NIAC Phase I Grant "Mars Ecopoiesis Testbed" NNX14AM97G Final Progress Report.

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Abstract/Executive Summary

Mars surface conditions where liquid water is absent were simulated for the purposes of laboratory research. A pressure-temperature (P-T) profile was maintained in which no combination of pressure or temperature corresponds to the liquid region of the water phase diagram. The triple point of pure water occurs at T = 0.1^{o}C and P(H_2O) = 6.01 mbar; therefore all temperatures and pressures must be kept below these values, respectively. A 35-day test was performed in a commercial planetary simulation system (Techshot, Inc., Greenville, IN) in which the minimum night-time temperature was -80^{o}C, the maximum daytime temperature was +26^{o}C, the simulated day-night light cycle in earth hours was 12-on and 12-off, and the total pressure of the pure CO_2 atmosphere was maintained below 11 mbar. Any water present was allowed to equilibrate with the changing temperature and pressure. The gas phase was sampled into a CR1-A condensation-mirror low-pressure hygrometer, which uses liquid nitrogen (down to 77^{o}K) to determine the dew point (Buck Technologies, Boulder, CO). Dew point was measured once every hour and recorded on a data logger, along with the varying temperature in the chamber, from which the partial pressure of water was calculated. The resulting calculated daily cycles were tracked on the water P-T diagram, and no points were found to fall within the liquid-phase region of the diagram. It is concluded that there was no liquid water present throughout the test except during the initial pump-down period when aqueous specimens were introduced on the first day (less than 1 hour). Mars regolith simulant was present during this test, and further investigation is needed to determine whether liquid water could have been present or absent in the regolith in the form of brine. Biological samples consisting of Cyanobacteria: *Anabena sp., Chroococcidiopsis* CCMEE171, *Plectonema boryanum;* Eubacteria: *Bacillus subtilis*, *Pseudomonas aeruginosa,* and Eukaryota: *Chlorella ellipsoidia* were maintained in the simulator under the above-described conditions. The exposed specimens were tested for intracellular esterase activity, chlorophyll content (where appropriate) and reproductive survival. All tests yielded low-level positive results in all cases. In parallel to these terrestrial studies a planned design study was undertaken for the proposed test bed . Design requirements include compact assembly for transport and installation on the planetary surface (multiple units per mission would be expected), protective internal package for the release of organisms, a means of atmosphere exchange, access to sunlight, a means of penetrating the planetary surface, and most importantly a means of acquiring regolith while meeting the requirements of planetary protection. In consultation with advisers a design was created, and a large-scale mock-up of this design was fabricated by additive manufacturing at Techshot, Inc. with moving parts that simulated the components of the design. The mock-up assembly has been demonstrated to interested parties. A means of detecting live metabolism will also be included in the test bed. Several options were reviewed, and it is concluded that, by the time the ecopoiesis test bed is ready for testing the optimum instrument will be the equivalent of a hand-held mass spectrometer for metabolic gas analysis. This will maximize versatility and reveal much more information than could a detector of a single product (such as molecular oxygen), and the simple output signals will be compatible with telemetry. The objectives of this project, (1) Model and test the

availability of liquid water in Techshot's Mars simulator facility, (2) Identify current candidate pioneer organisms for testing and initiate a selection program, (3) Create a mechanical and electronic design concept for Mars surface shallow penetrator with planetary protection, and (4) Identify electronic biological activity tests, were fulfilled by the completion of the Phase-1 research described in this final report.

Introduction

The cover of *Astrobiology* , September 2015, "Celebrating 15 Years" features, in bold color, photosynthetic cells reacting with extraterrestrial water to form hydrogen ions – the initial process in photosynthesis – within a microbial fuel cell. The molecular oxygen produced is reduced to water at the cathode generating an electric current proportional to the diurnal levels of insolation as a proposed means of detecting extraterrestrial life [Figueredo et al., 2015]. Active life cannot be sought in the absence of liquid water and cannot be implanted in the absence of liquid water. Mars orbital photographic evidence for the slow movement of perchlorate evaporites down slopes at Garni and Gale craters is currently taken as evidence that flowing brine may be responsible for recurring slope lineae on these steep slopes [Martin-Torres et al., 2015]. The high salinity and low temperatures that correspond to this transient liquid condition (certain times of day) may not be hospitable toward terrestrial extremophiles. These recent findings further set the stage for searches for microbial life and ecopoiesis research.

What is a Mars Ecopoiesis Test Bed?

The term Ecopoiesis was introduced by Bob Haynes and Chris McKay, in collaboration with Carl Sagan in the 1970's [Sagan, 1973], and conferences were held on this subject [Haynes, 1992; McKay, 1989, 1991, 2004]. McKay and Averner and others performed early calculations concerning the use of dark materials on the Mars polar caps, enhanced insolation and/or artificial greenhouse gases to initiate a runaway greenhouse effect that would result in conditions allowing liquid water to exist in abundance on the Red Planet [Averner, 1976; MacElroy, 1976; Haynes, 1992; Fogg, 1995; Gerstell, 2001; McInnes, 2006]. Our proposed concept emphasizes *bio*-ecopoiesis [McKay, 1989, 1997; Thomas, 1995], in which contained pioneer organisms will eventually be tested for bio-activity in a suitable location on the Martian surface (low latitude and low altitude, where pressure and temperatures combine to flirt with the possibility of metastable liquid water [Hecht, 2002; Heldmann, 2005; Carr, 1996; Jakosky, 1992; Levin, 2003]) using a robotic mechanism on a future rover.

The proposed concept is illustrated diagrammatically in Figure 1.

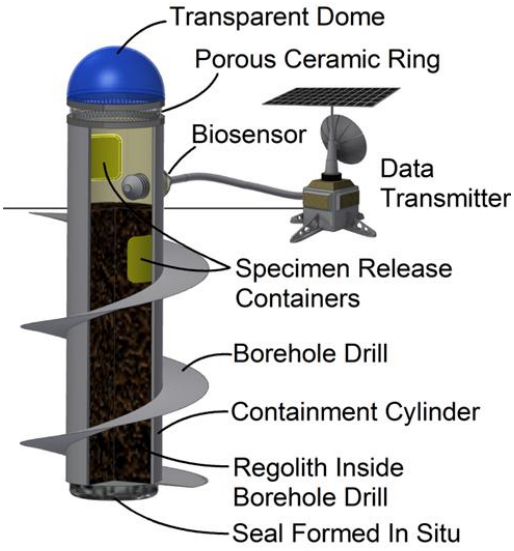

Figure 1. Rendering of the Mars surface shallow penetrator as originally proposed showing, from top to bottom: transparent dome for the admission of light for photosynthesis, porous ceramic ring that allows free exchange between the internal vapor phase and Mars atmosphere while providing planetary protection, sensor of biological activity (TBD) connected to datalink to a Mars orbiter for relaying signals, specimen containers that robotically release one set of contents onto the surface and one set of contents below the surface, threads that cause the penetrator to penetrate the regolith when rotated, the containment cylinder which constitutes the entire shell of the penetrator and which contains the sample of regolith to be tested, and at the very bottom the planetary protection seal that will be formed in situ after the penetrator is at full depth and before the release of specimens.

Relevance to NASA Astrobiology Roadmap

The objective of NASA Astrobiology Roadmap Goal 6.2 is *Adaptation and evolution of life beyond Earth,* and the following tasks are enumerated: "Explore the adaptation, survival and evolution of microbial and other organisms under environmental conditions that simulate conditions in space or on other potentially habitable planets. Identify survival strategies to evaluate the potential for interplanetary transfer of viable organisms and to establish requirements for effective planetary protection. Identify and validate roles that microorganisms might play in life support and resource acquisition during human missions envisioned by US Space Policy. Develop tools to track the function and adaptation of microbes and other organisms to extraterrestrial environments during mankind's exploration efforts." **Example** investigations are listed as: "Document the effects of the space environment upon microbial ecosystems. Examine the survival, genomic alteration, and adaptation of microbial ecosystems in a wide range of simulated Martian environments. Interpret the significance of these experiments regarding the potential for the forward biological contamination of Mars and for utilizing microorganisms to support the needs of human exploration. Examine the effects of the space environment upon the biosynthesis and utilization of biomolecules that play key roles in biogeochemical processes and also upon the viability of microbes that might be transferred between planets by natural processes (*e.g.,* impact ejection). Develop automated assay tools to monitor the adaptation of organisms in lunar and Martian

environments, especially those areas most likely to be visited by human explorers over the next century." Components of nearly all of these goals and tasks are included in the research being conducted on this project "Mars Ecopoiesis Test Bed".

Team Members and Their Contributions

A core scientific and engineering team was assembled in Phase I. The Phase-I activists have been:
PI: Dr. Eugene Boland, Chief Scientist Techshot, Inc.
Project Scientist: Dr. Paul Todd, Chief Scientist Emeritus, Techshot, Inc.
Project Engineer: Mr. Michael (Andy) Kurk, Techshot, Inc.
Project Astrobiologist: Prof. David J. Thomas, Professor, Lyon College
Project Adviser: Dr. Lawrence Kuznetz, Principal, Spinoff, NASA, Retired
Additional participating engineers were drawn from Techshot's space-hardware-experienced staff, and the additional advisory scientists were drawn from Drs. Thomas' and Todd's circle of colleagues and were Dr. Chris McKay, NASA Ames Research Center and Dr. Chris House, Penn State University.

Objectives of the Phase I Project

The Phase I objectives, exactly as briefly stated in the proposal, were as follows:

Objective 1. Model and test the availability of liquid water in Techshot's Mars simulator facility. A low-temperature hygrometer will be acquired, and conditions surrounding 7-10 mbar will be sampled by interrupting experiments at specific times in Martian sols and measuring regolith moisture and by monitoring the formation of mineral evaporites.

Objective 2. Identify current candidate pioneer organisms for testing, and initiate a selection program.

Objective 3. Create a mechanical and electronic design concept for Mars surface shallow penetrator with planetary protection.

Objective 4. Identify electronic biological activity tests (O_2 sensor, for example); initiate testing in a laboratory simulator.

Progress on Phase I Objectives

Objective 1. Model and test the availability of liquid water in Techshot's Mars simulator facility. A low-temperature hygrometer will be acquired, and conditions surrounding 7-10 mbar will be sampled by interrupting experiments at specific times in Martian sols and measuring regolith moisture and by monitoring the formation of mineral evaporites.

