# PHASE II FINAL PROGRESS REPORT

## July 6, 2006

# *ROBOTIC LUNAR ECOPOIESIS TEST BED*

**USRA Research Subcontract 07605-003-026**
**NASA Prime Contract NAS5-03110**

**Presented to**
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# Introduction

SHOT is pleased to present this year-end progress report to NIAC on the "Robotic Lunar Ecopoiesis Test Bed". This report is written in compliance with the subcontract between USRA and SHOT, Inc. signed August 6, 2004 and modified July 25, 2005.

The long-term concept is to let a living ecosystem create itself in an engineered enclosure on the moon under controlled Mars-like conditions. Under robotic control a community of organisms creates its own environment that is no longer hostile to living things. The Ecopoiesis Test Bed is an architecture that causes the environment to evolve on its own, starting with water and nitrogen and spores or inactive cells of appropriate prokaryotes, seeds, and eggs of organisms that eventually occupy the test-bed module. Experimental ecopoiesis is a new field, so experiments that were designed in Phase I were begun in the laboratory in Phase II and are to evolve to ISS or another orbiting carrier before a lunar module is to be considered. A gradual, stepwise multi-year approach was proposed, in which Phase I was a feasibility study, Phase II consisted of laboratory experiments and space flight planning, and Phase III is expected to be a multi-institution undertaking of indefinite duration culminating with a robotic lunar ecopoiesis laboratory. The originally proposed concept is sketched in Figure 1. Progress during the entire Phase II research period is described in this report.

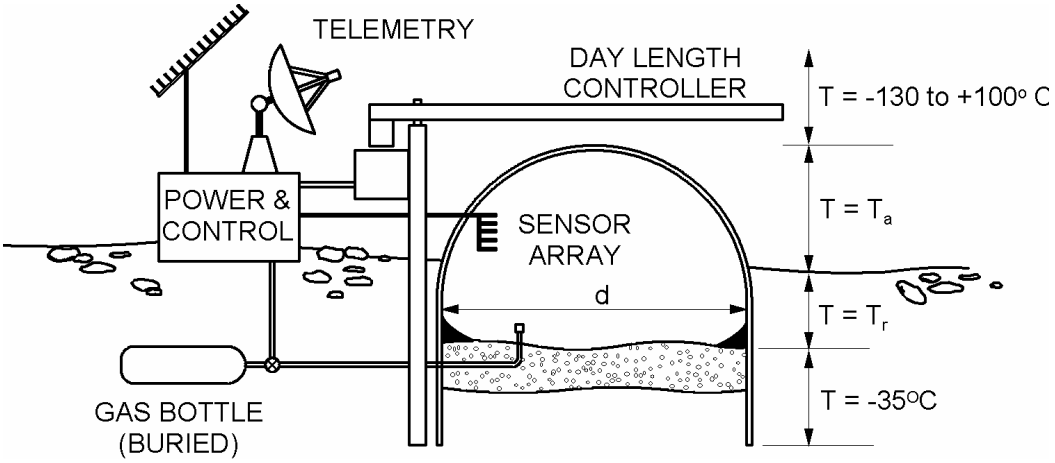



**Figure 1 – Original artistic concept of a robotic lunar ecopoiesis test bed. The long-range goal of the proposed program, showing positioning of the in situ polymerized inflated dome to take advantage of lunar thermal and gravitational characteristics.**

The report will follow the following outline specified by NIAC in the above-mentioned contract, which requires the following four elements:

1. All of the activities performed under the Phase II contract
2. A complete technical description of the concept and its operational principles
3. Identification of enabling technologies
4. Plan for future technical development of the concept

Also requested are "Copies of any briefings, presentations or professional society technical papers pertaining to the proposed Phase II area of study". These items are collated as appendices to this report.

The four objectives designated in the workscope, as described in the proposal and contract are

Objective 1

*Engineering component of the Architecture. Build ecopoiesis chamber for experiments at 1g.* A low-temperature freezer at 1 atm will be built, or acquired, and it will contain a transparent low-pressure 10L chamber based on the design derived from Phase I, objective 2. Follow-on support for this objective will be sought in the form of designing similar equipment for sales into customer laboratories.

Objective 2

*Experimental science component of the architecture. Run tests using communities selected in Phase I.* A multi-institutional biological research team will be assembled for the acquisition and preparation of a test community of organisms for inoculation into the "regolith" component of the ecopoiesis chamber built under Phase II objective1. After a limited number of experiments during Phase II, follow-on support will be sought through federal agency funding such as NASA's Office of Space Science (OSS "NRA mechanism") or Astrobiology Institute.

Objective 3

*Large-scale science component of the architecture. Build a community of prototype ecopoiesis-chamber users and produce designs for future experiments.* To further the widespread performance of feasibility experiments on Earth and on ISS using SHOT's existing variable-gravity space centrifuge technology, a low-volume (80 ml) chamber design will be completed. Concurrent and follow-on funding for the actual construction of such chambers will be sought through Federal SBIR programs and NASA's Office of Educational Affairs.

Objective 4

*Future science components of the architecture. Refine requirements for extraterrestrial test beds.* Plan tests at 1 g. Enlarge the participating community by creating an organized structure in the form of a distributed ecopoiesis research "center" under an existing relevant umbrella.

Using the outline specified above, progress is reported on each of these objectives. Objectives 1 and 2 were stressed during year 1, and all objectives were addressed as planned during the two years of the project. The original terminology of the four Objectives is preserved in the above paragraphs, although terminology has changed owing mainly to government re-organizations.

# Work Period

August 2004 to July 2006 (23 months).

# 1. All of the activities performed under the Phase II contract

## 1.1 Objective 1. Laboratory Test Bed

This objective occupied most of the effort during the first several months of the project. The Laboratory Test Bed became operational on schedule with all functions satisfactory and most of them automated. There was a lengthy delay in obtaining the "AM0" optical filter which is required for the simulation of the solar spectrum beyond the Earth's atmosphere. Its delivery marked the final capital purchase and the completion of the Test Bed in May 2005. The following paragraphs summarize key events in the development of the Laboratory Test Bed (which, for a time, was named "MARS-LTB").

Components that had to be purchased were selected and priced. The purchase list was submitted to the sponsor for approval, and all items were purchased. Table 1 is a version of the components list. The most expensive item was the solar illumination simulator.

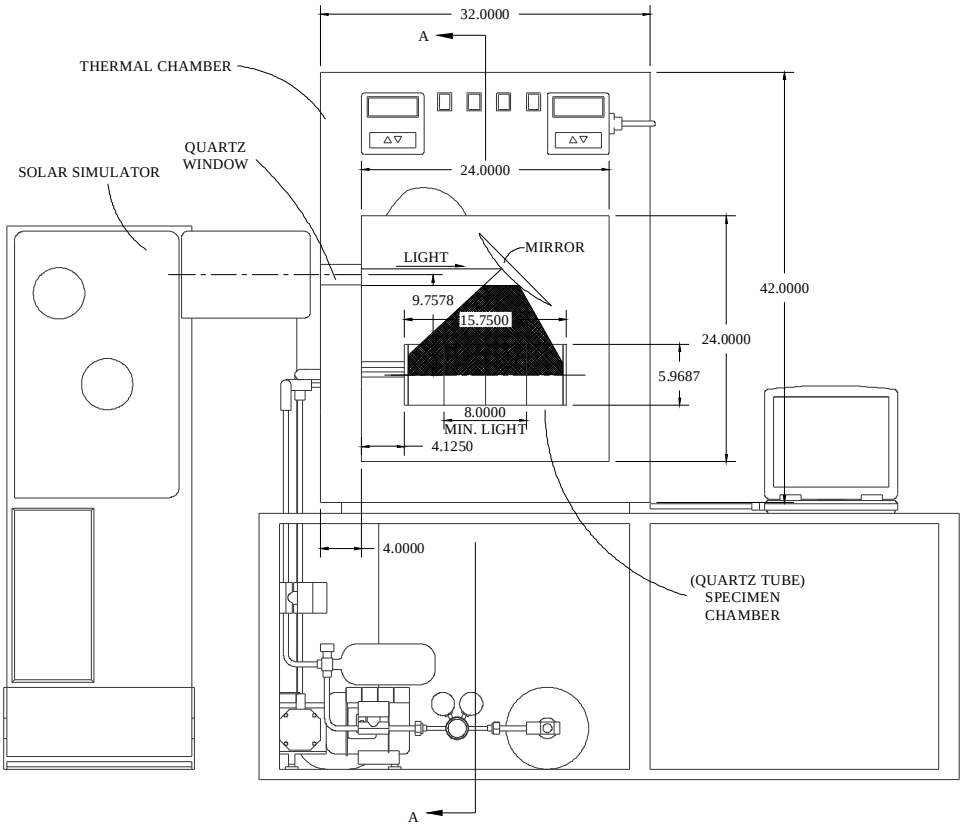
**Table 1. Capital purchase items list for the Laboratory Test Bed (sales tax not included)**

| Item | Vendor | Qty | Budgeted Price (Total) | Price (Each) |
|---|---|---|---|---|
| **1.5KW Solar Simulator System** | Sciencetech | 1 | $ 22,260.00 | $ 23,812.00 |
| **Mars Regolith Simulant** | NASA-JSC | 1 | $ 55.00 | $ - |
| **Gas bottle with custom mixture** | | 1 | $ 411.00 | $ 346.25 |
| **Fused silica cylinder** | Quartz Scientific, Inc. | 2 | $ 1,020.00 | $ 510.00 |
| **Freezer-Heater combination** | Associated Environmental Systems | 1 | $ 12,200.00 | $ 9,260.00 |
| **Vacuum pump 2581D-24** | Welch | 1 | $ 1,407.00 | $ 1,468.20 |
| **2-stage regulator** | Victor High Purity and Instrumentation | 1 | $ 267.00 | $ 267.00 |
| **Notebook computer** | Dell | 1 | $ 3,224.00 | $ 200.00 |
| **LN2 (bulk, setup, rental, install)** | Welding & Therapy Service, Inc. | 1 | $ 450.00 | $ 10,557.00 |
| **Table, test** | Process Equipment | 1 | $ 2,150.00 | $ 100.00 |
| **Pressure Control Valves (3)** | ASCO Red Hat | 3 | $ 600.00 | $ 600.00 |
| **Low-pressure gas storage tank** | Leisurepro | 1 | $ 280.00 | $ - |
| **Aluminum Coated Pyrex Mirror** | Esco Products | 1 | $ - | $ 395.00 |
| | **TOTALS:** | | **$ 44,324.00** | **$ 47,515.45** |

A description of the development of each of the major subsystems (solar simulator, thermal cabinet, sample container ("Mars jar") is given in the following paragraphs.

### 1.1.1 Solar Simulator

When final vendor selection was made for the solar simulator (Sciencetech) it was customized by the vendor so that the light beam line passes into the chamber through a 4" x 4" (10 x 10 cm) opening and is expanded by a front-surface mirror inside the chamber to illuminate the quartz Mars jar. The vendor provided a set of drawings for this purpose, and these are consistent with the ray-tracing diagrams determined by SHOT and shown in Figure 2. Trade-offs were considered for the control of light intensity for high-fidelity of simulation and in terms of biological requirements. The final configuration of the quartz window in the thermal cabinet was two 4" x 4" quartz panels with 3" of dry air sealed between them (with silica gel desiccant) and a stainless steel housing that penetrated the insulation of the thermal cabinet.



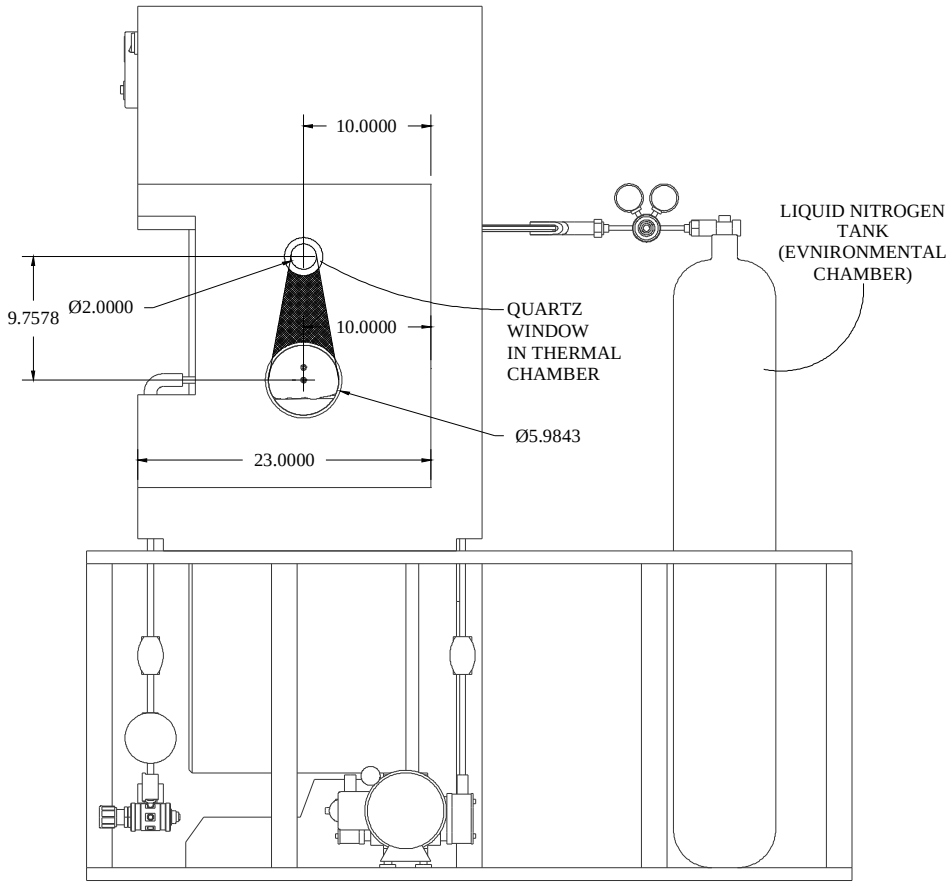



**Figure 2. Internal light path for illumination of the MARS vessel though a 4" diameter window (the final configuration was a 4" square – Figure 5). Top: Front elevation. Bottom: Side elevation.**

Figure 3 is a photograph of the installed solar simulator, the most expensive component of the Test Bed, and Figure 4 shows the AM0 filter in place.

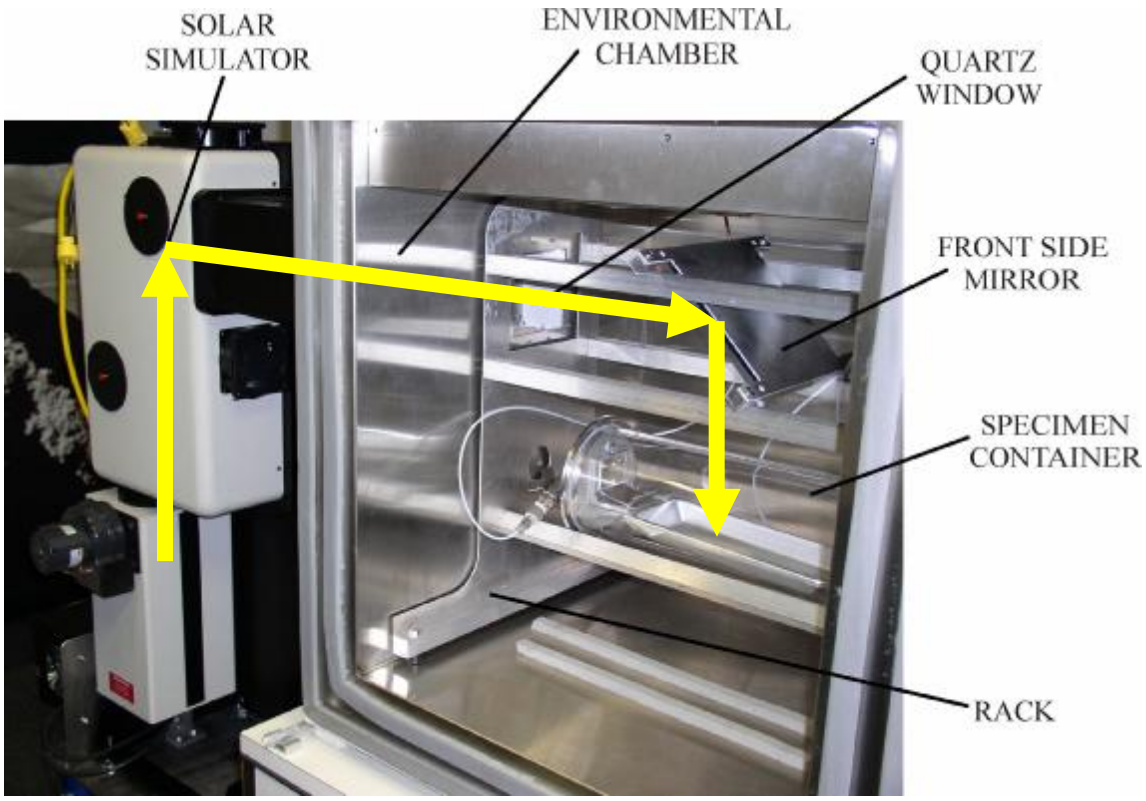



**Figure 3. Photograph showing the installation and use of the solar simulator. The yellow arrows indicate the path of the light from the bulb to the specimen.**

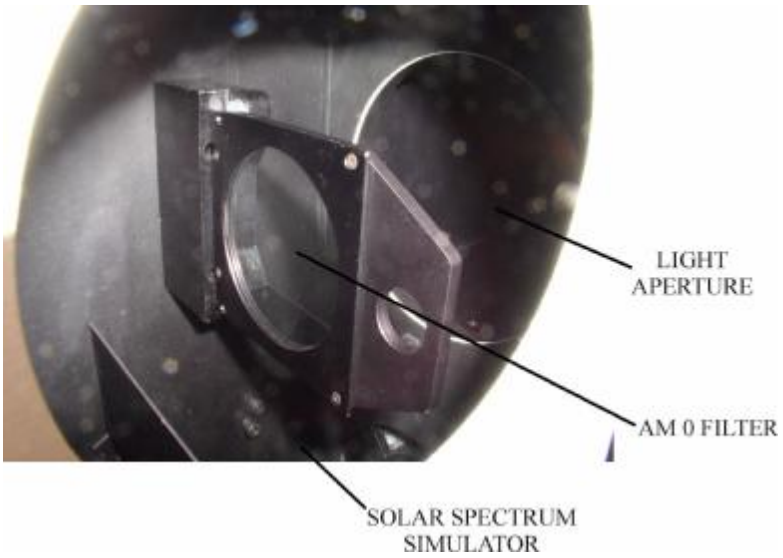



**Figure 4. Photograph of the AM0 filter installed in the solar simulator.**

The light path penetrates the thermal enclosure (Figure 5) and is reflected downward onto the specimen (as seen in Figure 2 & 3). The window is double-paned with desiccant to minimize moisture collection. The solar simulator was designed with wheels such that it can be removed and this window be used as an observation port when necessary.

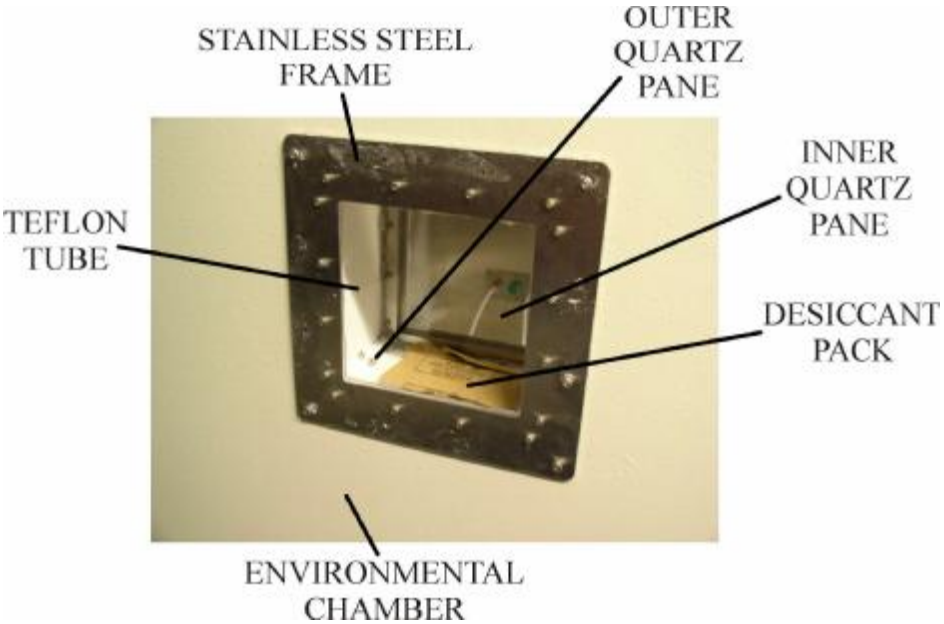



**Figure 5. Window in thermal chamber for light path.**

There is a required procedure for starting the solar simulator. When the Xenon lamp is cool, the power supply for it must be set to about 1100W and tuned back to 1000W once it has lit. The power supply sends three voltage spikes (arcs) to the lamp in an effort to start it. It is noted that the lamp often does not light on the first attempt. To automate

this lighting process, SHOT engineers had two possible solutions. The first was to splice into the electronics of the power supply and the second was to mechanically override the user controls. Due to the high power and unfamiliar electronics, the latter was found to have the least number of technical risks. Two miniature servos are used to interface with the user controls on the power supply (Figure 6). These are driven by a SHOT-designed control board and commanded from a PC-based application (Figure 7). The PC's Graphical User Interface (GUI) was custom designed by SHOT. This GUI is helpful to the operator because it has a visible clock and informs where it is in the timeline. Solar cycles and power settings are programmable by editing a simple timeline "text" file on the PC.

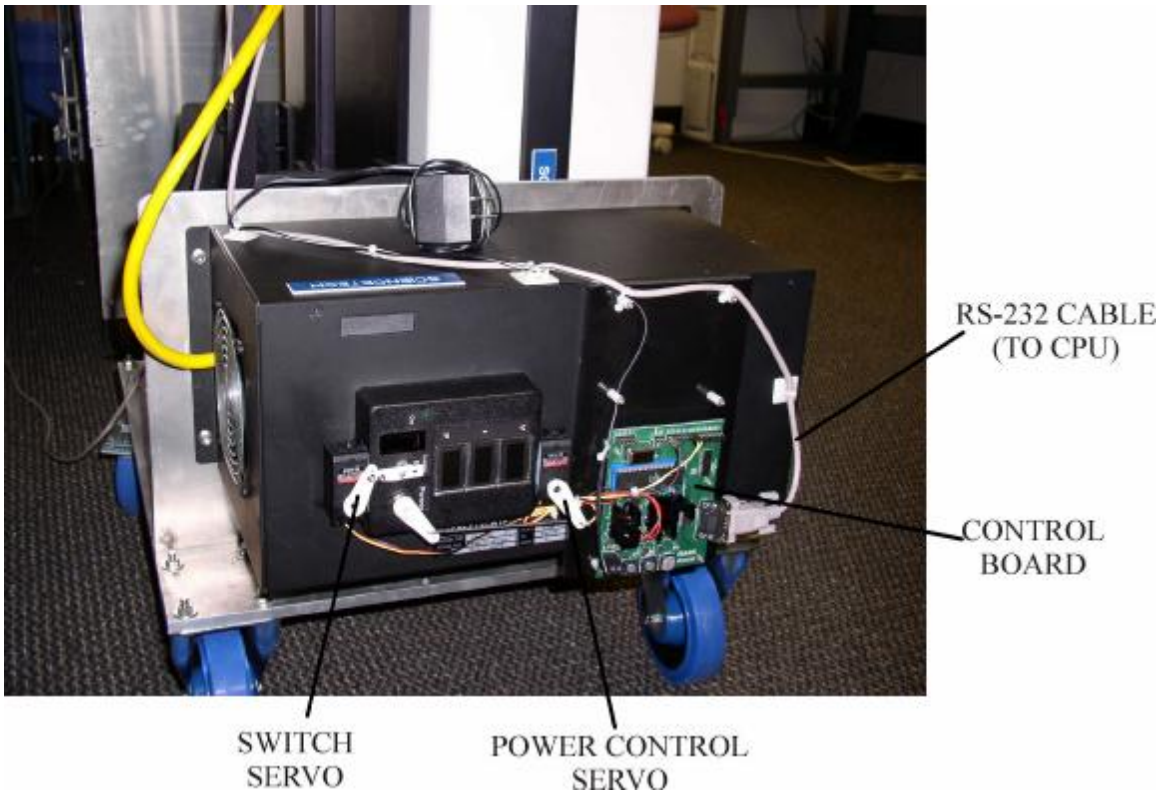



**Figure 6. Automation of the MARS-Laboratory Text Bed light source power supply using electromechanical actuators.**

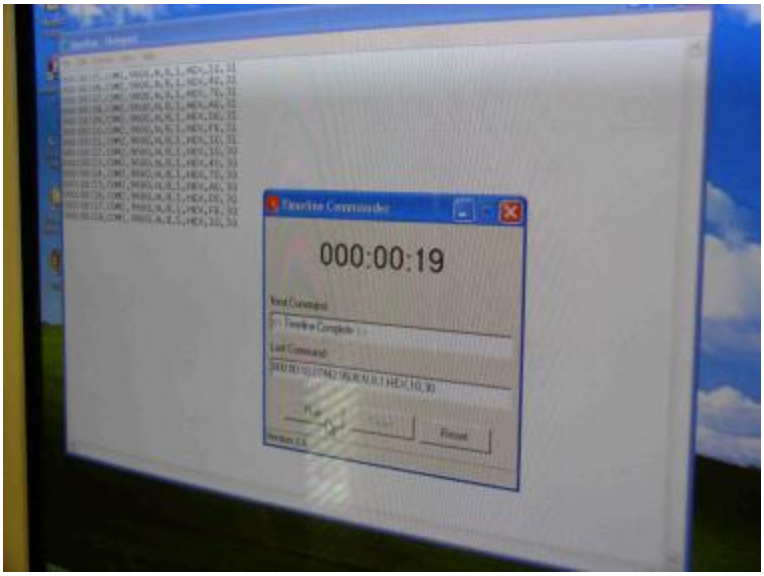



**Figure 7. Graphical User Interface (GUI) developed by SHOT for controlling the solar simulator.**

The amount of photosynthetically active radiation (PAR) at the regolith, inside the sample chamber was measured and found to be 1100 μmoles/$m^2$-s, corresponding to 237 W/$m^2$, which is in agreement with that used by Dr. Andrew Schuerger, namely 242 W/$m^2$ derived from Mars atmospheric models. This also translates closely to the 590 W/$m^2$ total irradiance at the Martian surface. The flux outside the light beam inside the chamber was found to be 12 μmoles/$m^2$-s or about 1% of that in the direct light. In some tests it is desirable to have some samples maintained in indirect light only. It is likely that the UV-B and UV-C components are attenuated further yet.

The advertised spectrum of the xenon arc lamp with its AM0 filter is shown in Figure 8. The xenon emission lines at longer visible wavelengths are somewhat suppressed by the AM0 filter.

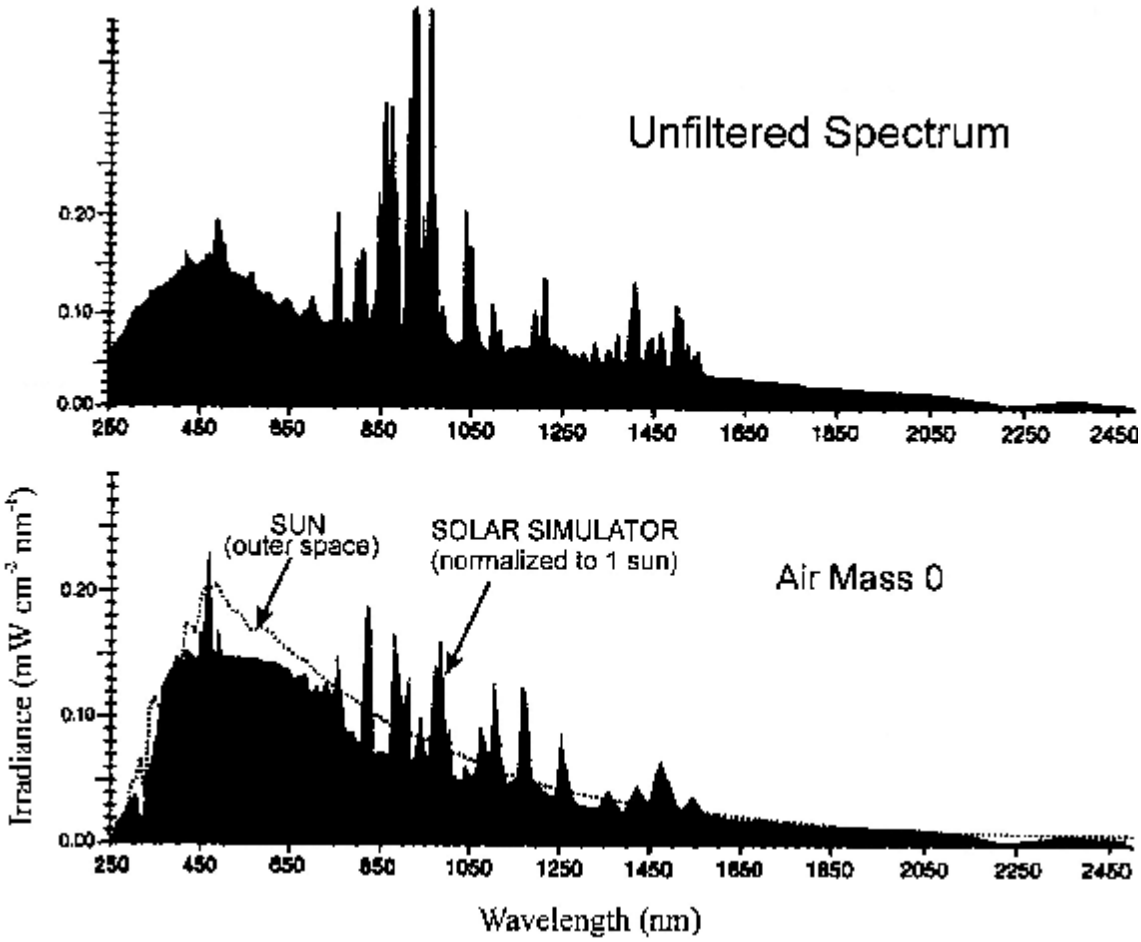



**Figure 8. Top: Unfiltered output of xenon arc lamp. Bottom: Spectrum with AM0 filter. Source: Sciencetech Inc.**

### 1.1.2 Thermal Cabinet

A trade study of Laboratory Test Bed refrigeration systems was undertaken. SHOT engineers performed thermal analysis and product evaluations on the three following alternatives:

1. Cryogenic heat transfer block

This concept would be implemented by SHOT engineers and would consist of a solid metal block thermally regulated by heat-transfer coils bearing liquid nitrogen. This alternative was found to be about 1.5 to 2.0 times as efficient as commercial products but would cost more to implement. This alternative was eliminated on the basis of costs but could be taken seriously were the production of multiple units for sale to become a reality.

2. Ultralow compressor laboratory freezer

Upright and chest-type refrigeration-cycle freezers capable of reaching the required low temperatures are available (from Revco, for example). While these freezers are excellent for storage they do not tolerate daily freeze-defrost cycles (the compressors are not built

to tolerate such frequent and prolonged operation), and the prevention of water-frost build-up is extremely difficult.

3. Cryogenic thermal chamber (in original design)

The Phase I design specified a cryogenic thermal chamber that is a highly insulated cabinet 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. However, at steady state there is 8 $ft^3$ of gaseous nitrogen in the chamber, and this is flowing at a rate that depends on the controlled temperature level at the time. It is estimated that this flow rate could be up to 5 gal of $LN_2$ in a single hour at the highest cooling rate.

The final selection, which was ordered, has the specifications given in Table 2.

**Table 2. Liquid Nitrogen Cooled Environmental Test Chamber – ZBD-108 (Customized)**

| | |
|---|---|
| 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 |

18 Gauge cold rolled steel with two coats of textured epoxy paint
Watlow F4 Programmable Controller with RS232 communications
Dry Nitrogen Purge
Dimensions are given in the elevation view of Figure 9.

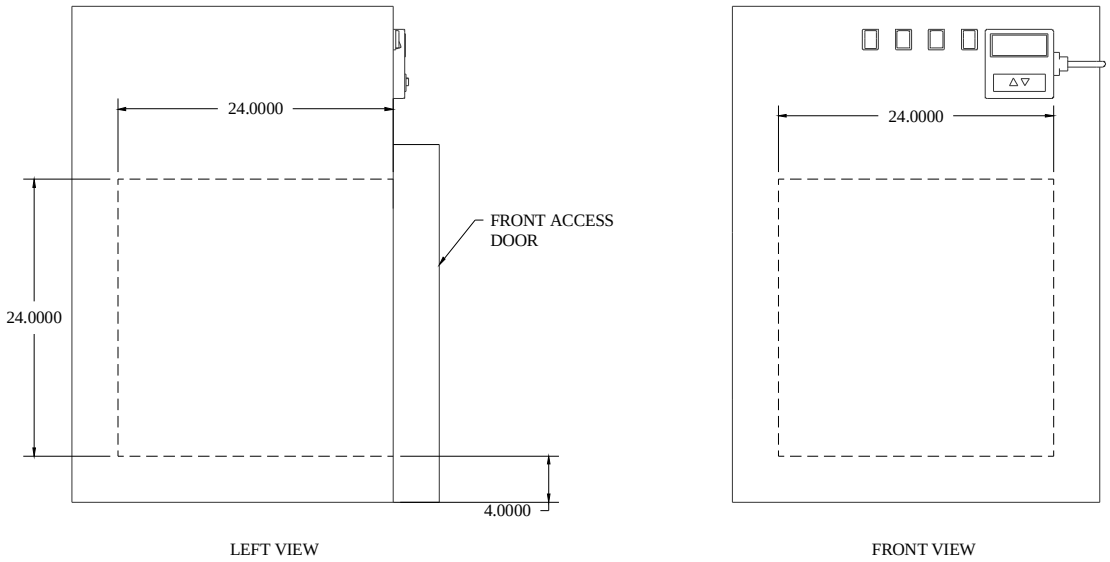



**Figure 9. Elevation views and dimensions of thermal chamber (inches).**

Test bed operations showed that a single Mars-sol cycle consumes 80-100 gallons of liquid nitrogen. This fills the chamber as gaseous nitrogen and is vented directly to the exterior of the building. A cryogenic safety briefing was presented to SHOT staff by the cryogenic contractor with whom liquefied gas requirements were also estimated.

Subsequent to the trade study on Laboratory Test Bed refrigeration systems undertaken during the first reporting period, the cryogenic thermal chamber (in original design) was ordered, and delivery occurred in early December 2004, as scheduled. The thermal chamber was installed and tested. In order to meet liquid nitrogen requirements SHOT leased a 500-gal vacuum-insulated cryogenic tank, which has been installed at the exterior of the SHOT building on a new concrete pad that was poured for this purpose. This will be filled monthly at a rental-plus-supply cost of about $500 per month. A vacuum-insulated pipe carries liquid nitrogen through the building wall into the dedicated simulator laboratory. Installation was completed in December, 2004, and the system was successfully tested in January, 2005. Figure 10 depicts the installation of the external tank.

**Figure 10. Left: Delivery of the leased 500-gal $LN_2$ tank. Right: Installation of the tank at the exterior wall of the dedicated MARS-LTB simulation laboratory. Subsequent to installation a safety fence with a locked gate was installed around the tank.**

After installation and testing of the $LN_2$ system and the thermal chamber (shown in Figure 11), the planned series of initial tests was begun. The chamber was manually programmed for a Mars daytime temperature and a night-time temperature, and the impact of an internal heat load equivalent to that of the solar illuminator was measured. The chamber readily met the requirements consisting of reaching a lower temperature of -135°C and an upper temperature of +26°C. This represents the capability for a high-fidelity simulation of the Mars-sol temperature cycle.

Framework construction was completed, and a panel for penetrating the building wall from the exterior was installed, and all penetrating facilities ($LN_2$ lines, fan opening, exhaust line) were installed and operated (also seen in Figure 11).

**Figure 11. Thermal chamber installed on reinforced support and connected to $LN_2$ inlet from the outdoor tank and exhaust to the outdoors. The disk-shaped object at the left is the safety exhaust fan that is actuated by the low-$O_2$ sensor that has been installed and tested. The fan can also be switched on manually for room air control.**

Software was developed to exploit the existing Watlow F4 Programmable Controller with RS232 communications provided with the thermal environmental chamber. The Watlow controller was tested and found to be satisfactory.

Safety requirements were always met. The manual supplied by the vendor of the environmental chamber contains safety specifications that SHOT implemented prior to running the above tests. The excess nitrogen gas after use for cooling is discharged to the exterior of the building via an exhaust pipe that is a component of the thermal environment chamber. Thus the closed chamber and its exhaust have no contact with the laboratory air. A duct and louvered fan (also shown in Figure 11) were installed and linked to an alarms system. These will exhaust room air to the exterior for emergency exhaust of the laboratory that houses the facility. The fan is activated by a low-$O_2$ detector that also has an audible alarm. This detector system was installed and tested prior to performing the above tests.

Thermal cycling of the Test Bed according to the Mars daily cycle was accomplished by programming the Watlow F4 Controller (Figure 12.) that was provided as a standard component with the thermal chamber. This controller is programmed with a timeline that can include thermal ramping functions. The programming can be performed either on the user panel or with a GUI on the PC.

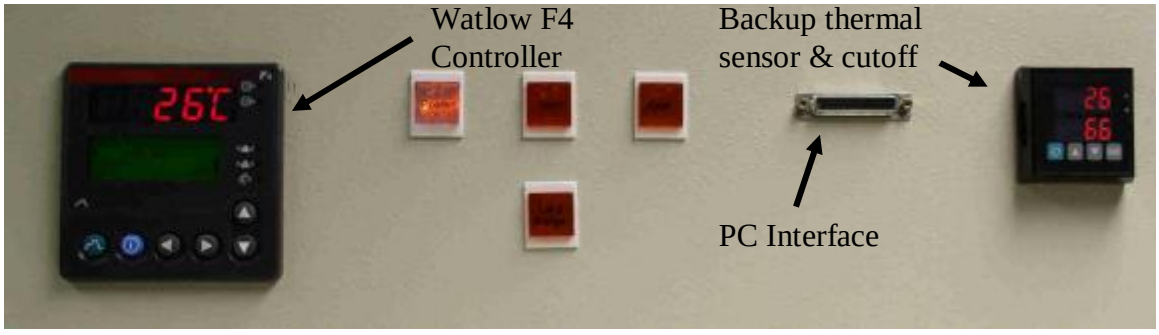



**Figure 12. Thermal chamber panel interfaces.**

Temperature programming was based on Mars daily cycles, as described by Carr (1996) (Figure 13). Although the control Unit will control the temperature of the test chamber down to -135°C economically on a daily basis, it would also be possible to follow at least one typical Martian daily profile, such as one of those shown in Figure 13 for the equator and mid-southern latitudes, where the minimum temperature could be as high as -80°C.

