0 The Requirements

The team shall complete a functioning Balloon Satellite ready to launch on a high altitude balloon to 25,000 – 30,000 meters by 11/6/2010. The Balloon Satellite shall not weigh more than 1.5kg total or cost more than \$1000 dollars, minus the provided hardware.

The instruments of the spacecraft shall remain intact and functional during a 90 minute ascent to an altitude of 30 km and a 45 minute descent including landing. The Balloon Satellite shall be configured to allow for attachment via a string, with several other satellites, connected to the balloon launch vehicle.

The Balloon Satellite shall measure the temperature on both the heated and unheated sides of the payload using the HOBO Datalogger and an attached probe. The Balloon Satellite shall carry 70m plastic nylon tube with the inside coated with agar. The Balloon Satellite shall provide unique environments for tube to harness bacteria. The Balloon Satellite shall maintain a temperature in the heated side above 0°C at all times.

The mass and financial budget shall be divided among all structural and science components and shall allow for the purchasing of spare parts. The entire systems of the Balloon Satellite shall be completed and fully tested by 11/04/2010.

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The Balloon Satellite shall be constructed from aluminum wrap and nylon tubing, resulting in a circular cylinder. It shall be sturdy enough to contain and keep intact all necessary hardware found inside. It shall be tethered through the middle by the flight string to achieve optimal balance. The Balloon Sat shall be tethered for the entirety of the flight without severing the string or obstructing the functionality of other Balloon Satellite also attached to the balloon.

The Balloon Satellite shall be functional at an exterior temperature as low as -80 degrees Celsius. The constructed heaters will act to keep the heated side of the Balloon Satellite above 0 degrees Celsius. The heaters will be located directly above and below the coiled plastic tube. The heaters are meant to ensure that adequate temperatures are maintained towards the focus of the experiment. The most vital part of our system, the batteries, will be located in carved pockets in the central insulation which divides the heated and unheated sides of the payload. The HOBO Datalogger will be located in the heated side of the payload in another carved pocket in the central insulation. The inside components on the heated side of the payload shall not be exposed to the outside conditions, but rather kept on the inside of the foam core structure.

Using the HOBO Datalogger, the temperature of the heated side of the payload during the flight shall be measured. The HOBO will be located along the insulated dividing wall central to our structure.

The temperature on the cold side of the payload will be measured using a temperature probe connected to a HOBO Datalogger. It will be drilled through a small hole in the insulation dividing the heated side from the unheated side of the payload, and will be located near the nylon plastic tube.

3.0 Design

The satellite that we built to collect microbes in the atmosphere included the following components: 200 m plastic tubing (963.36gm), three 9vlt batteries(128.31gm), electronic wires (82.5gm), a top and bottom cover (99.08gm), flight tube (17.9gm), two hobos (132.16gm), and aluminum wrap (77.28gm).