Rationale

Ecopoiesis will require water. That means maximizing the chances of liquid-phase water being transiently present in the test bed with the most likely sites being found at Mars' lowest altitudes and latitudes [Kuznetz, 2006]. A preliminary identification of these "landing" sites, already considered for certain past and future robots, is given briefly in Table 1. The tidal pressure swings of $\pm$0.5 mbar need to

be considered. These sites are also thought to contain evaporites, possibly including nitrates (all of which are water soluble) to provide nitrogen and magnesium salts [Tosca, 2006]. Recent results from the Curiosity Rover in Gale Crater are encouraging with regard to the availability of minerals to support autotrophic life [Navarro-González, 2013]. The big question of course has to do with the thermodynamics and transport processes of water in real and simulated Martian environments. Even at 11 mbar, the vapor pressure of water is well below the 6.1-mbar triple point, where, at increased temperature ice will normally sublime. However, speculative calculations modeling the diffusion of water vapor from ice surfaces during sublimation indicate a local (within a few mm of ice) increase in water vapor concentration to some 60%, or the required 6.1 mbar in the 11 mbar environment [Levin and Weatherwax, 2004]. Therefore, early proposed research has used the Techshot simulator [N. Thomas et al., 2006] to test such hypotheses.

Table 1. Characteristics of potential Martian test venues.

SITE NAME	LATITUDE	MAX DEPTH, m	MAX P, mb	MAX T ^{O}C
Elysium Planitia	3^{O}N	45	6.2	32
Isidis Planitia	3.0-12.9^{O}N	3,600	7.5*	26
Valles Marineris	13.9^{O}S	7,000	11.0	26
Gale Crater	4.5^{o}S	4,500	7.8	28
Triple Point			6.1	0.02

*Approximate, based on linear interpolation.

Improved simulator facility

The Mars simulation facility has been upgraded with an automated power supply that can control the Martian sol by automatically firing the arc lamp (and confirming it lit) and tying the power and temperature output to a data logger. Previously this was a manual process to confirm the arc lamp fired. We attained the use of a cryogenic hygrometer to monitor moisture continuously and subjected it to testing to verify that we can produce the pressures and vapor pressure requirements set forth for the research.

To maintain a Martian atmospheric environment, the sample is loaded into a 140 mm ID x 430 mm long quartz-tube chamber featuring a single open end. Once loaded, within the tube, the open end of the tube is sealed with a stainless steel end cap that features all of the ports and valves necessary to maintain a vacuum, monitor temperature and pressure, and input a simulated Martian atmosphere or liquid water through external automated valves. A photograph of the Mars Simulator Chamber within the environmental cabinet is shown in Figure 2.

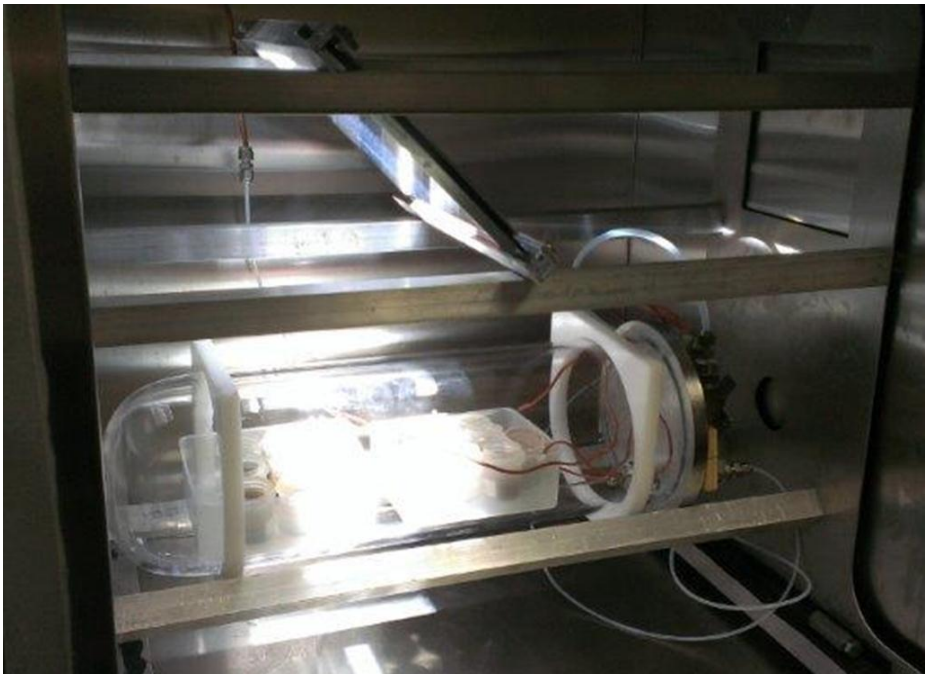

Figure 2. Interior of the Mars simulator environmental cabinet showing reflecting mirror, quartz chamber, sample tray, eighteen samples with twelve of them in direct illumination and six of them in shade, thermistor leads for temperature measurement in and out of regolith, end plate for pressure and electrical access, and cradle to hold quartz-tube Mars chamber. This configuration was used in an uninterrupted five-week simulation campaign.

Once the experiment loading is complete the quartz chamber is secured within a thermal cabinet and all external mechanical and data interfaces are connected to the sealing end plate. Upon completion of this process, the inner volume of the simulator chamber is drawn down to the desired Martian atmospheric pressure by a Welch Reitschle Thomas vacuum pump. To accommodate most very low pressure experiments the vacuum pump is required to run continuously. If minor pressure adjustment is required, a ball and needle valve are located downstream of the experiment between a low pressure hygrometer and the vacuum pump. When needed, the valves can be adjusted to obtain the desired pressure.

In addition to control and measurement the Mars Simulator Chamber end plate contains a port through which liquid water or a gas can be periodically introduced to the test volume throughout the experiment. Periodically CO_2 or a specially mixed gas resembling the Martian atmosphere is introduced into the volume to flush any dry nitrogen that may have intruded into the volume and maintain an atmosphere consistent with that of the simulation objectives. This function is fully automated and its frequency is programmed into the thermal cabinet's Watlow controller as part of the experiment profile. In this case, pure, dry CO_2 was used as the chamber gas.

The downstream hygrometer, a CR1-A condensation-mirror low-pressure hygrometer that uses liquid nitrogen (down to 77^{o}K) to determine the dew point (Buck Technologies, Boulder, CO), is used to continuously sample the moisture content of the atmosphere within the Mars simulator chamber. The hygrometer data are monitored along with pressure and temperatures and are recorded using a Fluke 1586A Super DAQ data logger.

The Martian thermal environment is simulated by a highly insulated, modified, cryogenic thermal cabinet (Model ZBD-108 LN_2 Cooled Chamber, Associated Environmental Systems, Ayer, MA) that is thermally maintained by the evaporation of liquid nitrogen. Since the chamber is filled with dry nitrogen there is very little water present, and heavy frosting is avoided. Thermal cycling of the chamber according to the Mars daily cycle is accomplished by programming a Watlow F4 Controller that is a standard component of the commercial thermal chamber. This controller is programmed with a timeline that enables thermal ramping functions that can be programmed either on the user panel or with a GUI on the PC.

Table 2. Customized Liquid Nitrogen Cooled Environmental Test Chamber Features

Working Volume:	24" x 24" x 24"
Insulation:	4" Fiberglass
Power Requirements:	120 VAC, 1 phase, 60 Hz
Refrigeration System:	LN_2 Cooled
Temperature Range:	-135^{o} C to + 177^{o} C
Temperature Stability:	+/- $1/2^{o}$ C at sensor
Temperature Rise Time:	Ambient to upper limit – 20 minutes
Temperature Pull Down Time:	Ambient to lower limit – 20 minutes
Interior:	18 Gauge 304 Stainless Steel
Illumination:	Double-paned quartz window

18 Gauge cold rolled steel with two coats of textured epoxy paint
Watlow F4 Programmable Controller with RS232 communications
Dry Nitrogen Purge

Simulated Solar energy is provided to the sample by an automated Sciencetech Solar Simulator that has been equipped with an AM0 filter to more closely resemble the mildly filtered solar radiation which reaches the Martian surface. A diagram of the unfiltered (top) output of the xenon arc lamp and the output spectrum after filtering with an AM0 filter (bottom) is shown in Figure 3.

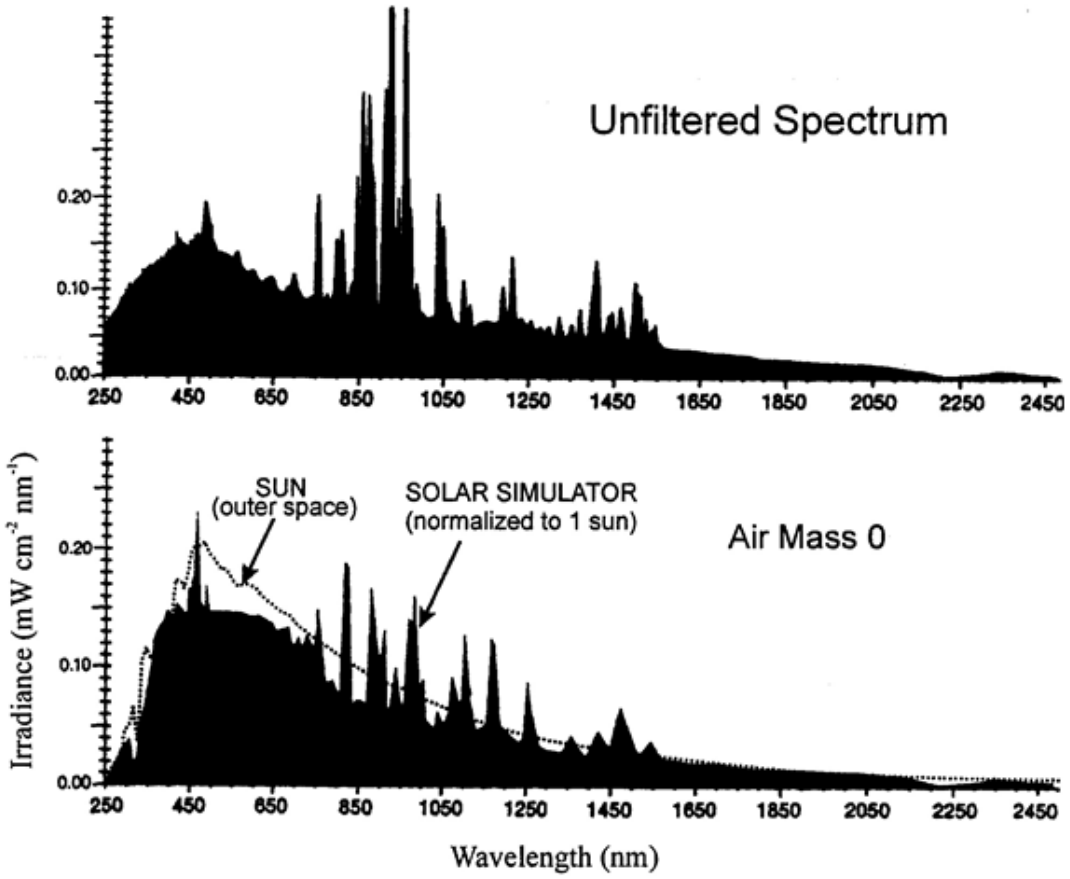

Figure 3. Light spectrum produced by the 1,000 W xenon arc illuminator without (above) and with (below) AM0 (Air Mass zero) light filter. The similarity to the solar spectrum below about 300 nm is important in terms of its known biologically damaging action.