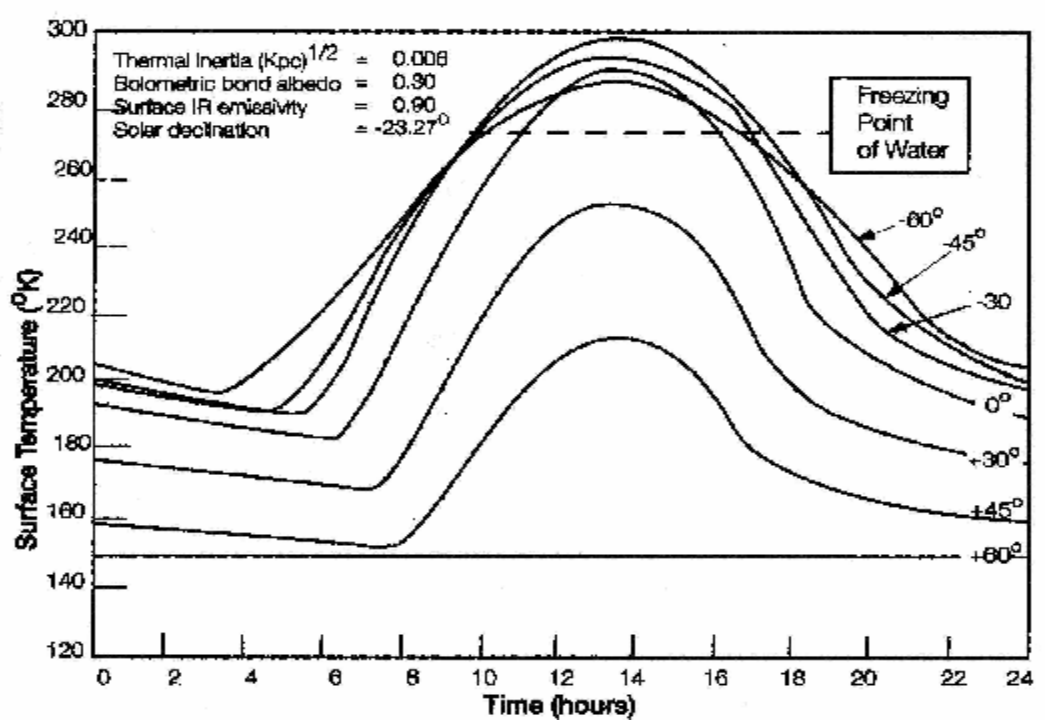



**Figure 13. Daily temperature profile at the surface of the Mars regolith at various latitudes. At a depth of 1 m the temperature is a steady -20°C [Carr, 1996].**

The chosen temperature cycle for early experiments is shown in Figure 14, in which points are plotted for every hour. Each point represents a set-point on the Watlow controller, so that each hour a new set-point causes the temperature to change until the set-point is reached at a rate that is also programmable in the Watlow controller. The steeply rising and steeply falling curves in Figure 14 show that the heating rate and cooling rate achievable were more than sufficient to meet the Mars-simulation requirements.

The chamber was operated at maximum heating and cooling rate with a heat source inside. The resulting heating curve, starting at -135°C, was found to be very steep, as shown in Figure 14, which also includes the target heating and cooling rates in the form of a Martian sol temperature profile at mid-latitude in summer (minimum -83°C, maximum +26°C). Likewise, the cooling curve, perhaps more critical, showed more than adequate cooling rate, as seen in Figure 14 – the steep curve on the right. It was concluded that, as predicted, the cooling and heating dynamics requirements of the MARS-Laboratory Test Bed could be easily met. Thus software can simulate the temperature profile of a Martian sol at any latitude at any season. Initial experiments utilized the profile shown in Figure 14.

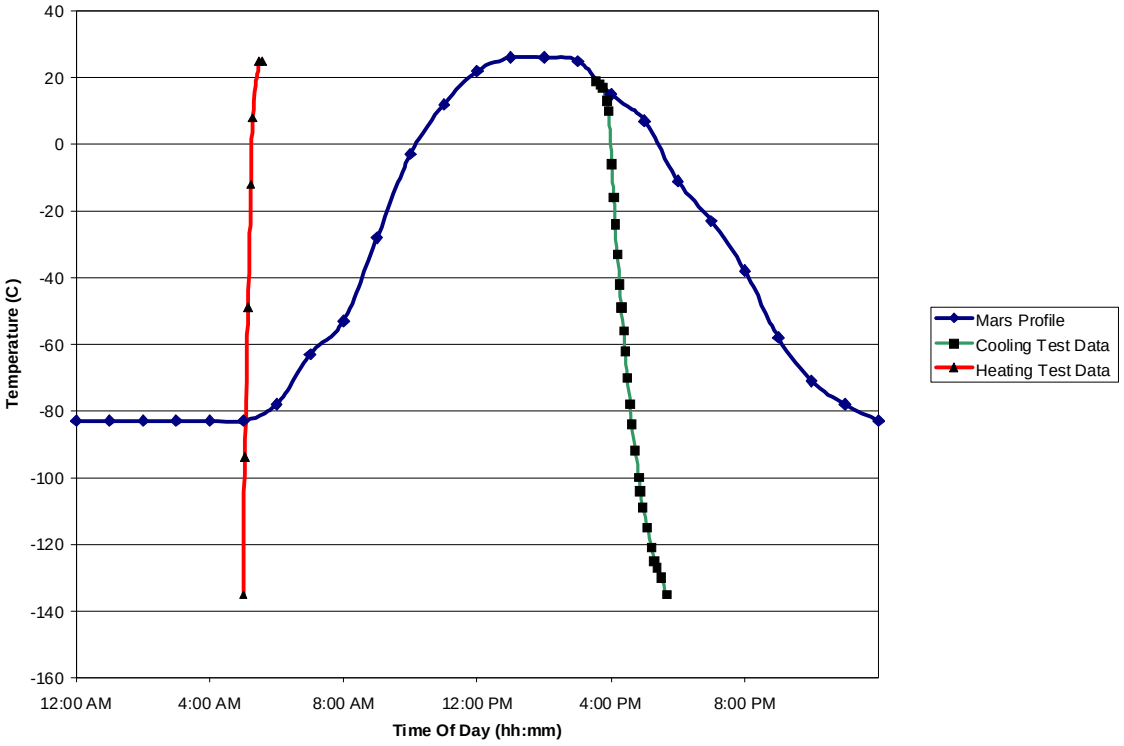



**Figure 14. Heating (left vertical line, triangles) and cooling (right vertical line, squares) curves for environmental chamber compared with a summer temperature profile of a Martian sol (diamonds) at mid-latitude.**

### 1.1.3 Atmosphere Control, Specimen Chamber

Two fused silica cylinders were purchased. These were fitted with aluminum or stainless steel end plates that were custom machined for the purpose. These are attached to the quartz cylinder so that the two end caps are pulled toward the flat rims of the cylinder and sealed with a flat low-temperature silicone rubber gasket at each end. The end caps include threaded holes for gas/vacuum fittings allowing the vacuum pump to draw in Mars-atmosphere gas and to hold pressure down to 10 mbar. A partially assembled view of the cylinder is shown in Figure 15. End-plates with airlocks and manipulators will not be used initially but are being designed for application during later stages of the project.

**Figure 15. Photograph of quartz cylinder fitted with end-plates, gaskets and inlet and outlet fittings and vacuum gauge. The cylinder is approximately 45 cm long and has a volume of approximately 5.6 l. In normal operation the vacuum gauge is outside the thermal cabinet.**

Through a series of valves and plumbing, the user regulates the pressure (Figure 16.) and Mars gas content (Figure 17.) within the specimen chamber. The thermal chamber has been modified to have an access panel for the gas tubing and sensor wires (Figure 18). Internal to the thermal chamber is a special rack which holds the specimen container and mirror in alignment (Figure 19,). The gas composition supplied by the vendor closely resembles that reported to be on the surface of Mars (Table 3), namely 2.760% $N_2$, 0.139% $O_2$, 1.615% Ar, balance $CO_2$ by analysis.

**Table 3. Composition of the Mars atmosphere at the planetary surface [C. McKay, 2004].**

| | | |
|---|---|---|
| **p** | Carbon Dioxide (CO2) | 95.3% |
| **p** | Nitrogen (N2) | 2.7% |
| **p** | Argon (Ar) | 1.6% |
| **p** | Water Vapor (H2O) | 0.03% - 0.1% (saturated in places) |
| **p** | Oxygen (O2) | 0.13% |
| **p** | Carbon Monoxide (CO) | 0.07% |

It has been customary, for ecopoiesis experiments, to operate the cylinder at 100 mbar rather than the actual 10-mbar Mars pressure. The end plates are not perfectly sealed at the lowest temperatures. At 100 mbar there is a leak rate of about 0.8 g/hour of $N_2$ entering the cylinder during continuous pumping. A once or twice hourly "puff" of about 1.0 g of Mars gas is used to replace this using a valve control within the controller/driver complex supplied with the thermal cabinet.

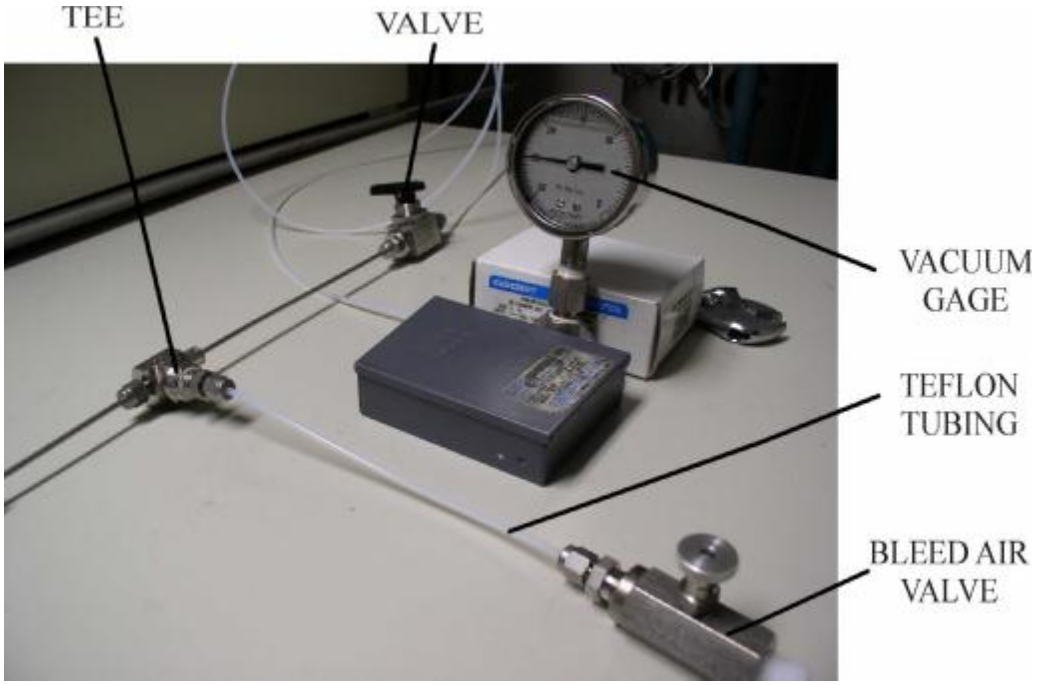



**Figure 16. Plumbing and valves for controlling pressure and gas content in specimen chamber.**

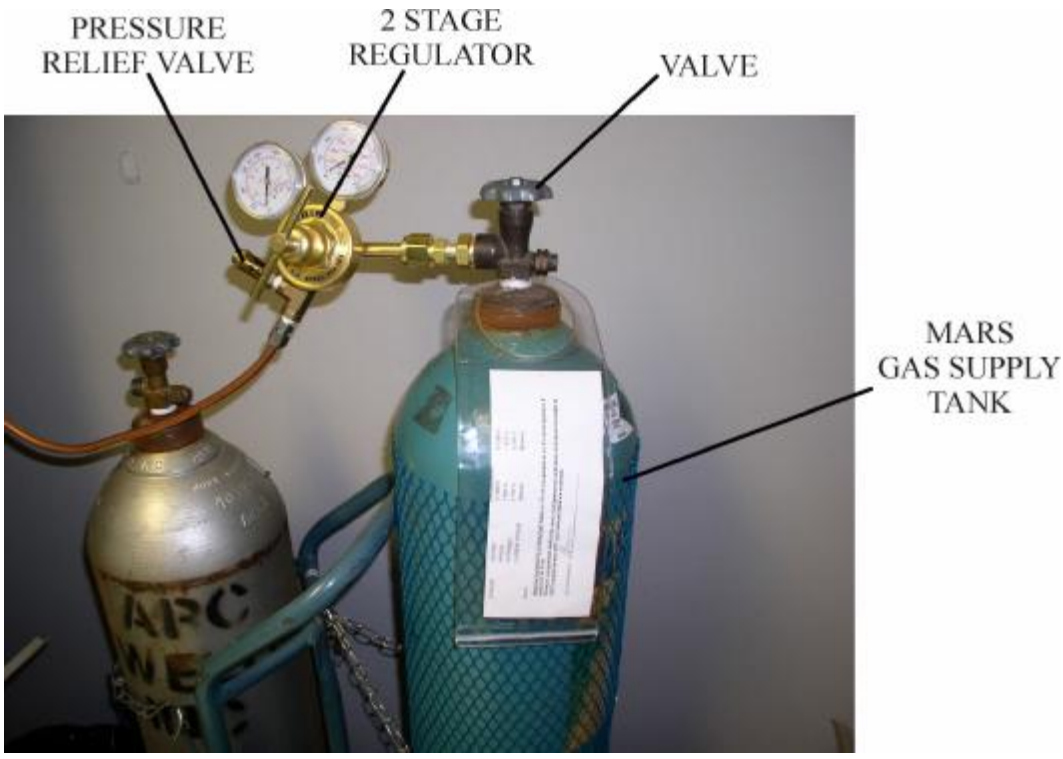



**Figure 17. Simulated Mars gas supply.**

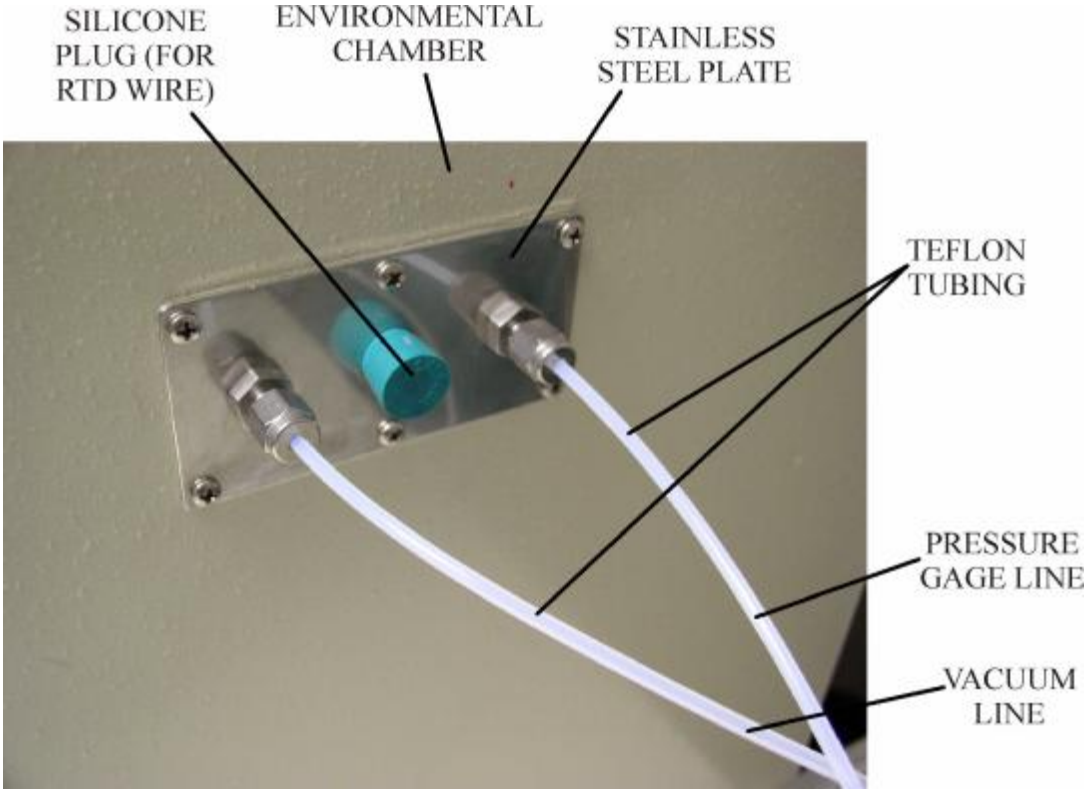



**Figure 18. Access panel for tubing and wires to penetrate the thermal chamber.**

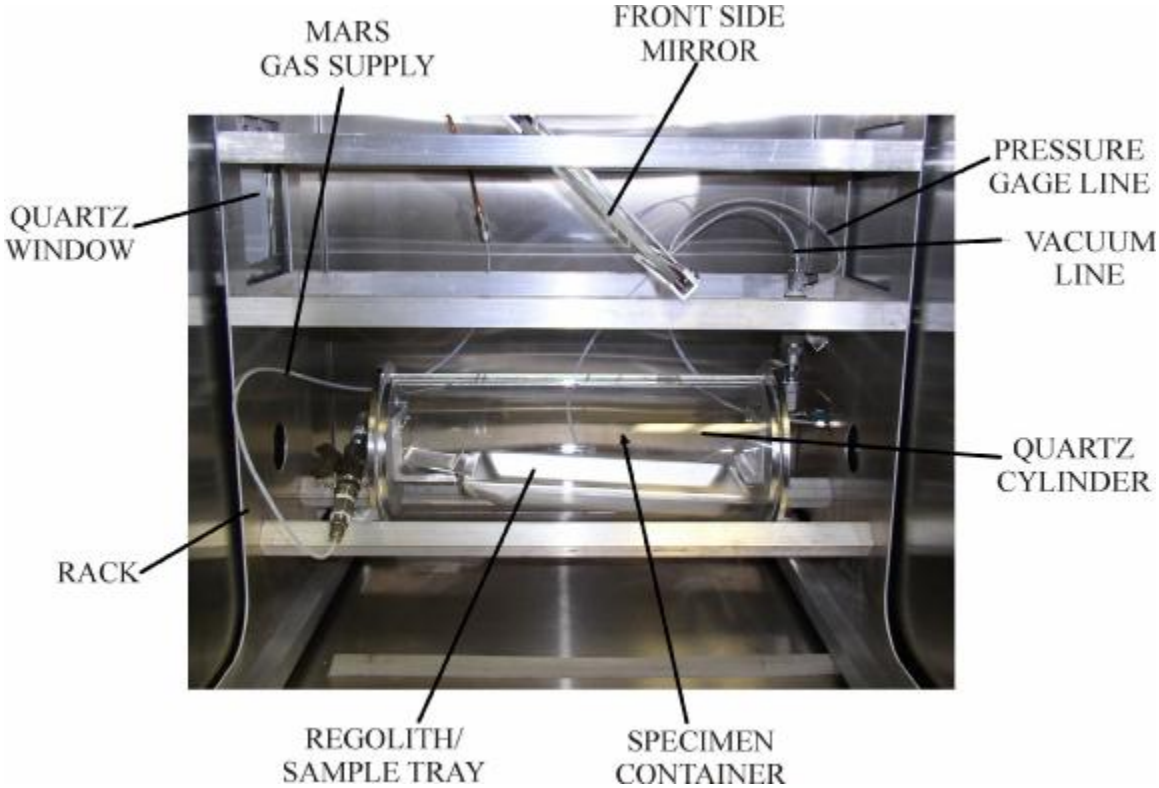



**Figure 19. Photograph of internal rack and plumbing.**

In the first protocol using the chamber, a thermal sensor was placed within the specimen regolith (Figure 20) and the data collected (Figure 21). This information was used to program the Watlow controller to control the cabinet temperature to be 12 – 14$^{o}$C below that of the regolith while the illuminator is on, since the regolith is heated by the absorption of the light.

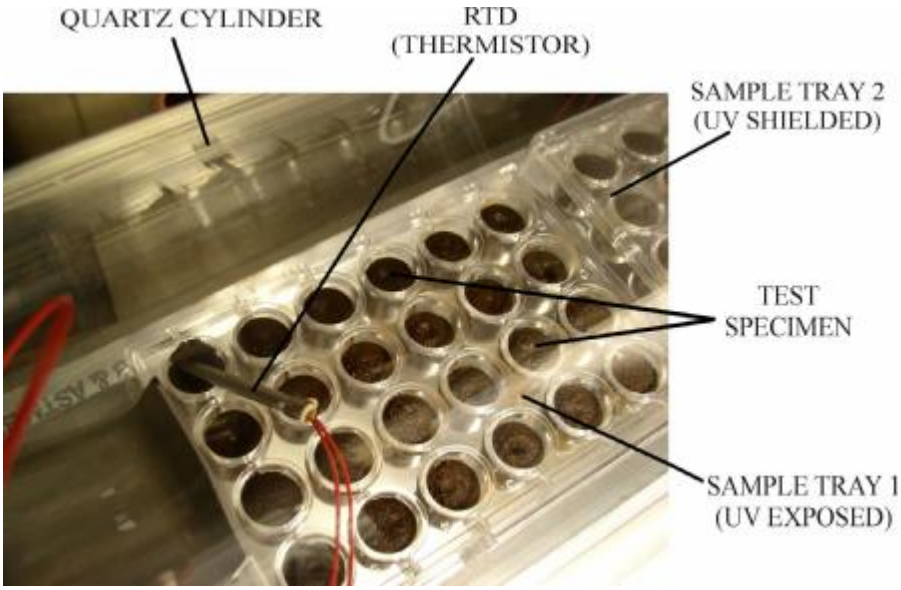



**Figure 20. Photograph of the specimen chamber loaded with (2) 24-well Titer plates. A RTD sensor is placed within the regolith to measure temperature during a protocol.**

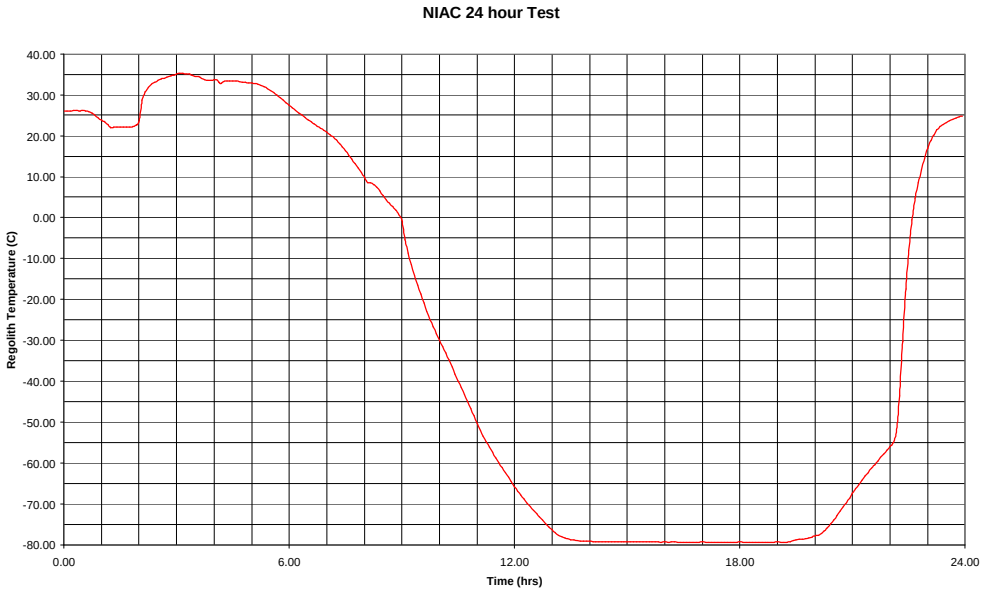



**Figure 21. A plot of the RTD temperature readings collected in the regolith during an initial protocol. This temperature does not exactly match the chamber profile due to heating of the sensor when the illuminator is on.**

### 1.1.4 System Integration

Control of the system was not fully integrated into a single graphical user interface (GUI). Temperature in the cabinet is monitored by the cabinet circuitry; temperature in the regolith is monitored using a H-P digital datalogger; and light on-off is used as a control in the illuminator GUI (Figure 7). All displays and gauges and the exterior of the system are monitored by a wide-field digital camera the images of which are updated every 0.5 h and displayed on the company web site www.shotscientific.com/webcam so that biological users can monitor progress from their own institutions, and SHOT engineers can view conditions in off hours. A result of this overall monitoring approach can be seen in Figure 22, which is a plot of the measured parameters vs. time in a 7-day experiment.

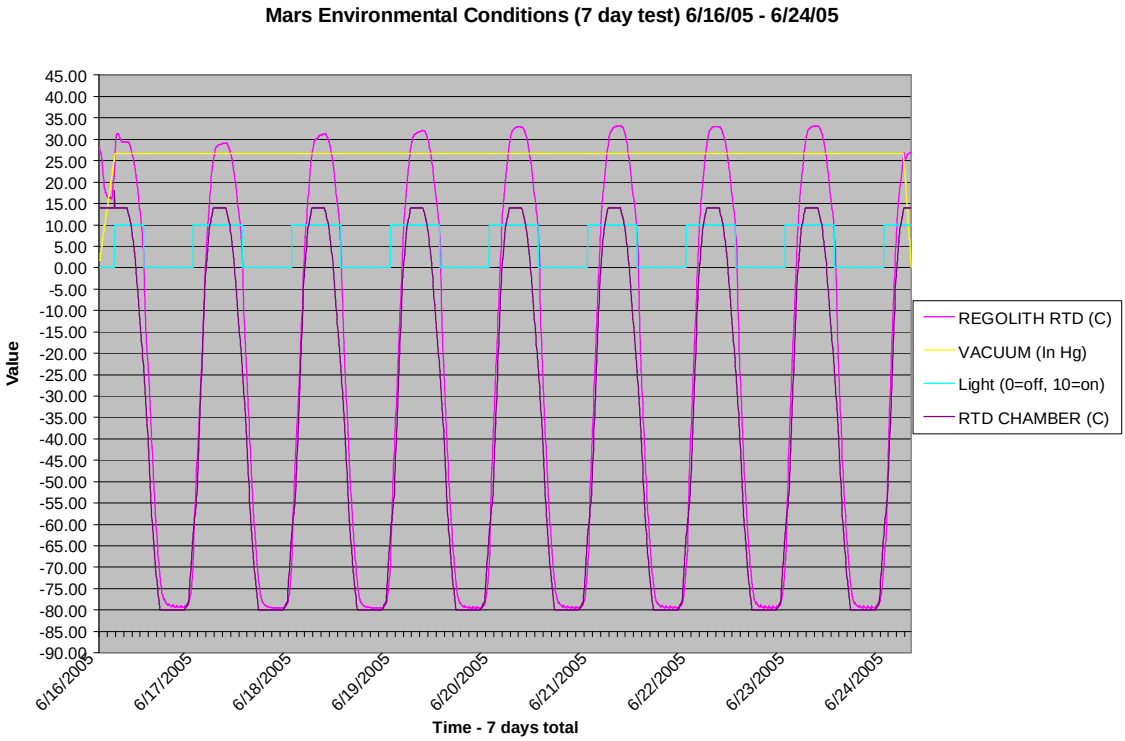



**Figure 22. Data record for a 7-day experiment. There are two thermal sensors (RTD), one is for thermal chamber control and the other is buried in the sample regolith. The temperature read by the sensor in the regolith is higher than that in the chamber due to heat absorbed from the light.**

Figure 23 is an example of the webcam display that can be viewed on the web site. The camera view allows reading of the vacuum gauge, cabinet temperature, sample temperature, time of day, time of run, and condition of the illuminator.


**Figure 23. View of the Laboratory Test Bed recorded by the web cam showing parameter reading that can be read on the internet on the half-hourly updates.**

### 1.1.5 Test Bed Venue

Design parameters were established for the Laboratory Test Bed venue – a dedicated laboratory favorably situated within SHOT's facility. The venue is in an attractive environment easily accessible to visitors that occupies a ground-floor location that conveniently facilitates liquid nitrogen transfer to the Test Bed using outdoor tanks that are readily accessible to the liquid nitrogen vendor. Portions of the venue are visible in Figure 23.

## 1.2 Objective 2. Experimental science

Experimental ecopoiesis research was conducted using the Laboratory Test Bed mainly by SHOT's two external investigators, Dr. David Thomas and Dr. Penelope Boston.

### 1.2.1 Preliminary Research and Test Organism Selection

The first test conditions were identified in collaboration with Dr. David Thomas. It was determined that a cautious approach would be used, and a certain amount of Mars-environment fidelity would be initially reduced in rigor to improve the possibility that the test cyanobacteria would adapt to more slowly changing conditions. Brief personal meetings were held with Dr. Larry Kuznetz and Dr. Chris McKay. In discussions with the latter concerning guidance on the range of desired and acceptable physical parameters for the tests, Dr. McKay suggested that a minimum night-time temperature of -80$^{o}$C should be acceptable if it were to be impossible or difficult to achieve temperatures below

-100°C as planned. Upon exploring the $CO_2$ phase diagram, it was determined that the formation of $CO_2$ frost from the Martian atmosphere simulant would occur at about -130°C, which, as can be seen in Figure 13, occurs only at polar latitudes. For most biology it was suggested that a lowest temperature of -40°C would be adequate. Thus the SHOT engineering team chose -80°C as a reasonable target and tested this as soon as the Laboratory Test Bed was operative.

Dr. Thomas pointed out that some researchers have hypothesized that life may have arisen on Mars at roughly the same time that it did on earth and that the search for high $CO_2$ tolerant microorganisms is relevant to life on the early earth as well as on early Mars. Ironically, the evolution of an antioxidant system is also considered critical. His laboratory studies related to this project therefore focused on cyanobacterial species and strains that tolerate very high (100%) $CO_2$ levels and are capable of deactivating reactive oxygen species. Those experiments concentrated on cyanobacteria: ***Anabaena, Plectonema boryanum, Synechococcus*** PCC7942, and ***Synechocystis*** 6803 ***Chroococcidiopsis sp.***. Electron transport experiments in increasing atmospheric $CO_2$ were also performed in his laboratory at Lyon College, as were chlorophyll fluorescence experiments under the same conditions. Species capable of surviving very dry conditions are vital to the ecopoiesis project, and rRNA sequence identification of denitrifying bacteria isolated from the Atacama Desert of Chile was undertaken, at least six isolates were acquired, and three were classified by other laboratories during the project. Student work on the project in the summer of 2005 cointributed significantly, and three students worked in Dr. Thomas' laboratory and participated in visits to SHOT at appropriate times.

Desert varnish, cave, and lavatube organisms were selected for study in the Laboratory Test Bed by Dr. Boston. The organisms listed in Table 4 below were selected from the culture collection based on their metabolic capabilities of both direct and indirect relevance to ecopoiesis goals. Their abilities to produce mineral precipitates of possible use in ecopoiesis or to accelerate the breakdown of bedrock are of direct utility. Breakdown of rock materials to enhance soil production and liberation of nutrients is a capability of great interest. Ability to precipitate manganese, iron or other oxides as albedo-enhancing or damping materials is significant to terraforming. Our work on desert varnish and those of other investigators is showing a possibly significant role of the microorganism communities in production of at least some (if not most) of the desert varnish rock coatings that cover the surfaces of rock in vast areas of arid terrain on Earth. Such a large scale surface phenomenon can materially alter the reflectance and absorbance properties of the surface, and thus, a planet's energy budget. Indirect relevance of the capabilities of these organisms includes the basic metabolic machinery that allows them to metabolize metal compounds, their overall adaptation to very low nutrient levels, their ability to thrive in extreme environments like ultraviolet blasted deserts or in the Earth's subsurface, and their tendency to produce a large number of unusual chemical compounds.

The laboratory trials in the ecopoiesis Test Bed were designed to include pre-growth of these slow-growing organisms on a selection of prepared media (with and without

regolith simulant) and then exposure of actively growing cultures to the ecopoiesis simulation conditions for a period of days to weeks. These exposures were followed up with measurements of relative viability (via live/dead staining, DAPI uptake, etc.) in comparison to a non-exposed control set of cultures. Further growth of exposed organisms were monitored to see if metal oxidation and crystal precipitation capabilities and other properties are affected.

Complex communities were taken from ongoing work in caves, mines, lavatubes and surface rock coatings such as desert varnish. These organisms exhibit a wide variety of chemical transformations and other behaviors that might be useful in the ecopoiesis context (Boston et al., 2004). These include the ability to precipitate minerals on rock surfaces to darken albedo, ability to fix $CO_2$ as organic carbon using inorganic compounds in the form of gases or mineral sources, and photosynthetic organisms that are adapted to osmotically challenging endolithic (within rocks), chasmolithic (in fractures), sublithic (beneath rocks), or cryptogamic soil environments that hold desert pavements together with algal filaments and fungal hyphae. These behaviors are also being studied as part of other funded projects in Dr. Boston's laboratory.

The organisms that inhabit desert varnish rock coatings are adapted to extremes of temperature, both diurnally and seasonally, and capable of dealing with high intensity ultraviolet radiation. Of course, the ultraviolet environment of Mars involves shorter wavelengths and higher intensities than anything experienced on Earth (Cockell et al., 2000; De Angelis, et al., 2004), nevertheless it is useful to investigate these organisms as potential future Martian candidates. In contrast, the Fe/Mn oxidizing counterparts in caves are likely to be more susceptible to UV than typical surface organisms and we have included them in our experiments as a test of that notion. The gypsophilic or gypsotolerant cyanobacteria from evaporite outcrop fractures have been included because of their relevance to reports of sulfates at the MER Gusev Crater site and from the Mars Express OMEGA experiment (Gellert et al., 2004; Gendrin et al., 2005). Since the Mariner missions of the early 1970's (Collins, 1971; Ezell, 1984), Mars has been known to be well endowed with volcanic landforms of many sorts so that organisms capable of colonizing such surfaces are of significant interest. The actinomycete and streptomycete dominated communities isolated from lavatube surfaces in northwestern New Mexico and manganese-rich biotic crusts from andesite flows in central New Mexico have been chosen because of their ability to exist in volcanic terrain and their mineral-precipitating abilities.

With the above considerations in mind, desert microorganisms and cave microorganisms were selected from a variety of sites that had been previously characterized (Boston et al., 2001, 2005; Hose et al., 2000; Melim et al., 2001; Northup et al., 2000, 2003; Spilde et al., 2005). These include Fe/Mn oxidizing bacteria both from speleosols (soils formed in caves, Spilde et al., 2005) and surface desert varnish, gypsum fracture-inhabiting cyanobacteria, lavatube wall microorganisms, and organisms that metabolize copper sulfides to copper oxides. During the last experiment, we added acid saline mine jarosite-precipitating organisms from the Soudan Iron Mine in Minnesota. Several different types of inocula were exposed. The first was derived from actively growing organisms taken

from the environments just described. In many cases, these had been growing in the laboratory for protracted periods of time (months to years). These cultures are on agar surfaces either in plates or in tubes. The other sample types consisted of actual environmental samples from the sites, e.g. desert varnish or cave biogenic mineral deposits collected by sterile means but not subjected to any processing in the laboratory.

Organism identities are still under investigation via molecular techniques including DGGE and RFLP, however, only one strain can be confidently assigned to a known organism, namely, *Pedomicrobium manganicum*. The other strains are apparently novel, but further analyses are underway to determine their nearest relatives via construction of phylogenetic trees. An important characteristic of the isolates from these various environments is a pronounced tendency to be hard to separate into individual strains. Many of these are maintained in mixed culture as their loss rate is less than when attempts are made to maintain pure cultures. This apparent preference for community association is not surprising in light of their extreme environments where collaboration, rather than simply competition, may be a valuable survival strategy. Organisms available (identified and unidentified) and their characteristics are listed in Table 4.

***Table 4. Desert varnish, cave, and lavatube organisms selected for study in the ecopoiesis Test Bed***

| ***ORGANISM*** | **ORIGIN** | **MEDIUM** **(with & without Mars regolith simulant)** | **REMARKS** |
|---|---|---|---|
| | | | |
| 1) Pedomicrobium manganicum | Desert Varnish, Hanksville, Utah | Mn/Fe acetate<br>Mn low carbon<br>Mn no carbon | Mn oxidizer, also other metals are oxidized (e.g. gold, iron) |
| 2) DVU 06-02-16 | Ditto | Mn/Fe acetate<br>Mn low carbon<br>Mn no carbon | Produces black crystal bundles at ends of filaments throughout agar medium |
| 3) DV U 02-03-14 | Ditto | BG 11<br>1/2 R2A<br>Fe low carbon | Dark cyanobacteria growing widely on desert varnish under Mn/Fe crust. Maybe a significant primary producer. |
| 4) DVLL 04-02-02 | Luis Lopez Desert Varnish, Socorro, NM | Mn/Fe acetate<br>Mn low carbon<br>Mn no carbon | Produces black surface xtals |
| 5) RR 04-09-22 | Lechuguilla Cave, Carlsbad Caverns National Park, NM | Mn/Fe acetate<br>Mn low carbon<br>Mn no carbon | Produces black patches of Mn oxide that crystallize through an organized mineral sequence. |
| 6) EF 06-12-14 | Ditto | 1/2 R2A<br>Hyphomicrobium medium<br>Pseudomonas medium | Small cells with long filamentous processes found in the decemented punky bedrock. |
| 7) FS 02-02-01 | Ft. Stanton Cave, Capitan, NM | Mn/Fe acetate<br>Mn low carbon<br>Mn no carbon | Pristine cave passage covered with thin black layer that yields organisms. Unique calcite “river” formation. Now closed. |
| 8) FW 06-02-09 | Four Windows Lavatube, El Malpais Nat. Mon., NM | Actinomycete medium<br>Oatmeal agar<br>Basalt medium | Actinomycete that precipitates calcite xtals on basaltic lava tube surfaces. |
| 9) FW 07-01-01 | Ditto | Actinomycete medium<br>Oatmeal agar<br>Basalt medium | Streptomycete-like organism that precipitates calcite xtals on basaltic lava tube surfaces. |
| 10) PA 01-01-01 | Pahoehoe Lavatube, El Malpais Nat. Mon., NM | Actinomycete medium<br>Oatmeal agar<br>Basalt medium | Possibly involved in precipitation of cave “moonmilk” calcite deposits. |

### 1.2.2 Test Bed Experiments at SHOT

Biological testing began as planned. Dr. David Thomas and three students (Figure 24) spent three days at the SHOT facility and completed a 1-day survival experiment with 11 species of microorganisms.

**Figure 24. Dr. David Thomas and undergraduate students from Lyon College, Batesville, Arkansas, during introduction of specimens into the Test Bed. Left to right: Laura McWilliams, Dr. Thomas, Tiffany McSpadden, John Boling.**

The first one-day exposure experiment consisted of placing specimens from 11 species into JSC Mars-1 regolith by pipetting 1.3 ml of organism suspensions into 10 grams of regolith in each well of a 24-well plate. Three plates were prepared for each experiment: one inside the chamber in full sunlight with the lid removed, one inside the chamber in indirect light with the lid left on, and one wrapped in foil and stored in a cooler at about 4$^{o}$C. A Mars sol temperature cycle was completed with the Test Bed pressure held at 100 mbar with the pump operating continuously with the addition of 10 sec of Mars atmosphere simulant flowing at 2-3 psi each hour. The disposition of the samples within the Test Bed is depicted in Figures 25 and 26.

Organisms chosen for the first study included 5 strains of cyanobacteria: *Anabaena, Plectonema boryanum* Utex45, *Synechococcus* PCC7942, *Chroococcidiopsis* CCMEE171, and *Synechocystis* 6803 and six isolates from the Atacama Desert of Chile, currently named Rock Garden-1, Rock Garden-2, Rock Garden-3, Rock Garden-4, Yungay-2, and Atacama 2002-1. These 72 specimens were returned to the laboratory and

analyzed for chlorophyll, esterase activity and viable cells. At the same time an identical set of specimens were exposed in the Test Bed, continuously at 100 mbar for a period of 8 days. During this time, moisture was added at the rate of a few grams per day until saturation was evident on the basis of standing water at the bottom of the sample container (water boils at 49.4$^{o}$C at 100 mbar).