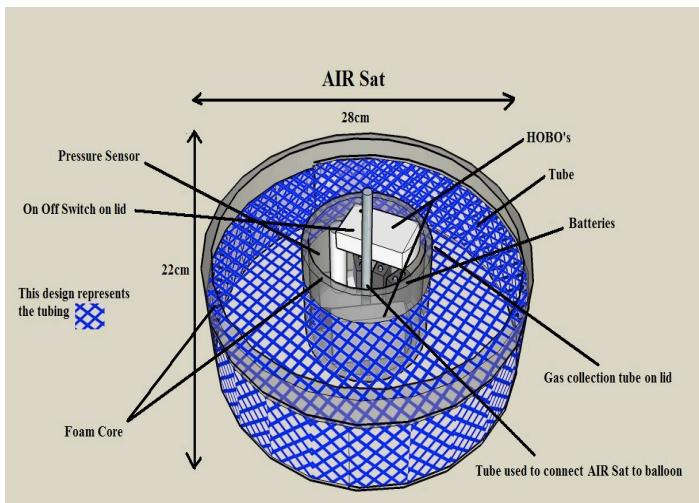


Figure 2: Functional block diagram

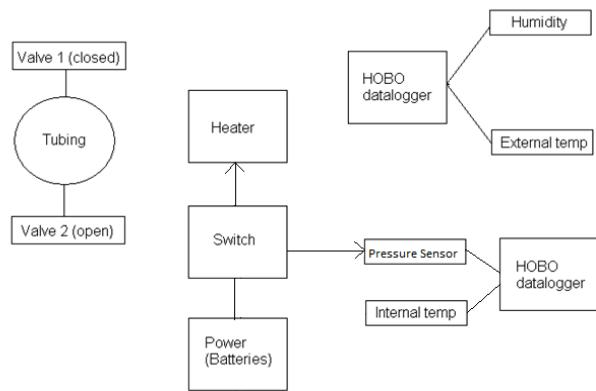


Figure 3: System interface

4.0 Test Plan and Results

The tests that we performed on the satellite included the following experiments: 1. Testing (agar coverage in tubing and viability, sterility). 2. Mission simulation testing –cooler (temperature and humidity) test. 3. Functional testing – stair test and whip test.

1. Sterilize tubing-flush with boiling water then soak with bleach for 30min. Repeat a boiling water flush to tubing and fill with sterilized agar. Finally clamp the ends of the tube. Hopefully, this will keep any unwanted microbes out of the sterilized tube. To see if sterilization has occurred in the agar tube, we will incubate the tube for

7days to a maintained temp of 115degrees F. Results have shown no contamination.

2. The modified “cooler” test was a cold impact test, and it was undoubtedly the most important test for our project. The reason for this is because the tubing may become brittle or crack at low temperatures and the Balloon Satellite will be subjected to an extremely cold environment. We took our fully functional and integrated flight payload and cooled it to a temperature simulating that of launch day by placing it in a cardboard cooler with 7 to 10 pounds of dry ice. The dry ice was uniformly distributed within the cooler. We used the HOBO data logger to obtain the temperature reading that the payload experienced on the exterior and interior while in the cooler. Results have shown that the internal heater, batteries, and Hobos maintained integrity.
3. The stair test on the structure simulated the most extreme forces acting on the Balloon Satellite during flight, especially those occurring at burst. The whip test involved swinging the structure with simulated flight masses by a line (approximately 1 m) running through its central tube. This test replicated the gravitational and tension forces acting on the structure as it twists and spins during the flight. Results have shown no serious compromise to the system.

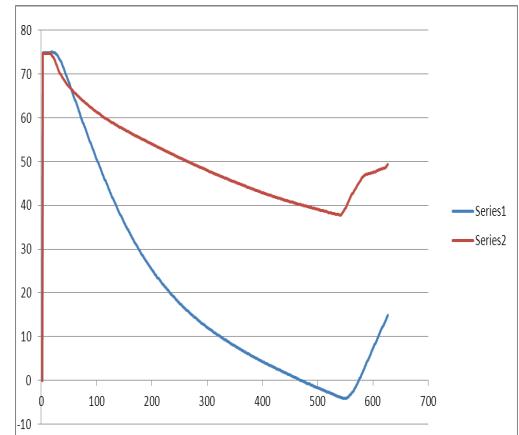


Figure 4: “Cooler test” (temperature of internal and external reading)

5. Data Results from Flight

The two HOBOs were programmed to take data during the flight; these will record internal temperature, external temperature of

the tubing, relative humidity, and pressure for each atmospheric level.