As part of the improved solar simulator automation package the user can preprogram a light cycle and intensity. For this experiment a 12 hour, 1000 W cycle was preprogrammed prior to initiation. The ScienceTech illuminator and the added control technology is shown in Figure 4. Modifications to the stock liquid nitrogen cooled thermal chamber, enable the solar simulator's light beam line to pass into the thermal chamber through a 4" x 4" (10 x 10 cm) double paned opening after which it is reflected by a front-surface mirror inside the cabinet to illuminate the quartz Mars jar chamber as shown in Figure 2. A photograph detailing the light path from the solar simulator is shown in Figure 5.

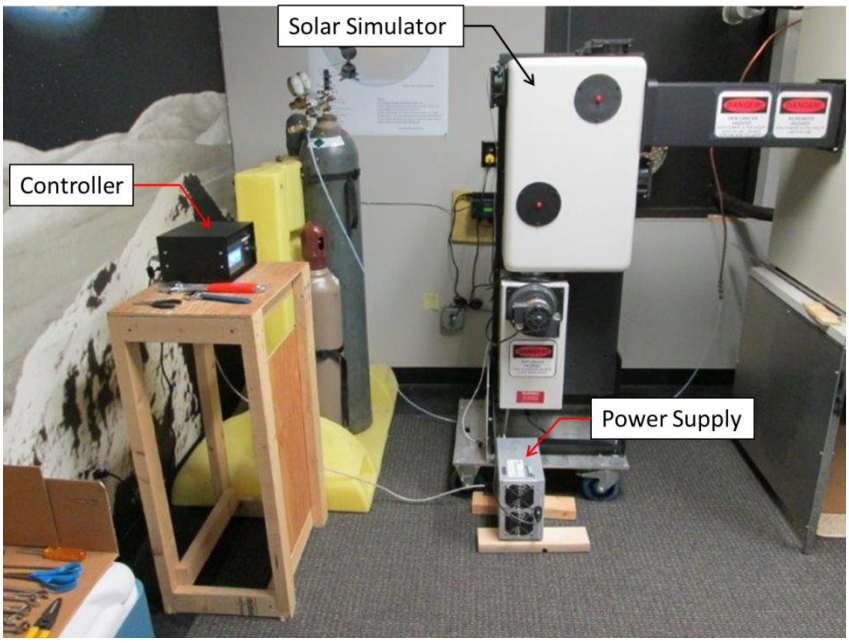

Figure 4. ScienceTech solar simulator with power supply (UPS) and Techshot controller for automatically re-igniting the 1,000-W xenon arc lamp. Also seen are the chamber gas supply cylinders (left) and environmental chamber (far right). See also Figure 5.

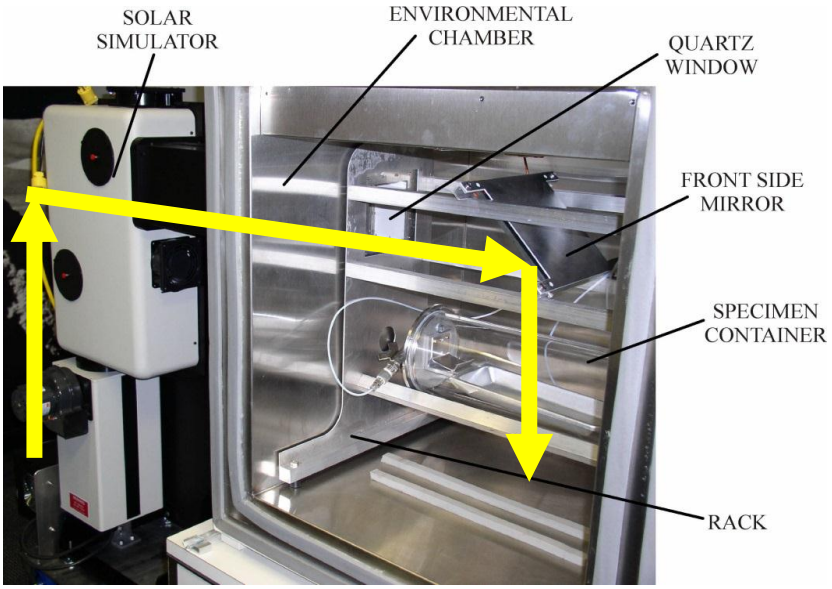

Figure 5. Light path from the solar simulator's xenon arc lamp to the specimen chamber via double-paned quartz window, front-surface mirror and quartz-tube chamber. See also Figure 2.

Nine variables were measured and logged as a function of elapsed time every 5 minutes for 5 weeks. These were ambient laboratory temperature, Mars-jar pressure, cabinet temperature, regolith temperature, Mars-jar temperature, hygrometer pressure, dew/frost point, illuminator on/off, and water concentration.

An example of measured values during days 10-20 is given in Figure 6. The pressure pattern is seen to have spikes due to the hourly introduction of fresh CO_2 to maintain the atmospheric composition. The purpose of this operational procedure was to prevent cabinet nitrogen from entering the simulated atmosphere inside the chamber.

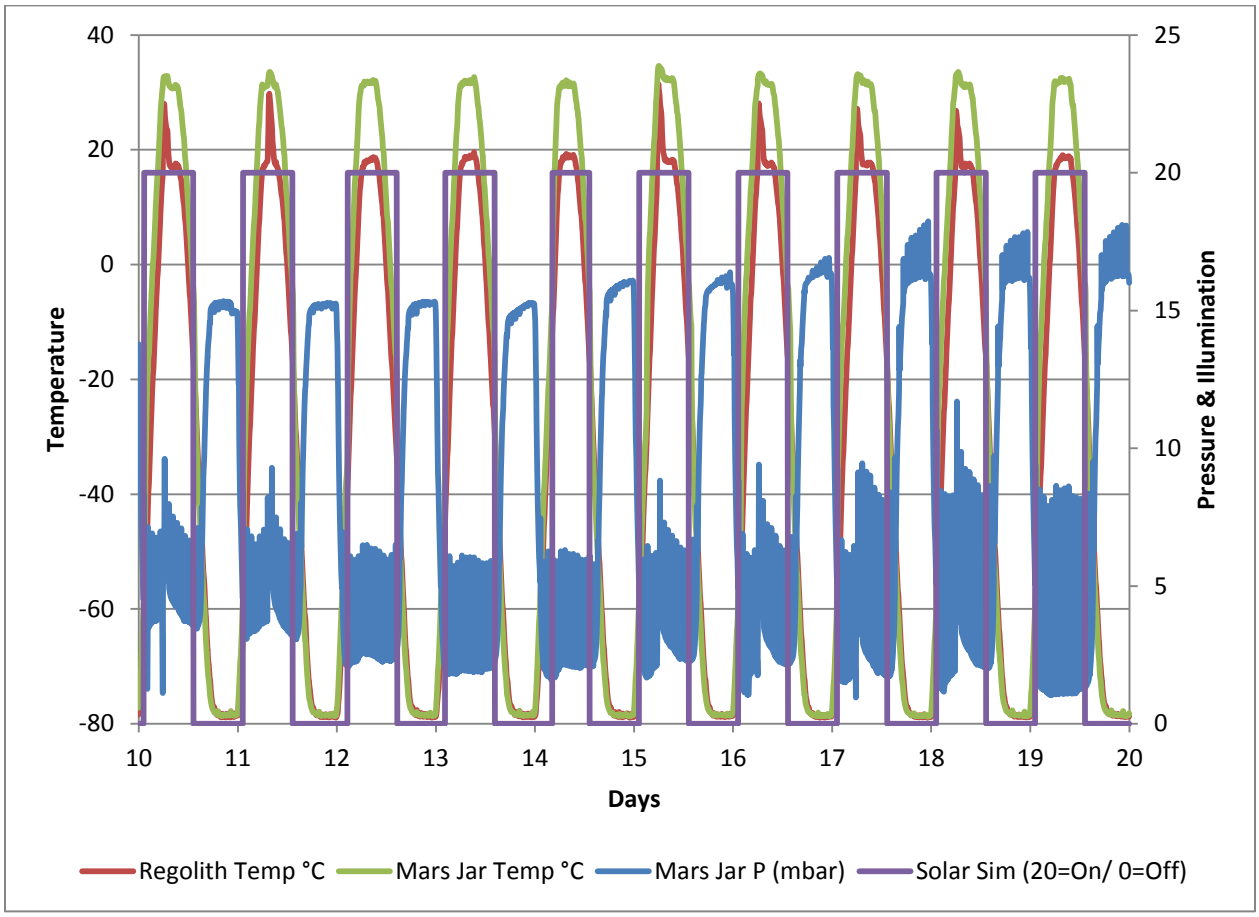

Figure 6. Example of environmental data logged over a 10-day period, showing four measurements: regolith temperature (red), chamber internal temperature (green), chamber internal pressure (blue), and solar simulator on/off (violet). The "Days" on the abscissa are earth days and not Martian sols.

Moisture Measurement and Control in the Laboratory Test Bed

Nine parameters were logged during test and experimental operation of the laboratory test bed. Of these, the CR-1A cryogenic hygrometer (Buck Research Instruments, LLC, Boulder, Colorado) recorded temperature, pressure and dew/frost point in extracted chamber atmosphere. Figure 7 is a chart record of nine logged parameters during days 11-14 of a 35-day campaign. The dew/frost point never exceeded the regolith temperature except when a pulse of liquid water was injected when the temperature was below -75 C. At all other times humidity was well below saturation and the partial pressure of water was below the triple point (see Figure 8).