Initial test runs with the chamber indicated that all systems were working properly. Figure 25 and Figure 26 are photographs taken at the beginning of the first qualifying test.

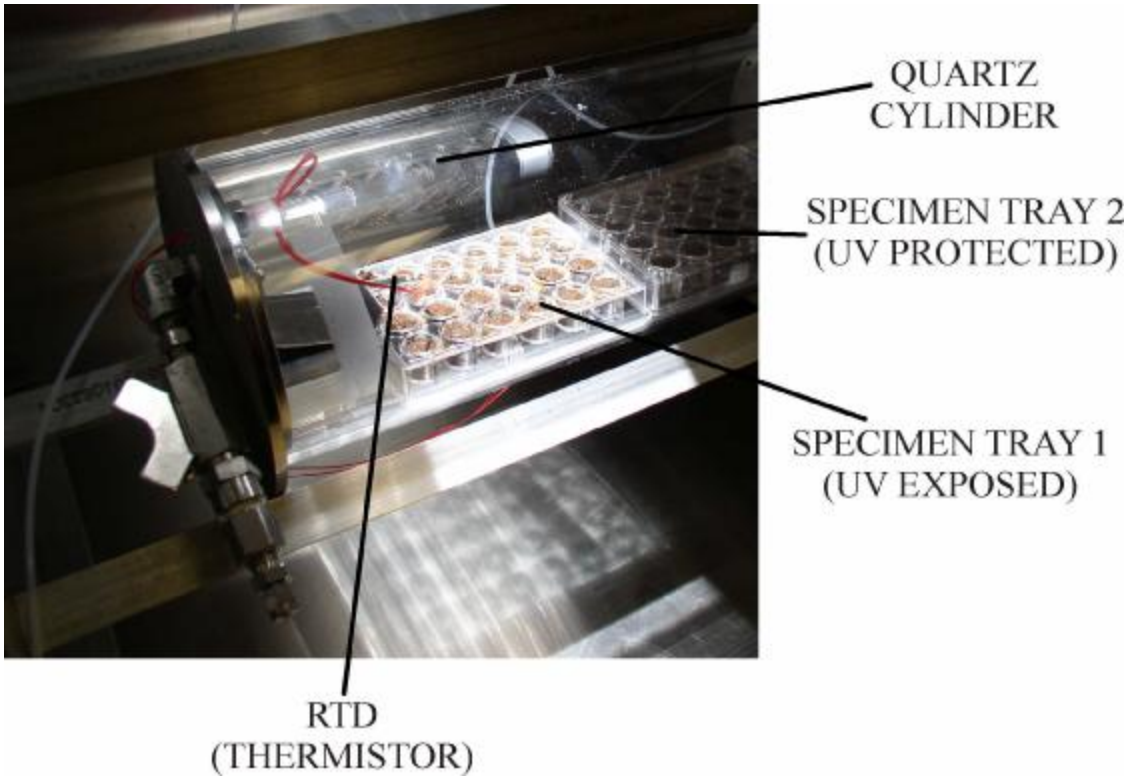



**Figure 25. Photograph taken at the beginning of the first test using live specimens. The date of this photo is May 31, 2005.**

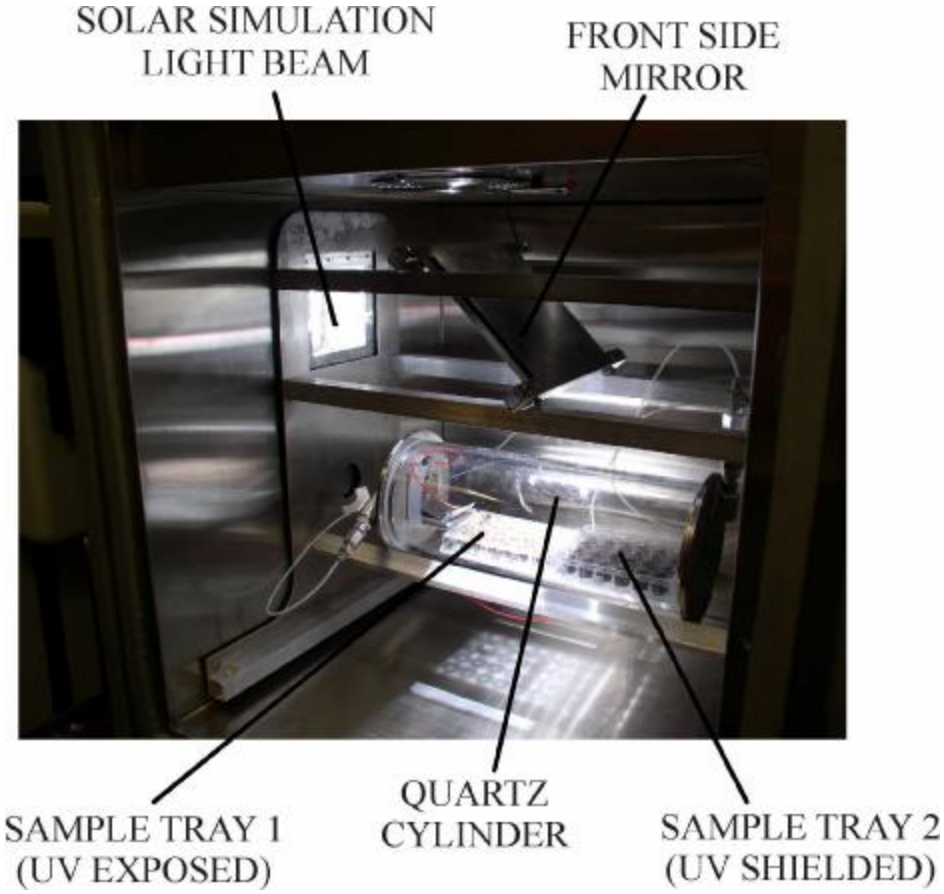



**Figure 26. Photograph taken at the beginning of the first test using live specimens. The date of this photo is May 31, 2005.**

To date nine biological test runs have been completed in the Laboratory Test Bed. These are listed in Table 5.

**Table 5. Experiments performed date in the MARS Laboratory Test Bed.**

| EXPERIMENT | DATE | DURATION | SPECIMENS | P(mbar) |
|---|---|---|---|---|
| 1 | May 31 | 23 hours | Thomas | 100 |
| 2 | June 1 | 14 days | Thomas | 100 |
| 3 | June 18 | 8 days | Thomas | 100 |
| 4 | June 23 | 22 hours | Boston | 100 |
| 5 | June 25 | 5 weeks | Boston & Thomas | 100 |
| 6 | Oct 15 | 5 weeks | Boston & Thomas | 25 |
| 7 | Jan 28 | 5 weeks | Thomas | 1000 |
| 8 | Mar 6 | 5 weeks | Boston & Thomas | 500 |
| 9 | May 3 | 5 weeks | Boston & Thomas | 300 |

Gross biochemical assays were performed to determine extractable chlorophyll and esterase activity before and after exposures in the test bed. The initial set of results of experiments using cyanobacteria, Atacama desert bacteria and local soil are given in Figures 27 and 28. These data are presented without interpretation, as viability data were

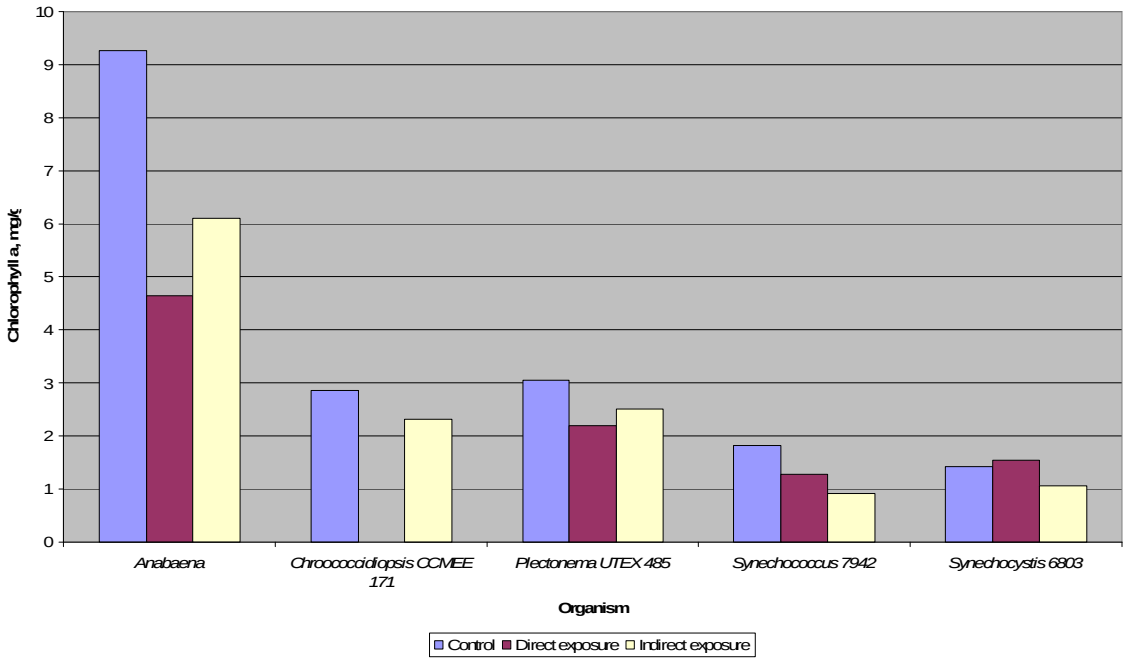



**Figure 27. Extractable chlorophyll (O.D. units) from regolith containing organisms tested in Experiment 2, 14 day exposure. In each case the left bar represents control cultures, middle bar is for cultures in the Test Bed under full illumination, and the right bar represents cultures in the Test Bed but shaded from direct light.**

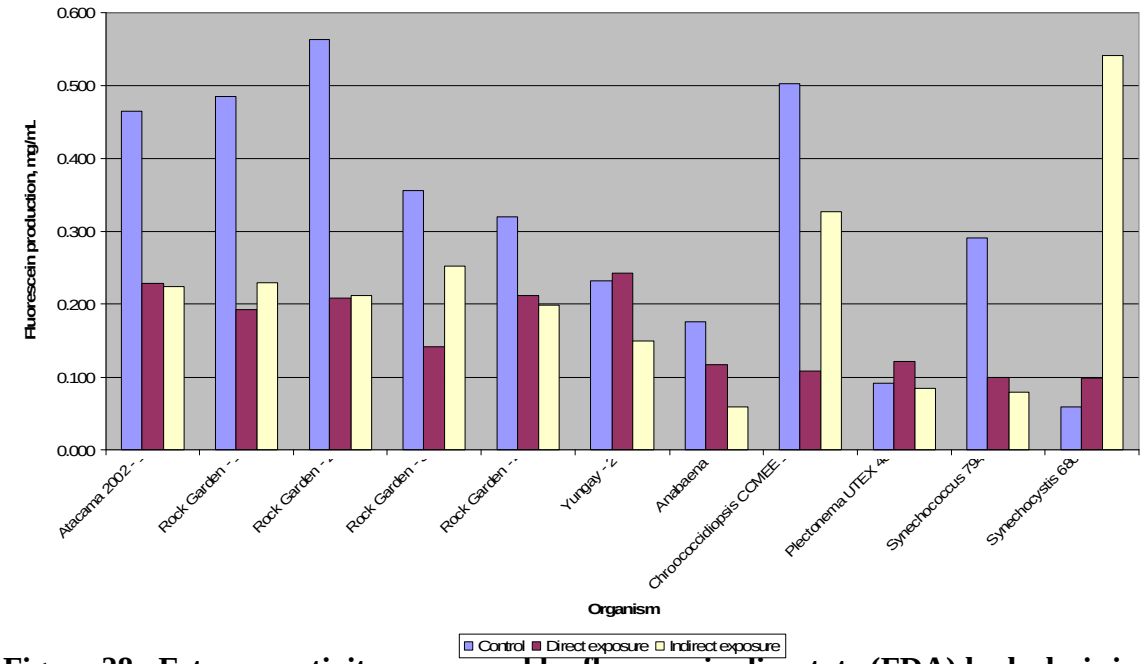



**Figure 28. Esterase activity as assayed by fluorescein diacetate (FDA) hydrolysis in regolith containing organisms tested in Experiment 2, 14 day exposure. Bars represent the same three conditions as described in Figure 27 caption.**

not available at the time of presentation. The reproductive growth assays required additional time owing to the slow rate of growth of the organisms. Perhaps surprisingly, one of the least robust organisms in terms of extractable chlorophyll and esterase activity was *Chroococcidiopsis*, originally assumed to be the most robust. Later results were generally consistent with this finding.

Cave and desert-varnish organisms also demonstrated some form of survivability under the conditions of the tests. The samples were exposed in open 1.5-ml cryovials clustered in jars. In some cases cultures were used and in others native communities were kept in their original endolithic habitats. Figure 29 is a photo of a typical sample configuration in the Laboratory Test Bed.

**Figure 29. Placement of rock community samples. Each vial contains two chips of rock that contain microbial communities. One chip was buried under 2-5 mm of JSC Mars-1 simulant; the second chip was placed on the surface of the simulant.**

Desert varnish and cave bacteria selected from the list in Table 6 were inoculated into individual containers of soil simulant and exposed for 5 weeks at 100 mbar. Figure 30 shows the FDA analysis results for six organisms.

**Table 6. Culture materials for inoculation (P. Boston) from cave and desert samples**

| 1 SAMPLE # | 2 DESCRIPTION | 3 MEDIA | 4 DATE OF LAST TRANSFER | 5 COMMENTS |
|---|---|---|---|---|
| 6 | 7 | 8 | 9 | 10 |
| 1 (plate) | Cyano Lith 2 UT Lith Canyon, MDRS Site, Hanksville, UT | BG11 | 10/28/04 | Partially damaged in transit, but most colonies still discrete. Visible cyanobacterial growth from sub-oxide layer. |
| 2 (plate) | Cyano Lith 3 UT Lith Canyon, MDRS Site, Hanksville, UT | BG11 | 10/28/04 | Intact, good colony separation, some bacterial colonies visible including a few Fe oxidizing spots (dark red-brown). |
| 3 (plate) | Cyano Lith 4 UT Lith Canyon, MDRS Site, Hanksville, UT | BG11 | 10/15/04 | Damaged in transit. Agar entirely flopped on lid. But lots of cells to harvest. Visible colonies are cyanobacterial. |
| 4 (plate) | Cyano AA1 UT From limestone cobbles with heavy black coating. | BG11 | 10/29/04 | Agar dislodged during transport. Still colonies intact on surface, much cell dissolution in fluid and relocation under loose agar slab. |
| 5 (plate) | DV Quartz 2 UT Large (8 m diam) shattered quartzite outcrop about 0.5km from the MDRS Hab. | Fe-Carb frm Fe Lo-Carb | 9/07/04 | Damaged, agar flopped on lid. Harvestable growth & xtals on surface and lid. Significant black and orange crystallization. |
| 6 (plate) | DV Yellow Stripes UT | Fe-Carb frm | 9/23/04 | Perfect condition. |

| | | | | |
|---|---|---|---|---|
| | Yellow and white striped sandstone boulders. | Fe No-Carb | | Clumped xtals and growth on surface. Under scope, heavy Fe precipitation on filaments including very long filaments under agar. |
| 7 (plate) | DV Yellow Stripes UT Yellow and white striped sandstone boulders. | Fe/Mn acetate frm No-Carb Mn | 9/9/04 | Intact. Small, discrete black dot sized colonies along streak track. Several large diffuse filamentous black colonies. Several rust colored colonies. |
| 8 (plate) | DV Mottled Striples UT Red stripes and cross hatching on sandstone. | Mn-Carb frm Mn No-Carb | 6/30-04 | Intact. Beautiful diffuse filamentous growth patchs with "lanes" between patches. Under scope, black precip very globular along filaments |
| 9 (plate) | DV FB UT Red and black patches on sandstone outcrop near Factory Butte access road. | Mn-Carb frm Mn No-Carb | 9/07/04 | (#61 on DGGE runs) Minor damage, half agar flopped on lid but growth mostly intact. Much black xtal precip but many rust "colonies" look xtally under scope. |
| 10 (plate) | Lech Rainbow Rm IB7. Purple-red speleosol deposits in Lechuguilla Cave. | M n R2A frm Mn Lo-Carb | 2/01/01 | Mn xtal producer from Lech. Harvested multiple times. Many large cells diffusely through agar, xtals on surface. |
| 11 (plate) | Harvard Chalcocite 134225 Fuzz growing on copper sulfides in Harvard Mineral Museum collection. | 1/2 R2A Chalcocite | 11/15/01 | Original streak from museum specimen. Extensive discrete fungal colonies, diffuse subsurface growth into agar possibly unrelated. Not much clear |

| | | | | |
|---|---|---|---|---|
| | | | | mineral precip on surface. |
| 12 (tube) | DV Stripes UT Black sheen on red & white sandstone boulders. | Fe/Mn glucose | 05/30/04 | Original inoculum w/ DV chunk. Significant black band and haloing around chunk. Xtals and filaments through scope. |
| 13 (tube) | DV Lith 2 UT. Lith Canyon, MDRS-Hanksville | Fe/Mn glucose | 05/30/04 | Original inoculum w/ DV chunk. More filamentous than 12. |
| 14 (tube) | DV Lith 3 UT. Lith Canyon, MDRS-Hanksville | Fe/Mn glucose | 06/01/04 | Original inoculum w DV chunk. #8 on the DGGE screening. Coloration is diffuse in small "clouds". |

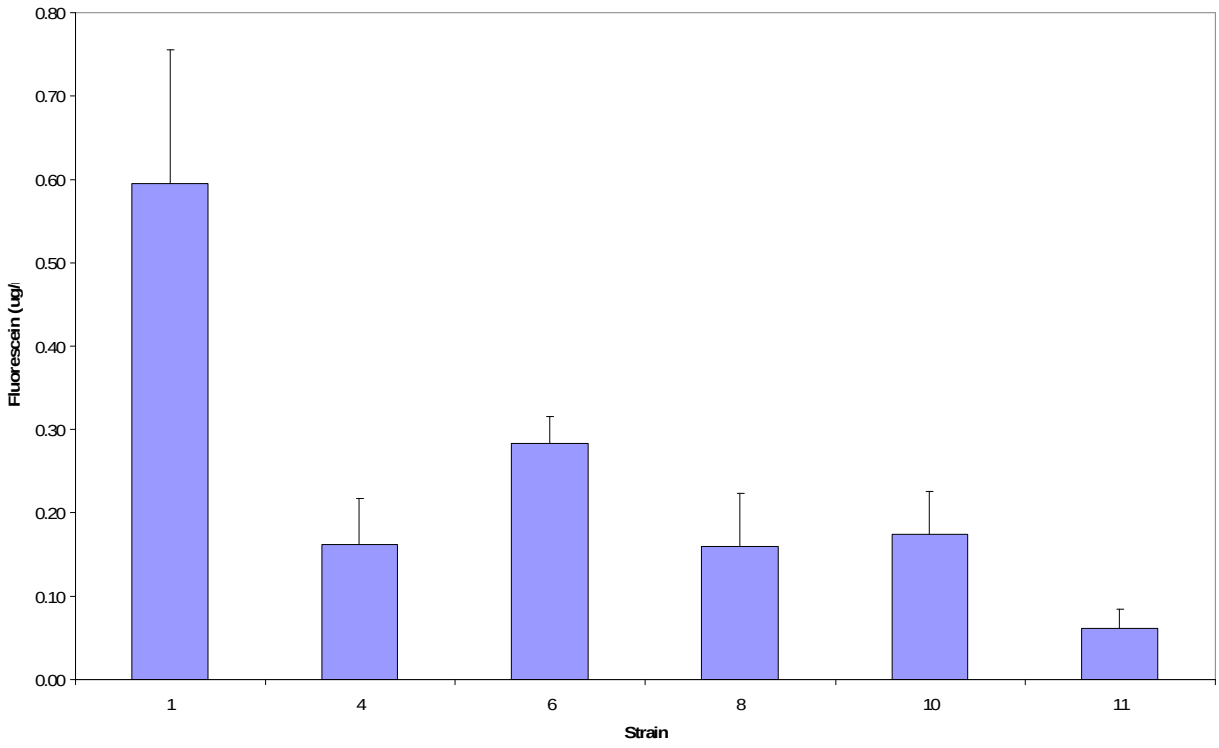



**Figure 30. Fluorescein production in desert varnish and cave bacteria samples. Individual cultures were mixed into JSC Mars-1. Subsamples were measured in triplicate for FDA hydrolysis activity. The bars represent esterase activity (thus viable biomass) measured as mg fluorescein $g^{-1}$ soil $h^{-1}$. The identities of the strains are given in Table 4.**

A key indicator of potentially useful species and communities for ecopoiesis is the ability to function normally after exposure to Mars conditions. We have completed the first step of this assessment for a number of potentially useful strains. The most interesting survivor by far has been desert varnish community DV8 from the Mars Desert Research Habitat site near Hanksville, Utah. This community was exposed in each of three simulations and has produced significant survivorship in each one. The number of individual strains within this community diminished after the Mars chamber experience; however, two very significant organisms have emerged: one is a cyanobacterium and the other is a manganese oxidizing bacterium. Both of these organisms grow robustly in culture after their exposure. They appear to do significantly better when maintained in mixed culture. The cyanobacteria are very prolific and the manganese oxidizer is still capable of manganese utilization after exposure. We employed 2-(*p*-iodophenyl)-3-*p*-(nitrophenyl)-5 phenyltetrazolium chloride and 4′,6′-diamidino-2-phenylindole assays (King & Parker, 1988) to assess the metabolic state of organisms shortly after retrieval from the Laboratory Test Bed. The relatively high numbers of metabolically active cells (~20-32% of total cell count) leads us to conclude that organisms are maintaining themselves at some level of activity even when in the chamber. Rapid growth immediately upon post exposure inoculation strengthens this interpretation of their metabolic state. Relevant photomicrographs demonstrating various post-exposure metabolic activities are shown in Figure 31.

One microobial community survival experiment was performed. A mixture of cyanobacteria and heterotrophic bacteria was inoculated into a broad soil simulant sample (Figure 32, inset): *Klebsiella oxytoca*, *Bacillus licheniformis*, *Bacillus subtilis*, *Chrooccidiopsis* sp. CCMEE171, *Chrooccidiopsis* sp. CCMEE662, *Anabaena* sp., and *Plectonema boryanum*. Survival of cyanobacteria was lower than in previous, shorter-duration experiments, but the desert autotroph survival after 5 weeks at 100 mbar was surprisingly high. Attempts to perform plate counts have been unreliable. Instead, we used measurements of esterase activity—fluorescein diacetate hydrolysis—and chlorophyll extractions as proxies for microbial biomass. The fluorescein hydrolysis data from this experiment are given in Figure 32.

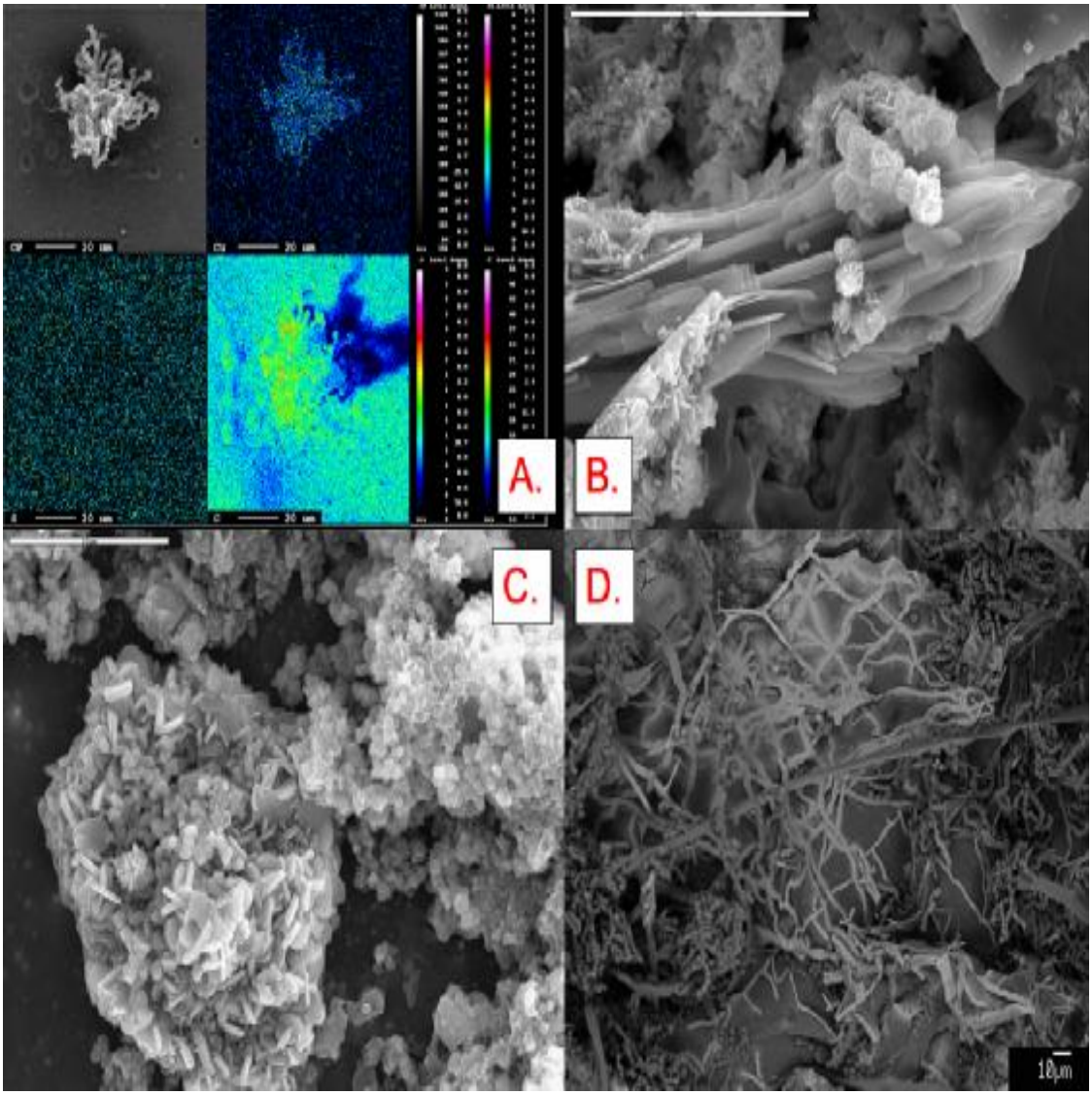



**Figure 31. Images showing production of normal biogenic minerals by strains after exposure in the Mars Laboratory Test Bed. All of these are from laboratory growth experiments following the first long duration Mars exposure experiment in June of 2005 (P = 100 mbar, 5 weeks). A: Organisms that take up copper from the mineral bornite are still taking up copper and depositing it intracellularly as can be seen from this electron microprobe elemental map. B: Spherical rosette crystals are manganese oxides produced by a strain related to the genus *Pedomicrobium*. These have been isolated from both surface desert varnish and cave environments. Production of dark oxides biologically may serve as an albedo adjustment tool for ecopoiesis. C: Platy iron oxides produced copiously in culture and in nature are still produced by organisms after exposure. D: Organisms that use the sulfide groups in the mineral chalcocite and take up copper still perform this process after Mars simulation exposure.**

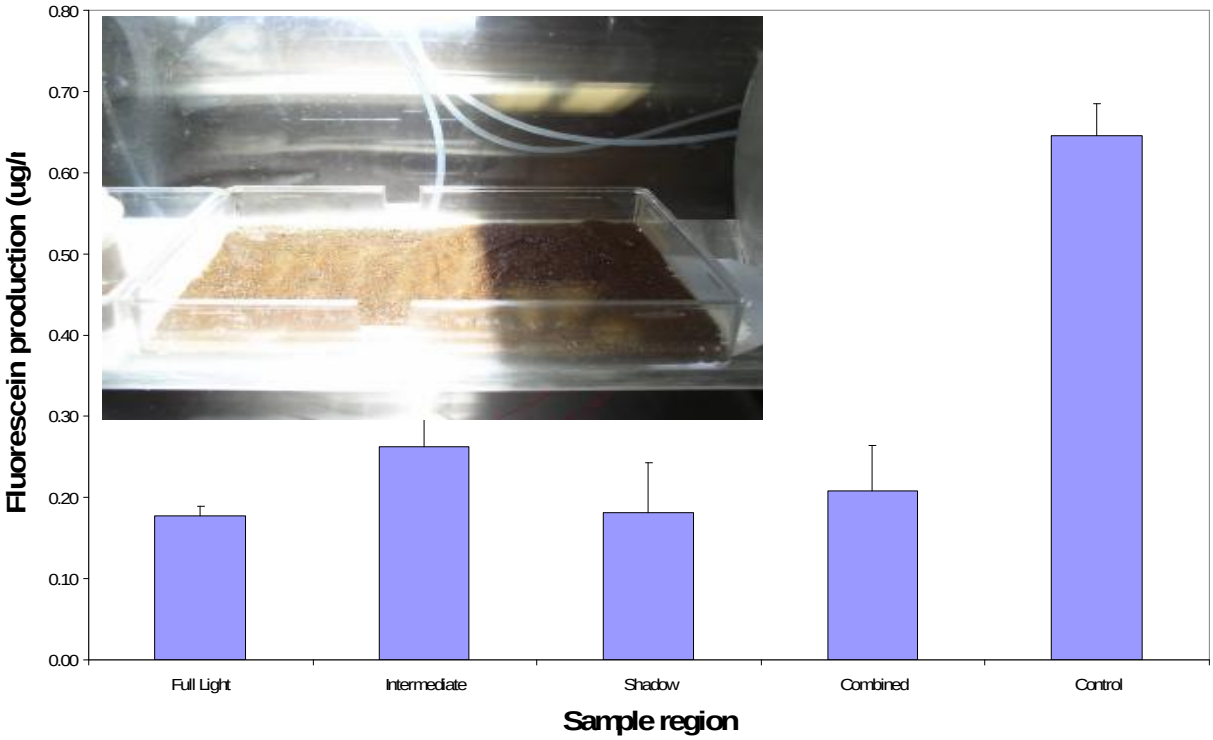



**Figure 32. Fluorescein diacetate hydrolysis in an aggregate simulated soil containing multiple microbial species. The inoculated Mars soil simulant was sampled in triplicate at intervals across the light gradient. The bars represent esterase activity (thus viable biomass) measured as mg fluorescein $g^{-1}$ soil $h^{-1}$.**

After these preliminary "looks" a systematic study of atmospheric pressure in pure $CO_2$ occupied the final year of experimentation. This was undertaken with the advice and guidance of the Science Advisory Committee. In ecopoiesis, the early planetary variable to be controlled, of course, is atmospheric pressure; on Mars it must be elevated. The tolerance of pioneer organisms to various total pressures of $CO_2$ needs to be understood. An example of daily condition profiles during a 1,000 mbar, 5-week experiment is given in Figure 33.

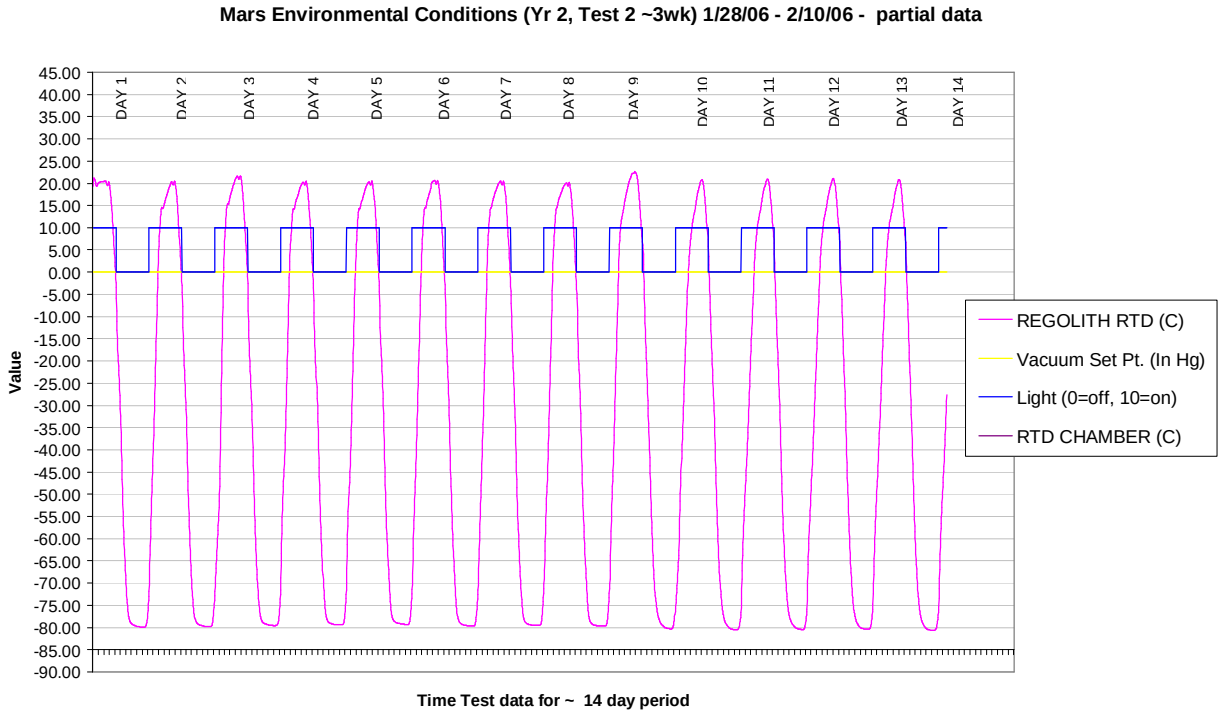



**Figure 33. Temperature, light and pressure profile data for an experiment operated for 5 weeks at 1,000 mbar (1 atm) pure $CO_2$.**

Using the same life detection assays as above (FDA hydrolysis and cholorophyll determination), three species of cyanobacteria, *Anabaena sp.*, *Chroococcidiopsis sp.* strain CCMEE2171, *Plectonema boryanum* strain UTEX485 and one species of alga, *Chlorella ellipsoidea* strain YCC002, were exposed for 5 weeks using a standard protocol at 25, 100, 300, 500 and 1,000 mbar pressure using pure $CO_2$. In the standard protocol 25-ml polypropylene jars contained 7 g of JSC Mars-1 regolith simulant and 10 ml of culture as a starting "mud puddle". Control samples were placed on ice in the dark, and then maintained in the dark at 4°C at Lyon College. They were analyzed alongside the experimental samples at the conclusion of each experiment. All atmospheres were water-saturated, and moisture content of each sample at the end of experiments was highly dependent on the amount of light absorbed – shaded samples retained moisture while illuminated samples became dry (Figure 34).

**Figure 34. Differences in sample moisture. Examples of 25 ml polypropylene jars containing samples at the end of an experiment. By the end of each experiment, samples located toward the ends of the chamber (left) contained more water than samples in the middle of the chamber (right). Sample jars were arranged such that each triplicate series experienced the full range of moisture within the simulator chamber.**

The results of the life detection assays for the five experiments are summarized in Figures 35 and 36. Since the control samples for each experiment were stored in the dark at 4°C, and were otherwise treated identically, these data were combined for all five experiments. Among the three cyanobacteria, the highest FDA hydrolysis activity was found at 100 mbar, but the highest chlorophyll content was found at 300 mbar. The alga, *Chlorella*, had its highest FDA activity and chlorophyll content at 300 mbar. Outside of the 100-300 mbar range, both extractable chlorophyll and FDA hydrolysis levels were very low. Previous research with cyanobacteria in high $CO_2$ atmospheres and ambient pressure (Thomas et al. 2005) showed significant growth inhibition of *Anabaena* and *Plectonema* at $CO_2$ concentrations of 40% or more. Another cyanobacterium, *Synechocystis*, was inhibited by 20% $CO_2$. At ambient pressure, this corresponds to a partial pressure range of $CO_2$ ($pCO_2$) of 200-400 mbar—which overlaps the survival range shown in Figures 33 and 34. The low survival rates in the 500 and 1000 mbar experiments may be due to $CO_2$ toxicity. While *Anabaena* and *Plectonema* can survive in 100% $CO_2$ under culture conditions, the added stresses of other parameters of the ecopoeitic conditions result in inhibition and death.

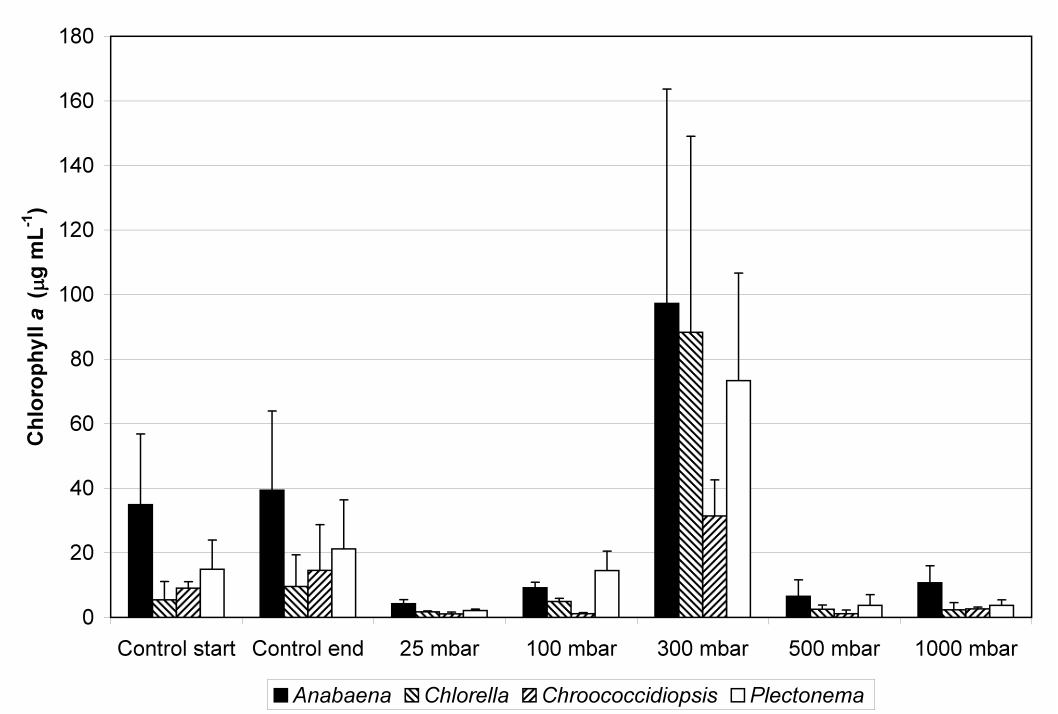



**Figure 35. Chlorophyll extracts. 1 mL of liquid was removed from each sample, centrifuged and extracted in 80% ethanol in the dark for 24 hours at -20°C. Extracts were measured spectrophotometrically at 664 nm. Controls were combined from all experiments. Error bars equal standard deviations (n = 10-15 for controls, n = 3 for experimental groups). For all samples, the highest amount of extractable chlorophyll *a* was found in the 300 mbar experiment.**

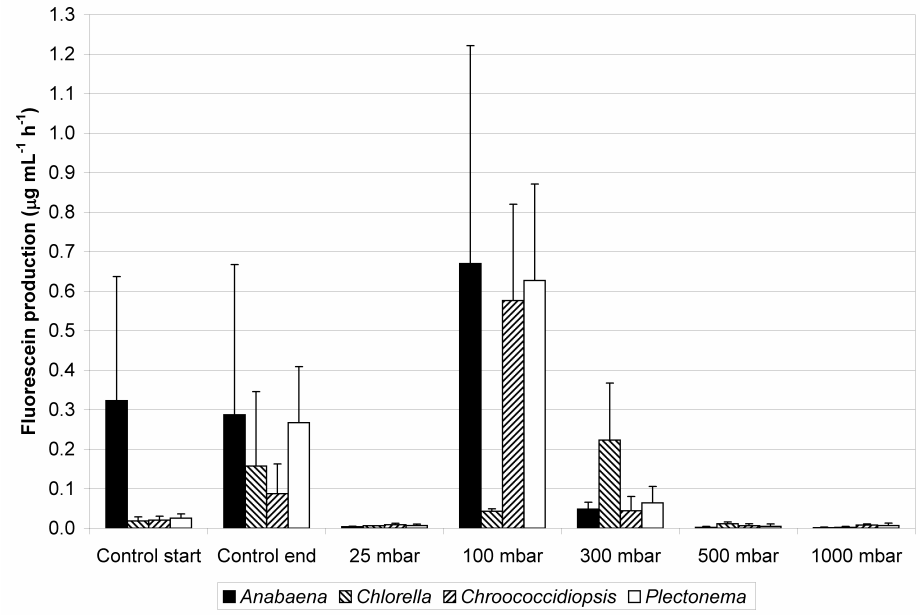



**Figure 36. FDA hydrolysis assays. 1-2 mL of liquid was removed from each sample and brought to 5 mL with the addition of pH 7.6 phosphate buffer. 10 mL of 5 mg mL$^{-1}$ FDA solution was added to each sample, followed by incubation for 2-6 hours**

**at 25°C. Samples were extracted in 2:1 chloroform:methanol. The methanol:buffer fractions were measured spectrophotometrically at 490 nm. Controls were combined from all experiments. Error bars equal standard deviations (n = 10-15 for controls, n = 3 for experimental groups). For the three cyanobacteria, the highest amount of FDA hydrolysis activity was found in the 100 mbar experiment, but the alga, *Chlorella ellipsoidea*, had more activity in the 300 mbar experiment.**

Besides the inhibitory effects of $CO_2$, atmospheric pressures below 100 mbar may have both physically and physiologically inhibitory effects. At 25 mbar, water boils at 9°C. Since the diurnal temperature ranges from -80°C to 26°C, the microbes are boiled and then deep-frozen every day. Our results are consistent with research on *Bacillus* spp. that shows little or no growth at 25 mbar (Schuerger et al. 2006a, Schuerger and Nicholson 2005).