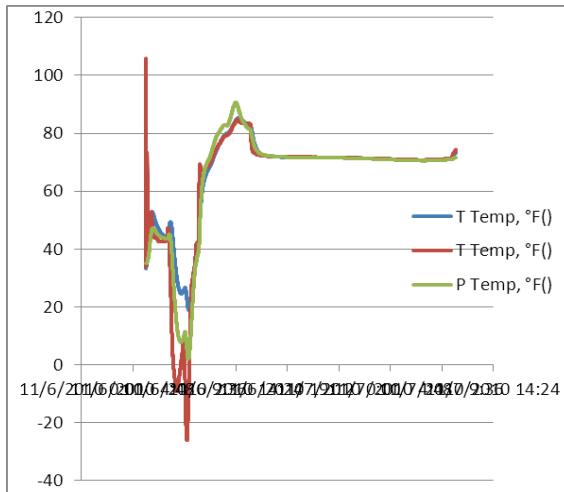


Figure 5: Flight path of Balloon Satellite (internal and external readings-pressure)

6. Results, Analysis, and Conclusion

The Balloon Satellite had a safe launch and recovery. The plastic tubing was intact and sealed after landing of the Satellite. We clamped the tube into 28 sections. The agar inside of the tubing was transferred to petri dishes. We used 20 sections of the 28 sectioned tubing. A set of three petri dishes for the 20 sectioned tubing was made. One set of regular nutrient-N, anaerobic-A, and aerobic-O is shown below (Figure 6). Also shown are samples from the middle and last section of the tube where agar was transferred in petri dishes (Figure 7 and 8).



Figure 6: Three petri dishes after incubation. O-1-2 shows white-colored growth.

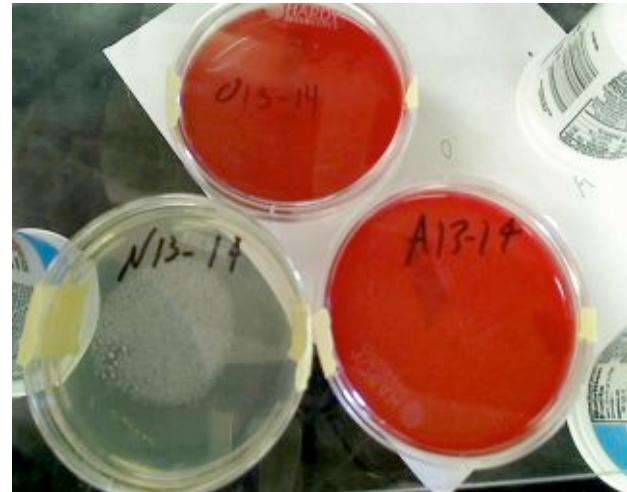


Figure 7: No growth is shown in these petri dishes



Figure 8: O (aerobic) 27-28 shows white colored growth.

The petri dishes that showed the most colonies of growth at room temperature incubation were sections N3-4, O4-5, N5-6, O6-7, O8-9, O21-22, N22-23, O22-23, N23-24, O26-27, and O27-28. These were best suitable for gram stain. The results after gram stain showed that O1-2, N22-23, O22-23, O26-27, and O4-5 had the most growth under microscope. O1-2 did test gram positive for bacteria. It has not been identified and photographs are pending.

Our results have not proven a definitive conclusion that we collected an extremophile or microbe from the flight path of our Balloon Satellite. Although we have successfully cultured colonies of growth within many of our petri dishes, cross contamination is the major concern. The issue of sterile field problems included the sterility of the agar from its insertion into the tube, its journey to the launch site, and the agars safe transfer into the petri dishes without contamination.

7. Ready for Flight

Our Balloon Satellite design allowed for easy access and retrieval of data. We did not experience any critical problems. The greatest problem we faced was coating the interior of the plastic tube with agar. Maintaining a sterile environment within the plastic coated agar was also a challenge.

8. The Benefits

New discoveries in the way life may thrive in harsh conditions are the greatest benefit behind collecting extremophiles. Application in the field of Biology (Taq Polymerase) and Astrobiology (development of life on distant planets) is where extremophiles have proven their significance.

9. Potential Follow-on Work

Because the benefits of discovery new extremophiles are significant, this mission is worth continuing. We may have also overlooked bacteria on our gram stains so there is possibility for a new outcome to our results.

10. Lessons Learned

The most important lesson learned was in the difficulty of creating the means to create a sterile environment to obtain micro-organisms. We still have not found a better method or technique.