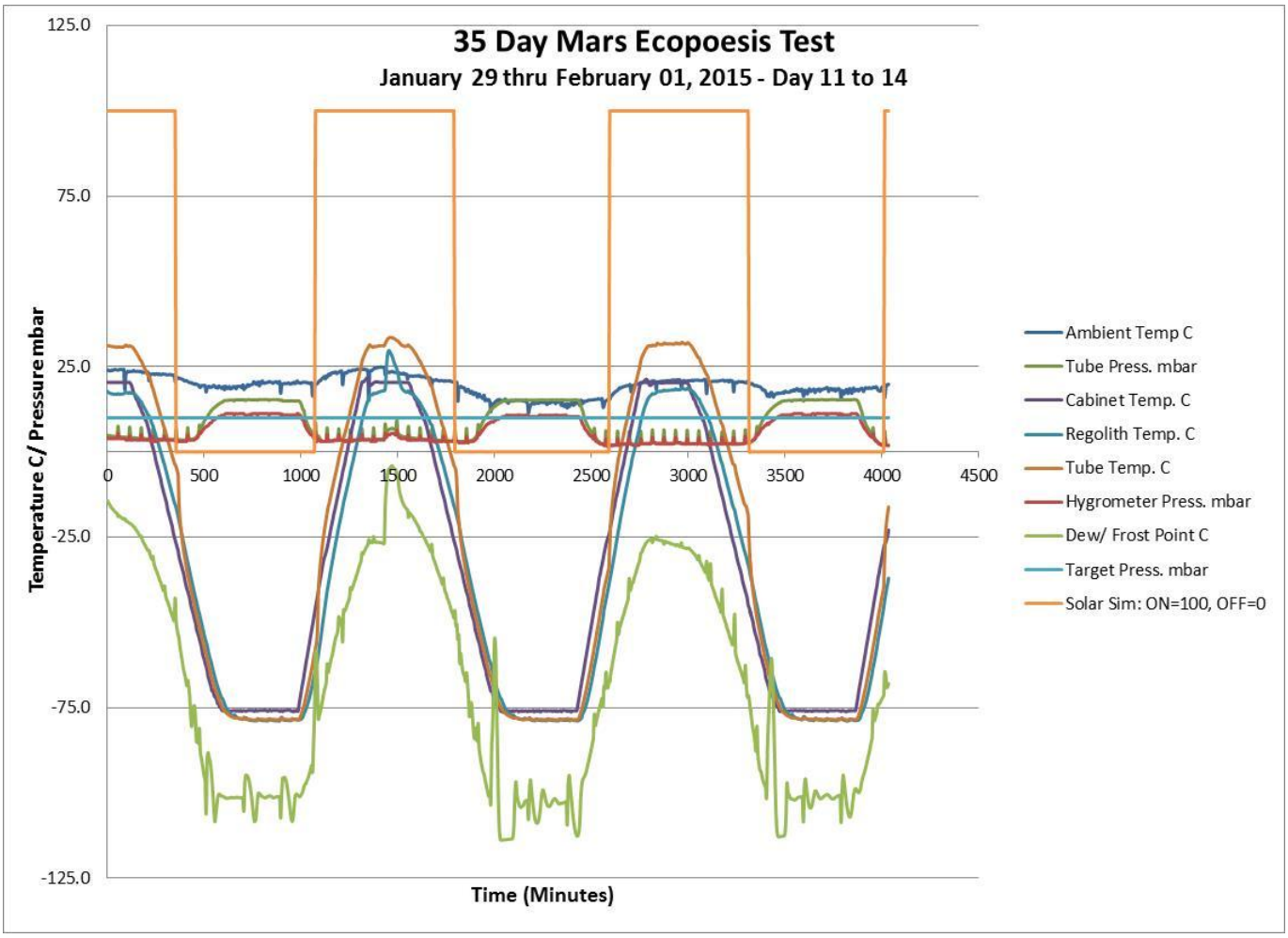

Figure 7. Chart record of nine logged parameters during days 11-14 of a 35-day campaign. The dew/frost point never exceeded the regolith temperature except when a pulse of liquid water was injected when the temperature was below -75 C. At all other times humidity was well below saturation and the partial pressure of water was below the triple point (see Figure 8).

Spikes in pressure and dew/frost point that appear on the chart are due to the periodic injections of fresh dry CO_2 for maintenance of atmosphere composition. From the data set of Figure 6 were extracted correlated time points for chamber pressure (as measured by the CR-1A hygrometer) and regolith temperature. Figure 8 represents the repeated journey around the P-T diagram through three daily cycles. The total pressure never exceeded values measured on the Martian surface.

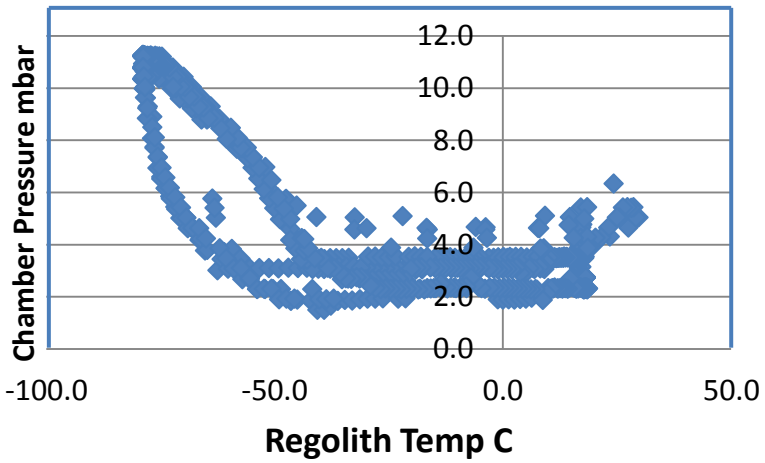

Figure 8. Trace of chamber pressure and regolith temperature over three daily cycles ending 03-01-15 on days 11-14 of a 35-day campaign.

The CR-1A Cryogenic Hygrometer measured, and the data logger recorded dew/frost point at each sampling time. From the recorded dew/frost points the partial pressure of pure water was calculated using the empirical formula

$$p_w = 6.11*10^{[7.5*T_d/(273.3+ T_d)]}$$

using p_w = water partial pressure in mbars and T_d = dew/frost point in ^{o}C. For 814 time points accumulated over days 11-14 of a 35-day campaign this partial pressure is plotted against temperature on a traditional P-T diagram, on which the liquidus line for pure water is also shown (Figure 9).

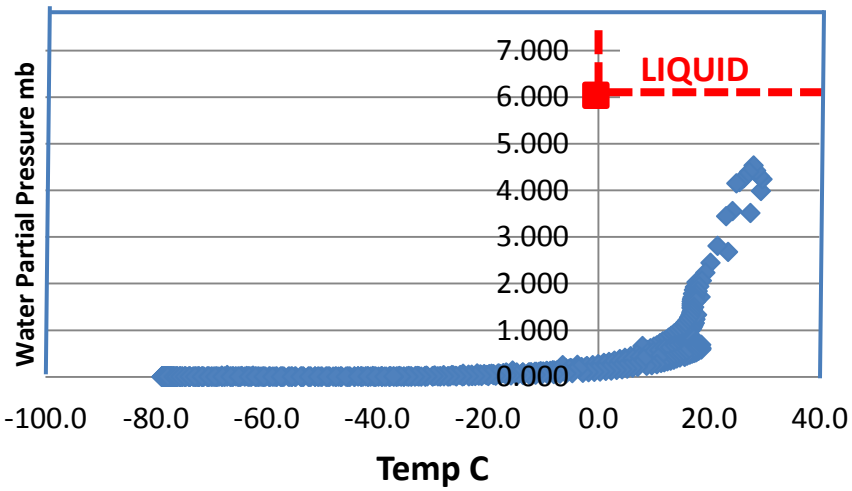

Figure 9. Water partial pressure during the period ending 03-01-15, days 11 through 14 of a 35-day campaign (814 data points). The liquidus line for water on this P-T diagram is shown in red, with the triple point shown as a red square.

Performance record: A review of all data sets was made to create a list of "bumps in the road". The CR-1A hygrometer must be cooled below the chamber temperature at all times, and cooling was lost on a small number of occasions during the 35-day campaign; however, the maintenance of environmental conditions did not depend on continuous hygrometer readings, and its temperature and pressure readings are redundant. The hygrometer only contributed data, and hygrometer-related events are noted by an asterisk in Table 3. Some maintenance of the solar illuminator, including xenon arc lamp replacement was required toward the end of the campaign.

Table 3. Descriptions of individually recorded data sets during 35-day Mars surface simulation campaign.

DAYS	OBSERVATIONS
1,2	Breaking in. Chamber pressure rose to 14-20 mbar for 5 min during hourly CO_2 injection
2-4	Pressure maintained <10 mbar. Hygrometer cooling lost; hygrometer data not taken*
4,5	Same as days 11-14, which were used as model for detailed report data
5-8	Loss of hygrometer function on day 8*
8	Hygrometer recovery period; tube pressure spike to 70 mbar for 2 h*
9-11	Same as days 11-14
11-14	Chosen as source for model data
14-16	Same as days 11-14; spike in measured dew/frost point on day 15
16-18	Same as days 11-14
18-21	Same as days 11-14
21-24	Loss of hygrometer cooling, days 23,24*
25-30	Loss of hygrometer cooling, day 29*
30-36	Loss of light day 31, ½ of day 32, day 35. Repression recorded when campaign ended.

*Losses of hygrometer function have no impact on environment control.

The data above demonstrate the continued presence of water in the solid and vapor phases throughout this 35-day campaign. At the end of the campaign the chamber was repressurized for sample recovery, at which time two observations on liquid water were made. There had been an accumulation of some 10 cm^3 of liquid water, melted from the solid and condensed from the vapor phases of the chamber upon its return to earth-ambient conditions, and the regolith simulant in the shaded specimen jars was dark due to moisture while the regolith in the illuminated specimens was the color of dry regolith.

Objective 2. Identify current candidate pioneer organisms for testing, and initiate a selection program.

The project officially kicked off internally with the convening of the advisory panel consisting of Dr. Larry Kuznetz (Advisory council chair), Dr. Christopher House and Dr. David Thomas. Unfortunately, Dr. Chris McKay was unable to attend the first meeting because of commitments with Curiosity Rover results.

The first key outcome of the meeting was the identification of test organisms. Due to the size of our Mars simulator and past experience, we decided to proceed with 3 cyanobacteria and an alga. Space

permitting, we would add two species of heterotrophic eubacteria, which actually was done. The final selections are given in the following list.

Organisms:
Anabaena sp. (cyanobacteria)
Chroococcciopsis sp. CCMEE171 (cyanobacteria)
Plectonema boryanum UTEX485 (cyanobacteria)
Chlorella ellipsoidea (algae)
Bacillus subtilis (bacteria)
Pseudomonas aeruginosa (bacteria)

A single test was designed for a 5-week period, to start after simulator improvements and physical tests were completed.

A single 5-week simulation was performed using 100% CO_2 at pressures between 3 and 10 mbar and water supplementation as described in Figures 2-10. Multiple samples of each species were subjected to four conditions for 5 weeks for comparison: Mars simulator, -80 C in darkness, +4 C in darkness and 25 C in diurnal illumination.