Detailed results of this study sequence and have been prepared for publication. The formatted two-column manuscript is Appendix 2.6.

## 1.3 Objective 3. Community of users

### 1.3.1 Outreach and Presentations

The NIAC annual meeting in Seattle, October 19-20, 2004, was put to good use in the generation of interest, including that of student participants. The presentation that was made was submitted to NIAC for archiving in the form of a digital file, which was also shared with Dr. Wirt Atmar, President of AICS Research Inc. for conversion, with audio to a QCShow file for internet access. This turned out to be an excellent internet product, quite flattering to the presenter. Productive discussions were held with Dr. Sharon Garrison, NASA's Contracting Officer's Technical Representative (COTR) for the NIAC program, and these were followed up during the immediate subsequent weeks with efforts to raise agency consciousness of this project.

A presentation was made at the American Society for Gravitational and Space Biology (ASGSB) annual meeting held in Brooklyn, NY, November 10-13, 2004. The abstract is given as Appendix 1.1. This presentation generated considerable audience interest, including interest in purchasing the technology. Simulator sales is considered to be a critical part of the architecture of ecopoiesis research and an important near-term goal for SHOT. At this meeting the PI was elected to the office of President-Elect of the Society.

Under a new publication policy, an "expanded abstract" of this presentations was published in *Gravitational and Space Biology* 14 (2) (2005), with the content given in Appendix 1.2.

Some of Dr. Thomas' work on this project was presented at the 14th Western Photosynthesis Conference in January, 2005. A copy of the abstract of this presentation is given as Appendix 2.1.

In addition an article demonstrating cyanobacteria function in 100% $CO_2$ has been published in *Astrobiology*. The abstract of this article is found as Appendix 2.2.

As a companion paper, Dr. Thomas' group published a description of their inexpensive laboratory technology, which materially extends, at minimum expense the versatility of experiment design for this project. This work also extends Mars biology into the realm of undergraduate education. The abstract is Appendix 2.3.

The annual Space Technology and Applications International Forum is a venue for regular representation of this ecopoiesis project. Dr. Boston spoke on behalf of the project during the 2005 year's Forum, and the abstract submitted for this presentation follows is Appendix 2.4.

Additional efforts went into the broadening of the audience and user community. A visit to NASA Glenn Research Center (at no expense to the project) resulted in the stimulation of considerable interest. Although GRC has some of the world's largest and best vacuum test facilities these are not generally available at reasonable cost for high-fidelity Mars environment simulation. Therefore, scientists encountered during the Glenn ExPO – Exploration of Partnership Opportunities in Cleveland yielded significant levels of interest, and these were pursued in greater depth during a visit of GRC personnel to SHOT in May, 2005.

The Principal Investigator as ASGSB president-elect is expected to assume the presidency of the Society in calendar year 2006. In a previous report it was stated that "It is intended that this will be a platform for, among other things, integrating the space biology and astrobiology/exobiology communities and strengthening ties between astrobiology and the wider research world." At its March meeting, the Governing Board of the ASGSB authorized a membership campaign targeting, among others, the astrobiology community and a plenary symposium at the November, 2005 annual meeting of the society titled "Planetary Biology and Terraforming".

Dr. Thomas has presented work of the project at national meetings and continues to do so. An abstract in anticipation of results from research using the Test Bed was submitted for presentation at the Eighth International Mars Society Convention in Boulder, CO, August 11-14, 2005. The text of this abstract is given in Appendix 2.5.

Student work on the project during the summer is underway. Three Lyon College students (Figure 24) will work overlapping schedules from May through early August, including visits to SHOT at appropriate times, one of which has occurred and has been a successful experience for the students.

Additional efforts went into the broadening of the audience and user community. The visit to NASA Glenn Research Center (at no expense to the project) resulted, not only in the stimulation of considerable interest, but also a visit to SHOT by GRC key personnel. Although GRC has some of the world's largest and best vacuum test facilities there was interest expressed in reasonable-cost high-fidelity Mars environment simulation. This

and other interested pursued in greater depth during the visit of GRC personnel to SHOT which occurred in May, 2005.

The Principal Investigator as president-elect of ASGSB was asked to organize and chair a plenary symposium on Planetary Biology and Terraforming. Five speakers were invited to participate, and the detailed program will be added to the and is expected to assume the presidency of the Society in calendar year 2006. In the last report it was stated that "It is intended that this will be a platform for, among other things, integrating the space biology and astrobiology/exobiology communities and strengthening ties between astrobiology and the wider research world." At its March meeting, the Governing Board of the ASGSB authorized a membership campaign targeting, among others, the astrobiology community and a plenary symposium at the November, 2005 annual meeting of the society tentatively titled "Planetary Biology and Terraforming". Invitations to join ASGSB were issued to individuals and plenary groups at the biannual Astrobiology Scientific Conference AbSciCon sponsored by NASA SMD, where a poster was presented, the abstract for which is Appendix 1.3.

An abstract was submitted for the forthcoming meeting of the Mars Society in Washington DC, August 3-6, 2006. It is Appendix 2.4.

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As mentioned in the previous report a symposium on ecopoiesis was held at the annual meeting of the American Society for Gravitational and Space Biology (ASGSB) 1-4 November, 2006, a chairman's introduction by the Principal Investigator was submitted and has been reviewed for publication in the forthcoming issue of *Gravitational and Space Biology* and is reproduced as Appendix 3.1.

The project team conducted a plenary symposium "Planetary Biology and Terraforming" at the annual meeting of ASGSB held in Reno, NV, November 1-4, 2005. The results of biological testing, as they arose and became interpretable, are the subject of ready to publish archival scientific articles.

### 1.3.2 Modular Test Bed

Objective 3 includes the design of portable modular test beds ("MARS-MTB"), and it was stated that "Concurrent and follow-on funding for the actual construction of such chambers will be sought through Federal SBIR programs" (see Introduction, above). A proposal for Modular Test Bed design and construction was submitted in response to NASA's Ominibus SBIR solicitation and was submitted September 9, 2004. As a reminder, an illustration of the proposed MARS-MTB is shown in Figure 37. The funding of NASA SBIR Phase I applications was announced on November 19, 2004. SHOT's application was not on the list of projects funded, and it appears that the proposed "Miniature Planetary Environment Simulator" will not be funded by the NASA SBIR program during the current fiscal year. A briefing concerning the criteria for non-selection eventually became available, and SHOT will commence the preparation of revised proposals.

Related activities described in previous reports continued. Opportunities continued to be sought for supporting the proposed MARS-MTB (modular test bed for laboratories and classrooms). This included intensified contacts with SBIR officers at NASA field centers. While there did not seem to be any negative features of the reviews of the proposal submitted in response to last year's NASA SBIR solicitation, further wisdom is being sought in light of the drastic changes about to take place in NASA's SBIR program. As previously, in summary, support for this component of the architecture will continue to be pursued.

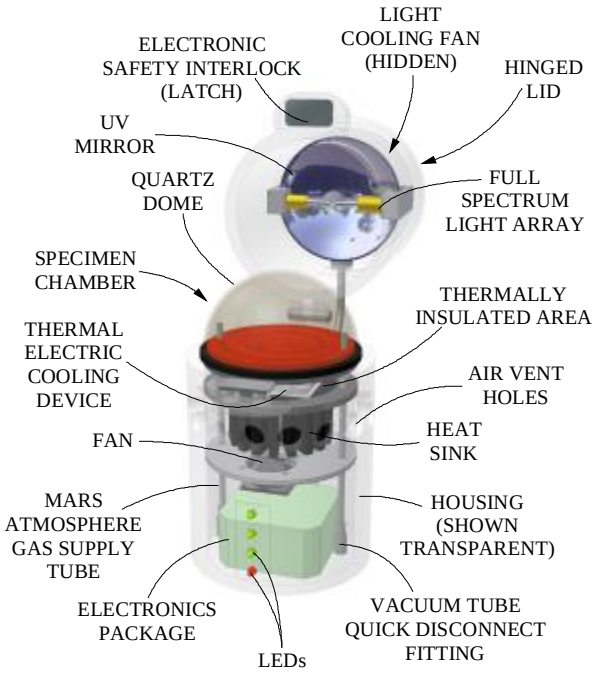


**Figure 37. Rendering of the proposed modular test bed (MARS-MTB). Showing principal subsystems.**

Detailed renderings and safety considerations have been developed and are presented in the paragraphs that follow.

The proposed Modular Test Bed has two major components: The modular Simulator (A.K.A. "Mar Jars") and the Recharging Station for multiple Simulators. Preliminary planning for both has been initiated, as can be seen in the detailed descriptions that follow. The extreme conditions associated with both components require special safety considerations, and these are also outlined at the end of this section.

The Miniature Planetary Environment Simulator is a compact, comprehensive, stand-alone assembly. The housing is a one-piece cylindrical shaped, insulated aluminum body. Most of the subsystem components are located inside the housing. Inside the dome-shaped quartz hemisphere that is located on top of the housing is the simulated regolith and experimental organisms. A hinged lid attached to the top of the housing covers the quartz dome. The hinged lid houses the light array that is the solar spectrum

generator. These and the remaining components are called out in Figure 37. Each potential component of the Simulator has been researched as discussed in the following paragraphs.

*Quartz / Zeon Plastic Dome* – Specimen chamber: The specimen chamber that is formed by a flat aluminum bottom plate and an upper quartz or Zeon plastic hemisphere houses the specimen, atmosphere, and simulated regolith. It is in this chamber that the living organisms are introduced and all the environmental conditions are controlled. The quartz or Zeon plastic dome allows the full spectrum of light from the light array to pass through into the controlled environment. While both Quartz and Zeon plastic have excellent light passing characteristics, Zeon plastic may be chosen over the quartz for its thermal insulation properties.

*Hinged Lid – Light array*: Directly above the specimen chamber is a hinged aluminum cylindrical lid. The hinged lid has a heavy duty hinge and a magnetic latch that is a safety interlock. The hinged lid remains closed and locked during normal operations. The lid may be opened for specimen viewing during the day cycle of Mars once all environmental condition set-points have been met. An LED on the housing of the Simulator indicates that the day cycle on Mars is in process and tells the user that it is safe to open the lid. The high-intensity Xenon arc light and its special housing is installed at the top of the inside of the hinged lid. Prices range from $600-$1000 per lamp. The radiation spectrum of a xenon lamp is continuous over the ultraviolet, visible, and infrared light ranges. The fused silica bulb radiates a strong spectrum in the ultraviolet light range down to 185 nm. The output radiant intensity is approximately proportional to the current flowing to the lamp so a stabilized power supply for the lamp is included. The xenon lamp surface temperature will be maintained at less than 750 $^{o}$C and the metal base surface temperature (anode side) at less than 200 $^{o}$C. Forced air cooling with a fan in the lid will maintain temperature. Care will be taken so that the fan does not stop during operation or for several minutes after switching the lamp off. High quality insulation materials will be used to avoid leakage of trigger high voltage when the lamp is switched on. Since the xenon arc lamp operates at very high pressures and temperatures and emits ultraviolet radiation, it must operate with a fully enclosed housing installed in the lid. A safety interlock will be used to prevent access to the lamp when it is on or cooling off thus preventing operator exposure to the radiation and to protect against inadvertent contact with the hot lamp. There will be a thermal interlock that turns the lamp off in the event the temperature within the housing exceeds safe operating levels. In addition, there will be a safety interlock that turns the system power off in the event of cooling-fan failure. If these safety interlocks fail when the hinged lid is opened the lamp automatically shuts off. This safety interlock latch prevents the users from damaging their eyes by exposure to the UV light. During the simulated day cycle of Mars, the temperatures are close to ambient temperatures in the lab. Allowing the hinged lid to open during the time that the temperature difference between the lab and the controlled experiment volume is small prevents condensation or frost from forming on the quartz dome.

*Thermal System*: A combination of staged Thermal Electric Devices (TEDs), a heat sink fin, control electronics and high performance insulation provide the specimen chamber with the desired thermal control. TEDs are positioned below the aluminum base plate of the experiment volume for removing heat from the chamber. Thermoelectric devices utilizing the Peltier effect have been demonstrated to be an effective means of producing low-capacity refrigeration and heating. These solid state heat pumps have no moving parts, fluids or gases, which makes them ideal for small compact laboratory equipment. Temperatures within the specimen chamber should range from - $80^{O}$C during simulated Mars night to $26^{O}$C during the simulated day cycle of Mars (see Section 1.1).

The hinged aluminum lid is insulated with high performance insulation to minimize heat loss through the structure. The lid will remain closed during low temperatures to prevent heat loss and for preventing human exposure to extremely cold surfaces. The TEDs are sandwiched between the base of the controlled volume and a heat sink. The heat sink's material is manufactured from highly thermally conductive aluminum. Two pancake style fans move cool lab air from the bottom of the housing across the heat sink and out the vent holes in the side of housing removing heat conducted from the TEDs to the heat sink fin. The air volume that would be surrounding the TEDs is filled with high performance, moisture proof insulation to minimize heat transfer in that area. The moisture proof quality of the insulation prevents water from getting to the TEDs and prevents corrosion from forming.

*Electronic Package*: The electronics package is located beneath the heat sink fans at the bottom of the modular simulator housing. The electronics of the unit is powered by 115VAC. Maximum power consumption is estimated at 373 watts. Power converters, filters and programmable chips are packaged to supply the proper voltage and current to various electronic components of the modular simulator (Figure 38). The following list is an initial rough estimate of the power requirements of the stand-alone unit.

| | |
|---|---|
| Xenon Lamp | 75 watts |
| TEDs/Cooling Unit | 250 watts |
| Data Management System (DMS) | 8 watts |
| Light Cooling Fan | 5 watts |
| Heat Sink Fans | 15 watts |
| Power Supply Unit | 20 watts |
| **Total:** | **373 watts** |

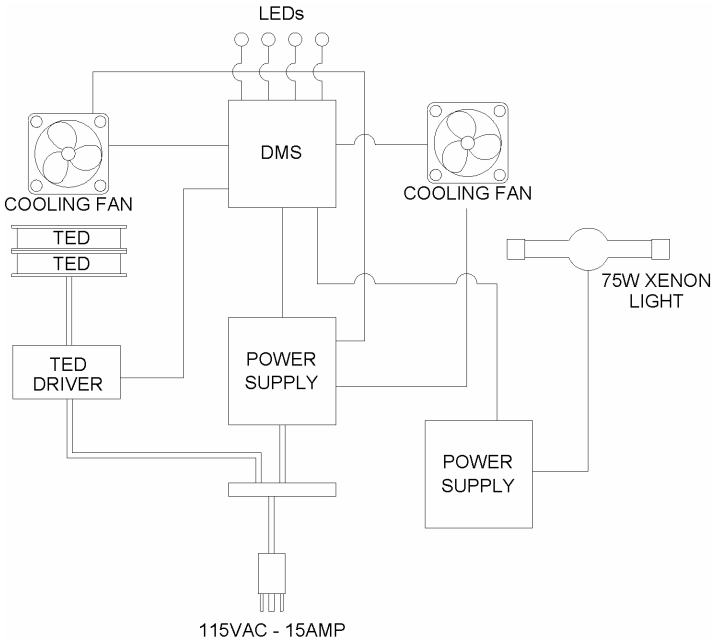


**Figure 38. Miniature Simulator Electrical diagram**

*Gas system:* Pressure as well as atmospheric gas content is regulated within the controlled volume of the Simulator. Pressure is maintained at levels of 10-15mbar. The quartz dome above the housing is sealed to the base. The base has two portals that allow high pressure stainless steel tubing to penetrate the base and terminate in the control volume. Each tube has a quick-disconnect fitting at one end near the bottom of the Simulator. The tubes connect by seating the unit on the recharge station. Atmosphere is evacuated from the controlled volume after installation on the recharging station. A vacuum pump located in the base of the recharging station is activated until the sensors indicate that the desired vacuum has been obtained in the dome. The simulated gaseous mixture of Mars is created by activating the control valve connected to the gas supply bottle in the recharge station. The gas mixture is added to the control volume while the vacuum system maintains the desired pressure. The gas mixture is 95.57% $CO_2$, 2.7% $N_2$, 0.13%$O_2$, and 1.6% Ar.

*Recharging Station*. The recharging station for the MPES is a bench top unit. It has a high fidelity graphical user interface. Silicone rubber buttons are used to toggle between status and control screens. Inside the recharging station's protective base resides a gas supply bottle that contains the Mars atmosphere in a compressed state. The gas supply bottle is connected to the quick disconnect fitting by high pressure tubing. A two stage reservoir placed in line between the tank and the quick disconnect fitting regulates the input and output pressure. The base of the recharging station houses a small vacuum pump capable of evacuating the specimen chamber to 10mbar. A rendering of the exterior of the recharging station is shown in Figure 39.

*Data Management and Monitoring Module (DMMM):* The control of the Recharge Station is maintained using the DMMM. The DMMM consists of several main parts:

information processing circuitry (embedded 16-bit processor), input and selection buttons, graphical interface, speaker and housing. Information processing circuitry is the brains of the recharge station. The electronics circuitry processes inputs from the user as well as monitors and controls system performance. The input and selection buttons are used for moving to different sections of the graphical interface screen. The buttons are also used to toggle between different screens as well as entering data. The input and selection buttons are actually all of one larger single molded silicone button pad. The molded silicone button pad has built in carbon pills behind each "button". When a button is depressed the carbon pill shorts out exposed traces on the circuit board directly behind the button pad causing an operation to be performed. The graphical interface (GUI) used in the recharge station is a high quality organic LED (OLED) display. The OLED is used to display information about the status of the Miniature Simulator. The OLED display also displays status information on the recharge station's systems. The fine resolution and brightness of the OLED display allows the screen to be used for displaying high resolution graphics as well as textual information. A speaker is located on the front face of the recharge station to allow for audible messages and alarms to be presented to the user.

*Gas Analyzer:* The recharging station also contains a gas analyzer unit. The gas analyzer tees off of the vacuum line. The gas analyzer will evaluate levels of carbon dioxide, methane, ammonia and oxygen in the simulated atmosphere. Gas content is sampled by accessing the gas sampling graphical user interface (GUI) page. The gas sampling GUI page will allow gas level values to be stored in the recharging station's onboard memory. A simple screen on the GUI allows the user to study real time information, or averages of values across a set time period. Gas sampling can be initiated in real time or can be programmed via a timeline to record gas level values on preset intervals or activated manually as each stand-alone unit gets is gas analyzed.

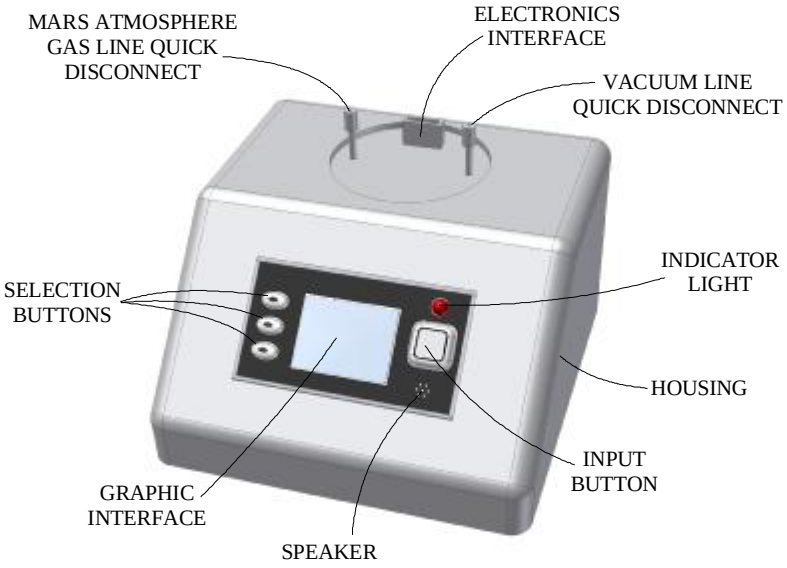


**Figure 39. Rendering of proposed Recharging Station for Miniature Planetary Environment Test Beds.**

*Safety.* Several features of the Miniature Test Bed are potentially hazardous. General safety precautions need to be followed during its use. SHOT personnel will use SHOT document 900SP497 Industrial Health and Safety Plan for general safety guidelines. Hazards specific to the Miniature Simulator described in this section.

*Environmental Control Unit:* The safety precautions are listed associated with each identified hazard.

*Pressurized Liquid Nitrogen Tank and line*

- Extremely cold temperatures
  - Personal Protective Equipment (PPE) will be worn
- Depletion of Oxygen in breathable air
  - Low oxygen alarm will be located in the laboratory
- Pressurized tank – potential projectile
  - Tank will be secured to stand
- Electrical Shock
  - Equipment will be properly grounded
  - Shock hazards will be guarded and labeled

*Solar Spectrum Generator*

- High Intensity full spectrum light
  - UV blocking goggles will be worn
- Hot Surfaces
  - Hot surfaces will be guarded and labeled
- Electrical Shock
  - Shock hazards will be guarded and labeled

*Gas Supply System*

- Pressurized Mars simulated gas tank
  - Pressurized tank – potential projectile
    - Tank will be secured to stand
  - Depletion of Oxygen in breathable air
    - Low oxygen alarm will be located in the laboratory
- Vacuum Pump
  - Hot surfaces
    - Hot surfaces will be guarded and labeled
- Quartz Specimen Chamber Cylinder
  - Evacuated chamber – risk of implosion, glass cuts
    - Quartz cylinder structure analysis completed for proper thickness of cylinder wall.
    - Cylinder is contained within the ECU
- Test organisms
  - Contamination of lab, spreading of organisms
    - Organisms will be loaded using sterile transfer hood
    - Proper PPE will be worn

*Simplified, Low-Cost Version.*
A novel low-cost version of the Modular Test Bed incorporates an original idea of Prof. David Gan of the University of California. The planned design of these "Mars Jars" is as follows:

The SHOT Miniature Planetary Environment Simulator "Mars Jars" for classrooms replicates many of the extreme environmental conditions on Mars. Temperature, atmospheric composition and pressure and even soil (regolith) are physically present in the classroom in a compact unit that students can see and help operate. A wide range of biological and geological experiments can be conducted in the unit.

The "Mars Jars" will approximate on a very-limited basis the temperature profiles, atmospheric and regolith composition present on the Mars planetary surface. Design calls for a shallow regolith simulant bed into which test organisms can be buried. The Mars Jars will utilize dry ice (solid $CO_2$) to provide a limited simulated Martian environment (-78$^{o}$C) for the test organisms, which represents the lowest surface temperature near the Martian equator. When dry ice is added to the mars jars it will simulate the Martian night, and as the dry ice changes directly from a solid to a gas the regolith will warm up to simulate the Martian day. The dry ice will sublimate at a rate of five to ten pounds every 24 hours. Therefore, the number of Martian days will depend upon how many times the user replaces the dry ice. The Mars Jars will be connected to a vacuum pump (see Figure 40) to simulate the Martian pressure. As atmosphere is evacuated from the control volume of the Mars Jars $CO_2$ is drawn into the Specimen Chamber giving an inexpensive Mars-like atmosphere at Mars-like pressure.

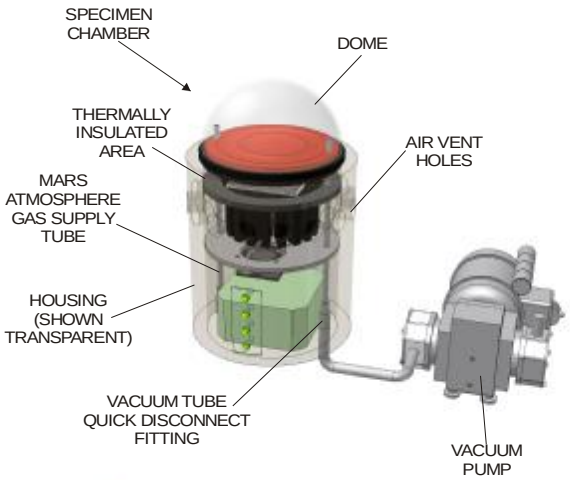


**Figure 40. The Mars Jars will be connected to a vacuum pump to simulate the Martian pressure. The box at the bottom of the housing is loaded manually with solid $CO_2$.**

When the review of the 2005 proposal for the MARS-MTB was obtained there did not seem to be any negative features of the review, which stated, among other things:

> "The proposed Miniature Planetary Environment Simulator details the development of a novel research tool that can be used by researchers at NASA, as well as those in educational institutions of all ages. The proposal explains the idea, construction and electrical wiring of the device in a technically accurate way, and addresses technological concerns associated with accurately simulating a planetary environment such as Mars. The proposal also delineates safety concerns with high and low temperature, vacuum creation and electrical shock, and will develop the device to minimize such safety hazards. The proposal describes a feasible and novel device, which can add a significant utility to those interested in learning about life in extreme conditions.
>
> "The commercial merit of the Miniature Planetary Environment Simulator is a targeted, specialized audience. Such a device can only be useful in laboratories that research extremophiles as well as educational institutions at the middle, high and college levels. This device does not have any applications to the mainstream community; however a successful development and marketing to educational institutions and national laboratories can prove to be quite profitable. Simulating multiple environments other than Mars will make this device more versatile."

Safety concerns were voiced by one reviewer:

> "The safety of such a device should take precedence in the development phase. Because extreme environments are being created inside the chamber, extreme accidents can occur outside the chamber, significantly injuring one or many researchers. Therefore, every precaution should be addressed when designing the system for maximum performance. I recommend the Miniature Planetary Environment Simulator for SBIR Phase I funding. Because it is an educational device, reception for this type of innovation is looked favorably upon."

Opportunities continued to be sought for supporting the proposed MARS-MTB (modular test bed for laboratories and classrooms). This included intensified contacts with SBIR officers at NASA field centers. We have been pointing out that there did not seem to be any negative features of the reviews of the proposal submitted in response to last year's NASA SBIR solicitation

In summary, support for this component of the architecture will continue to be pursued. Discussions are underway with NASA personnel to determine optimal proposal content for a re-submission in response to NASA's 2005-2006 SBIR/STTR solicitation.

### 1.3.3 Laboratory Test Bed User Community

Investigators have expressed considerable interest in becoming users of the existing facility at SHOT or purchasers of a facility of their own. The SAC suggested that the facility be used as a means of generating standards of fidelity for planetary environment simulators. Specifically, the Indiana University Astrobiology Center has expressed

intense interest in using the facility for mineralogical studies, and preliminary testing has begun. Astrobiologists at NASA Ames Research Center are very interested, and Dr. Lynn Rothschild will definitely perform a series of DNA damage studies possibly during the second Phase II year of the project.

The economics of utilization of the Test Bed are being determined. With sponsor permission SHOT anticipates a customer fee of $300 per day, which covers everything except customer transportation but especially including personnel services and consumables. SHOT anticipates selling installed units to customers at a price slightly greater than $100,000 not including renovations and cryogenics. A separate additional price for renovations and cryogenics done by SHOT is under consideration. Early Year-2 activity will include development and refinement of a SHOT business model built around the Laboratory Test Bed.

A large proposal was submitted to the NASA Astrobiology Insitute (NAI) titled "Astrobiology Planetary Simulator Consortium". This is a 5-year plan involving the placement of five Laboratory Test Beds around the U. S. in laboratories primarily of NAI investigators. The executive summary of this proposal is given in Appendix 4.1.

## 1.4 Objective 4. Future science components of the architecture

### 1.4.1 Spaceborne ecopoiesis research facilities

During Phase II an evolution of concepts for spaceborne ecopoiesis research facilities occurred, starting with an extended version of SHOT's environmental control facility known as "Avian Development Facility" [Vellinger et al., 2003] -- ADF. In its most general principal it would accommodate four Modular Test Beds (see previous section) and be carried as an internal payload on ISS or the Space Shuttle as shown in Figure 41. However, these venues are no longer available, and autonomous environment-control hardware will be needed. A concept was presented to the Robotic Lunar Exploration Program (RLEP) for use on the lunar surface as sketched in Figure 42. Finally in the place of either of these two possibilities a more versatile design is envisioned as shown in Figure 43.

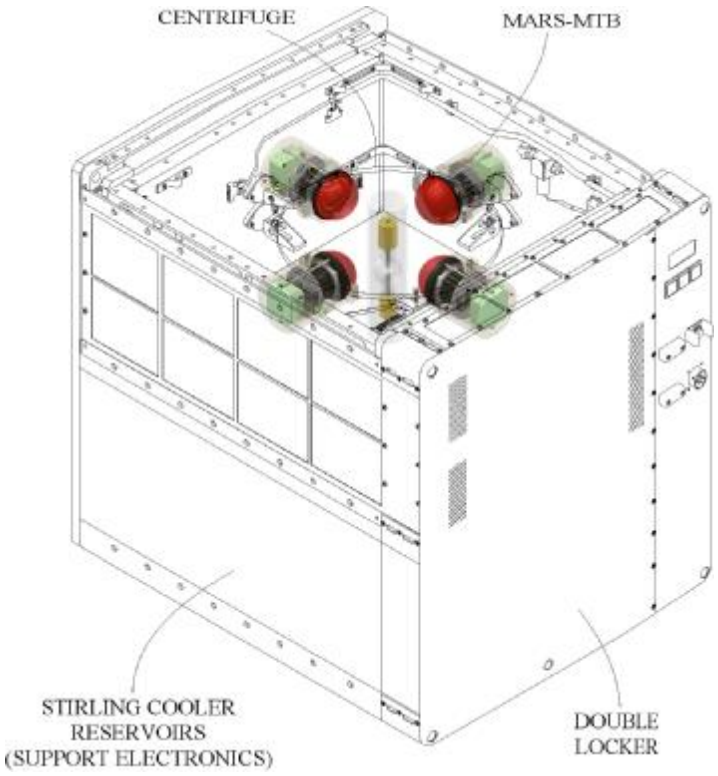


**Figure 41. Original concept for a spaceborne ecopoiesis test bed. The environmental box and avionics are placed in a double middeck locker equivalent with a Stirling cooler and a 0.38-g rotor carrying four test beds modeled after the Modular Test Bed described in the previous section. The design concept has been superceded by that described in Figure 41.**

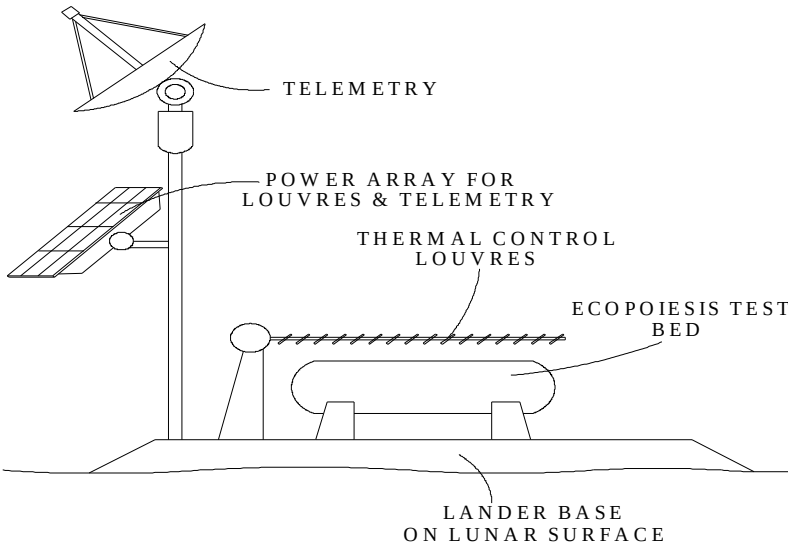


**Figure 42. Sketch created to explain potential hardware components for a secondary-payload Robotic Lunar Ecopoiesis Test Bed for the Robotic Lunar Exploration Program (RLEP). Louvres control temperature to within Mars limits and can optionally cycle solar illumination to resemble 14 Martian sols during the lunar day, for example.**

This concept builds on the notions sketched in Figure 42 but includes additional necessary technical detail and has realistic versatility built in. With small design modifications it should be capable of functioning in orbital space flight or on the moon while providing Mars gravity. Illumination is based on movable mirrors, so that intrinsic illumination is not required, as is the case in the configuration of Figure 41.

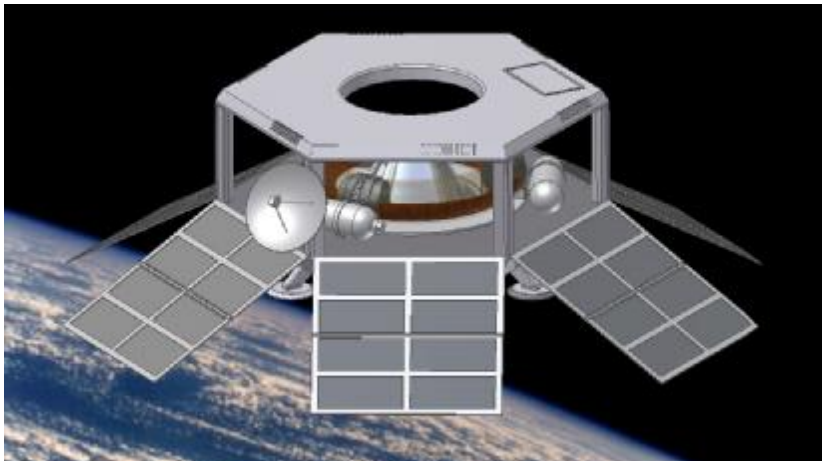

**Figure 43. Engineering rendering of most recent Robotic Ecopoiesis Test Bed concept, including Mars gravity in Earth orbit or on the lunar surface provided by a rotating three-sectored toroidal sample container.**

The features of this version can be appreciated in the exploded view in Figure 44, which shows three transparent toroidal sectors for three ecopoiesis experiments, each with an autonomous low-pressure gas supply. The transparent sectors are illuminated by a set of sunlight-reflecting mirrors that can be positioned to provide the lighting program. These sectors form a toroid that rotates at various speeds depending on the g-level desired. To create Mars gravity at a radius of 15 cm, for example, the rotation frequency is 50 rpm (about 1 Hz). A very small motor can drive the nearly weightless rotor using power from the photovoltaics. On the moon, the entire system would rest during the 14-day lunar night. A small antenna communicates commands and data via relay satellites, whether in orbit around Earth or around the moon.

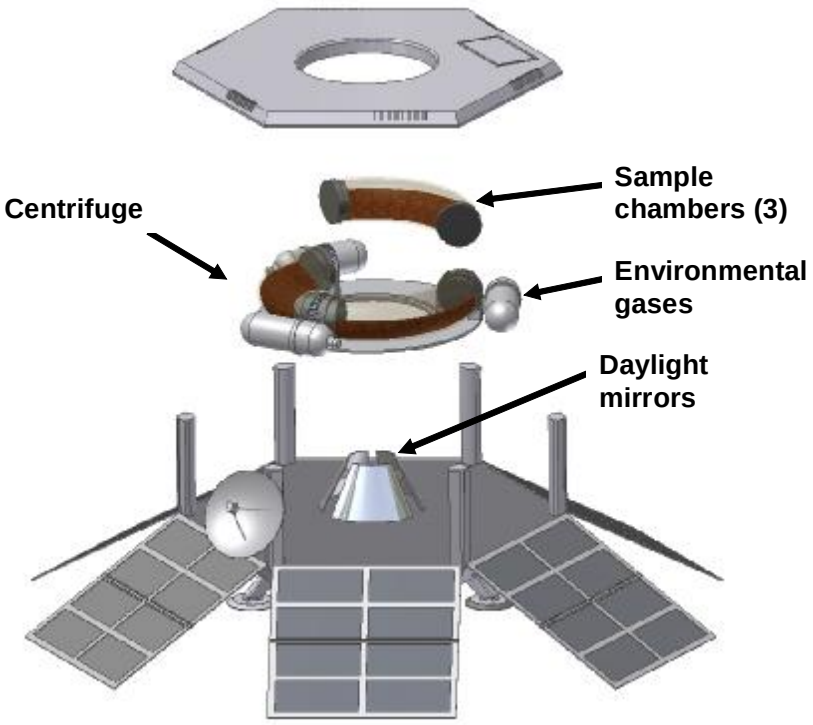


**Figure 44**. **Engineering rendering of most recent Robotic Ecopoiesis Test Bed concept, exploded view. Significant components include three experiment toroidal sectors, movable mirrors, autonomous gas supplies for each sector, and a rotor.**

The role of mirrors is to create day and night cycles or to control the lighting program in general. In Figure 45 the mirrors are shown in illuminating mode on the left and in non-illuminating mode on the right.