The following test organisms were used:
Cyanobacteria: *Anabena sp., Chroococcidiopsis* CCMEE171, *Plectonema boryanum*
Eubacteria: *Bacillus subtilis*, *Pseudomonas aeruginosa*
Eukaryota: *Chlorella ellipsoidia*

As in previous work [Thomas et al., 2008] the production of fluorescence by samples resuspended in aqueous solution was used as a test for the presence of intact cells containing esterases using the fluorescein diacetate (FDA) test. Figure 10 is a summary of the resulting measurements on suspended regolith samples.

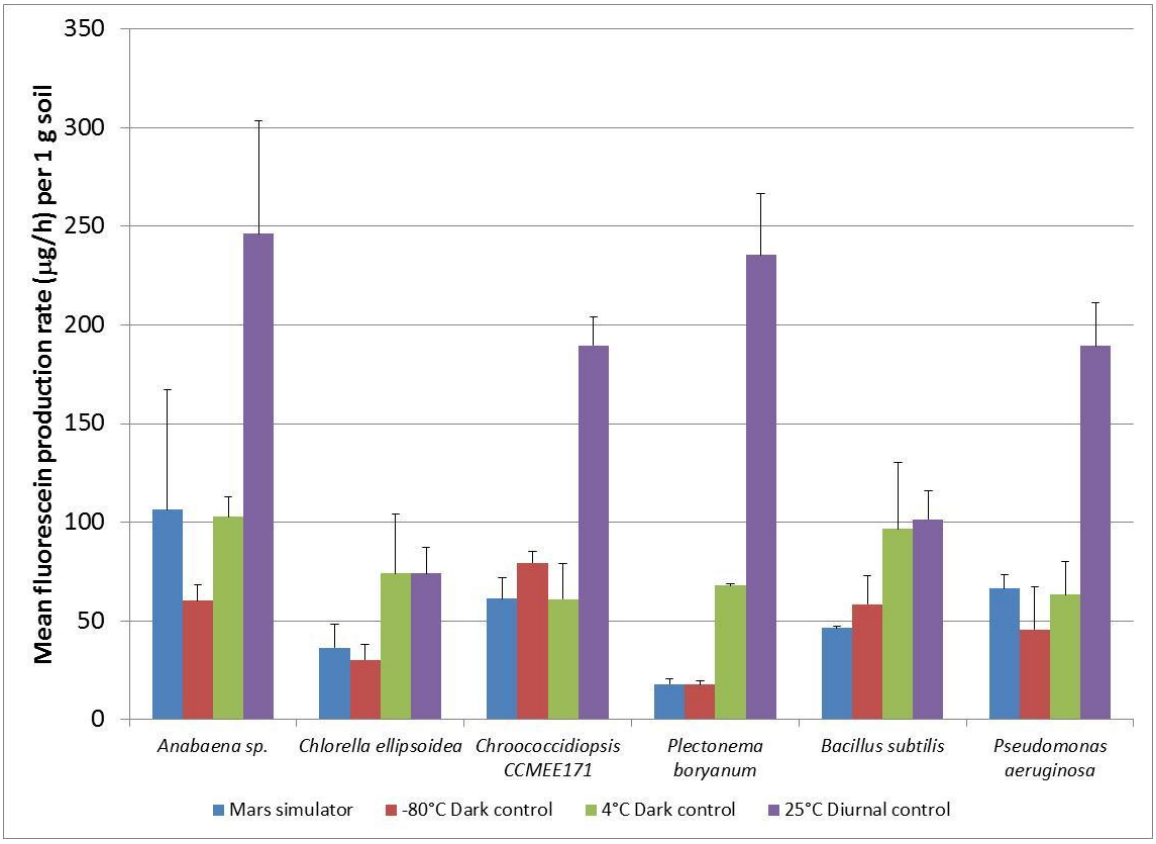

Figure 10. Rate of FDA hydrolysis by 1 gram of regolith containing each of the six species exposed, measured as rate of increase in fluorescence intensity.

When specimens were returned to ambient temperature and pressure liquid water present in the samples was tested for biological activity. Figure 11 is a summary of the resulting measurements of FDA conversion in liquid water samples.

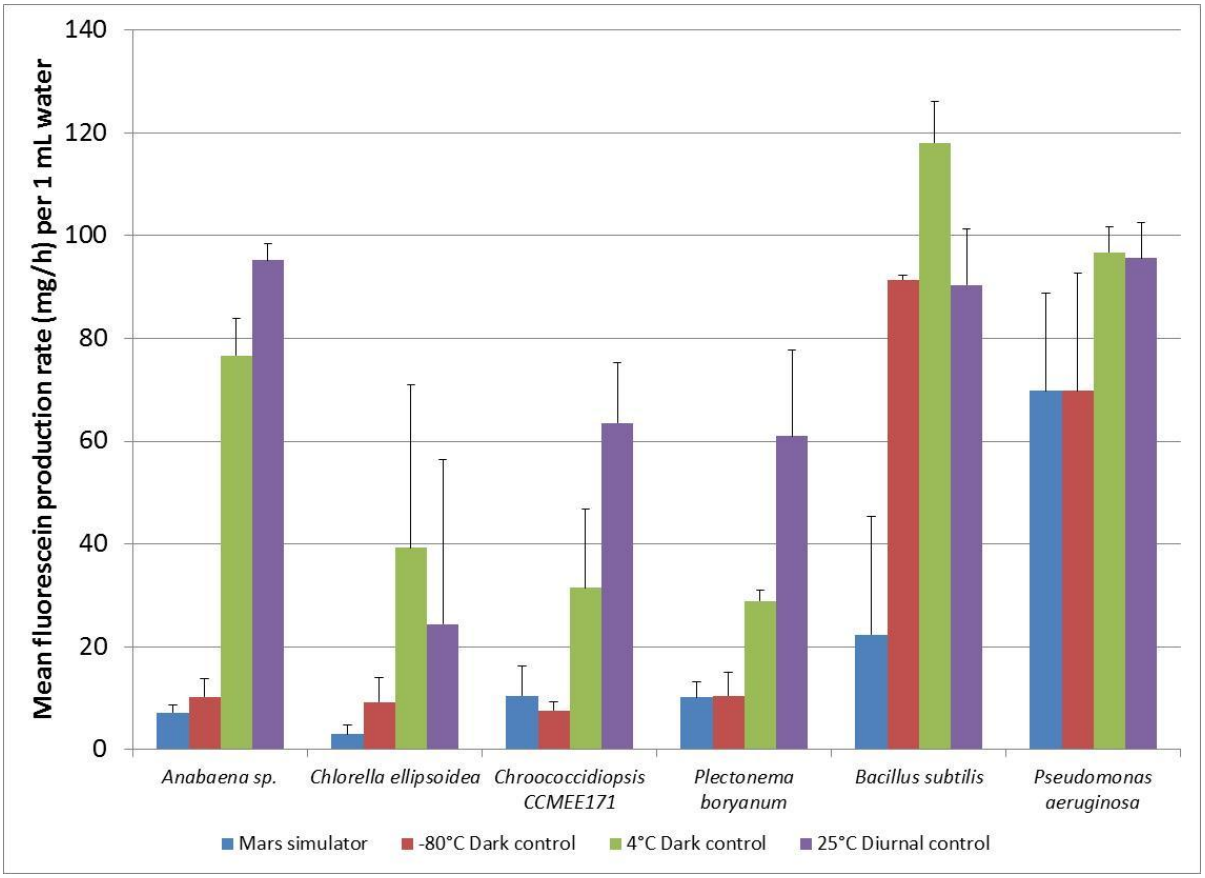

Figure 11. Rate of FDA hydrolysis by 1 mL of water residue obtained from each of the six species exposed, measured as rate of increase in fluorescence intensity.

Samples were streaked for growth on semisolid media and assessed for colony counts on a 4-unit scale. Many plates had too many colonies to count, so an area-coverage scale of 0 – 4 was used to characterize growth, using 25 C diurnal samples as control, identified as a score of 4. These results are summarized in Figure 12.

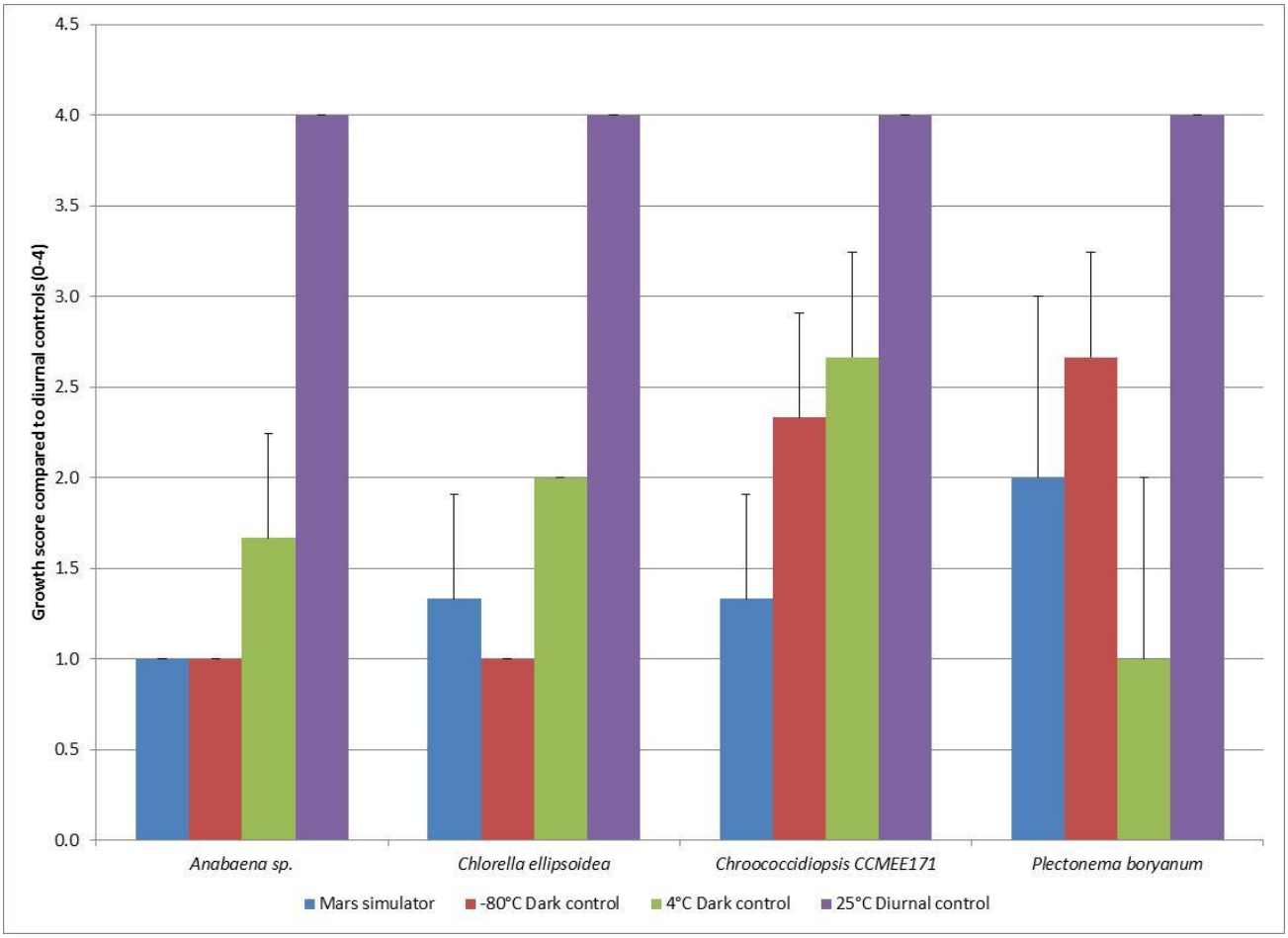

Figure 12. Relative phototrophic growth of cyanobacteria on nutrient plates based on a 4-unit scale with 25 C diurnal controls defining a score of 4.

The colony-forming ability of the two heterotrophic bacteria was assayed directly on the basis of colony counts on nutrient agar. The results are shown in Figure 13 and indicate the survival of reproducing cells under all treatment conditions.

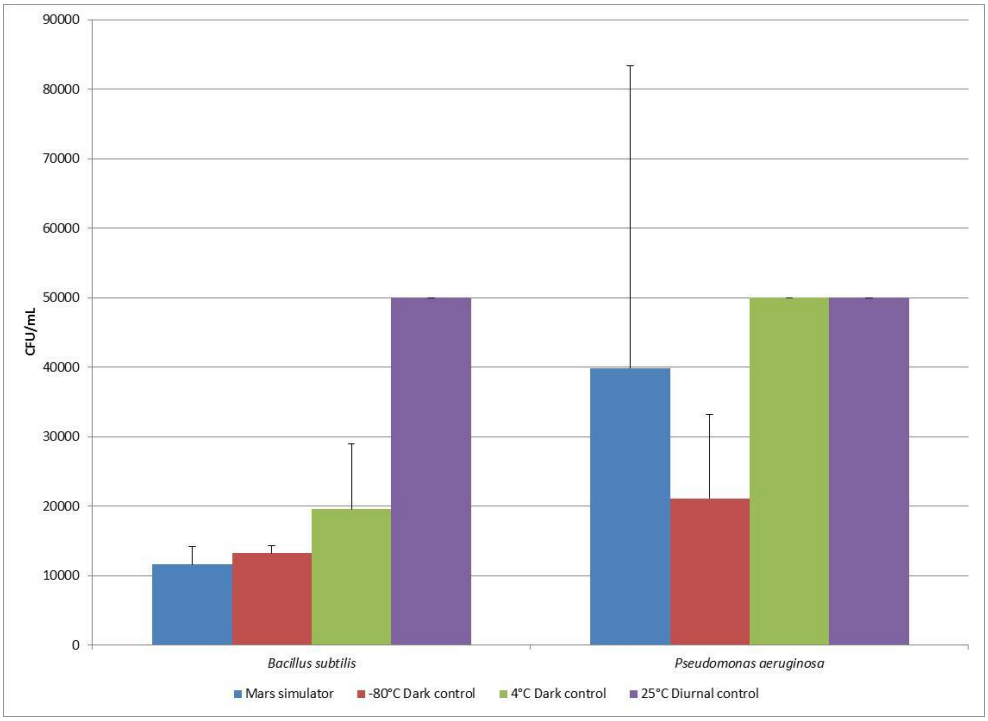

Figure 13. Counts of cfu/mL of two species of heterotrophic eubacteria.

Chlorophyll was extracted from samples of the four autotrophs and measured spectrophotometrically. Figure 14 indicates that chlorophyll could be detected in all specimens under all treatments, and Mars simulation was found less destructive of chlorophyll than the other two test treatments.

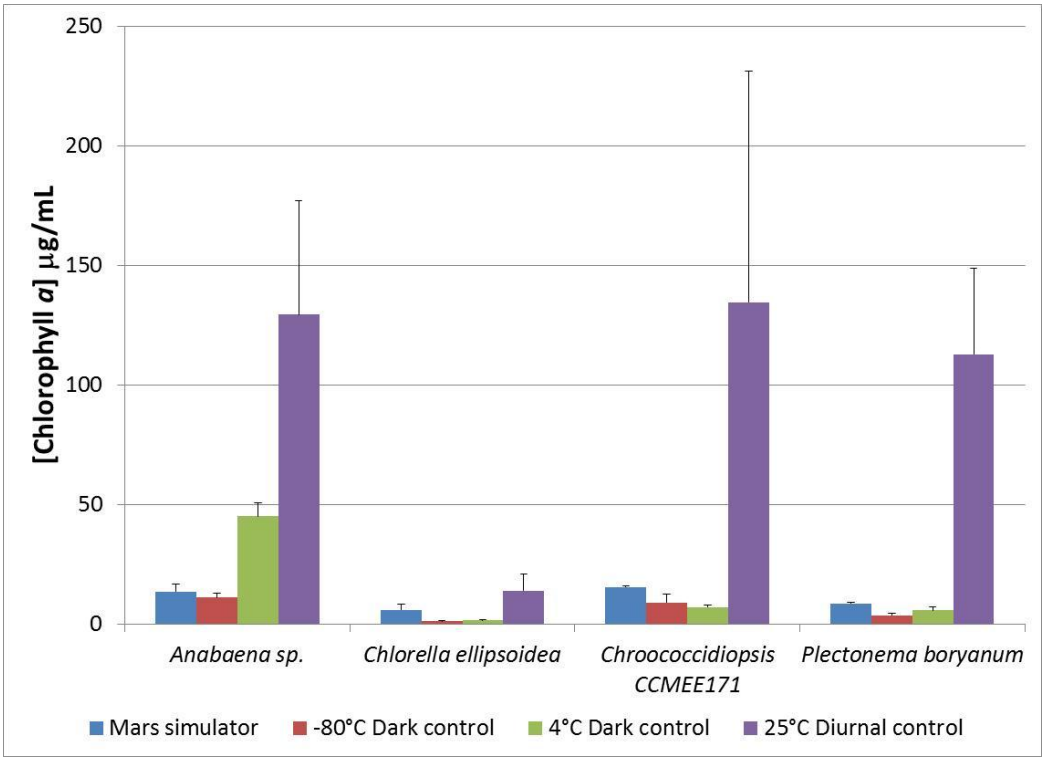

Figure 14. Chlorophyll concentrations in extracts of phototrophs after treatments.

Nitrite tests were performed to determine remaining functions for denitrification and converting nitrate to nitrite. Table 4 summarizes the results of nitrite tests (vs. optical standards) for all six species tested. Only the heterotrophic eubacteria retained denitrification ability after exposure to Mars conditions.

Table 4. Nitrate reduction and denitrification by the tested microbial species.

Organism	Mars simulator	-80°C Dark control	4°C Dark control	25°C Diurnal control
Anabaena sp.	None	None	None	NO_2
Chlorella ellipsoidea	None	None	None	NO_2
Chroococcidiopsis CCMEE171	None	None	None	NO_2
Plectonema boryanum	None	None	None	NO_2
Bacillus subtilis	N_2	None	N_2	N_2
Pseudomonas aeruginosa	N_2	NO_2	N_2	N_2

No nitrate reduction	None
Nitrate → Nitrite reduction	NO_2
Denitrification	N_2

Images of the streaked plates all showed evidence of surviving reproducing cells after exposure to the simulated Mars conditions. Figures 15 and 16, respectively, consist of images of plates streaked with phototrophic and heterotrophic cells from the Mars exposure (bottom row) compared with plates streaked with cells from cultures kept at 25 C with diurnal lighting (top row).

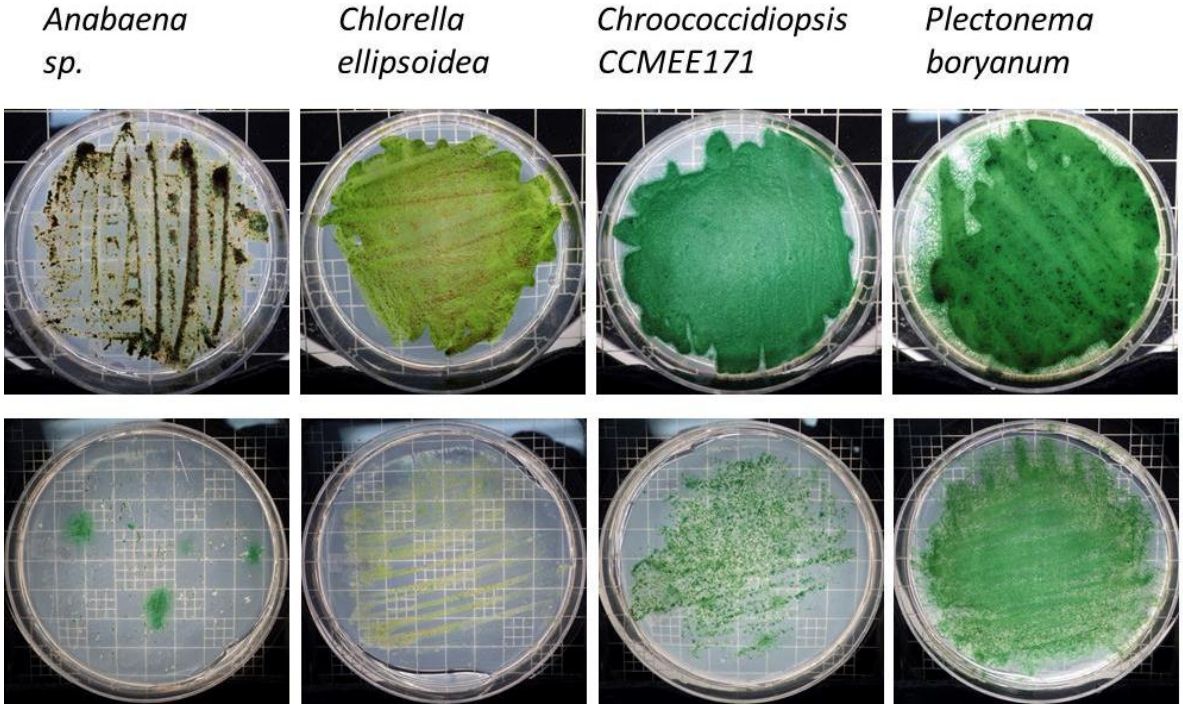

Figure 15. Images of plates streaked with phototrophic cells from the Mars exposure (bottom row) compared with plates streaked with cells from cultures kept at 25 C with diurnal lighting (top row).