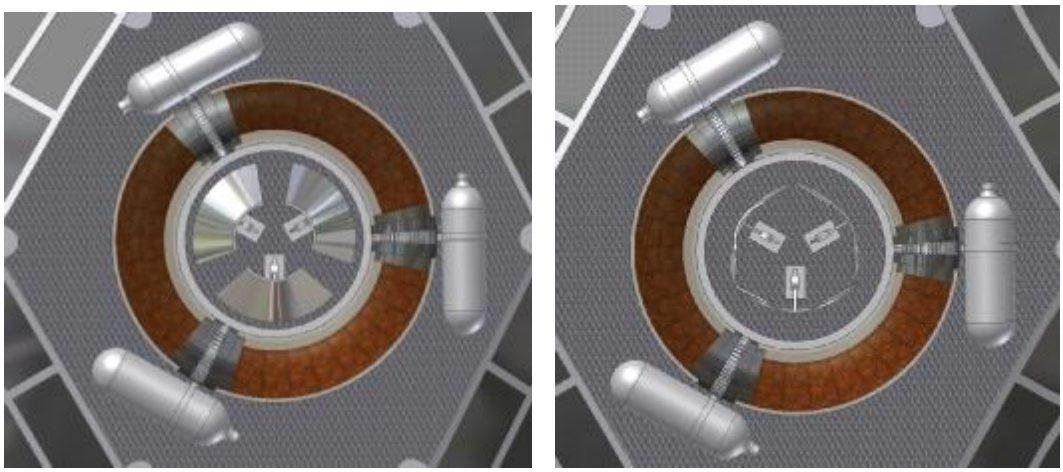

**Figure 45. Illustration of mirror operation. In reflecting position on the left, non-reflecting, on the right.**

### 1.4.2 Seeking sponsorship for ecopoiesis research in space

A response to the NASA Request for Information for the Robotic Lunar Exploration Program (RLEP) was submitted by October 12, 2004, and it led to a statement of interest by the RLEP leadership. The Principal Investigator visited with the RLEP leadership at Goddard Space Flight Center on January 11 and 12, 2005, and continued to maintain contact by telephone and via Lunar Exploration Advisory Group (LEAG) activities as an appointed member of LEAG. It should be added that the RLEP is also interested in other SHOT products.

For background, the RLEP is a program of the recently established Exploration Science Mission Directorate (ESMD, mostly composed of the former Office of Biological and Physical Research). In parallel the Science Mission Directorate (SMD, mostly composed of the former Office of Space Science) is working together with ESMD to establish early lunar research priorities through a joint forum known as Lunar Exploration Analysis Group (LEAG), which met in Maryland in January, 2005. The Principal Investigator is an appointed member of LEAG.

The SMD issued a separate solicitation for 2-page "White Papers" on lunar exploration, to which SHOT submitted a response that included the content given in Appendix 3.2.

As the Robotic Lunar Exploration Program (RLEP) progressed in its planning, a Specific Action Team (SAT) was assembled at the Lunar and Planetary Institute in Houston March 18-20, 2005, in which the principal investigator participated. After the Lunar Reconnaissance Orbiter (LRO) mission in 2008, the RLEP is expected to consist of three missions to the lunar surface between 2009 and 2015. These missions are highly constrained and, according to current policy, dedicated to gathering information that is critical to the safe landing of human missions, most likely at a polar site. Under this policy it appears unlikely that the RLEP would provide a venue for astrobiological research including experimental ecopoiesis. The search for ecopoiesis research opportunities at extraterrestrial venues will therefore need to target later opportunities, such as may be associated with human exploration when it begins, no earlier than 2015.

The NASA Science Mission Directorate (SMD) has not responded to the above "White Paper" that was submitted by SHOT in response to its request for lunar surface demonstration concepts. The goals of the SMD remain unclear at the moment, since most attention is being given to critical paths toward safe, successful human exploration missions with relatively less attention to scientific goals. SHOT continues to assess policy changes that impact ecopoiesis research, and the assurance of continuation of the development of this architecture (expansive program design) continues to be pursued.

The Robotic Lunar Exploration Program (RLEP), despite extensive work by a Specific Action Team (SAT), in which the principal investigator participated, the RLEP missions are expected to include little if any life science experimentation. Even the two possibilities considered, a biosentinal for radiation and a lunar dust hazard experiment,

unlikely to be considered. Under the existing policy it appears unlikely that the RLEP would provide a venue for astrobiological research including experimental ecopoiesis.

A response was submitted to NASA Request for Information (RFI) NNH06RFI001R "Request for Information: Developing a Strategy for Future Exploration of the Moon and Beyond". Selected components of this response are summarized in Appendix 3.3.

It is to be expected that further rounds of RFI's will appear, and it is our intention to respond to them until the locus of responsibility for RLEP settles. In a single year, the RLEP leadership assignment has traveled from Goddard Space Flight Center (to which RFI's were sent) to Ames Research Center (to which the above RFI was sent) to Marshall Space Flight Center, where responsibility, according to recent news items, now resides. Another issue to consider is pricing. The budget for the first RLEP mission, the Lunar Reconnaissance Orbiter (LRO), as LRO has matured, has progressed from about $350 million to potentially over $1 billion, partly due to escalation of scope and partly due to revised choice of launcher. It is a certainty that Robotic Lunar Test Bed will not be a part of RLEP2, which is planned as the first lunar lander in the series.

The pace of working toward extraterrestrial venues for ecopoiesis research was expected to increase, owing to disciplinary shifts in research emphasis in several circles. However, the rapid movement of the RLEP and the formation of LEAG have impacted the role of science in national space exploration planning. At first there was a great deal of hope that this hastening of exploration of the Moon and Mars would be favorable to the project. Despite active efforts on the part of the Principal Investigator and his colleagues around the country, with the shifts in emphasis that are currently occurring, SHOT's plans for using extraterrestrial research venues may be delayed by several years.

Continued participation in lunar planning at NASA by the Principal Investigator is expected – at no cost to the project. Further opportunities to propose research at extraterrestrial venues in the long term will, despite current trends, still be sought.

# 1.5 Project Management

### 1.5.1 Research Team

The project team at SHOT was assembled and tasks were assigned to individuals with a kickoff meeting held August 25, 2004. Initial SHOT participants were:

- Dr. Paul Todd – Principle Investigator, Chief Scientist
- Nathan Thomas – Project Manager, Electrical Engineering
- Bill Metz – CAD, Mechanical Design, Assembly
- Heidi Platt – Chemical Engineering
- Alan Constance – Mechanical Engineering, Thermal Design
- Darrell Masden – Mechanical Engineering, Structural Design
- Bill Johnson – Software Engineering
- Lara Deuser – Lab Scientist
- Ken Barton – Facility Support (Lab, Network, Utilities)

Recently Alan Constance was replaced by Andy Kurk, and Bill Johnson was replaced Doug Hudson, owing to internal project reassignments at SHOT. As is normally the case at SHOT, these replacements were seamless.

Dr. Penny Boston and Dr. David Thomas performed preparatory research in their laboratories at New Mexico Institute of Mining and Technology and at Lyon College, respectively, until May 2005, after which they initiated experiments using the Test Bed at SHOT.

### 1.5.2 Gantt Chart

The originally proposed schedule is repeated in the Gantt chart (Figure 46). Test bed operations began about 6 weeks later than scheduled on the chart, owing to delayed delivery of the solar simulator and AM0 optical filter. The biological runs are shorter than originally planned, so the 5$^{th}$ biological run has been executed. The first meeting of the SAC at SHOT was delayed owing to delays in Test Bed operation. The performance of the detailed MTB design had to wait until the LTB was operating, since the same personnel were involved. Most of the rest of the project continued to follow this schedule.

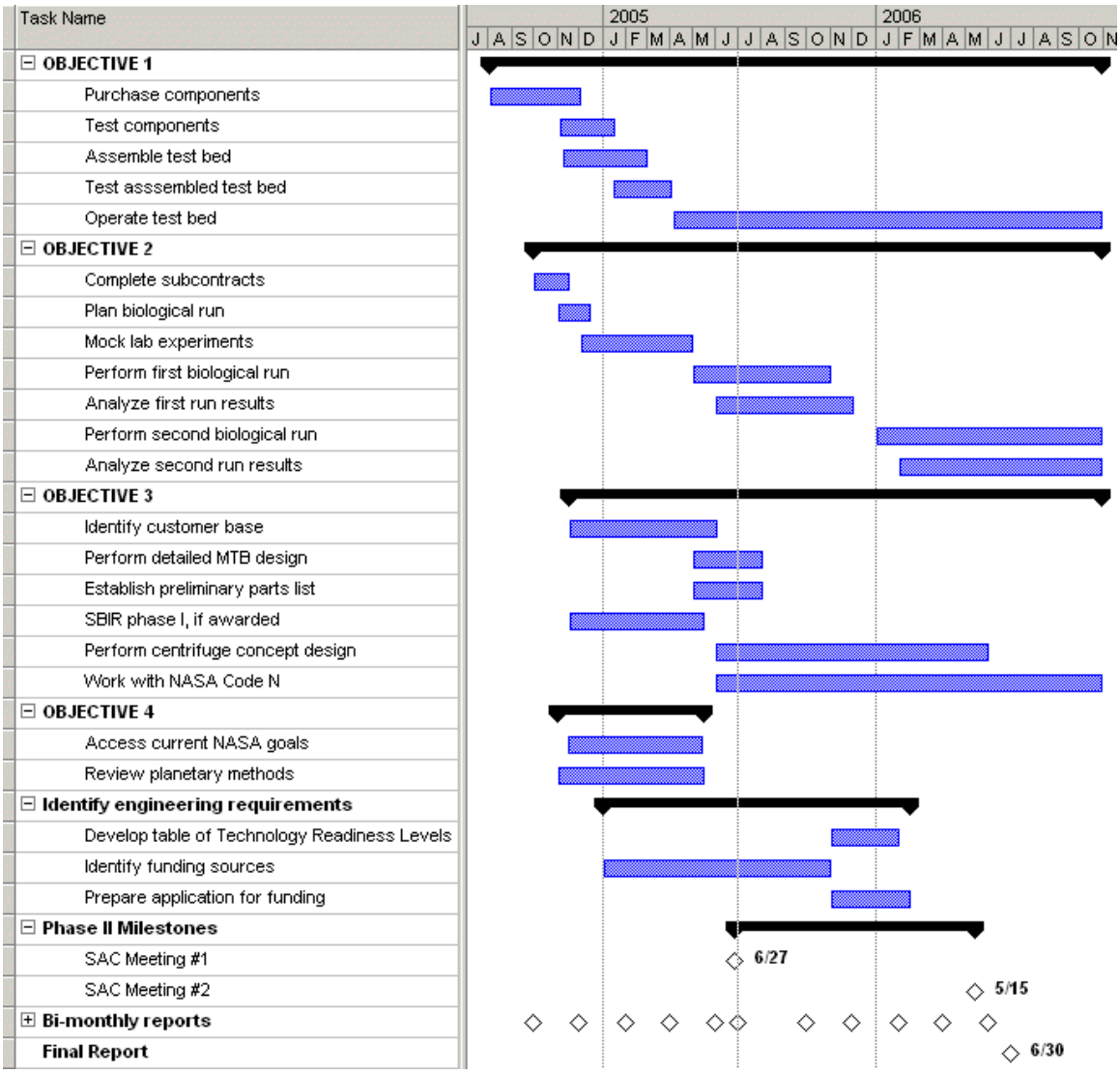


**Figure 46. Gantt chart as originally planned. The only changes represented are SAC meeting dates.**

### 1.5.3 Science Advisory Committee

Prior to Phase I of the project several U. S. leaders in astrobiology research were canvassed for their availability and suitability to serve on the project SAC. After carefull selection the following membership was established, and these members have been of great value to the project throughout its life:

Dr. Penelope J. Boston, New Mexico Institute of Mining and Technology
Dr. Lawrence Kuznetz, NASA Space Biomedical Research Institute (NSBRI) and Johnson Space Center
Dr. Christopher P. McKay, NASA Ames Research Center
Dr. Lynn Rothschild, NASA Ames Research Center
Dr. Andrew Schuerger, Florida International University
Dr. David J. Thomas, Lyon College

Dr. Chris McKay, member of the Science Advisory Committee (SAC), visited SHOT on November 9, 2004. Dr. Lawrence Kuznetz, also a member of the SAC, was paid a visit at Johnson Space Center in January, 2005. He assisted in establishing contact with Dr. Geoffrey Landis of NASA GRC and Dr. David McKay at JSC. Chris and Larry were unable to attend the on-site meeting of the SAC in June, 2005 (agenda below). Both were able to participate in the SAC meeting held August 13, 2005, at which Dr. Schuerger and Dr. Rothschild were absent, and at which time the final components of the Phase II research outline were established.

After the Test Bed became operational at SHOT's facility a meeting of the Science Advisory Committee (SAC) occurred at SHOT during the week of June 27. The agenda included the following items:

Review of SHOT
Review of test-bed engineering, including tour
Review of test-bed applications
Review of plans for the remainder of the project
Review of plans and future funding possibilities
Tour of SHOT facilities, opening of the test bed for sample removal
Executive meeting of the SAC
Preparation for NIAC site visit

The members present were Lynn Rothschild, Andrew Schuerger, Penelope Boston and David Thomas.

The SAC expressed extreme enthusiasm for the project and offered numerous ideas for sustaining it, especially in the context of the Laboratory Test Beds and the Modular Test Beds. As a result of the executive session they will produce a report advising the future of the project. This report is expected to be available to the NIAC site visit team on July 6, 2005. Advice that will be incorporated includes recommendations for analytical capabilities to be added to the Test Bed during Year 2. SHOT intends to follow this advice and acquire optimized analytical capabilities for the atmospheric gases and spectral measurements.

The following approximate calendar was developed for planning purposes for the science of the final first-year reporting period:

| | |
|---|---|
| May 31 | Dave Thomas begin 1-day survival experiment at SHOT |
| Jun 1 | Bimonthly interim report to NIAC |
| Jun 2 | Dave Thomas start multi-day experiment |
| Jun 9-13 | Finish multi-day experiment |
| Jun 23 | Penny Boston begin experiment |
| Jun 24-31 | Dates for SAC meeting |
| Jun 23-31 | Penny Boston research, combine with D. Thomas<br>Initiation of a possible 6-week experiment. |

| | |
|---|---|
| Jul 1 | First-year report due (30 days before option date) |
| Jul 6 | Site visit by NIAC |
| Aug 1 | Second-year of project begins |
| Aug 11-14 | Presentations at Mars Society International Convention |
| Sep 1 | Bimonthly interim report to NIAC |

## 2. A Complete Technical Description of the Concept and Its Operational Principles

The matters contained in the title of this required section are thoroughly covered in the material contained in Sections 1 and 3. A brief synopsis suitable to this subheading is provided as follows:

The Concept represents a research program architecture, as required of NIAC-fellow applicants. Therefore, the engineering description of the enabling technologies is found in sections 1 and 3 while this section is devoted to the scientific rationale for the Concept, which is the establishment of a technical foundation for ecopoiesis research and identifying the moon as one of various potential venues for such research and identifying robotics as the enabling means. A specific refinement of the terraforming idea dubbed "ecopoiesis" by Robert Haynes refers to the process of naturally evolving a physical and biological environment on a previously lifeless planet by the deliberate introduction of terrestrial-type ecosystems on remote celestial bodies such as planets, moons and asteroids.

The operational principle is to proceed in a stepwise fashion, following the Haynes-McKay paradigm: "…. if it is decided to implement such a program of planetary engineering, a slow and conservative approach is essential. Sufficient time must be allowed for a wide range of studies of Mars as it exists at present, and for careful planning, modeling and 'pilot-plant' trials (where possible) of all successive steps in the enterprise." This challenge calls for research that includes biological experiments and theoretical modeling directed at the implementation of the enabling notions in terraforming.

The operationsl principles, therefore, consist of stepwise introduction of enabling technologies and their implementation as "pilot plants" for ecopoiesis research. This means, first, high-fidelity ground-based simulation of planetary environments (Section 1.1) followed by the use of these environments in the search for pioneer organisms (Section 1.2). This step is followed by the move into space, where gravity can be treated as a variable, and space-based technology can be practiced in Earth orbit and on the moon (Section 1.4), closer to Earth than on the surface of Mars, for example. Such undertakings require the participation of numerous investigators who must be equipped, educated and enthused for participation in the terraforming adventure (Section 1.3).

## 3. Identification of enabling technologies

Section 1.1 of this report is dedicated to the primary components of this subject, providing requirements, design principles, and final designs and operations where implemented. Section 1.3 contains technical descriptions and designs for the proposed community of terrestrial users, and Section 1.4 indicates proposed enabling technologies for studies in space, including Earth orbit and the lunar surface. A synopsis is provided here, using summary diagrams representing each technology: Laboratory Test Bed, Modular Test Bed and Space Test Bed.

### 3.1 Laboratory Test Bed

The detailed description given in section 1.1 is summarized in the original design scheme reflected in the flow diagram of Figure 47 and shown as a rendering in Figure 48, in which each of the components of the Laboratory Test Bed are called out.

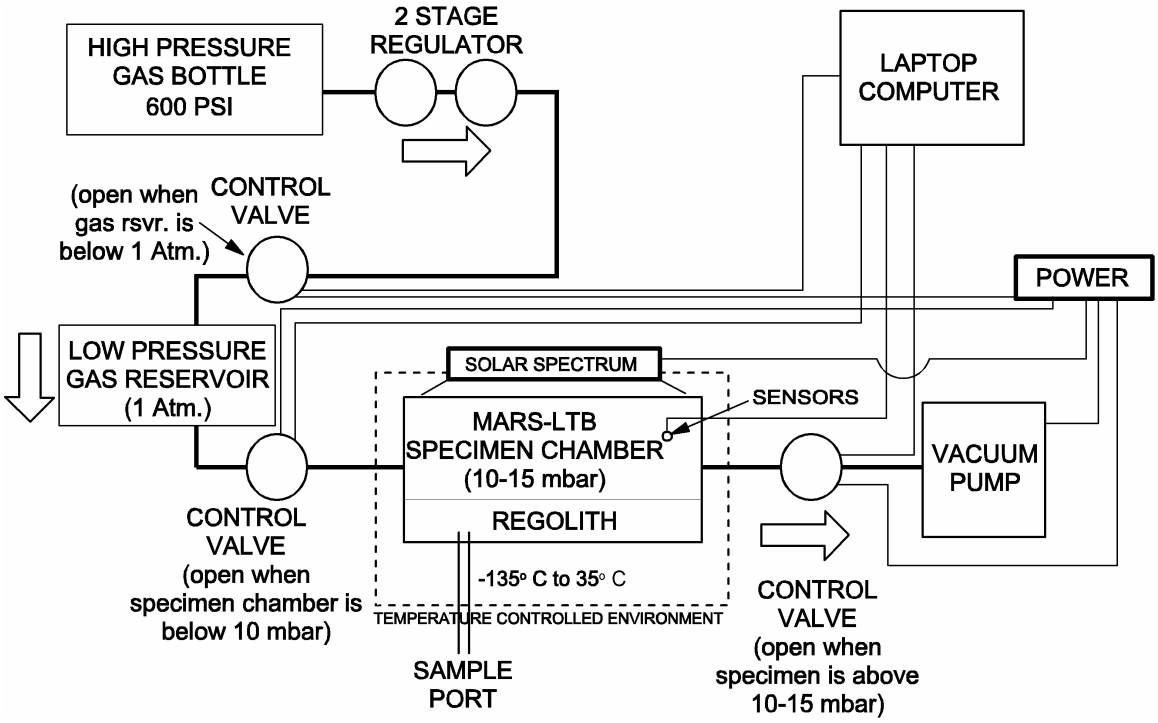


**Figure 47. Flow diagram for the initial enabling technology, the Laboratory Test Bed.**

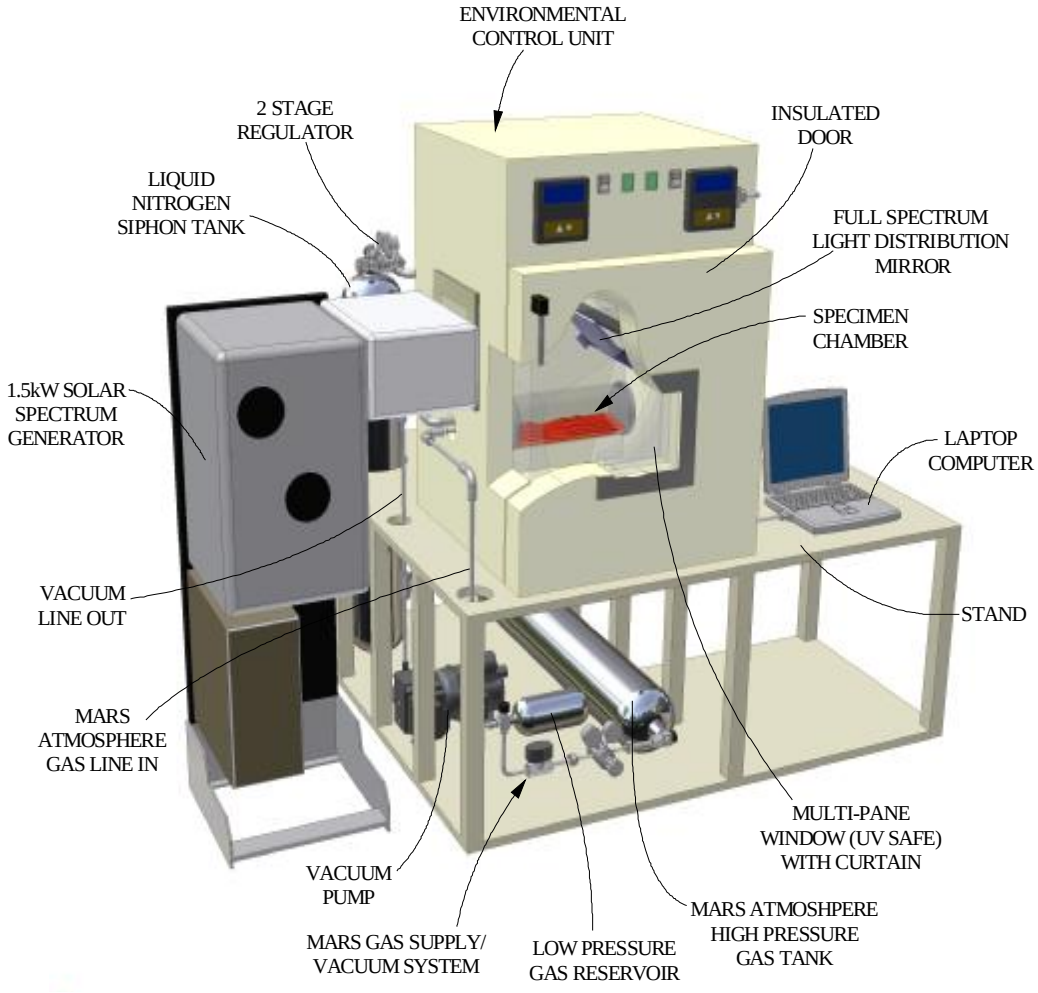


**Figure 48. Rendering of the enabling technology design that was implemented, as described in detail in Section 1.1.**

### 3.2 Modular Test Bed

The second enabling technology is the Modular Test Bed, designed to increase the community of users and the level of interest in ecopoiesis research and planetary biology in general. The full details are given in Section 1.3, and summary rendering is shown in Figure 49. In section 1.3 it has been seen that frozen $CO_2$ is a feasible alternative to thermoelectric cooling as a means of temperature control in this enabling technology. This design was never fabricated, but, in the absence of SBIR funding, SHOT has volunteered to undertake development if the project "Planetary Simulation Consortium" is to be funded by the NAI.


**Figure 49. Rendering of the enabling technology for proposed Modular Test Beds.**

### 3.3 Space Test Bed.

Figure 50 illustrates a general view of the proposed technology described in greater detail in Section 1.4. The lunar test bed should be capable of functioning in orbit or on the surface of the moon.

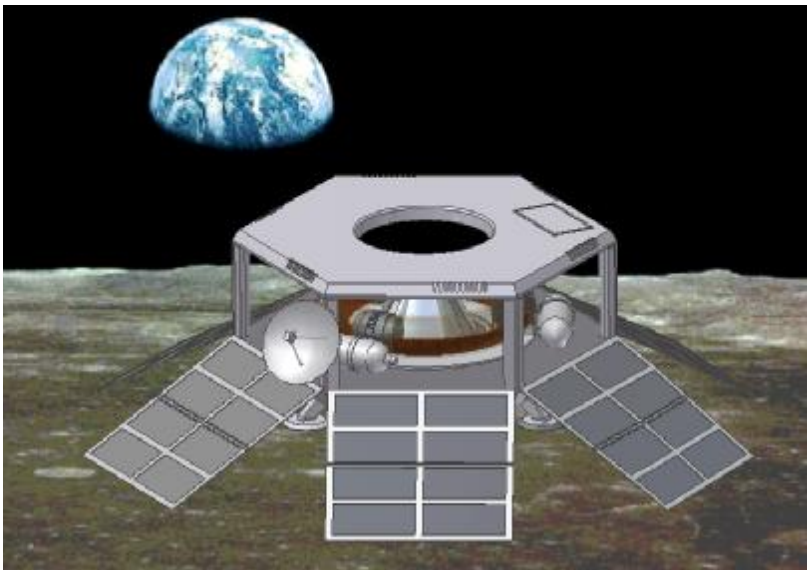

**Figure 50. Artistic concept of the enabling technology for space-based ecopiesis test bed potentially usable in slight or on the lunar surface.**

## 4. Plan for the Future Technical Development of the Concept

### 4.1 Original long-range plan

Figure 51 is a reproduction of the plan presented in the Phase I proposal and to NIAC at Fellows' meetings. As has been seen above (section 1.4) modifications to SHOT's Avian Development Facility (ADF in the figure) to provide an environment for on-orbit study is no longer under consideration. The volume of the proposed very large chamber for external orbital studies is greatly reduced.

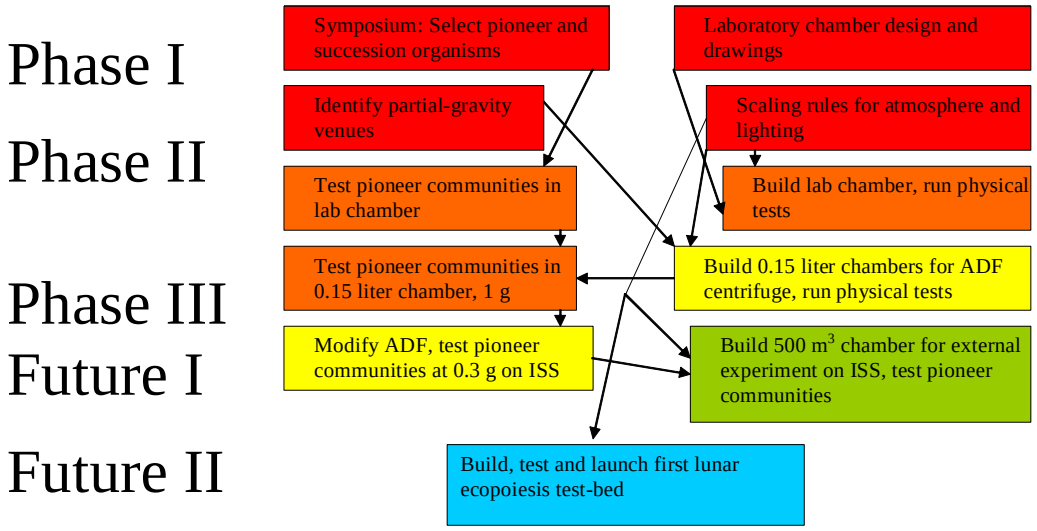


**Figure 51. Reproduction of the long-range program plan presented in the Phase I proposal. The only modifications are the replacement of the ADF-based environmental container for controlled-g experiments and the reduction in the size of final test beds.**

### 4.2 Implemented plan to date

To date the plan was implemented according to design, staying very close to the Gantt chart in Figure 46. All four objectives were addressed, and the progress made on each was as expected in the initiation of a concept implementation expected to take at least 10 years. The plans for the future of each objective are given in the following paragraphs.

### 4.3 Plans per objective

#### 4.3.1 Earth-based facilities

SHOT will serve astrobiology and space hardware customers using the existing Laboratory Test Bed. SHOT will continue to seek funding and to help others seek funding for the placement of Laboratory Test Beds in the laboratories of astrobiology

researchers. As mentioned in Section 1.3, a proposal to the NAI to place Test Beds in five key laboratories is pending. If this project is funded SHOT will complete development of low-cost $CO_2$-cooled "Mars Jars" mainly for educational use. Funding for Modular Test Beds (Section 1.3) will continue to be sought.

### 4.3.2 Ecopoiesis research on Earth

Initially, research in planetary biology will continue at SHOT's facility ona pay-as-you-go basis. An experiment on Martian evaporites and mineral processing in Martian brines is beginning approximately 1 hour before the closing of the NIAC-sponsored project for which this final report is written. Two new proposals have been submitted by collaborating principal investigators Prof. Lisa Pratt of Indiana University and Prov. Tullis Onstott of Princeton University (see next section). The future of test-bed based ecopoiesis research on earth appears to be off to a good start.

### 4.3.3 Community of users

During this reporting period interest among the user community rose considerably, and two groups have sought funding to become users of the laboratory simulator at SHOT. They are members of the Indiana-Princeton-Tennessee Astrobiology Institute (IPTAI), and their proposals were submitted in response to NASA solicitation NNH06ZDA001N-MFRP "Mars Fundamental Research". Prof. Tullis C. Onstott of Princeton University has proposed to perform simulation experiments in the SHOT Simulator as a component of a project titled "Degradation and Release of Volatile Organic Molecules in a Simulated Martian Environment". Prof. Lisa M. Pratt of Indiana University, Department of Geological Sciences, has proposed to perform simulation experiments in a project under the same NASA program involving amino acid volatility, synthesis and degradation in a variety of mineral environments.

Both of these investigators are interested in the SHOT simulator because it can provide a water-saturated environment along with otherwise authentic Mars surface conditions. It is worth mentioning that the original Simulator design was focused on ecopoiesis (as is the project as a whole), and water must be treated as a controlled variable in ecopoiesis research.

The proposal for the MARS-MTB (modular test bed for laboratories and classrooms) will be prepared for re-submission, and this activity already includes due attention to identifying interested divisions/branches within NASA. The proposal will also be prepared for submission for sponsorship in the educational context, and dialogue with educational interests will be continued. The great news of the operation of the Laboratory Test Bed has been posted on the SHOT web site and linked to the "Marsbugs" web site and will be the subject of a press release soon. This increase in visibility will be exploited to generate support or financial interest in the MARS-MTB.

Whether support for development of the MARS-MTB is obtained or not, a synopsis of its design status will be prepared for the NIAC final report as specified in the Phase II proposal.

### 4.3.4 Space-based Test Beds

The Robotic Lunar Exploration Program (RLEP), a central feature of the Vision for Space Exploration, has been exchanged among three NASA field centers during its short life and is advised by an eclipsing scientific advisory group. It is SHOT's intention at present to follow up on the submitted responses to the RFI: "DEVELOPING A STRATEGY FOR FUTURE EXPLORATION OF THE MOON AND BEYOND", Reference Number NNT06DSFEMBL. SHOT will a\d engineering detail to the concepts for extraterrestrial test bed design & implementation for forwarding to NASA Headquarters, Exploration Systems Mission Directorate (ESMD).

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## List of Publications and Presentations

At NIAC's request a cumulative list of publications and presentations resulting from this project to date was prepared and submtted to the Director.

PUBLICATIONS

P. J. Boston, P. Todd and K. R. McMillen. Robotic Lunar Ecopoiesis Test Bed: Bringing the Experimental Method to Terraforming. *Space Technology and Applications International Forum – STAIF 2004*, Ed. M. S. El-Genk, American Institute of Physics, Washington, DC (2004).

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# Appendices

Also requested within the final report are "Copies of any briefings, presentations or professional society technical papers pertaining to the proposed Phase II area of study". These items are collated as appendices to this report.

**1. Articles, Presentations, Abstracts and White Papers about the Test Beds**

1.1. Environmental simulatiors for experimental ecopoiesis.
1.2. A Versatile Tested Planetary Environment Simulator for Biological and Mineral Research (abstract)
1.3 A Versatile Tested Planetary Environment Simulator for Biological and Mineral Research
1.4 Performance Characteristics of a Laboratory-Based Simulator of the Mars Surface Environment

**2. Articles, Presentations, Abstracts and White Papers about Ecopoiesis Research**

2.1 SURVIVAL AND GROWTH OF COMMON CYANOBACTERIA IN PRIMORDIAL ATMOSPHERES
2.2 Common freshwater cyanobacteria grow in 100% $CO_2$.
2.3 An inexpensive apparatus for growing photosynthetic microorganisms in exotic atmospheres.
2.4 Ecological community development in a lunar ecopoiesis test bed facility: Key concepts
2.5 EARLY RESULTS OF ECOPOESIS EXPERIMENTS IN THE SHOT MARTIAN ENVIRONMENT SIMULATOR
2.6 EFFECTS OF ATMOSPHERIC PRESSURE ON THE SURVIVAL OF PHOTOSYNTHETIC MICROORGANISMS DURING SIMULATIONS OF ECOPOESIS

**3. Articles, Presentations, Abstracts and White Papers about Ecopoiesis Science and Extraterrestrial Research**

3.1 PLANETARY BIOLOGY AND TERRAFORMING
3.2 Ecopoiesis Biology Surface Demonstration
3.3 Robotic Lunar Ecopoiesis Test Bed

**4. Proposal abstracts**

4.1 Miniature Planetary Environment Simulator
4.2 Astrobiology Planetary Simulation Consortium

**5. Power-Point Presentations**

5.1 STAIF, January 2005
5.2 NIAC Fellows Meeting, November 2005
5.3 Astrobiology Scientific Conference, Poster, March 2006

**Appendix 1.1**

A presentation was made at the American Society for Gravitational and Space Biology (ASGSB) annual meeting held in Brooklyn, NY, November 10-13, 2004. The abstract follows:

"ENVIRONMENTAL SIMULATORS FOR EXPERIMENTAL ECOPOIESIS" by Paul Todd, Penelope J. Boston, Heidi Platt and George W. Metz.

The major issues of planetary biology consist of searching for life, planetary protection, and environmental life support for human visitors and terraforming. A potential sequel to the first three is terraforming, the conversion of a planetary surface to earth-like conditions hospitable to Earth life. A subset of terraforming is ecopoiesis, the early development of a living ecosystem. Despite approximately two decades of public discussion there has been little or no experimental research focusing on ecopoiesis. Initially, small experiments with terrestrial organisms in a planetary (Mars-like) environment are required. To facilitate such experiments an affordable Mars Atmosphere and Regolith Simulation Laboratory Test Bed (MARS-LTB) has been designed and is under construction. Its robotic controls can be programmed to effect a Mars night and day cycle at a variety of simulated latitudes effecting temperature swings between -165$^{o}$C and +26$^{o}$C at a pressure around 10 mbar in an atmosphere with Martian composition. The chamber is about 4 l in volume and contains about 1 kg of regolith stimulant. The regolith and atmosphere can be sampled with minimum disturbance to the contained environment. As an adjunct to the Laboratory Test Bed a portable Modular Test Bed (MARS-MTB) has been designed for distribution to research laboratories and educational institutions ("Mars Jars").

This research is supported by the NASA Institute for Advanced Concepts, subcontracts 07605-003-020 and 07605-003-026 under contract NAS5-03110 between Universities Space Research Association and NASA.

This presentation generated considerable audience interest, including interest in purchasing the technology. Simulator sales is considered to be a critical part of the architecture of ecopoiesis research and an important near-term goal for SHOT. At this meeting the PI was elected to the office of President-Elect of the Society. Under a new publication policy, an "expanded abstract" was published in *Gravitational and Space Biology* 14 (2) (2005), with the following content:

**Appendix 1.2**

Under a new publication policy, an "expanded abstract" of the above presentations was published in *Gravitational and Space Biology* 14 (2) (2005).

**ENVIRONMENTAL SIMULATORS FOR EXPERIMENTAL ECOPOIESIS.** P. Todd[1], P. J. Boston[2], H. Platt[1], G. W. Metz[1]. [1]SHOT, Inc., Greenville, IN, [2]Center for Cave and Karst Studies, New Mexico Institute of Mining and Technology, Socorro, NM.

The major issues of planetary biology consist of searching for life, planetary protection, environmental life support for human visitors, and terraforming. A potential sequel to the first three issues is terraforming, the conversion of a planetary surface to earth-like conditions hospitable to Earth life. A subset of terraforming is ecopoiesis, the early development of a living ecosystem [Haynes and McKay, 199x]. Despite approximately two decades of public discussion there has been little or no experimental research focusing on ecopoiesis. Initially, small experiments with terrestrial organisms in a planetary (Mars-like) environment are required. Such experiments simultaneously address issues of ecopoiesis and planetary protection. To facilitate such experiments in research laboratories an affordable Mars Atmosphere and Regolith Simulation Laboratory Test Bed (MARS-LTB) has been designed and is under construction for testing prior to its becoming available for distribution to research laboratories and educational settings. The purpose of this brief presentation is to report progress on the design and development of this research tool.

The requirements for high-fidelity simulation of the Mars environment are based on current knowledge or understanding of the pressure and composition of the Mars atmosphere, the Mars daily temperature profile, the light spectrum at the surface and the composition and physical properties of the Mars regolith. Each of these features of Mars is described briefly.

Atmosphere. The atmospheric pressure on the surface of Mars varies between 7 and 10 mbar, depending on altitude (mountains vs. valleys) and season. This is about 1% of the pressure on the surface of the Earth. The atmosphere contains 95% $CO_2$, 2.7% $N_2$, 1.6% Ar, 0.13% $O_2$, 0.03% $H_2O$ and trace amounts of Ne, Kr, Xe and $O_3$. Since the trace $O_3$ does not contribute to the UV absorbance of the atmosphere it and the trace inert gases are not essential components of a simulated Mars atmosphere.

Temperature. The maximum temperature on the surface of Mars on a cloudless summer day at equatorial and mid-latitudes is about 26°C. The minimum temperature at night at high latitudes is -135°C. An average daily cycle under consideration for simulation purposes is -80°C minimum night time temperature and +26°C maximum mid-day temperature. This day-night temperature cycle must be repeated every 24.8 h, the length of the Martial sol (day).

Sunlight. The total intensity of sunlight on the surface of Mars is about 40% of that at the top of Earth's atmosphere, so that the Martian surface receives 590 W/$m^2$ of the essentially unattenuated solar spectrum. This spectrum includes ultraviolet light with wavelength below 300 nm, including down to 190 nm. This spectral range is photochemically damaging to nucleic acids and known to kill cells and is included in the spectral output of xenon arc lamps.

Regolith. The dust on the surface of Mars has a high content of oxides of iron, some of which were found to be oxidizing agents in the experiments on the Viking missions. In elemental composition the regolith is 12.5% Fe, 21% Si, 5% Mg, 4% Ca, 3.1% S, 3% Al, 2.3% Na, 0.7% Cl, 0.3% P, and contains traces of Mn, Co, Cu, I , Zn, B, etc. Thus, in addition to the aluminosilicates there is an ample supply of components required by living organisms.

A three-dimensional rendering of the physical layout of the MARS-LTB is shown in Figure 1. Its robotic controls can be programmed to effect a Mars night and day cycle at a variety of simulated latitudes with temperatures between -165°C and +26°C at a pressure around 10 mbar in an atmosphere with composition

resembling that of Mars. The chamber is about 10 liters in volume and contains about 2 kg of regolith simulant. The regolith and atmosphere can be sampled with minimum disturbance to the contained environment.

After several months of testing, models of the MARS-LTB can be made available to research workers and educators interested in astrobiology, planetary microbiology, planetary protection and ecopoiesis.

This research was supported by NASA Institute for Advanced Concepts, subcontract 07605-003-020 under contract NAS5-03110 between Universities Space Research Association and NASA.

REFERENCE Haynes, R. H., and McKay, C. P. "The implantation of life on Mars: Feasibility and motivation." *Advances in Space Research* 12, 133-140 (1992).