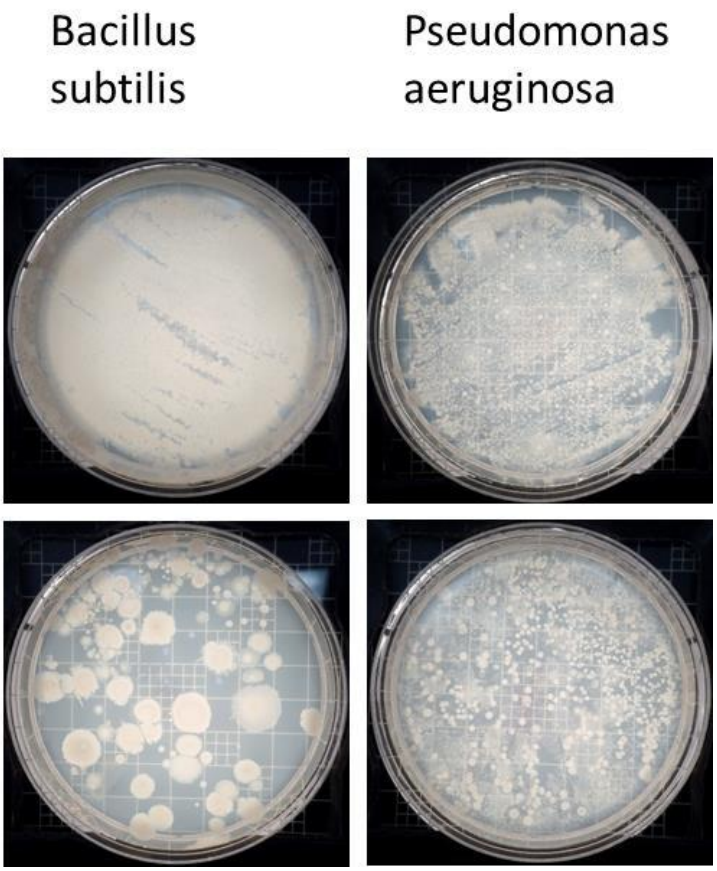

Figure 16. Images of plates streaked with heterotrophic cells from the Mars exposure(bottom row) compared with plates streaked with cells from cultures kept at 25 C with diurnal lighting (top row).

Objective 3. Create a mechanical and electronic design concept for Mars surface shallow penetrator with planetary protection.

Proposed details of this task are seen in Figure 1. The advisory team held substantial discussions on how we would seal the bottom of the testbed after boring it into the Martian regolith as well as understanding the size scale. Since the organisms of choice are photosynthetic, we anticipate depths of no more than 3cm are required so an overall depth of 15 cm was considered adequate. Requirements are clear that it must prevent bacterial escape but allow the hollow shaft to be filled with Martian regolith prior to sealing. Sealing concepts ranged from a chemical and civil engineering approach using regolith-based composite or concrete to mechanical approaches that lock the system closed after penetration and filling. The Techshot mechanical design team took this on and produced a number of CAD concepts. A design was selected, and 3-D printing of prototypes of all parts was undertaken in order to assemble a partially functional mock-up of the penetrator at an enlarged scale.

Selection of penetrator design

The overall design concept is understood by viewing the CAD model presented in Figure 17. It consists of, from top to bottom, a star-nose regolith drill to break up regolith, a threaded penetration shaft to drive the drill and shaft downward, four regolith-collecting scoops that pick up and deliver regolith to the interior of the shaft, an O-ring seal closure that closes the scoops for planetary protection, a flange that defines the depth of penetration, an internal means to release organisms, a ceramic or steel microfilter for gas exchange with the Martian atmosphere, a transparent dome to allow illumination, an "avionics" module with photovoltaic cells to provide power for telemetry, and a means for detecting metabolic activity of cells (Objective 4).

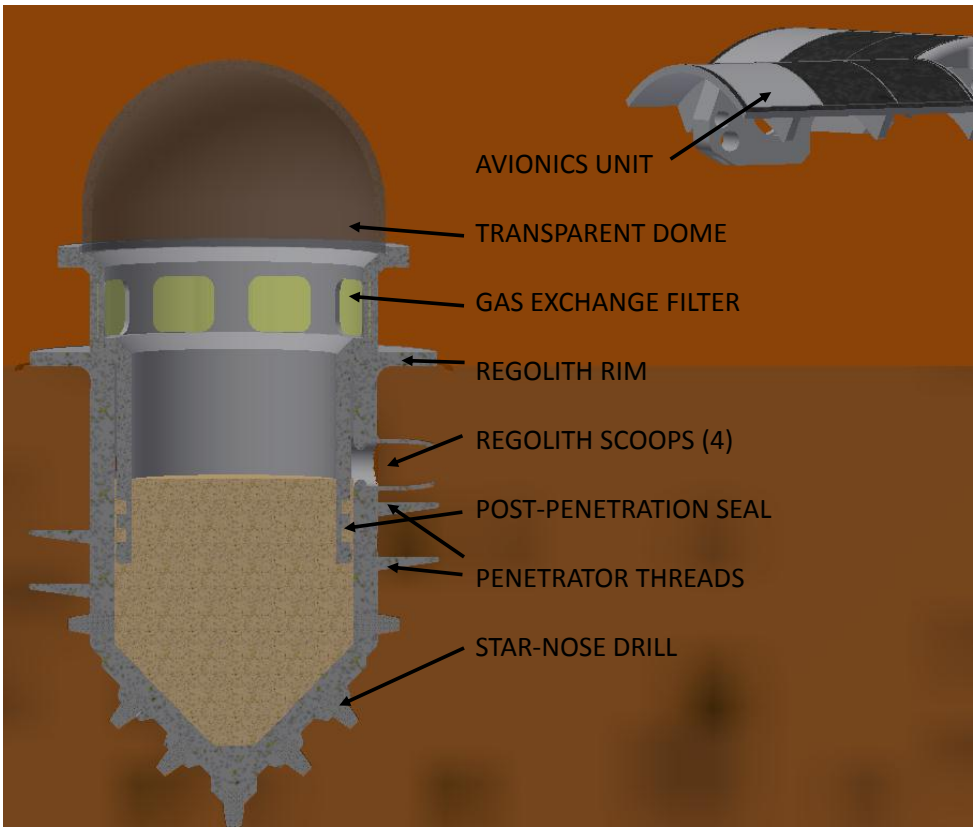

Figure 17. CAD model cross-section of Mars ecopoiesis test bed design with mechanical features labeled.

Figure 18 is an exploded view (on left) showing the individual modules accompanied by two frames (available in video) indicating how the test bed might look as it penetrates the regolith by rotating clockwise (center) and after it is deployed (right), connected to the avionics unit that receives signals from the proposed gas analyzer. The exploded view indicates how the inner cylinder fits inside the penetrator and seals off the regolith scoops by being pressed down inside the scoop holes. This act would prevent contamination of the planet exterior to the experiment.

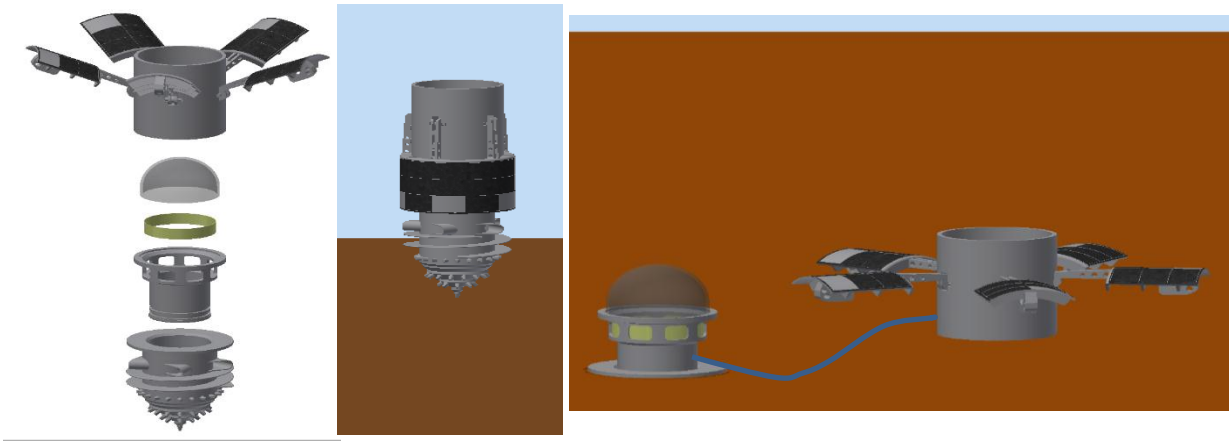

Figure 18. CAD models of Mars ecopoiesis test bed showing exploded view (left), test bed as it penetrates the regolith (center) and after it is deployed (right), connected to the avionics unit.

Penetrator Mock-up

A plastic mock-up of the CAD-designed mechanical components of the Mars ecopoiesis test bed was fabricated by additive manufacturing using the design depicted in figures 17 and 18. These components could be assembled as planned, and they were photographed in the expected configurations as shown in Figure 19. This shows a clearer view of how the inner cylinder is moved downward into the outer cylinder and engages the sealing mechanism, shown in black in the left-most photo. In parallel with Figure 18 the photos show the exploded view, the totally assembled system, the configuration that penetrates the regolith, the sealed volume and the deployed avionics package.