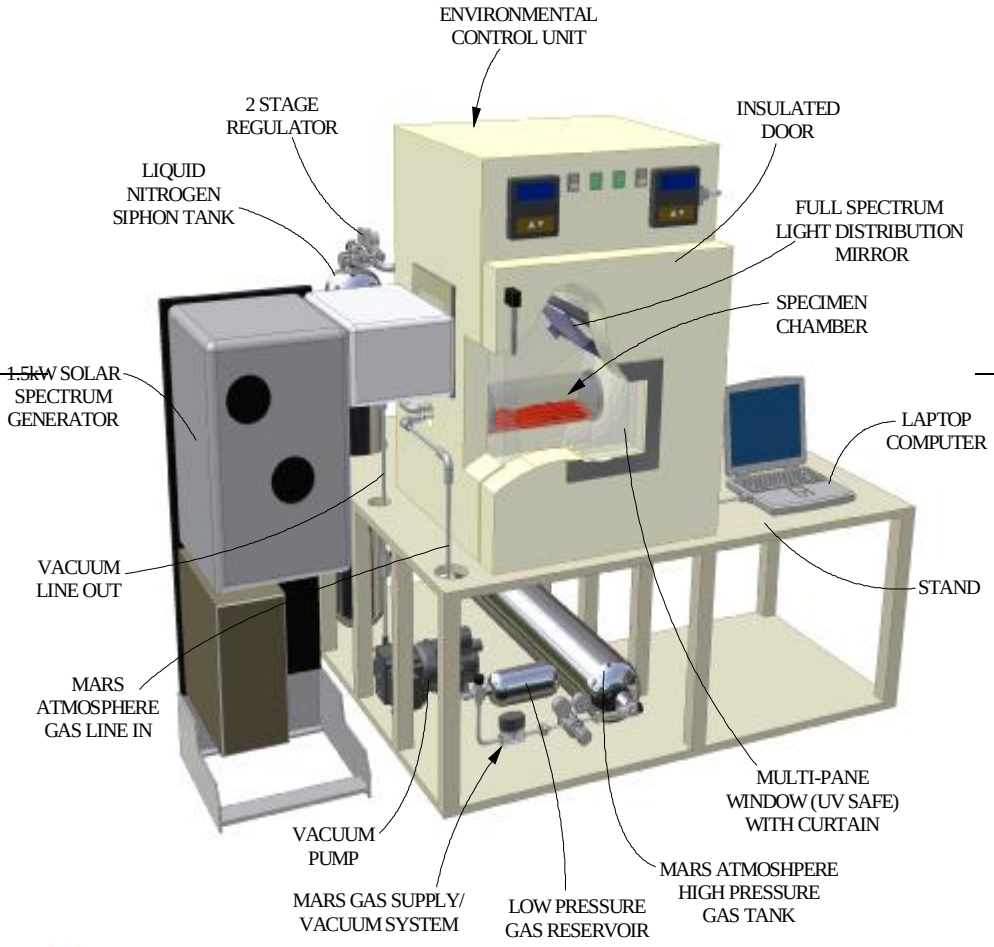


Figure 1. Mars Atmosphere and Regolith Simulator Laboratory Test Bed (MARS-LTB) showing components for illumination, temperature control, atmosphere supply, and experiment container ("Mars Jar").

**Appendix 1.3**
A poster was presented at AbSciCon in Washington DC, 26-30 March, 2006. The following is the abstract:

# A Versatile Tested Planetary Environment Simulator for Biological and Mineral Research

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Penelope J. Boston
*Cave and Karst StudiesProgram*
*New Mexico Institute of Mining and Technology*
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High-fidelity simulation of planetary environments is an obvious component of astrobiological research. Surprisingly few facilities are available to simulate planetary environments for this purpose. Simulators designed for the testing of space hardware tend to neglect certain biologically relevant variables and are seldom available to biologists on a scheduled basis. A commercial simulator suitable for research laboratories was therefore designed, built and tested. The simulator was designed to meet, minimally, requirements for high-fidelity simulation of conditions on the Martian surface: atmosphere composition and pressure (down to 7 mbar), daily temperature extremes (-135$^{o}$C to +40$^{o}$C), daily solar intensity cycle (up to 590 W-m$^{-2}$), regolith composition, and surface solar spectrum (down to 200 nm). To achieve these conditions a 6-liter quartz vacuum vessel containing regolith simulant and test samples is evacuated to the desired pressure, which is maintained using a user-selected support gas (including water if desired). This vessel is contained within a cryogenic chamber cooled by evaporating nitrogen and heated by resistive heaters and programmable to any desired temperature cycle. The solar spectrum is simulated by an automatically controlled 1,000-W xenon arc lamp with filters. Biological (ecopoiesis) experiments up to five weeks long have been conducted in this simulator. This research was funded as project 07605-003-026 of NASA's Institute for Advanced Concepts (NIAC), a program of Universities Space Research Association (USRA) funded by NASA contract NAS5-03110.

**Appendix 1.4**

An abstract was submitted for the forthcoming meeting of the Mars Society in Washington DC, August 3-6, 2006.

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**Performance Characteristics of a Laboratory-Based Simulator of the Mars Surface Environment**

Paul Todd[1], Nathan A. Thomas[1], Michael A. Kurk[1], G. W. Metz[1] and David J. Thomas[2].
[1]SHOT, Inc., Greenville, IN, USA and [2]Lyon College, Batesville AR, USA.

High-fidelity simulation of the Mars environments is an obvious component of fundamental Mars research. Surprisingly few laboratory facilities are available for this purpose. Simulators designed for the testing of space hardware tend to neglect certain scientifically and biologically relevant variables and are seldom available to scientists and biologists on a scheduled basis. A commercial simulator suitable for research laboratories was therefore designed, built and tested. The simulator was designed to meet, minimally, requirements for high-fidelity simulation of conditions on the Martian surface: atmosphere composition and pressure (down to 7 mbar), daily temperature extremes (-135$^{o}$C to +40$^{o}$C), daily solar intensity cycle (up to 590 W-m$^{-2}$), regolith composition, and surface solar spectrum (down to 200 nm). To achieve these conditions a 6-liter quartz vacuum vessel containing regolith simulant and/or test samples is evacuated to the desired pressure, which is maintained using a user-selected support gas (including water if desired). This vessel is contained within a cryogenic chamber cooled by evaporating nitrogen and heated by resistive heaters and programmable to any desired temperature cycle. The solar spectrum is simulated by an automatically controlled 1,000-W xenon arc lamp with an "AM0" filter to produce a spectrum resembling the solar spectrum outside the Earth's atmosphere. This simulator has been operated for more than one year, and biological (ecopoiesis) experiments from 24 hours up to five weeks long have been conducted using pressures of 25, 50, 100, 300, 500 and 1,000 mbar and atmospheric compositions consisting of Mars atmosphere simulant or pure $CO_2$. Temperature records are maintained as temperature is cycled between -80$^{o}$C and +26$^{o}$C once per 24 hours following the published Mars atmospheric and surface heating model applicable to equatorial or mid-latitudes around the Martian vernal equinox. Any thermal and lighting cycle is available and programmable. This research was funded as project 07605-003-026 of NASA's Institute for Advanced Concepts (NIAC), a program of Universities Space Research Association (USRA) funded by NASA contract NAS5-03110.

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**Appendix 2.1**

Some of Dr. Thomas' work on this project was presented at the 14th Western Photosynthesis Conference in January, 2005. Appendix 2.1 is a copy of the abstract of this presentation.

# 14th Western Photosynthesis Conference

6-9 January 2005

Asilomar Conference Center

Pacific Grove, California, USA

**SURVIVAL AND GROWTH OF COMMON CYANOBACTERIA IN PRIMORDIAL ATMOSPHERES.**

David J. Thomas.
Science Division, Lyon College, Batesville, Arkansas 72501, USA.

Life appeared on Earth approximately 3.5 billion years ago. At this time, Earth's atmosphere probably consisted of nitrogen and carbon dioxide with no free oxygen. During this same period, Mars may have had a thicker atmosphere than it does at present, which primarily consisted of carbon dioxide with some nitrogen and argon. Many researchers have hypothesized that life may have arisen on Mars at roughly the same time that it did on Earth. Research in our lab focuses on the ability of cyanobacteria to grow under primordial conditions. We have successfully grown four strains of freshwater cyanobacteria under atmospheric conditions resembling those of primordial Earth (20% $CO_2$): *Anabaena* sp., *Synechococcus* PCC7942, *Synechocystis* 6803 and *Plectonema boryanum*. We also found that *Plectonema boryanum* grows (slowly) under a hypothesized primordial martian atmosphere of 100% $CO_2$. We are also investigating the physiology and evolution of the antioxidant system under primordial conditions. Results from current experiments will be presented. (This research is supported by grants from the NASA/Arkansas Space Grant Consortium, and the NASA Institute for Advanced Concepts.)

**Appendix 2.2**

An article demonstrating cyanobacteria function in 100% $CO_2$ has been published in *Astrobiology*. The abstract of this article is Appendix 2.2.

D. J. Thomas, S. L. Sullivan, A. L. Price, and S. M. Zimmerman. Common freshwater cyanobacteria grow in 100% $CO_2$. Astrobiology 5(1) 66-74, 2005.

Cyanobacteria and similar organisms produced most of the oxygen found in Earth's atmosphere, which implies that early photosynthetic organisms would have lived in an atmosphere that was rich in CO2 and poor in O2. We investigated the tolerance of several cyanobacteria to very high (_20 kPa) concentrations of atmospheric CO2. Cultures of *Synechococcus* PCC7942, *Synechocystis* PCC7942, *Plectonema boryanum*, and *Anabaena* sp. were grown in liquid culture sparged with CO2-enriched air. All four strains grew when transferred from ambient CO2 to 20 kPa partial pressure of CO2 (pCO2), but none of them tolerated direct transfer to 40 kPa pCO2. *Synechococcus* and *Anabaena* survived 101 kPa (100%) pCO2 when pressure was gradually increased by 15 kPa per day, and *Plectonema* actively grew under these conditions. All four strains grew in an anoxic atmosphere of 5 kPa pCO2 in N2. Strains that were sensitive to high CO2 were also sensitive to low initial pH (pH 5–6). However, low pH in itself was not sufficient to prevent growth. Although mechanisms of damage and survival are still under investigation, we have shown that modern cyanobacteria can survive under Earth's primordial conditions and that cyanobacteria-like organisms could have flourished under conditions on early Mars, which probably had an atmosphere similar to early Earth's.

**Appendix 2.3**

As a companion paper, Dr. Thomas' group published a description of their inexpensive laboratory technology, which materially extends, at minimum expense the versatility of experiment design for this project. This work also extends Mars biology into the realm of undergraduate education. The abstract follows.

D. J. Thomas and S. K. Herbert.
An Inexpensive Apparatus for Growing Photosynthetic
Microorganisms in Exotic Atmospheres. ASTROBIOLOGY 5(1), 75–82 (2005).

Given the need for a light source, cyanobacteria and other photosynthetic microorganisms can be difficult and expensive to grow in large quantities. Lighted growth chambers and incubators typically cost 50–100% more than standard microbiological incubators. Self-shading of cells in liquid cultures prevents the growth of dense suspensions. Growing liquid cultures on a shaker table or lighted shaker incubator achieves greater cell densities, but adds considerably to the cost. For experiments in which gases other than air are required, the cost for conventional incubators increases even more. We describe an apparatus for growing photosynthetic organisms in exotic atmospheres that can be built relatively inexpensively (approximately $100 U.S.) using parts available from typical hardware or department stores (*e.g.*, Wal-mart or K-mart). The apparatus uses microfiltered air (or other gases) to aerate, agitate, and mix liquid cultures, thus achieving very high cell densities (*A*750 _ 3). Because gases are delivered to individual culture tubes, a variety of gas mixes can be used without the need for enclosed chambers. The apparatus works with liquid cultures of unicellular and filamentous species, and also works with agar slants.

**Appendix 2.4**

The annual Space Technology and Applications International Forum is a venue for regular representation of this ecopoiesis project. Dr. Boston spoke on behalf of the project during the 2005 year's Forum, and the abstract submitted for this presentation is Appendix 2.4.

**Space Technology and Applications International Forum**
STAIF-2005
Albuquerque, NM
February 13-17, 2005

Session E05. Settlements & Planetary Terraforming
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**Ecological Community Development in a Lunar Ecopoiesis Test Bed Facility: Key Concepts**

P.J. Boston[1], P. Todd [2]
[1]*New Mexico Tech (NM Institute of Mining and Technology), Socorro, NM*
[2]*SHOT, Inc., Greenville, IN*

**Abstract.** Physiological and behavioral responses of individual species of plants, animals, fungi, and microorganisms in the reduced gravity of the moon as a proxy for the reduced gravity of Mars will be worthwhile phenomena to study. However, those responses will not represent the behavior and functioning of an integrated ecological community. Whole communities must be studied to provide the basis for the prediction of suitability of communities for future use as pioneer organism assemblages for Mars use. Key concepts toward this end were identified and explored at a workshop as part of a Phase II NASA Institute for Advanced Studies project. A long-term progressive sequence of experimental studies to span the next century were outlined. Small, Earth-based experiments ultimately culminate in a large scale ecopoiesis test bed facility for the Moon and ultimately for Mars.

**Appendix 2.5**

Dr. Thomas has presented work of the project at national meetings and continues to do so. An abstract in anticipation of results from research using the Test Bed was submitted for presentation at the Eighth International Mars Society Convention in Boulder, CO, August 11-14, 2005. The text of this abstract is given in Appendix 2.5.

EARLY RESULTS OF ECOPOESIS EXPERIMENTS IN THE SHOT MARTIAN ENVIRONMENT SIMULATOR.

**David J. Thomas**[1], Paul W. Todd[2], Penelope J. Boston[3], John Boling[1], Tiffany McSpadden[1] and Laura McWilliams[1].
[1]Lyon College, Science Division, Batesville, AR 72501.
[2]Space Hardware Optimization Technology, Inc., Greenville, IN 47124.
[3]Complex Systems Research, Inc., Boulder, CO 80301.

Humanity is on the verge of having the capability of directing environmental changes on a planetary scale. One could argue that we are making these changes on Earth today, but in a negative manner. Within the foreseeable future, we will have the technology to modify Mars' environment, and make it a habitable planet. However, we do not have enough information to determine the course of such an event. SHOT has designed and built a test-bed apparatus that can replicate most of Mars' environment conditions (with the notable exceptions of gravity and cosmic radiation) within a ten-liter chamber. We are currently performing experiments to determine the suitability of specific microorganisms as pioneering life-forms for Mars. Included among the potential pioneers are five genera of cyanobacteria (*Anabaena*, *Chroococcidiopsis*, *Plectonema*, *Synechococcus* and *Synechocystis*), and at least three partially-characterized eubacterial strains that were isolated from Chile's Atacama Desert. During these initial trials, we used a present-day mix of Martian atmsospheric gases, but at a pressure of 100 mbar (10 times Mars's current atmospheric pressure). Organisms were inoculated into samples of JSC Mars-1 soil stimulant and exposed to full-spectrum simulated Martian sunlight. Day/night temperature cycled from 26°C to -80°C and back.. Planned experiments include a 24-hour, brief-exposure trial, a longer 7-14 day trial, and a 6 week trial to determine the survival and growth of our potential Martian pioneers. Results of these experiments will be presented. (This research is funded under subcontract agreement 07605-003-026 with NASA Institute for Advanced Concepts (NIAC) supported through Universities Space Research Association contract NAS5-03110 with NASA.

## Appendix 2.6

# EFFECTS OF ATMOSPHERIC PRESSURE ON THE SURVIVAL OF PHOTOSYNTHETIC MICROORGANISMS DURING SIMULATIONS OF ECOPOESIS

David J. Thomas[1*], L. Michelle Eubanks[1], Carl Rector[1], Jaime Warrington[1] and Paul Todd[2].
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[2]Space Hardware Optimization Technology, Inc., 7200 Highway 150, Greenville, IN 47124, USA.
*Corresponding author.

Three cyanobacteria (*Anabaena* sp., *Plectonema boryanum* and *Chroococcidiopsis* CCMEE171) and an alga (*Chlorella ellipsoidea*) were grown under simulated martian ecopoesis conditions. A xenon arc lamp with a solar filter provided simulated martian sunlight, and temperature cycled diurnally from -80°C to 26°C. A Mars-like atmosphere of 100% $CO_2$ was provided at 25, 100, 300, 500 and 1000 mbar. The cyanobacteria and alga were inoculated into JSC Mars-1 soil simulant and exposed to each atmospheric pressure for five weeks. Survival and growth were determined via extractable chlorophyll *a* and total esterase (fluorescein diacetate hydrolysis) activity. Maximum survival occurred at 100-300 mbar. At 25, 500 and 1000 mbar, esterase activity was near zero, and extractable chlorophyll *a* was less than 10% of control samples. Overall, the cyanobacteria survived better than the alga. Low survival at 25 mbar was probably due to desiccation. Low survival at 500 and 1000 mbar may have been due to $CO_2$ toxicity.

Abbreviations: FDA, fluorescein diacetate; PAR, photosynthetically active radiation; $pCO_2$, partial pressure of $CO_2$; UV, ultraviolet.

### Introduction

Currently, life as we know it cannot inhabit the surface of Mars. The combination of low temperature, low pressure, high ultraviolet flux and extreme aridity would kill the hardiest organisms on Earth. However, in the future, humanity might alter Mars' environment to make it more habitable (Averner and Macelroy 1976, Graham 2004, Haynes and McKay 1992, McKay 1982, McKay 1998, McKay 1999, McKay et al. 1991, Thomas 1995). Briefly summarized, after initial geophysical modifications that would allow liquid water to exist on the surface, microorganisms could be introduced (ecopoesis). Photosynthesis and denitrification would slowly convert Mars' $CO_2$ atmosphere to an $O_2/N_2$ atmosphere similar to that of Earth (terraformation).

Initial experiments of 1 to 14 days duration indicated that a variety of autotrophic and heterotrophic bacteria could survive under simulated ecopoesis conditions of pure $CO_2$ at 100 mbar pressure and a diurnal temperature cycle of -80°C to 26°C (Thomas et al. 2006 in press-a, Thomas et al. accepted). Here we report on the survival of cyanobacteria and algae during simulations of ecopoesis where the atmospheric pressure was varied from 25 mbar to 1000 mbar.

### Materials and Methods

*Mars simulator*. All experiments were performed in a Mars environment simulator (Thomas et al. 2006 in press-b) at the facilities of SHOT, Inc. in Greenville, Indiana, USA (Figure 1). For logistical reasons, Earth-normal 24-hour days were used in place of Mars days ("sols"). Diurnal temperature ranged between -80°C and 26°C (Figure 2), which was similar to Mars' equatorial climate during the vernal equinox (Carr 1996). Illumination was provided by a xenon arc lamp (Sylvania 69263-0 Short Arc Lamp, XBO, 1000 W/HS OFR) fitted with a solar filter that provided a close approximation of solar radiation. Photosynthetically active radiation at sample level ranged from 15 μmol photons $m^{-2}$ $s^{-1}$ in the shaded region to 1000 μmol photons $m^{-2}$ $s^{-1}$ in direct light (Figure 3). Total ultraviolet radiation (250-400 nm) was 1.7 μmol photons $m^{-2}$ $s^{-1}$ in the shaded region and 50 μmol photons $m^{-2}$ $s^{-1}$ in direct light. An atmosphere of pure $CO_2$ was used in all experiments. Atmospheric pressure was decreased within the sample container

over a period of two hours. A like period was used at the end of each experiment to bring the pressure back to ambient. Up to 1 mL of water was added to the chamber daily to maintain water saturation of the atmosphere. Water addition was stopped when standing water appeared in the bottom of the sample chamber. Each simulation lasted for 5 weeks. Five atmospheric pressures were used: 25, 100, 300, 500 and 1000 mbar.

*Culture conditions.* Three cyanobacteria and one alga were used in these experiments: *Anabaena* sp. (Carolina Biological Supply), *Chroococcidiopsis* sp. strain CCMEE171 (Culture Collection of Microorganisms from Extreme Environments), *Plectonema boryanum* strain UTEX485 (University of Texas Culture Collection), and *Chlorella ellipsoidea* strain YCC002 (University of Wyoming). Cyanobacterial and algal stock cultures were grown at 25°C in liquid BG-11 medium (Sigma-Aldrich, St. Louis, MO), pH 7.5, amended with 2.5 mM $NaHCO_3$ and 20 μg $L^{-1}$ vitamin $B_{12}$ (final concentrations). Cultures were continuously illuminated with 50 μmol photons $m^{-2}$ $s^{-1}$ PAR from cool white fluorescent tubes.

**Figure 1. The SHOT Mars environment simulator (internal view).** Samples are contained within the 6 liter quartz cylinder. Simulated sunlight is reflected onto the samples via a movable, front-surface mirror. The xenon-arc light source is connected at the left side of the simulator (not shown). In this configuration, samples in the middle of the cylinder receive approximately 1000 μmol photons $m^{-2}$ $s^{-1}$ PAR, while the samples in the shaded regions receive approximately 15 μmol photons $m^{-2}$ $s^{-1}$. The horizontal glass tubes in the bottom of the chamber were used for other experiments not described here.

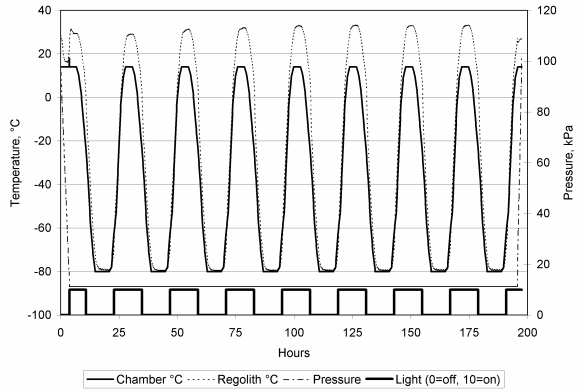


**Figure 2. Environmental conditions within the simulator.** These data are from an earlier, 7-day experiment, but they reflect the typical conditions within the sample chamber. In this example, the pressure was held at 100 mbar (~10 kPa).

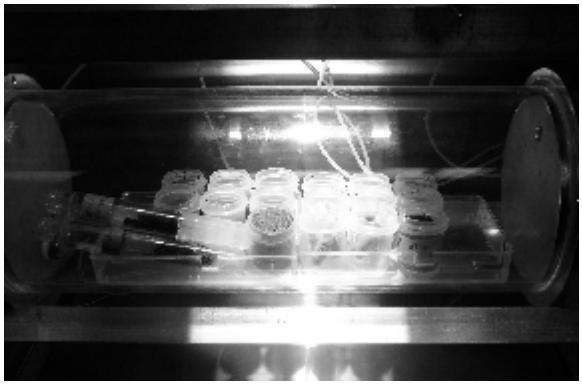

**Figure 3. Sample arrangements within the simulator chamber.** This fused silica (quartz) chamber allows full-spectrum transmission of light to the samples. The soil-filled jar at front-center contains a buried thermistor, which measures soil temperature. Each triplicate sample series had one jar in full light, another in full shade, and a third in between the two light extremes. The culture vials at the left and within the front row of jars were for other experiments not described here.

*Sample preparation.* Stock cultures were centrifuged for 20 minutes at 1000 x g. The cell pellets were resuspended in fresh BG-11 medium without added $NaHCO_3$ to an optical density of 0.15 at 720 nm measured with a Genesys 20 spectrophotometer (Thermo Electron, Waltham, Massachusetts, USA). Triplicate samples of each microorganism were prepared in 25 mL polypropylene jars. 10 mL of each culture was added to 7 g of sterilized JSC Mars-1 soil simulant, resulting in a simulated "mud puddle" with standing water above saturated soil (Figure 4). Samples were weighed before and after each experiment to determine water loss. Identical triplicate samples were kept in the dark at 4°C as controls. Samples were transported on ice between Lyon College and SHOT via automobile or overnight courier. Experimental samples were arranged so that each triplicate series received the continuum of available light—shade to full intensity (Figure 3). After each experiment, the sample jars were re-weighed; sterile water was added to return each jar to its original mass. The samples were capped and shaken to resuspend the microorganisms before sampling for life detection assays. Survival of the microorganisms was determined via extractable chlorophyll *a* and fluorescein diacetate (FDA) hydrolysis (esterase) activity.

*Chlorophyll extracts.* Chlorophyll *a* extractions were used to determine the relative abundance of photosynthetic organisms (Bowles et al. 1985, Myers et al. 1980). 1 mL subsamples were taken from each sample and centrifuged for 10 minutes at 10,000 x g. 800 µL of supernatant was removed and discarded. 800 µL of denatured ethanol was added to each subsample (resulting in 80% ethanol solution), which was then vortexed and placed in a -20°C freezer for 24 hours to extract the chlorophyll. After extraction, the subsamples were centrifuged again for 10 minutes at 10,000 x g. 800 µL of each extract was transferred to a polystyrene semi-micro spectrophotometer cuvette, and its absorbance was measured at 664 nm with an USB2000 diode array spectrophotometer (Ocean Optics, Dunedin, Florida, USA). Corrections for residual soil particles were made by subtracting nonspecific scattering at 720 nm from the $A_{664}$ measurements.

Known solutions of purified chlorophyll *a* (Sigma-Aldrich, St. Louis, MO, USA) were used to produce a standard curve (Figure 5).

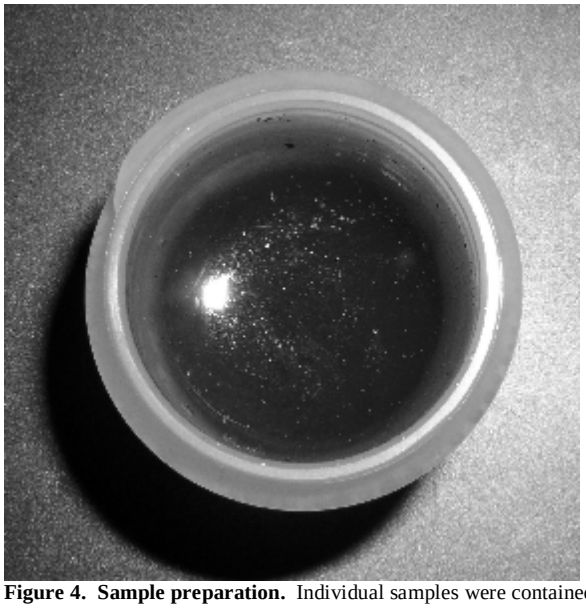

**Figure 4. Sample preparation.** Individual samples were contained within 25 mL polypropylene jars. Each jar contained 7 g of JSC Mars-1 soil simulant and 10 mL of culture, resulting in a miniature "mud puddle."

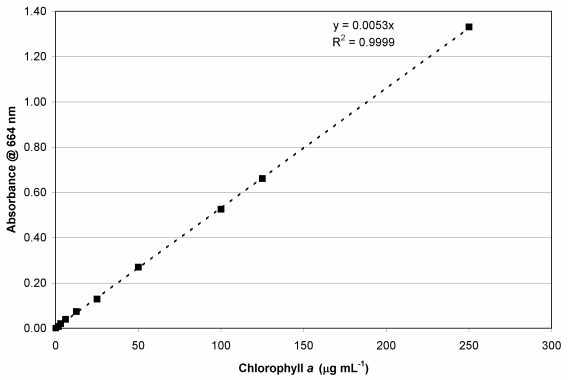


**Figure 5. Standard curve for chlorophyll *a*.** Purified chlorophyll *a* (from spinach) was dissolved in 80% ethanol.

*FDA hydrolysis assay*. Samples were analyzed for esterase activity via an assay of fluorescein diacetate (FDA) hydrolysis (Adam and Duncan 2001, Schnürer and Rosswall 1982) at the beginning and end of each experiment. The FDA hydrolysis assay indicates microbial metabolism across a wide variety of taxa, and correlates well with assays of respiration. Subsamples of 1-2 mL were taken from each sample before and after each experiment and transferred into 15 mL centrifuge tubes. 5 mL of 60 mM $K_2PO_4$ buffer (pH 7.6) was added to each tube, which was then briskly shaken for 10-20 seconds. Ten µL FDA in acetone (5 mg $mL^{-1}$) was added to each tube, and then all tubes were incubated for 3-5 hours at 25°C on a rocker table. Following incubation, the samples were extracted by adding 5 mL 2:1 chloroform:methanol. The samples were centrifuged for 10 minutes at 1000 x g, and the supernatant was measured spectrophotometrically at 490 nm with the USB2000 diode array spectrophotometer. Known solutions of fluorescein were extracted in the same manner as the samples, and were used to generate a standard curve (Figure 6).

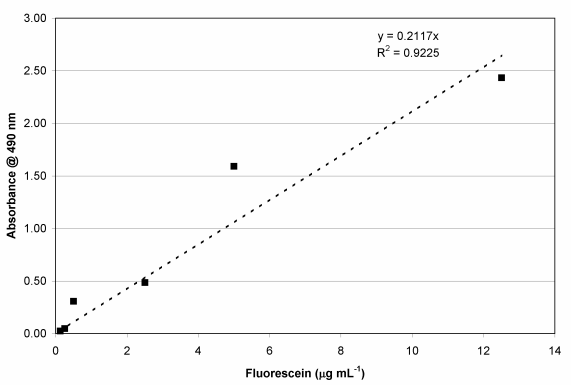


**Figure 6. Standard curve for fluorescein.** Fluorescein solutions were prepared in the same phosphate buffer used in the FDA hydrolysis assays, and were extracted in 2:1 chloroform:methanol. The concentrations shown represent the concentrations of fluorescein in the original solutions, not in the extracts.

## Results and Discussion

As was observed in earlier experiments (Thomas et al. 2006 in press-a, Thomas et al. accepted), a "water cycle" developed within the sample chamber. Water evaporated from the samples and from the bottom of the chamber during the day, and then condensed as dew and frost during the night. Most of the condensation occurred at the ends of the chamber, out of the direct light. Thus, the samples in the middle of the chamber were more desiccated than the samples at the ends (Figure 7). All samples were re-hydrated to their original states before the FDA hydrolysis and chlorophyll extraction assays were performed.

**Figure 7. Differences in sample moisture.** By the end of each experiment, samples located toward the ends of the chamber (left) contained more water than samples in the middle of the chamber (right). Sample jars were arranged such that each triplicate series experienced the full range of moisture within the simulator chamber. The culture tube at the bottom of the figure was for another experiment running simultaneously.

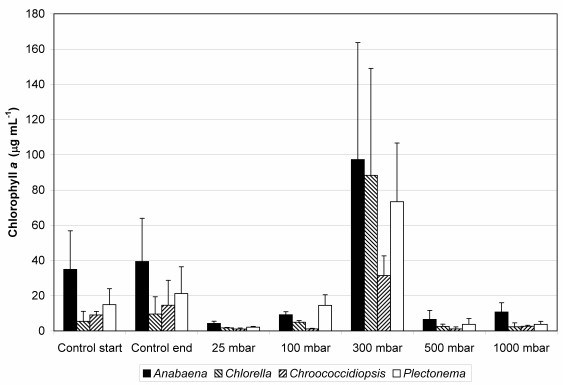


**Figure 8. Chlorophyll extracts.** 1 mL of liquid was removed from each sample, centrifuged and extracted in 80% ethanol in the dark for 24 hours at -20°C. Extracts were measured spectrophotometrically at 664 nm. Controls were combined from all experiments. Error bars equal standard deviations (n = 10-15 for controls, n = 3 for experimental groups). For all samples, the highest amount of extractable chlorophyll ***a*** was found in the 300 mbar experiment.

The results of the life detection assays for the five experiments are summarized in Figures 8 and 9. Since the control samples for each experiment were stored in the dark at 4°C, and were otherwise treated identically, these data were combined for all five experiments. Among the three cyanobacteria, the highest FDA hydrolysis activity was found at 100 mbar, but the highest chlorophyll content was found at 300 mbar. The alga, *Chlorella*, had its highest FDA activity and chlorophyll content at 300 mbar. Outside of the 100-300 mbar range, both extractable chlorophyll and FDA hydrolysis levels were very low. Previous research with cyanobacteria in high $CO_2$ atmospheres and ambient pressure (Thomas et al. 2005) showed significant growth inhibition of *Anabaena* and *Plectonema* at $CO_2$ concentrations of 40% or more. Another cyanobacterium, *Synechocystis*, was inhibited by 20% $CO_2$. At ambient pressure, this corresponds to a partial pressure range of $CO_2$ ($pCO_2$) of 200-400 mbar—which overlaps the survival range shown in Figures 7 and 8. The low survival rates in the 500 and 1000 mbar experiments may be due to $CO_2$ toxicity. While *Anabaena* and *Plectonema* can survive in 100% $CO_2$ under culture conditions, the added stresses of other parameters of the ecopoeitic conditions result in inhibition and death.

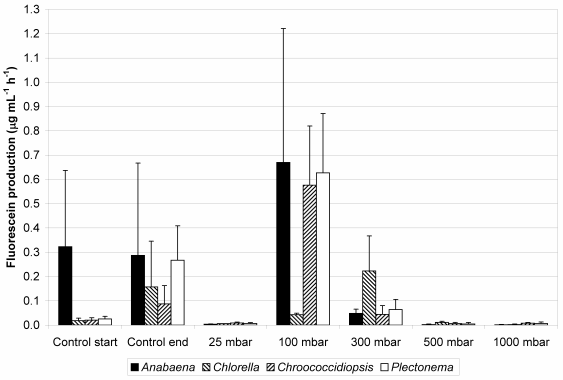


**Figure 9. FDA hydrolysis assays.** 1-2 mL of liquid was removed from each sample and brought to 5 mL with the addition of pH 7.6 phosphate buffer. 10 µL of 5 mg $mL^{-1}$ FDA solution was added to each sample, followed by incubation for 2-6 hours at 25°C. Samples were extracted in 2:1 chloroform:methanol. The methanol:buffer fractions were measured spectrophotometrically at 490 nm. Controls were combined from all experiments. Error bars equal standard deviations (n = 10-15 for controls, n = 3 for experimental groups). For the three cyanobacteria, the highest amount of FDA hydrolysis activity was found in the 100 mbar experiment, but the alga, *Chlorella ellipsoidea*, had more activity in the 300 mbar experiment.

Besides the inhibitory effects of $CO_2$, atmospheric pressures below 100 mbar may have both physically and physiologically inhibitory effects. At 25 mbar, water boils at 9°C. Since the diurnal temperature ranges from -80°C to 26°C, the microbes are boiled and then deep-frozen every day. Our results are consistent with research on *Bacillus* spp. that shows little or no growth at 25 mbar (Schuerger et al. 2006a, Schuerger and Nicholson 2005).

Ultraviolet radiation was the other major stress factor for our test organisms. Previous work showed that simulated martian UV levels quickly inhibited several *Bacillus* spp. and a strain of *Chroococcidiopsis* (Cockell et al. 2005, Schuerger et al. 2006b). Desiccation—such as that experienced by the organisms in this project—increased the rate of inhibition produced by UV radiation. The cyanobacteria and alga used in this project require light to survive, but also require a certain amount of shielding from UV radiation. This means that they probably only grow within a thin layer that is deep enough within the water/regolith column to be shielded from excess UV, but shallow enough to receive adequate amounts of PAR.

These experiments only begin to address the issues and problems associated with ecopoesis. At the beginning of these experiments, we wanted to discover which of these photoautotrophs would grow under martian conditions at various stages of ecopoesis. However, our results indicate that under most of the conditions tested, grow did not occur, and survival declined. Although the FDA assay showed increased activity in the cyanobacteria at 300 mbar, they are not significantly higher than the controls. Additional experiments in which surviving microbes are re-cultured and then put back under martian conditions may allow for the selection of hardier strains. A multitude of other potential pioneer martian organisms in culture collections and natural settings also awaits possible testing and selection. As interest in these problems increases, and additional test facilities become available, we will be able to answer more of the questions pertaining to the establishment and development of a new biosphere on Mars. At the same time, we will further our understanding of the functions and evolution of Earth's earliest ecosystems.

**Acknowledgements**

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**Appendix 3.1**

**Introductory Paper for Symposium on Planetary Biology and Terraforming at 21st meeting of the American Society for Gravitational and Space Biology, Nov 3-6, 2005.**

**PLANETARY BIOLOGY AND TERRAFORMING**

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Gravitational and Space Biology 19 (2), in press July 2006.

Abstract

Planetary biology can be considered in terms of four components: (1) planetary protection, (2) the search for life, (3) human life support and (4) ecopoiesis and terraforming. Initially, contamination of a planet with Earth life is to be minimized in order to facilitate a search for planetary life. Meanwhile, humans, if present, must be sustained. Subsequently, large-scale modifications of a planetary environment can be considered. "Ecopoiesis" is a term introduced by McKay and Haynes to describe the initiation of a living, self-sustaining ecosystem in a planetary (Mars) environment. "Ecosynthesis" refers to the development of an ecosystem that includes succession. "Terraforming" refers to creating an Earth-like world and includes planetary engineering. A considerable amount of thinking and writing has been devoted to these subjects, but, at least in the case of ecopoiesis, there has been very little, if any, experimental research. The speakers in this session address, in order, issues of planetary environments and habitability with reference to Earth analogues, the role of extremophiles as pioneer organisms in ecopoiesis, and the concept of succession as it applies to terrestrial ecology and ecosynthesis.

Key words: planetary biology, ecopoiesis, ecosynthesis, terraforming, Mars, extremophiles

## Introduction

A variety of claims have been made concerning the future of ecopoiesis (starting an ecosystem) and terraforming (creating an earth-like environment) – highly controversial subjects. Planetary protection is a significant component of the ecopoiesis debate. One of the significant outcomes of performing research in this area is expected to be the forcing of a scientific community consensus concerning these subjects. It is of great value to cause the visions of the prophets of the field to be extended an entire century forward. Meanwhile, a series of benign, but difficult experiments (laboratory, field and theory) can and should be performed.

Today's thinkers hold varying opinions concerning ecopoiesis, the process of evolving a physical and biological environment that can lead to "terraforming" – the deliberate introduction of terrestrial-type ecosystems on remote celestial bodies such as

planets, moons and asteroids (Haynes, 1990). On one extreme, principles of planetary protection prohibit the introduction of any living organisms onto Earth's neighbors in the solar system while, on the other extreme advocates of terraforming propose the early modification of Mars (for example) to initiate processes that will make the planet a more hospitable place for humans should they decide to go there (Zubrin and Wagner, 1996). Either way, knowledge is lacking, and experimental testing is non-existent, related to the basic understanding of the ecopoiesis process. Since the implementation of any concepts related to ecopoiesis would occur several decades in the future, the experimental study of ecopoiesis now would seem an ideal objective. To date, only individual components of ecopoietic systems have been investigated – a few species of microorganisms and certain primitive and vascular plants.

Although other possibilities exist, Mars is considered the ultimate target for terraforming. There are at least four levels of inquiry concerning biology and Mars:

(1) Planetary protection, contamination and quarantine issues (NRC, 1992),

(2) The search for life on Mars (Banin, 1989; Banin and Mancinelli, 1995; Ivanov, 1995; Koike et al., 1995; Biemann et al., 1977),

(3) Human expeditions to Mars and biological life support (Meyer & McKay, 1984, 1989, 1995) and

(4) The terraforming of Mars starting with ecopoiesis (Fogg, 1995; Haynes, 1990; McKay, 1990; Haynes and McKay, 1992; McKay et al., 1991,1994; Hiscox, 1993, 1995, 1998).

The undertaking of ecopoiesis/terraforming research can be considered a direct response to the challenge originally put forth by Christopher McKay and the late Robert Haynes: " . . if it is decided to implement such a program of planetary engineering, a slow and conservative approach is essential. Sufficient time must be allowed for a wide range of studies of Mars as it exists at present, and for careful planning, modeling and 'pilot-plant' trials (where possible) of all successive steps in the enterprise." (Haynes & McKay, 1995). This challenge calls for research that includes biological experiments and theoretical modeling directed at the implementation of the enabling notions in terraforming offered by Fogg (1995), McKay et al.(1991), Boston and Thompson (1992) among others.