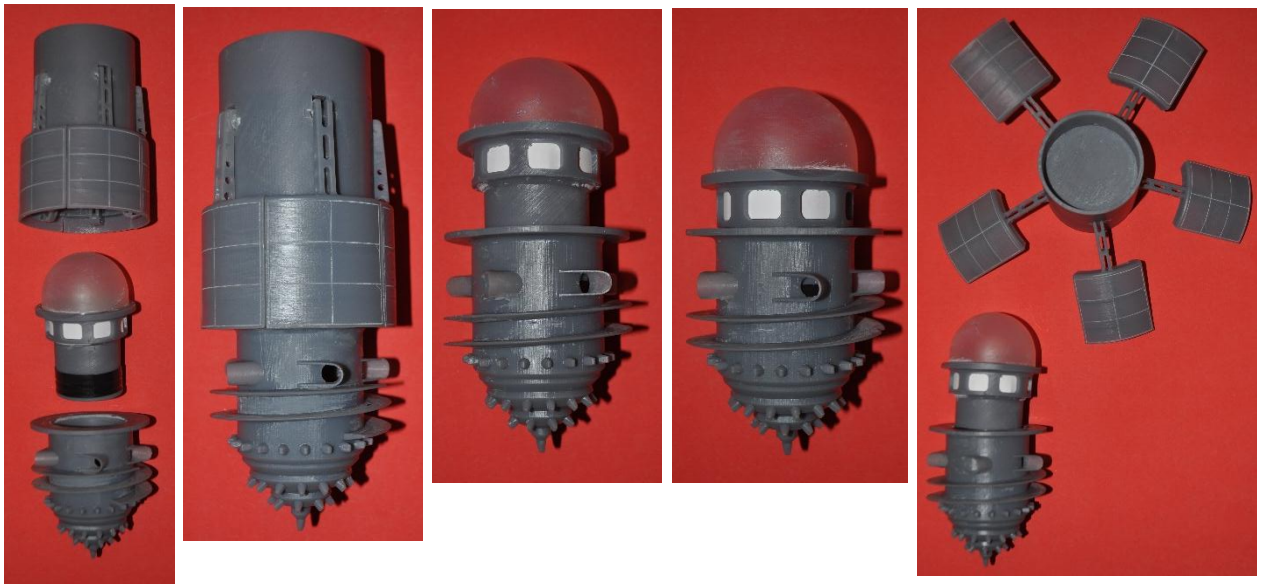

Figure 19. Photographs of Mars ecopoiesis test bed mock-up. From left to right: Exploded view, as packaged for deployment with photovoltaic arms furled, in position for regolith penetration, after sealing of inner cylinder, and as deployed with avionics module positioned and unfurled.

Selection of final components:
A ceramic filter that allows atmosphere into, but not organisms out from, the testbed was originally proposed. However, filter material with 0.5 µm pore size is also available in PEEK (polyetheretherketone) and stainless steel, and these materials are preferred because they are less frangible than porous ceramic filters. Identification of a miniaturized biosensor approach was the goal of Objective 4 study, which follows.

Objective 4. Identify electronic biological activity tests (O_2 sensor, for example); initiate testing in a laboratory simulator.

Lengthy discussions on sensor design and function to "detect" biological activity. Simply measuring O_2 may not be adequate so further discussions are scheduled to look into biomarkers that may serve as surrogates for O_2 production or an optical method of adding Fluorescein to detect the presence of liquid water enabling the production of O_2. It is the goal of the Phase 1 project to identify at least 1 method to move forward into phase 2. Further discussions led toward more versatile sensing of volatile biomarkers and the use of spectrophotometry, gas chromatography or mass spectrometry (MS).
A literature-based trade study was performed to select an approach to detecting biological activity within the test bed, according to Objective 4 and Task 4 as stated in the application from Techshot to NIAC. It was found early on that off-the-shelf commercial technology is not the way to think about instrumentation that is to be used some decade or more in the future. Potential methods include electrochemical oxygen sensing, spectrophotometry of the vapor phase, gas chromatography requiring a molecular detection system, and mass spectrometry. The merits and drawbacks of each were considered.

Oxygen sensing
The most likely test organisms are presently expected to have photosynthetic activity and to produce molecular oxygen as a metabolic product. Commercial electrochemical oxygen sensors, such as used in the headspace of bioreactors, for example, are suited to measuring molecular oxygen concentration in the vapor phase but not at the low concentrations expected in a Mars ecopoiesis experiment. The electrochemical sensing of oxygen production, heavily dependent on liquid water, has been proposed for the detection of extraterrestrial life [Figueredo et al., 2015]. There is a definite possibility, no matter what miniaturization and sensitivity progress is made in the next decade, that sensing of potentially minute oxygen concentration relative to the 0.13% already present in the Mars atmosphere would be neither feasible nor definitive. Furthermore sensing a single molecule as a "signature" is less likely to validate metabolic activity than a more versatile sensing system that reflects a pattern of biological products in the vapor phase.

Spectrophotometry
Seeking the appearance of new absorption peaks, and hence new molecular species in the vapor phase is more versatile than oxygen sensing and potentially more revealing about metabolic processes. However, either great sensitivity or long path length (not available within the test bed) would be required, and lower-level species would be completely missed.

Gas Chromatography
Gas chromatography is a highly sensitive, extensively practiced method for quantifying multiple analytes in the vapor phase. Extremely promising is the single-chip gas chromatograph developed and described by Akbar et al., [2015], which incorporates a photoionization detector and requires only a few mW of

electrical power. As with any gas chromatograph a support gas must be fed at a controlled flow rate – a complexity to be avoided in multiply deployed robotically operated miniature devices. Gas chromatography is nowadays frequently followed by mass spectrometry (GC-MS) to characterize vapor-phase mixtures with built-in "ground truth" in the form of precise molecular mass determination at the MS step.

Mass Spectrometry

The molecular mass of a compound is an almost completely unambiguous identifier, and the best hand-held technology for adaptation for bioproduct sensing in the Mars Ecopoiesis Test Bed is micro-manufactured MS. This is not current off-the-shelf technology but will be the technology of the time frame in which launching and operating the Mars Ecopoiesis Test Bed are expected to occur. Impressive recent advances in the miniaturization of MS point to MS as the most promising analytical technique. We refer specifically to the progress being made by an active research group led by L. F. Velásquez-García at MIT in which the development of an integrated hand-held micro-manufactured MS system, including sampling [Hill et al., 2014), ionization source [Dong et al, 2015; Chen et al., 2011], quadrupole separator and event detector and even an on-chip vacuum pump are presently becoming reality. Fortunately, the smaller the chip, the less vacuum required. One of the obvious drawbacks of MS is the requirement for high vacuum; however, with current advancements allowing ionization of gases in in the 0.1 mbar range [Fomani et al., 2014] it is quite possible that, within a decade, the Mars atmosphere itself could function as the vacuum environment for MS so that a postage-stamp-sized, multichip analyzer could simply be submerged, fully open, into the Mars atmosphere, and therefore the atmosphere of the Ecopoiesis Test Bed, for the unambiguous identification of all vapor-phase constituents. Therefore the best hand-held technology for adaptation for bioproduct sensing in the Mars Ecopoiesis Test Bed is micro-manufactured mass spectrometry. These tiny systems are suitable for mass measurements 1-400 Da and thereby cover the full range of biological volatiles that can be expected. The mass resolution that may be lost due to miniaturization is not expected to interfere with the interpretation of mass spectra [Taylor and France, 2009]. Therefore it was decided that Techshot will plan to collaborate, to the extent possible, with developers of microminiaturized MS on a parallel track to adapt this new and promising technology to bioproduct sensing on Mars. Parallel tracking of such development is also totally consistent with NIAC goals, which include the blending of future technologies for future research. The following concept is therefore considered:

Sampling. The pressure of the vapor phase to be sampled is already at 10 mbar or below, so the sampling step could be simplified to a passive microfluidic orifice sampler [Hill et al., 2014] or nanofilter, depending on vacuum requirements [Blain et al., 2009].
Ionization. The gas molecules could also be ionized at the Mars ambient pressure or slightly below [Fomani et al., 2014] using micro-manufactured field-emission technology [Dong et al, 2015; Chen et al., 2011; Blain et al., 2005].
Acceleration. The energy requirement is minimized by the short flight distances required, and all features could be operated using a few mW of power at a few volts.
Separation. Micro-manufactured (<1 cm length) quadrupole design [Taylor and France, 2009] or ion-trap arrays [Blain et al., 2005] can result in sufficiently high resolution even in a small space.
Detection. Detecting ion traps can be spaced a few micrometers apart providing high resolution in micro-space, and quadrupole separators may require only a single semiconductor element to record events or ion current. Their signals could be digitally converted on the same chip.

A block diagram of this concept is shown in Figure 20 – micro mass spectrometer schematic. By the time of initiation of phase 2 of this project

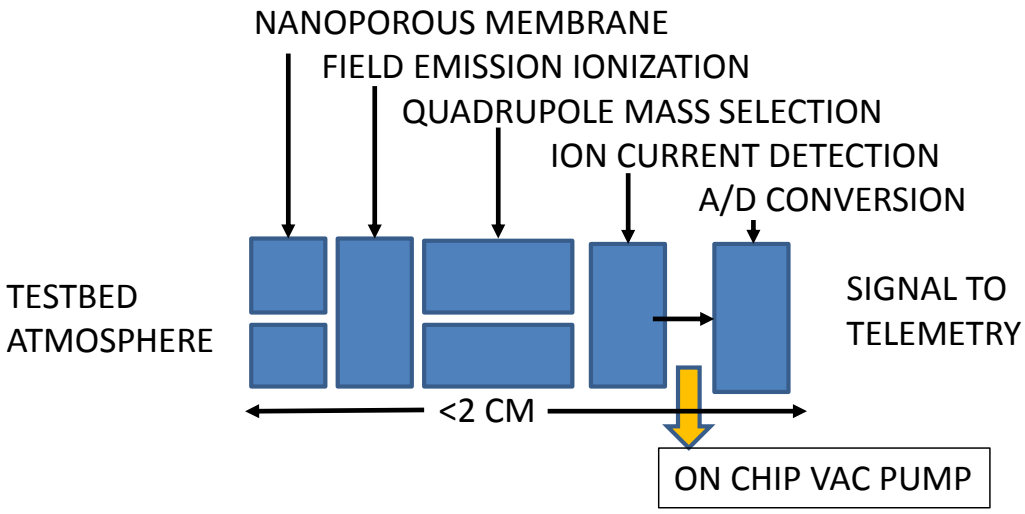

Figure 20. Schematic diagram of micro mass spectrometer architecture. Molecules from the test bed atmosphere enter through the nanoporous membrane into the field emission ionization stage, where DC and RF potentials drive them through the quadrupole electrode array where they are selected to exit to the detection plane. A microfabricated on-chip vacuum pump is likely to be adequate in the <10 mbar atmosphere. A miniature power supply, which may not need to generate more than 10 v, will cable power from the avionics unit to the mass spectrometer. Digital output signals will return using the same cable harness.

Conclusions

The objectives of this project, (1) Model and test the availability of liquid water in Techshot's Mars simulator facility, (2) Identify current candidate pioneer organisms for testing and initiate a selection program, (3) Create a mechanical and electronic design concept for Mars surface shallow penetrator with planetary protection, and (4) Identify electronic biological activity tests, were fulfilled by the completion of the Phase-1 research described in this final report. All of the outcomes point to the feasibility of successful Phase 2 research in which a functioning high-fidelity prototype of the Mars Ecopoiesis Test Bed can be designed, fabricated and tested under accurately simulated Mars-surface conditions prior to manifesting on a post-2020 planetary rover mission.

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http://www.nasa.gov/feature/planting-an-ecosystem-on-mars

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