**The Martian Environment and Planetary Engineering**

A set of ecopoiesis research principles is offered, and they are listed in the order in which they would logically be implemented in an integrated ecopoiesis research program:

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1. *Assume the ultimate problem to be addressed is the terraforming of the surface of Mars.*

Mars lies within the habitable zone of the solar system (Fogg, 1992, Kasting, 1993) and is second only in biocompatability to the Earth. If the laws of physics and chemistry are universal laws, and if the sequence of events that led to the origin of life on Earth occurs elsewhere, Mars may thus be considered a prime candidate for both the existence of life and human settlement (Ponnamperuma, 1995; Meyer & McKay, 1995,

1989, 1984; Zubrin 1995; Hiscox, 1997). The chemical-elemental inventory of Mars' surface (both absolute and relative concentrations, Table 1) is quite similar to the Earth's crust with the exception of a potentially low nitrogen content (Banin & Mancinelli, 1995; Stoker, 1991; Banin 1989, 1991; Lindsay, 1979).

The primary physical properties of the Martian environment that are a challenge for the survival and growth of terrestrial organisms include; low atmospheric pressure (~ 7 millibar), low termperature ranges (-100 ° C to - 5 ° C, with a rare high of 26 ° C), no observed liquid water to date (Soderblom, 1992), unfiltered ultra violet (UV) radiation, no significant ozone layer, no organic material, and high concentrations of surface oxidants (Kieffer, 1973; Rothschild, 1990; Banin & Mancinelli, 1995). The atmospheric pressure on Mars, mostly due to $CO_2$, varies from approximately 7.4 to 10 millibar (mbar). Extremely low pressure can damage organisms and affect efficient DNA repair (Ito, 1991; Koike, 1991).

2. *Starting conditions to be studied on Earth should resemble those expected to exist at the best possible location on the surface of Mars (a temperate location at low altitude or near potential water sources at the north polar cap).*

The physical and chemical properties of the planet are summarized in Table 1.

On Mars there are difficult trade-offs between water availability and temperature. In general, water is available only at or near polar caps because the temperature is sufficiently low to prevent sublimation at such low pressure. Liquid water may exist transiently in the Martian lowlands at mid-day in the summer months on occasions when the pressure (15- 20 mbar) and temperature (2-20$^{o}$C) are both above the triple point of water (Kuznetz and Gan, 2002).

3. *The regolith must be heated to a temperature compatible with the most robust form of terrestrial life at low temperature.*

Shortly after the Viking missions, Averner and MacElroy (1976) and Kuhn, Rogers and MacElroy (1978) launched a program to study the habitability and planetary ecosynthesis of Mars by designing an energy-balance model. The initial studies evaluated the effects of a Martian atmosphere on photosynthetic microorganisms by approximating equatorial equinox, parameters for solar and thermal radiation, convective and conductive energy transport and evaporative cooling. These early investigations took into consideration the effect of diurnal variation on the organisms temperature, transpiration and photosynthetic rates all of which are equally applicable to current ecosystem models. Initial heating of the regolith is to be achieved by the addition of black material to the dust in which the seed organism spores are buried, and heating of the atmosphere is achieved by the addition of fluorocarbon gas, a very effective greenhouse gas. Laboratory investigations of heating strategies will require a combination of experiment and modeling, since the laboratory scale differs significantly from the planetary scale.

4. *The first organisms must derive energy from the mineral content of the regolith and/or sunlight, and their metabolism must produce a net increase in greenhouse gas(es).*

Since the advent of MacElroy's program in 1975, several living systems on Earth have been identified as potential pioneer organisms for the Martian environment. These biota

may be classified as "extremophiles" and include classes of anhydrobiotes (Dose, 1995), cryophiles, thermophiles and halophiles, to name a few. Examples include cryophiles found in the ice-covered lakes in the Bunger Hills Oasis of Antarctica (Andersen et al., 1995; McKay, 1985, 1991, 1993; McKay & Boston, 1994) and in Arctic permafrost in the Kolyma lowland of Siberia, Russia (Ostroumov, 1955; Soina, 1995). Thermo- and halophiles including endolithic bacteria found in extreme arid deserts, high Alpine rocks, and hot springs (Boston, 1992, 1997; Friedmann, 1972, 1982, 1986, 1987, 1995), evaporite biota found in the hypersaline habitats (Rothschild, 1990, 1995) and cryptic microbial mats in the Guerrero Negro, Baja California Sur, Mexico.

An effective ecopoietic strategy requires knowing the limits of habitability for Earth organisms as we known them, especially under conditions of limited water and oxygen (Bannin, 1995; Carr, 1987, 1986; Fogg, 1995; Huffert et. al., 1981). To understand these limits, the broadest possible range of genetically encoded capabilities must first be considered, then, in a narrower way, conditions that are and are not destructive to Earth's most extremophilic organisms are to be identified. Using Mars regolith simulant (Allton et. al., 1985) and planetary environment simulation chambers (Sagan and Pollack, 1974), the vigor of such organisms can be tested (Koike et. al., 1991; Kuhn et. al., 1979; Hawrylewicz et. al., 1962; Thomas et al., 2006).

5. *The first organisms must be capable of withstanding, or be protected from, the ultraviolet and ionizing radiation present on the Martian surface and the high $CO_2$ of the atmosphere.*

The Martian atmosphere, devoid of an ozone layer, allows solar ultraviolet (UV) radiation to penetrate to the Martian surface in wavelengths ranging from 190 to 300 nm. In living cells UV radiation is absorbed by nucleic acids to activate the chemical formation of various adjuncts that inhibit replication and transcription of DNA and can be lethal (Lindberg & Horneck, 1991, 1992). The high concentration of atmospheric carbon dioxide (~95.3%) would, in most terrestrial organisms cause a low intracellular pH, resulting in damage to cellular components, proteins and metabolism.

6. *Organisms early in succession should produce significant amounts of $O_2$.*

At some point in the early stages of succession, $O_2$ will be essential to drive the dark reactions of photosynthetic organisms, which are $O_2$ concentration dependent and to support the metabolism of heterotrophic aerobes.

7. *Higher plants late in succession will need to be studied under conditions that pioneer organism that precede them can create.*

Cold-weather species that do not require pollination or can pollinate without insect assistance, that can function in low $O_2$ and high $CO_2$ and extreme drought and that are useful to humans are among those to be considered and investigated (Graham, 2005).

**The Presentations in This Series**

The subsequent presentations in this series address, in a similar sequence, the importance of and evidence for liquid water on Mars, the habitability of extraterrestrial and extreme

terrestrial venues for Earth life, the capability of extremophiles to serve as pioneer organisms in ecopoiesis and the succession of organisms in ecosynthesis required to fulfill the ultimate goals of terraforming.

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***Table 1. Physical properties of Mars***

| | |
|---|---|
| Pressure | 6 – 15 millibars (0.6 – 1.5 kPa) |
| Global temperature range | -133$^{o}$C -- +23$^{o}$C |
| Mean Solar irradiance | 589 W m$^{-2}$ (~50% of Earth's value) |
| Solar UV flux | 6500 ergs cm$^{-2}$ (190 – 300 nm) (<190 nm absorbed by $CO_2$) |
| Water Vapor | 0.03% of atmosphere mass (saturated at 8.1 mbar) |
| Gravitational constant | 0.0387g |
| Escape velocity | 5km s$^{-1}$ |
| Dau length | 24h 37min 22s |
| Year length | 687 Earth days |
| Equatorial diameter | 6788 km (~1/2 Earth) |
| Mass | 6.4 X 10$^{23}$ kg (1/10 Earth) |
| Water ice | North polar ice cap |
| Atmospheric composition | |
| $CO_2$ | 95% |
| $N_2$ | 2.7% |
| $O_2$ | 0.13% |
| CO | 0.07% |
| Argon | 1.6% |
| Neon | ppm |
| Krypton | ppm |
| Xenon | ppm |
| Lithosphere elemental composition | |
| SULFUR | 3.1% |
| MAGNESIUM | 5% |
| CALCIUM | 4% |
| IRON | 12.7% |
| POTASSIUM | 0.08% |
| CHLORINE | 0.7% |
| SILICON | 21% |
| ALUMINUM | 3% |
| SODIUM | 2.3% |
| PHOSPHOROUS | 0.3% |
| Mn, Co, Cu, I, Zn, B, V, Mo, F, Cr, Se, Tl, Br | Trace |

**Appendix 3.2**

The SMD issued a separate solicitation for 2-page "White Papers" on lunar exploration, to which SHOT submitted the following response.

Dr. Ghassem Asrar
Exploration Science White Papers
Science Mission Directorate
Suite 5E39-A
NASA Headquarters
Washington, DC 20546-0001.

Dear Dr. Asrar:

Enclosed please find our "Exploration Science White Paper" which is submitted in response to a message received from Dr. Jack A. Kaye, Director, Research & Analysis Program, Earth-Sun System Division. Our company, SHOT, Inc., has been working recently in the area of "ecopoiesis", the initial biological component of terraforming. The surface of Mars is the obvious eventual target; however, practical research on the earth and in near-earth venues (ISS, the Moon) should precede such research in view of, at the very least, goals of planetary protection and the prevention of forward contamination of Mars.

We would be very interested in your response to this concept, and we would certainly look forward to the opportunity to work with you in the near future in the field of planetary science.

# Ecopoiesis Biology Surface Demonstration.

## "White Paper"
## response to call for
## "Exploration Science White Papers"

**INTRODUCTION.** This response to the NASA's Science Mission Directorate call for "Exploration Science White Paper" addresses the subject of Biology surface demonstrations. The lunar surface environment presents a unique opportunity to investigate survival and function of terrestrial microbial populations directly without further forward contamination of Mars or the Moon. The objective is to test certain hypotheses associated with "ecopoiesis", the planting of pioneer organisms on a planet as an initial stage of eventual terraforming. To date there has been almost no experimental science in this field. This would be a precursor to terraforming studies (and planetary protection policy development) for Mars, but the venue is more accessible and controllable owing to the relative proximity of the moon. This approach differs from Closed Environment Life Support Systems (CELSS) approach in that the proposed "Ecopoiesis Test Bed" is a design that causes the environment to evolve on its own, starting with water and nitrogen and spores or inactive cells of appropriate prokaryotes. An enclosed system is required for several reasons. An atmosphere is essential, Mars regolith simulant is closer to Mars regolith than is lunar regolith, and analytical methods must be applied. Otherwise the lunar venue provides vacuum, solar spectrum and reduced gravity. Since the lunar day is 14 Earth days a solar modulator will be required

to simulate the 24.8-h Mars sol (day). Related research is currently taking place on the ground in which all of the extraterrestrial features are provided by artificial means (except chronic reduced gravity, which is inaccessible on Earth).

## BIOLOGY SURFACE DEMONSTRATION CONCEPT

The proposed biology surface demonstration concept would consist of a miniaturized Mars atmosphere and regolith simulant in an enclosed system for specific microbial species capable of photosynthesis at minimum atmospheric pressure. The demonstration would require a community of carefully selected organisms, remotely controlled or programmed light-level controls, telecommunications, power and power management systems, pressure vessel, thermal and environmental controls, and a gas analyzer.

Figure 1 is a sketch of a pressure-vessel concept that would serve as the core of a demonstration. The vessel ("Ecopoiesis Test Bed") contains Martian atmosphere simulant at about 7-10 mbar pressure, provided by an attached external tank, and simulated Mars regolith containing a mixture of carefully selected organisms. A gas analyzer is to sample for molecular oxygen, levels of $CO_2$ and nitrogen, and trace gases produced by organisms.

Figure 1 is also a sketch of the exterior architecture of the concept. It consists of the exterior of the pressure vessel, a louvered shade for controlling surface temperature during the lunar day, and a mast holding telecommunications antenna and primary photovoltaic array.

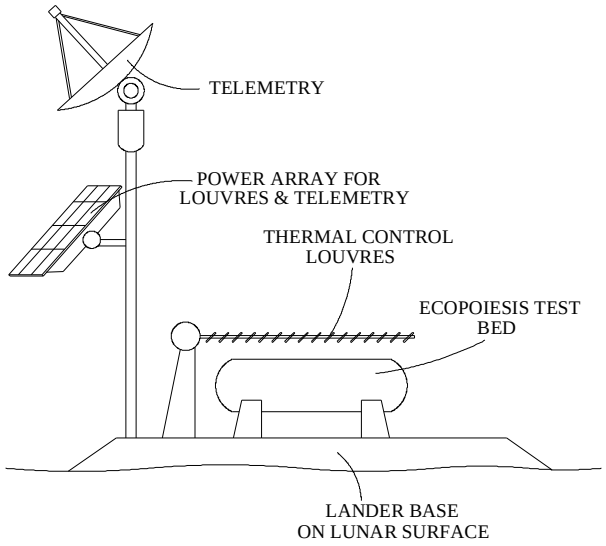



*Figure 1. Sketch of the exterior architecture of the biology demonstration concept as deployed on the lunar surface.*

Several steps must be completed in sequence with biological system development taking place in parallel with engineering design and testing. The corresponding pre-deployment steps may be:

(1) Utilize technology to test candidate microorganisms in the laboratory for their ability to grow in near Mars-like conditions using SHOT's Mars Atmosphere and Regolith Simulator Laboratory Test Bed (MARS-LTB) currently under construction in SHOT's laboratories.

(2) Select candidate pioneer organisms: Utilize SHOT's external extremophile team and internal MARS-LTB to evaluate a limited number of carefully selected candidates from the category of autotrophic extremophiles.

(3) Develop observation equipment primarily for the analysis of gases within the test-bed vessel (Figure 1), the gases being the input gases $CO_2$, $N_2$ and Ar and the biogenic gases $O_2$, methane and trace volatile organics.

(4) In parallel design, develop and test remote and robotic command and data systems, including electronic controls, for housing, manipulating, maintaining the test-bed vessel on the lunar surface.

(5) Develop and optimize a thin-film power system to be utilized on the surface of the Moon on a mast to provide power for telecommunications and mechanical shade control, in addition to the internal automated control functions.

(6) Design, construct and test a robust pressure vessel and the external components consisting of a power and telecommunications mast, and a louvered motorized shade.

**Appendix 3.3**

A response was submitted to NASA Request for Information (RFI) NNH06RFI001R "Request for Information: Developing a Strategy for Future Exploration of the Moon and Beyond". Selected components of this response are summarized below.

---

**Title: Robotic Lunar Ecopoiesis Test Bed**

*Objective*: To test concepts and operations of Martian ecopoiesis using lunar gravity, remoteness and temperature cycles.

*General Description*: The objective of the potential project is to exploit features of the lunar environment as an intermediate venue to test hypotheses concerning ecopoiesis on Mars. Ecopoiesis refers to the phenomenon of initiating the formation of a system of living organisms on a planet as a component of terraforming – giving a planet an earth-like environment as defined by habitability. The proposed biology surface demonstration would consist of a miniaturized Mars atmosphere and regolith simulated enclosed system ("Test Bed")for specific microbial species capable of photosynthesis at minimum Martian atmospheric pressure. The demonstration would require a community of carefully selected organisms, remotely controlled or programmed light-level (thermal) controls, telecommunications, power and power management systems, pressure vessel, thermal and environmental controls, and a gas analyzer.

*Value:* The lunar surface environment presents a unique opportunity to investigate survival and function of terrestrial microbial populations directly without forward contamination of Mars or the Moon. The objective is to test hypotheses associated with "ecopoiesis", the planting of pioneer organisms on a planet as an initial stage of terraforming. To date there has been almost no experimental science. This would be a precursor to terraforming studies and planetary protection policy development for Mars, but the venue is more accessible owing to the relative proximity of the moon. The proposed "Ecopoiesis Test Bed" is a design that causes the environment to evolve, starting with water and nitrogen and spores or inactive cells of appropriate prokaryotes. An enclosed system is required because a Mars-like atmosphere is essential, Mars regolith stimulant is required, and analytical methods must be applied. The lunar venue provides vacuum, solar spectrum and reduced gravity. Related research is currently taking place on the ground in which all of the extraterrestrial features are provided by artificial means except chronic reduced gravity.

*Intermediate Milestones*: Development is expected to span approximately 5-8 years including launch preparation. Lunar surface operations are expected to last at least 1 year after landing. Anticipated principal tasks are, by end of year 1 (2008): (1) initial selection of community of organisms, (2) Gas analyzer hardware design and processing protocols, (3) UV transparent dulture vessel and contents, by end of year 3: (4) Command & data subsystem, (5) Energy conversion and thermal control, (6) solar radiation detectors, (7) Pressure vessel mechanical design and testing, by end of year

4:(8) Integration and flight (9) Launch preparation, by end of year 5: (10) Lunar surface operations (>1 year), conclude in year 6, 7 or8.

*Ties to Mars*: As mentioned above, this would be a precursor to terraforming studies and planetary protection policy development for Mars, but the venue is more accessible owing to the relative proximity of the moon.

*Comment*: This concept has been submitted in much greater detail twice before for the RLEP. The Robotic Lunar Ecopoiesis Test Bed has been supported by NIAC (NASA Institute for Advanced Concepts) and has been brought to a significant level of maturity on the ground, including a high-fidelity planetary surface simulator. The Principal Investigator and Dr. Boston (team member) are NIAC fellows.

## Appendices 4. Abstracts of Selected Proposals

### Appendix 4.1

### Miniature Planetary Environment Simulator

Submitted to NASA SBIR. Status: Not funded

The Miniature Planetary Environment Simulator is a novel compact version of a larger laboratory test bed designed to simulate the temperature profiles, atmospheric and regolith composition and light spectra present on planetary surfaces, especially Mars. It is designed for distributed use in laboratories and/or classrooms. The Simulator will facilitate planetary biology experiments in a variety of venues from middle schools to NASA research labs. It provides a shallow regolith simulant bed distributed over a 2.7" diameter circular surface into which test organisms can be buried. The low-pressure gas volume in the quartz-domed specimen chamber, beneath a controlled solar-simulator lamp, is approximately 84 $cm^3$. A recharging station is required for refurbishment and collection of gases and control of atmosphere pressure. An overall view of the Simulator system is given in Figure 1. The use of several Simulators per recharge station will allow users to conduct multiple experiments simultaneously, for tests that compare how organisms react to different atmospheres, regoliths, temperatures or the composition of the atmosphere. Conditions on earth, the moon, or other planets may be simulated. A personal computer (PC) based tutorial will instruct the user on how to operate the system. The recharge station stores data on each experiment and allows the user to plan the experiment using the onboard software, store data reported during an experiment, and finally to download data.

**Appendix 4.2**

## TITLE: Astrobiology Planetary Simulation Consortium

Submitted to NASA Astrobiology Institute. Status: Pending

Executive Summary

A planetary simulation consortium will be formed in order to facilitate the wider exploration of hypotheses that require testing in simulated planetary environments. Planetary environment simulators are used to address experimentally the four main issues of planetary biology (a) planetary protection, (b) search for life, (c) human life support systems and (d) ecopoiesis and terraforming. SHOT, Inc. will provide technology and leadership in meeting the following four objectives of the proposed project:

1. Optimization and standardization of laboratory simulators ("test beds")
2. Optimization of existing test bed designs for manufacturing
3. Construction and distribution of laboratory test beds to NAI nodes and other laboratories engaged in planetary biology
4. Technical support of each test bed until the concluding date of the 5-year project.

The central focus of the project will be the construction and distribution of six laboratory test beds to the following institutions: New Mexico Institute of Mining and Technology, Lyon College, the SETI Institute, Indiana University, North Carolina State University, and SHOT, Inc. The laboratory test beds will be built on an optimized design based on SHOT's existing successful test bed. It will be capable of temperatures between -140$^{o}$C and +50$^{o}$C, pressures between 6 and 1,000 mbar; full spectrum solar illumination corresponding to the surface of Mars or Earth or interplanetary space; programmed cycling of temperature, pressure and illumination; and physical data logging. The active volume will be 6-10 liters. Test beds will be installed in the consortium laboratories at no capital cost and no sustaining engineering costs to the member users for the duration of the 5-year project. This is a very cost-effective way to expand the capabilities of the NAI in experimental planetary biology. A five-year program is planned, with deliveries of test beds to take place over a 15-month period beginning in the 2$^{nd}$ year.

## *Management*

SHOT, Inc. will assume all management responsibilities for the proposed project. To meet Objective 1 SHOT will utilize its outreach and engineering staff to establish user and technical requirements. To meet Objective 2, SHOT will utilize its engineering and design staff to arrive at an optimized plan that meets the established user and technical requirements. To meet Objective 3 SHOT's engineers, designers and assembly team will assemble six simulation test beds, ship them to the proposed venues, and utilize and pay for local site preparation in each case to effect quality installations of the test beds and their associated safety systems. To meet Objective 4 SHOT's sustaining engineering/technical support team will maintain all of the distributed test beds and provide supplies, especially including arranging and funding liquid nitrogen deliveries.

The timeline of events therefore consists of: Year 1, survey and design; Year 2 building and distribution, Year 3, distribution and service, Year 4 service and R&D, Year 5 service and close-out.

## *Education/Public Outreach*

There will be three public outreach elements incorporated into the project. (1) SHOT's own outreach activities have already emphasized its planetary simulation test bed, and this has attracted the attention of local educators in Indiana and Kentucky. Presentations to local community groups and leaders are and will continue to be frequent (about monthly). Press releases from SHOT's Business Development office will continue to announce progress in this exciting field. (2) Four of the simulators will be installed in institutions of higher learning (Lyon College, North Carolina State University, Indiana University, New Mexico Tech) where the co-investigators are committed to quality undergraduate teaching and the attraction of undergraduates to their research laboratories. (3) All of the test beds will be open to viewing, and in some cases experimentation, by elementary and secondary school students and teachers. An innovative program will be implemented so that participation of this age group is not passive. Students will propose "Experiments on Mars", and, twice each project year, a jury consisting of Consortium scientists will select the best experiment, and SHOT will provide simulator time for the proposed experiments.

## *Institutional Commitments*

Each of the five collaborating laboratories will receive, at no cost to their respective institutions, high-fidelity Planetary Simulators, each expected to be valued at about $160,000, installed at their venues and maintained by SHOT until the end of the proposed project in 2011. Until that time, no financial burden will be assumed by the collaborating laboratories' researchers, who will use the simulators for their research. SHOT's commitments to the investigators will be informal, and they will be invited to investigators' meetings at SHOT's expense (which is expected to be cofered by funds from the proposed NAI grant).

## *NAI Collaborative and Networking Concepts*

Two of the participating laboratories are members of the NAI: Indiana University and The SETI Institute. As with the other participants, these laboratories are at liberty to involve any collaborators they wish, especially including those in the NAI. It is a major purpose of the proposed project to provide planetary simulation capabilities to as many participants in the NAI as possible. The geographical distribution of the venues (east coast, west coast, mid-west, south, southwest) greatly facilitates such networking. Several criteria determined the selection of the five external sites, for example, geographical location, investigator enthusiasm, research relevance and existing NAI-related or –funded research programs.

**Appendix 5.1**

**STAIF, January 2005**

# Ecological Community Development in a Lunar Ecopoiesis Testbed Facility: Key Concepts

*A NIAC-funded Phase I and Phase II Project*


Penelope J. Boston
*New Mexico Tech, Socorro, NM*
*Complex Systems Res. Inc., Boulder, CO*

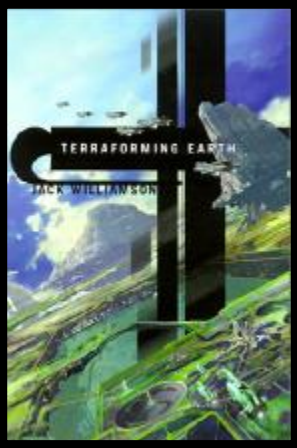
Paul Todd
*SHOT, Inc.*
*Greenville, IN*




# What is Terraforming?

*Altering a Planet's Environment As Suitable for Human Habitat*

- *"Terraforming"*
  *Coined by Science Fiction Author, Jack Williamson, 1942**
- First Seriously Proposed
  *Burns and Harwick*
  *Carl Sagan in 1973*




*"Collision Orbit", in *Astounding Science Fiction*, 1942

Terraforming is an *applied* science.

So far its just so much *yak yak yak*.

It's time to…

*"Shut up and play yer guitar"*

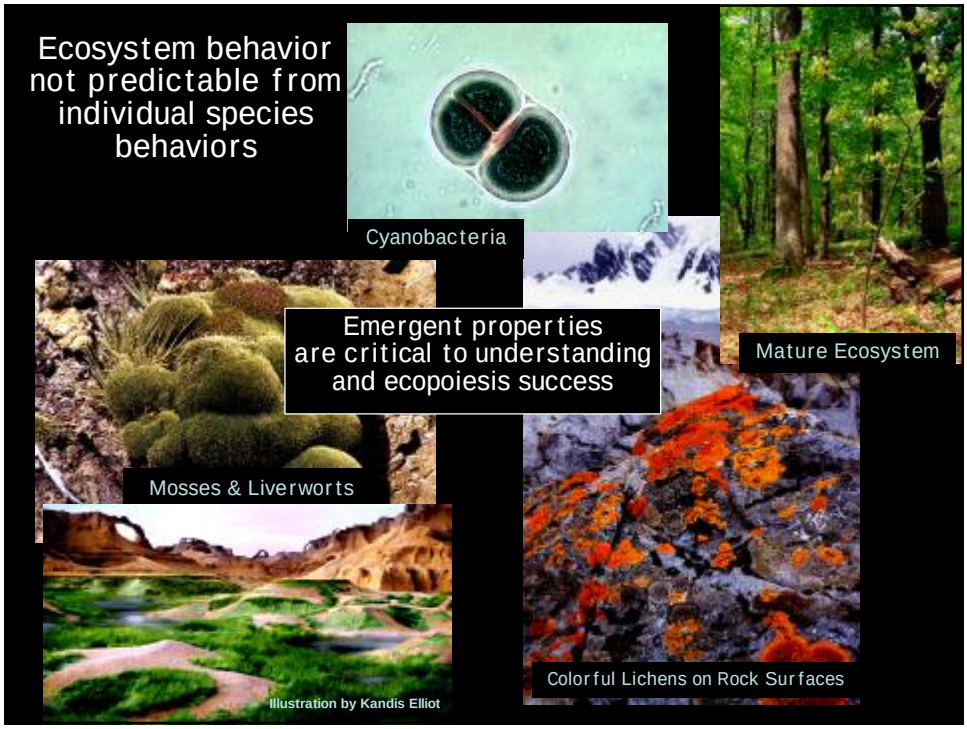
Frank Zappa

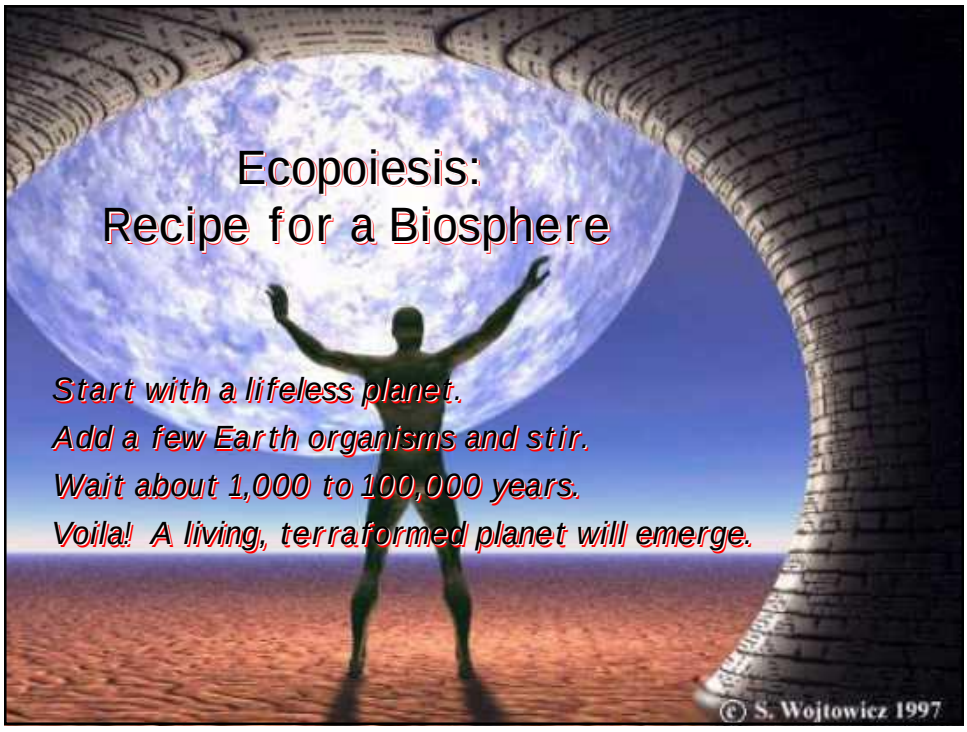
$$$ = Work

*Robotic Lunar Ecopoiesis Testbed Facility*

A NASA Institute for Advanced Concepts
Phase I Contract - Oct. 03- Mar. 04
Phase II Contract - Nov. 2004---

Paul Todd, PI


Ecosystem behavior not predictable from individual species behaviors
Cyanobacteria
Mature Ecosystem
Emergent properties are critical to understanding and ecopoiesis success
Mosses & Liverworts
Colorful Lichens on Rock Surfaces
Illustration by Kandis Elliot


Ecopoiesis:
Recipe for a Biosphere
Start with a lifeless planet.
Add a few Earth organisms and stir.
Wait about 1,000 to 100,000 years.
Voila! A living, terraformed planet will emerge.
© S. Wojtowicz 1997

## Ecopoietic Strategy

- Creation of a new biosphere
- Ecosystem-driven terraforming
- Presupposes simultaneous engineering for minimal habitability
- Requires knowing the habitability limits of Earth organisms

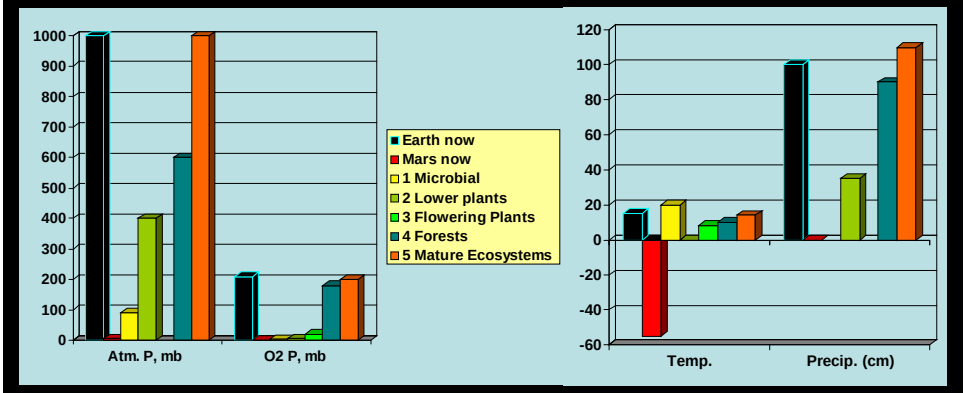
## Study of Earth Life Limits

- Step 1: Identify extremophile organisms from relevant habitats
- Step 2: Characterize individual traits
- Step 3: Study assemblage behavior
- Step 4: Selection of pioneer organisms and communities

## Modification of Pioneer Organisms

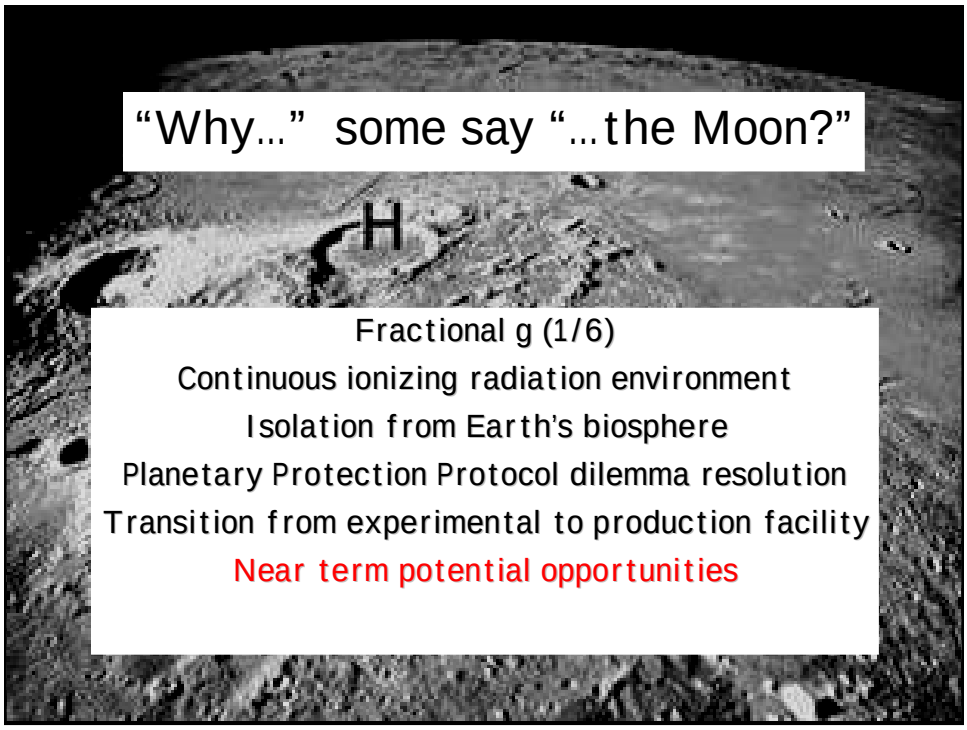
- Selective "breeding"
- Genetic modification
- Artificially assembled communities

## Bioclimatic Bootstrapping




Requires ecosystem succession processes


What *IS* an "Extreme Environment"?
• Homocentrically defined
• Extreme physical & chemical parameters
• Extremes on Earth may be average planetary conditions elsewhere
• Provides excellent starting point for candidate pioneer organism selection
Flema Spring, Cueva de Villa Luz, MX
Image courtesy of the National Geographic Society


"Why…" some say "…the Moon?"
H
Fractional g (1/6)
Continuous ionizing radiation environment
Isolation from Earth's biosphere
Planetary Protection Protocol dilemma resolution
Transition from experimental to production facility
Near term potential opportunities

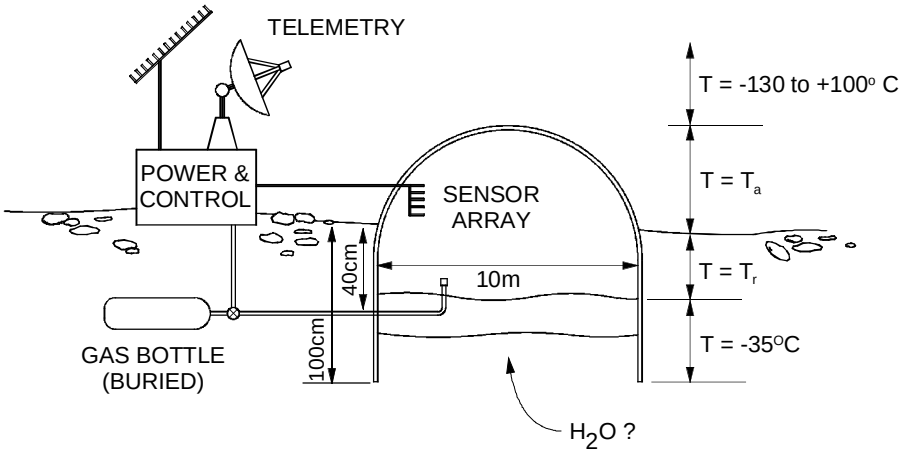

The Goal of Phase II
100 Year Strategic Plan for Experimentation & Development
of Ecopoietic Knowledge and Technology

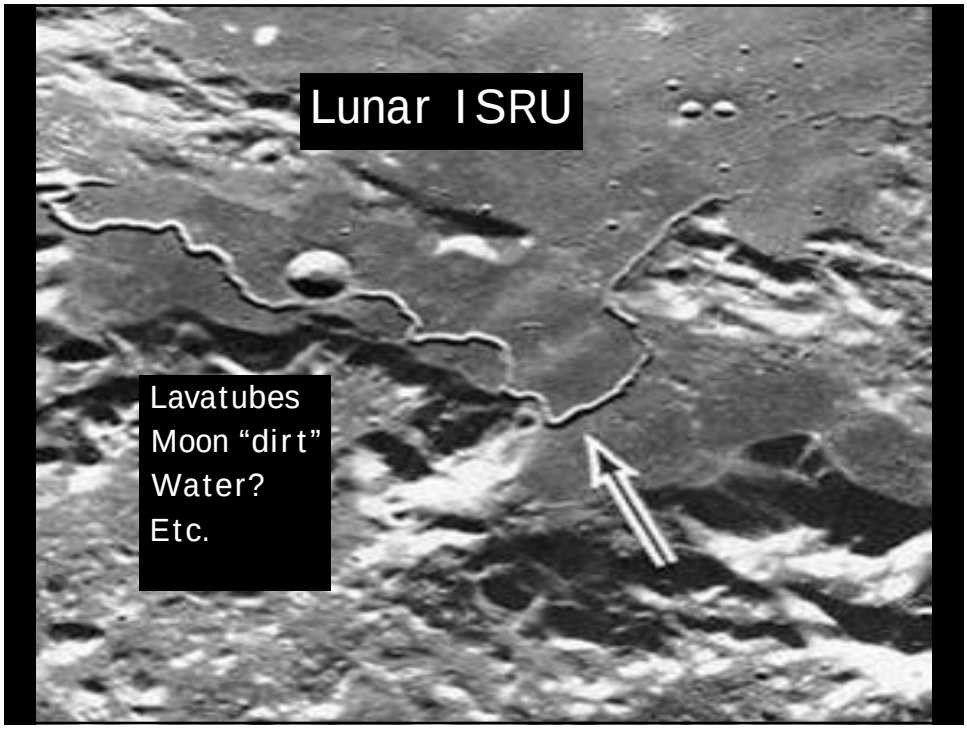

Lunar Disadvantages
14 day "day", 14 day "night"
More expensive than Earth-based facility
Less convenient than Earth-based facility
It isn't Mars

# A 100 Year Roadmap:
## *Phases Under Consideration*

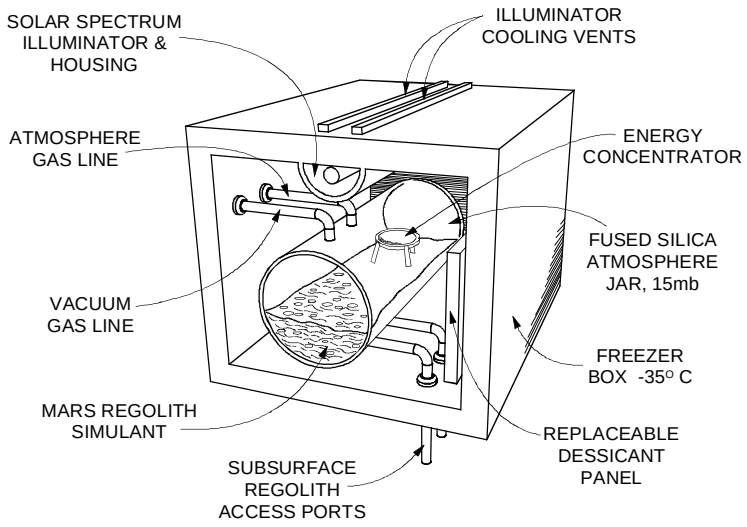
- Systematic multiple-investigator Mars Jar effort
- Large Earth-based facility
- ISS unit
- Small Moon Units
- Plant Growth Module to the Moon
- Large, whole ecosystem scale lunar facility
- Mars plant growth modules
- Mars artificial oases
- Large, whole ecosystem scale Mars facility

# Lunar Facility

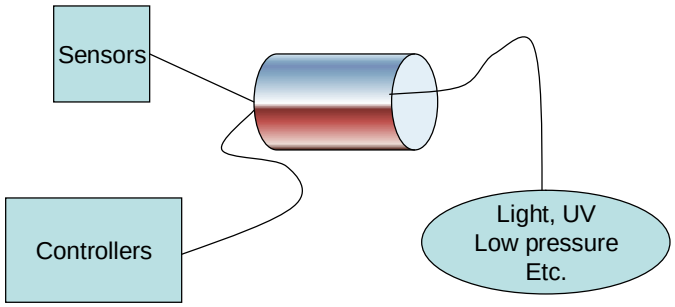





## Earth-based Benchtop or ISS?



## Sodapop Can Mars Jars?




## Sites of Potential Mars Habitability

Similar to Earth extreme environments

Water accessibility

Natural oases

Artificial oases

*different size scales*
*transient oases*

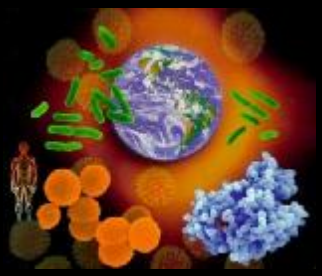
Painting by Pat Rawlings

## Sites of Potential Mars Habitability

Goals???

Terraforming?

Ecopoiesis?



Strategies???

Rapid “weedy” growth?

Stealth approach?

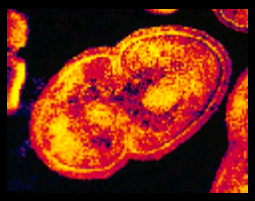
## Artificial Oases

Mini hotkaps – a la Bauman et al., 1979

Created oases in already protected spots

- *Canyon overhangs*
- *Natural small depressions*
- *Ice margin features and caves*

## Deep Science Questions



- Do all biospheres *start* with microbes?
- Do all biospheres *include* microbes?
- Can a biosphere be permanently microbial?
- Can biogeochemical cycling be done entirely by organisms?
- You pick your favorite ecosystem/biosphere questions…

## Appendix 5.2

## NIAC Fellows Meeting, November 2005

# Robotic Lunar Ecopoiesis Test Bed. Phase II NIAC

Paul Todd, Penelope Boston and David Thomas
Presented by Paul Todd
October 11, 2004


## Four Levels of Inquiry Concerning Biology and Mars

1. Planetary protection, contamination and quarantine issues (NRC, 1992),
2. The search for life on Mars (Banin, 1989; Banin and Mancinelli, 1995; Ivanov, 1995; Koike et al., 1995; Biemann et al., 1977),
3. Human expeditions to Mars and ecosynthesis (Meyer & McKay, 1984, 1989, 1995)
4. The terraforming of Mars, ecopoiesis (Haynes, 1990; McKay, 1990; Haynes and McKay, 1992; McKay et al., 1991, 1994; Hiscox, 1993, 1995, 1998).

# ECOPOIESIS

- **Term introduced by Haynes and McKay**
- **Terraforming = making another planet or object in the solar system like Earth**
- **Heating: (1) Greenhouse gases, (2) Mirrors and smoke, (3) Ecopoiesis**
- **Ecopoiesis = emergence of a living, eventually self-sustaining ecosystem**
- **Precedes terraforming**
- **Required step: experimental ecopoiesis**

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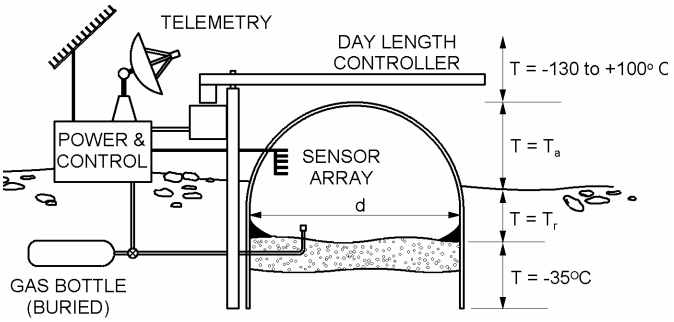
# Starting Position: Robotic Lunar Ecopoiesis Test Bed

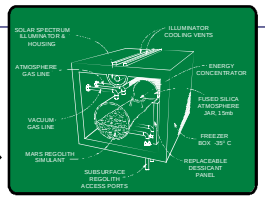
- Trenched, depressed site
- Sealed in all dimensions
- Inflatable dome solidifies
- Sealed interior controlled to Mars atmosphere
- Organisms & chemicals added to artificial regolith
- Control and data telemetry to earth




SHOT

# Project Architecture: Roadmap

Science base



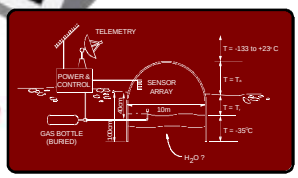

Laboratory test bed

Basic research


Distributed test beds

Low-gravity venues

Lunar test bed




Mars experiments

# Robotic Lunar Ecopoiesis Test Bed: Phase II Goals

1. ***Build and demonstrate Laboratory Test Bed.*** **Use preliminary chamber design from Phase I, purchase and assemble components, demonstrate operation.**
2. ***Evaluate pioneer organisms using Laboratory Test Bed.*** **Subject organisms identified in Phase I to various Mars-like ecopoietic conditions, test activity and viability.**
3. ***Develop ecopoiesis research community.*** **Design modular test beds for lab and classroom use. Develop user community.**
4. ***Refine requirements for extraterrestrial test beds.*** **Pursue opportunities within NASA programs such as ISS and RLEP.**

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## Robotic Lunar Ecopoiesis Test Bed: Science Advisory Committee


***Penelope Boston. New Mexico Institute of Mining and Technology; Complex Systems Research, Inc.***

***Lawrence Kuznetz, NASA Space Biomedical Research Institute***

***Christopher McKay, NASA Ames Research Center***

***Lynn Rothschild, NASA Ames Research Center***

***Andrew Schuerger, University of Florida***

***David Thomas, Lyon College***

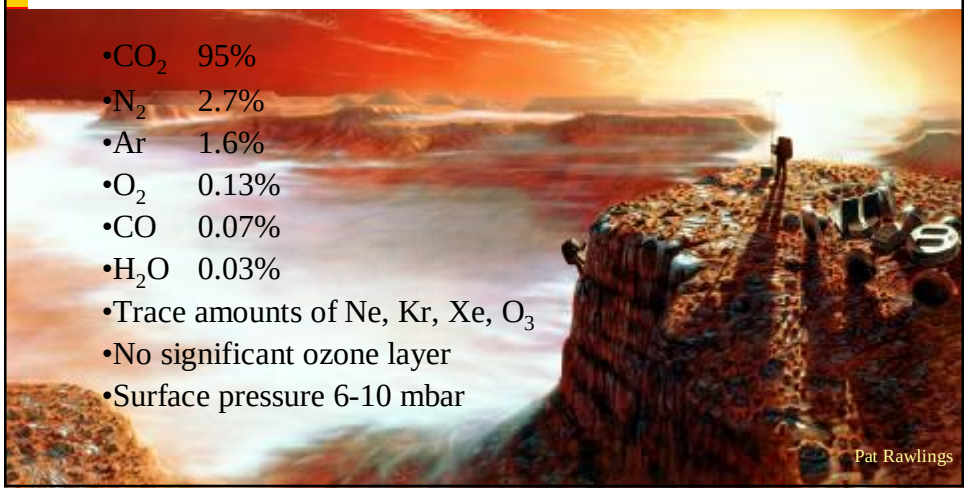


## Objective 1: Laboratory Test Bed

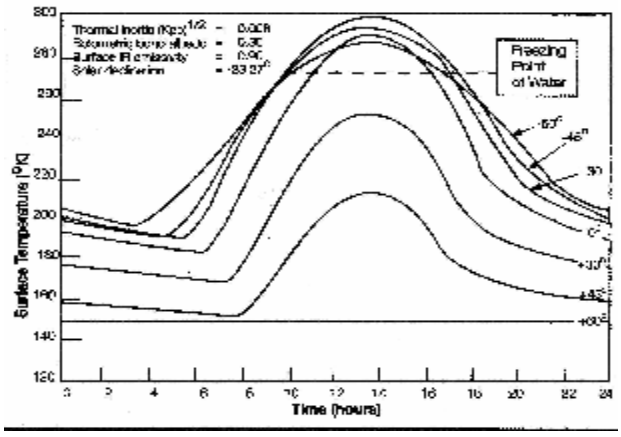
- **Requirements**
- **Design and Construction**
- **Performance**

# Mars' atmosphere today:

Assume that initial engineering efforts will increase atmospheric pressure and maintain the same relative abundances of gases or raise only $CO_2$.




# Temperature Cycles on Mars Surface




**M. H. Carr, 1996**

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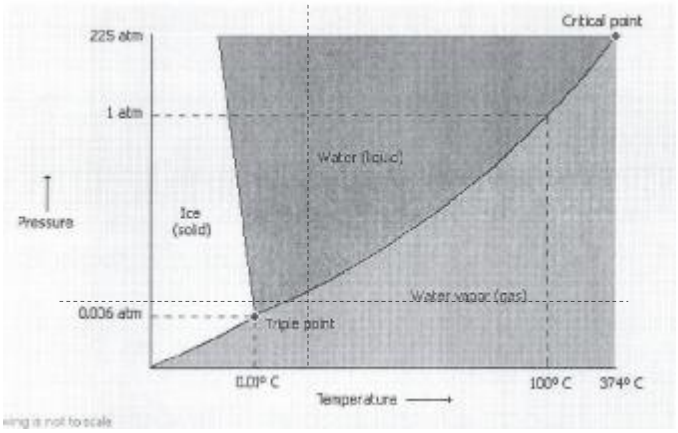
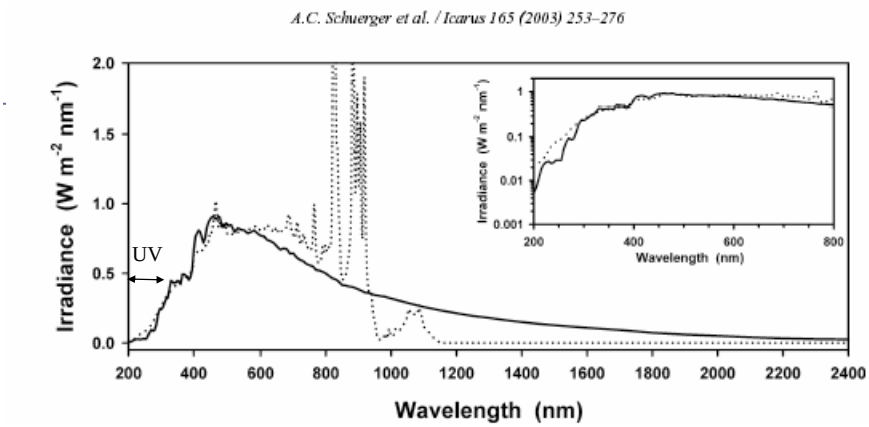
# Liquid Water on Mars (Sometimes)?

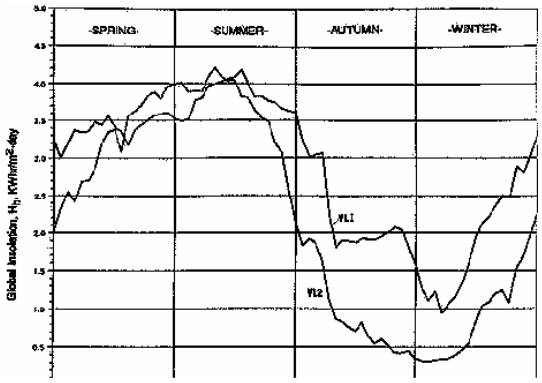



The main challenge for survival on the surface of Mars is the UV radiation between 200 and 300 nm.

# Lighting: Temporal Simulation

**Integral at mid-day is about 590 W/m²**




BY SEASON AT EQUATOR

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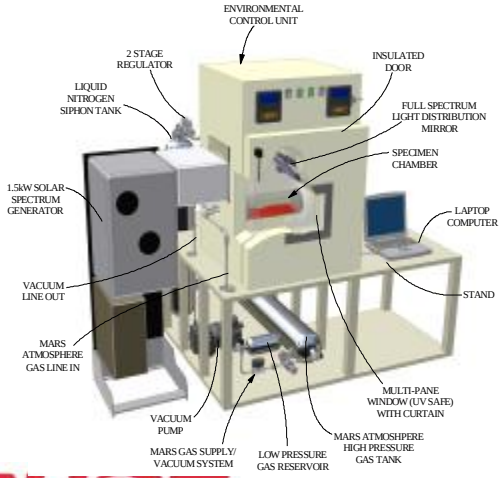
# Laboratory Chamber and Subsystems Design Drawings

- Outer housing controls temperature -130 to +26°C (dry nitrogen cryogenic)
- Sealed illuminator with housing & cooling vents
- Low-pressure "Mars Jar" held at >7 mbar
- Atmosphere composition analysis and control
- Regolith simulant
- Automated operation and data logging
- Affordable product for research laboratories

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# Laboratory Test Bed Thermal Control

***Continuous operation on Mars sol program (-80°C night; +26°C day) consumes >200 gal $LN_2$ /wk. A 500-gal supply tank was installed behind a locked safety fence. During dawn and evening, set-point is reset every 10 min to reproduce thermal profile on Mars at low latitude.***

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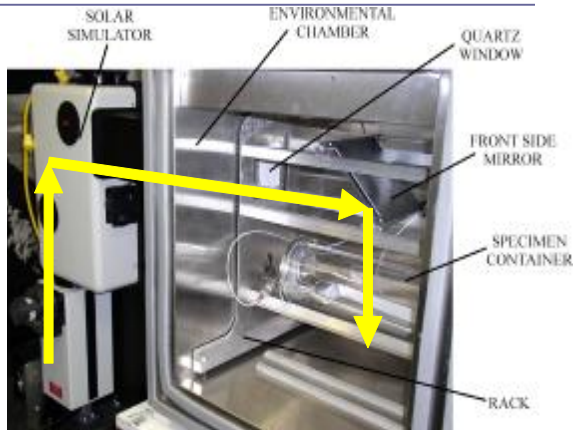
# Laboratory Test Bed Optical Path

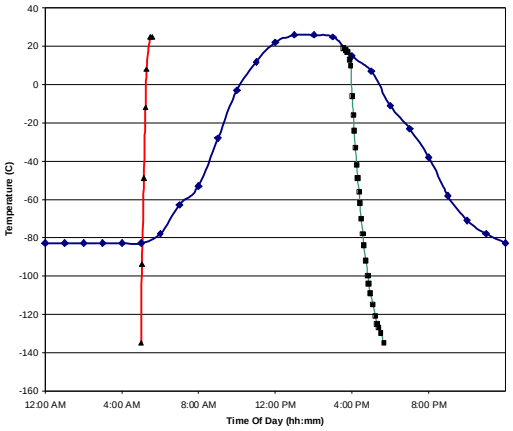
- **1000W Xenon arc**
- **Automated arc striking**
- **8" x 8" lighted area**
- **Fused quartz container**
- **Photosynthetically active radiation (PAR) = 1100 μmoles/m²-s = 237 W/m² (vs. 242)**
- **Translates closely to 590 W/m²**




SHOT

# Laboratory Test Bed
# Regolith Temperature Control

- **Rise rate (triangles) exceeds requirements**
- **Cooling rate (squares) exceeds requirements**
- **Watlow controller programmed to new set point every hour**
- **Simulates daily temperature profile at low latitude at vernal equinox**
- **Controlling sensor submerged in regolith simulant**

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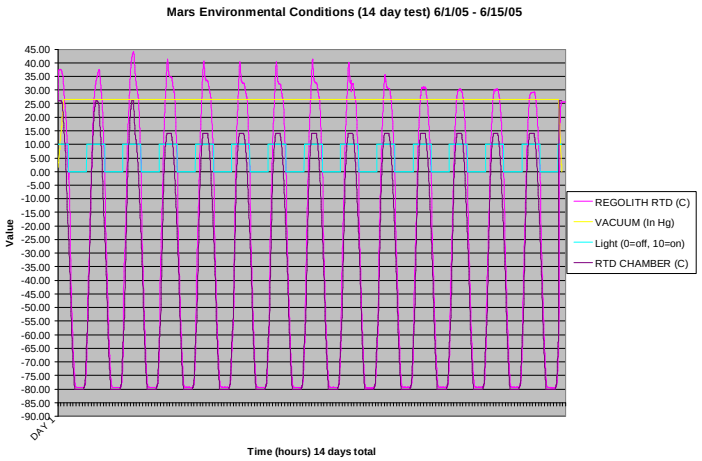
# Laboratory Test Bed
# Performance Data

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# Objective 2
# Evaluate Pioneer Organisms

- **Selection of organisms based on Phase I results**
- **Performance of experiments in test bed**
- **Preliminary results**

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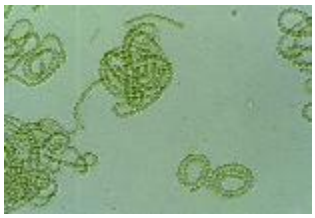
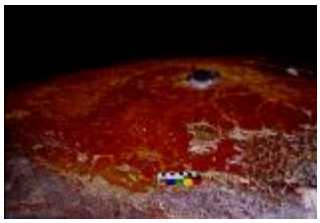
# Summary of Requirements for Pioneer Martians

- **Anaerobic**
- **UV resistant**
- **Low pressure**
- **Drought resistant**
- **Freeze resistant**
- **Phototroph**
- **Nitrogen fixing**

**C. McKay, 2004**





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# Physiological traits of engineered martian organisms ("Marsbugs"):

- Reactive oxygen tolerance (superoxides, peroxides, ozone, etc.).
- $CO_2$ tolerance.
- Intracellular acidification tolerance.
- Carbonate dissolution.
- Osmotic tolerance and adaptation.
- Ultraviolet radiation resistance and repair.
- "Switchable" genes for nutrient cycling (e.g., N-fixation, denitrification). (Hiscox and Thomas, 1995)

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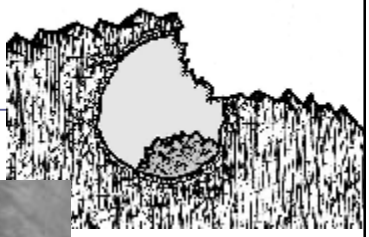
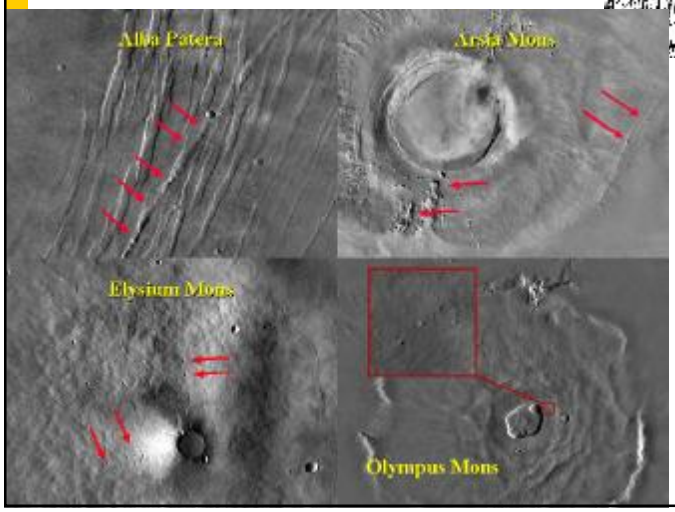
# Lava Habitats



Lavatubes



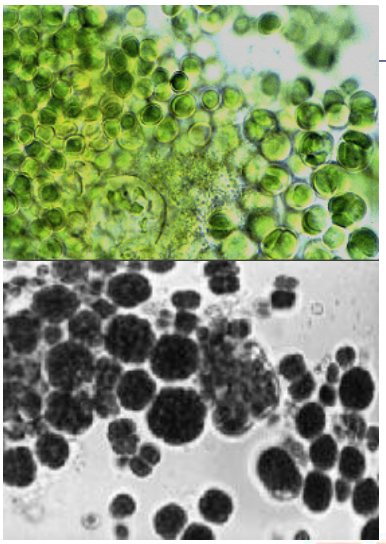

Bubbles

Squeeze-ups

Palagonitization

Dr. Penny Boston

# *Chroococcidiopsis*



- Primitive cyanobacterial genus.
- Unicellular, multicellular.
- Capable of surviving in a large variety of extreme conditions: aridity, salinity, high and low temperature.
- Sole surviving organism in hostile environments.
- Often endolithic.

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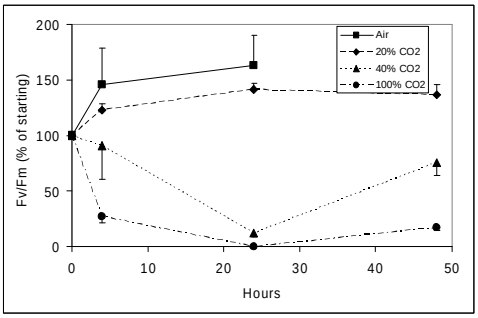
# High % $CO_2$ tolerance




**$CO_2$ effects on *Synechococcus*.** *Synechococcus* responds to high $CO_2$ similarly to *Anabaena*. PS-II activity increases at 20% $CO_2$, but is inhibited at 40-100% $CO_2$. At 100%, the photosystems do not recover after 24 hours in air (n = 4, bars = s.d.). Dr. David Thomas.

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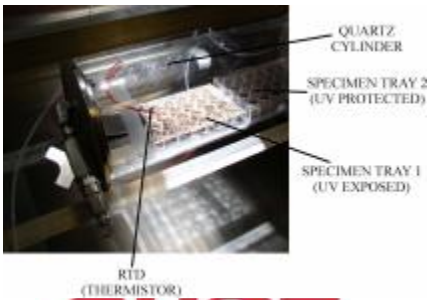
# Experiments with Microbial Specimens to Date

| EXPERIMENT | DATE | DURATION | SPECIMENS |
|---|---|---|---|
| 1 | May 31 | 23 hours | Dr. Thomas' |
| 2 | June 1 | 14 days | Dr. Thomas' |
| 3 | June 18 | 8 days | Dr. Thomas' |
| 4 | June 23 | 22 hours | Dr. Boston's |
| 5 | June 25 | 5 weeks | Dr. Boston's |
| | | | Dr. Thomas' |

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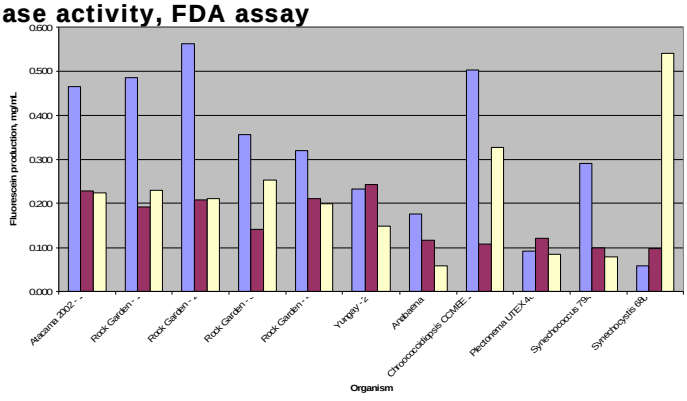
# Preliminary Results of Microbial Experiments

- **14 days at 100mbar, water saturaturation**
- **Cyanobacteria (5 strains), Atacama desert bacteria (6 isolates)**
- **Esterase activity, FDA assay**

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# Objective 3.
# Modular Ecopoiesis Test Bed

- **Controlled volume = 80 cc**
- **Several simulators per recharge station**
- **Temperature -80 -- +26ºC**
- **SHOT-designed computing hardware and software**
- **Thermoelectric cascade**
- **Solar spectrum simulator**
- **Classrooms and labs**
- **Funding applied for**


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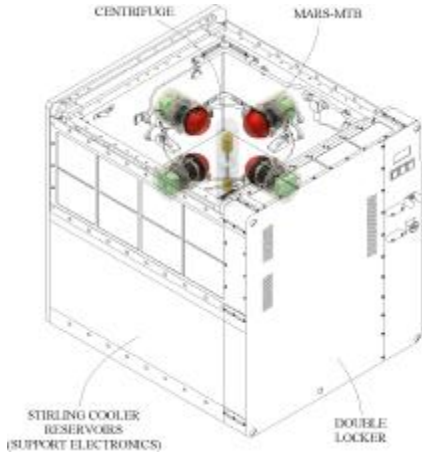
# Objective 4
# Extraterrestrial Test Bed Opportunities (Phase III)

- **Partial gravity on Shuttle or ISS?**
- **Modified Avian Development Facility and up to 4 low-pressure jars**
- **Test pioneer communities in MARS-chamber, 0.38 g**

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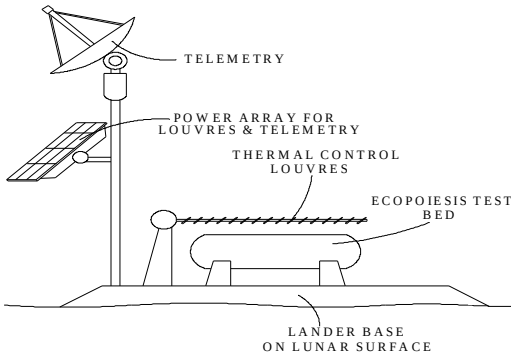
# Concept Proposed for RLEP

- **Test chamber same as laboratory test bed**
- **Mars gas pressure reservoir at 10 atmospheres**
- **Louvres for light and temperature control**

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# PROGRESS ON MILESTONES

- **Phase I completed; laboratory test bed design, extremophile selection initiated**
- **Phase I articles for publication**
- **Laboratory test bed completed, operated**
- **Modular portable test bed proposed**
- **Low-volume lunar test bed proposed**
- **Science Advisory Committee met twice**
- **Annual First report submitted**
- **Phase II presentations at conferences**

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## Year 2 Tasks

- Perform 4 additional 5-week biological experiments
- Create community of ecopoiesis and astrobiology simulation scientists
- Obtain funding for modular test beds
- Report biological results
- Stay connected to RLEP


## Thanks to HIAC and the SHOT EcopoiesisTeam

- Paul Todd, Principal Investigator
- Penny Boston, Co-Investigator (lithotrophs)
- David Thomas, Co-Investigator (cyanobacteria)
- Nathan Thomas, EE, Project Manager
- Bill Metz, MET, Mechanical design
- John Phelps, EET
- Bill Johnson, Software Engineer
- Darrell Masden, ME, Thermal Engineer
- Lara Deuser, ChE, Lab Scientist
- Heidi Platt, ChE

## Appendix 5.3

## Astrobiology Scientific Conference, Poster, March 2006

**A Versatile Tested Planetary Environment Simulator for Biological and Mineral Research**

Paul Todd, Nathan A. Thomas, Michael A. Kurk, G. W. Metz, David J. Thomas, Penelope J. Boston
SHOT, Inc., Lyon College, New Mexico Tech

**145** ABSTRACT

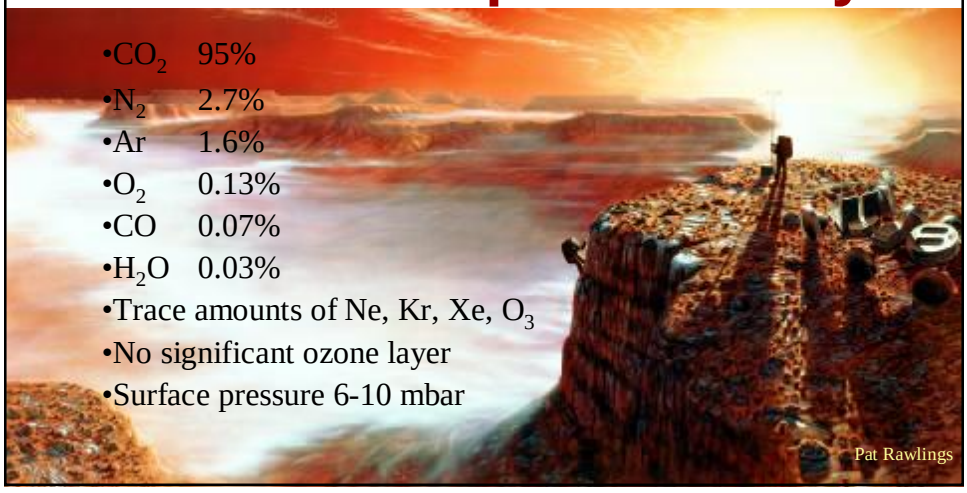
High-fidelity simulation of planetary environments is an obvious component of astrobiological research. Surprisingly few facilities are available to simulate planetary environments for this purpose. Simulators designed for the testing of space hardware tend to neglect certain biologically relevant variables and are seldom available to biologists on a scheduled basis. A commercial simulator suitable for research laboratories was therefore designed, built and tested. The simulator was designed to meet, minimally, requirements for high-fidelity simulation of conditions on the Martian surface: atmosphere composition and pressure (down to 7 mbar), daily temperature extremes (-135°C to +40°C), daily solar intensity cycle (up to 590 W-m$^{-2}$), regolith composition, and surface solar spectrum (down to 200 nm). To achieve these conditions a 6-liter quartz vacuum vessel containing regolith simulant and test samples is evacuated to the desired pressure, which is maintained using a user-selected support gas (including water if desired). This vessel is contained within a cryogenic chamber cooled by evaporating nitrogen and heated by resistive heaters and programmable to any desired temperature cycle. The solar spectrum is simulated by an automatically controlled 1,000-W xenon arc lamp with filters. Biological (ecopoiesis) experiments up to five weeks long have been conducted in this simulator.

## EXTREMOPHILE TEAM

Thomas, Kuznetz, Deuser, Binder, Kish, McKay, Boston, Russell, Todd, Friedmann, Ocampo-Friedmann,Turner

This research was funded as project 07605-003-026 of NASA's Institute for Advanced Concepts (NIAC), a program of Universities Space Research Association (USRA) funded by NASA contract NAS5-03110.

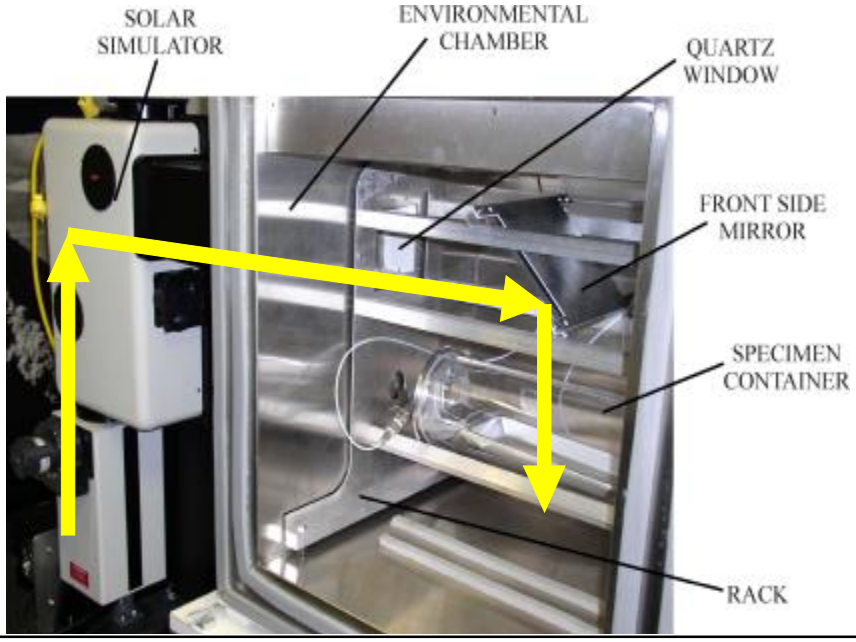
## Mars' Atmosphere Today

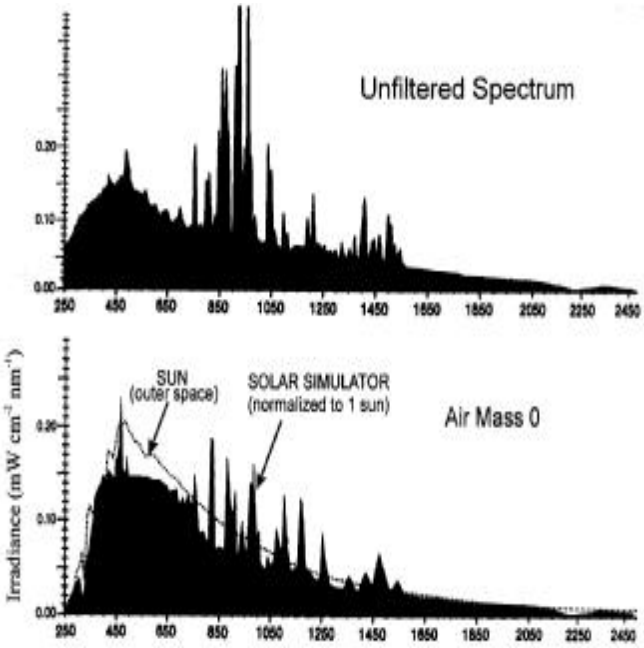


# Properties of Mars to Simulate

- Atmosphere: 95% $CO_2$, 7-15 mbar
- Sunlight: 46% of Earth, full UV
- Soil: Oxidizing regolith, high in Fe, dry
- Temperature: -135C at poles at night, +26C at equator and low latitudes at noon, -80C low latitudes at night
- Day length: 24 h 38 min per sol
- Year length: 669 sols
- Gravity: 0.38 Earth acceleration

# Laboratory Test Bed Optical Path






# ILLUMINATION SYSTEM

- 1000W Xenon arc
- Automated arc striking
- 8” x 8” lighted area
- Fused quartz container

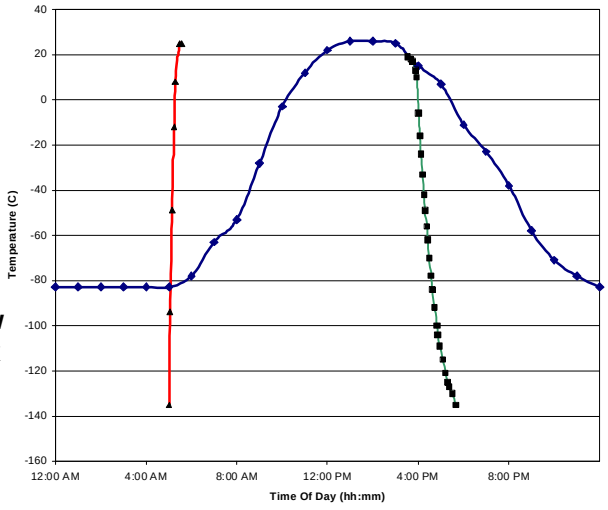
- Photosynthetically active radiation (PAR) = 1100 µmoles/m²-s = 237 W/m² (vs. 242 -- Schuerger)
- Translates closely to 590 W/m²
- UV = 50 µmoles/m²-s 250-400 nm

# Cryogenic Thermal Cabinet

- ***Volume = 8 cu ft***
- ***Evaporating LN2 and resistive heater***
- ***LN2 from external tank***
- ***LN2 vented to exterior***
- ***Fibreglass insulation***
- ***Watlow F4 Programmable controller***
- ***RS232 link to external control computer***
- ***Safety alarms for low $O_2$***

# Temperature Control

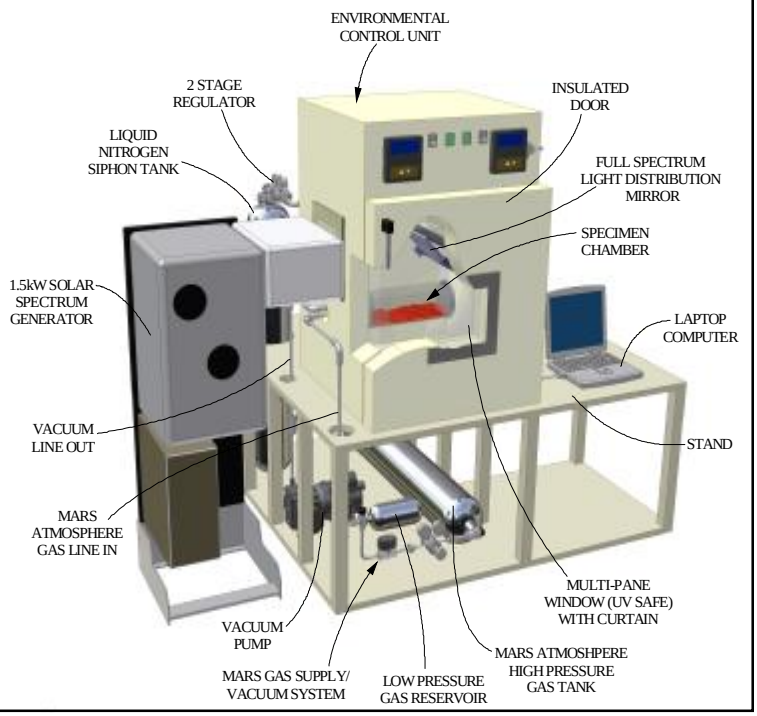
- **Rise rate (triangles) exceeds requirements**
- **Cooling rate (squares) exceeds requirements**
- **Watlow controller programmed to new set point every hour**
- **Simulates daily temperature profile at low latitude at vernal equinox**



# Low-Pressure Chamber

***COMPOSITION***

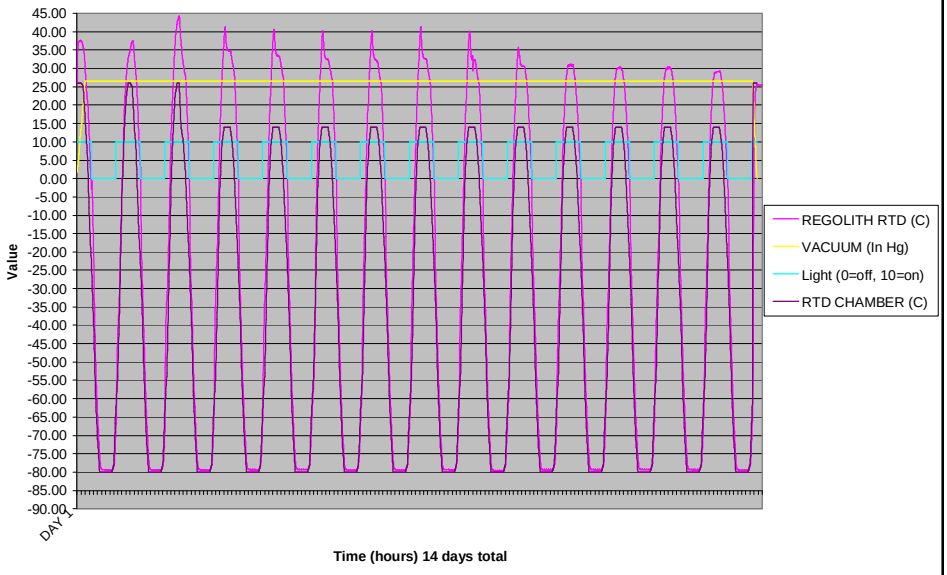
- ***Carbon Dioxide ($CO_2$) 95.3%***
- ***Nitrogen ($N_2$) 2.7%***
- ***Argon (Ar) 1.6%***
- ***Water Vapor ($H_2O$) saturated***
- ***Oxygen ($O_2$) 0.13%***
- ***Pressure 10-1,000 mbar***



# Integrated Laboratory Test Bed

## Affordable product for research laboratories

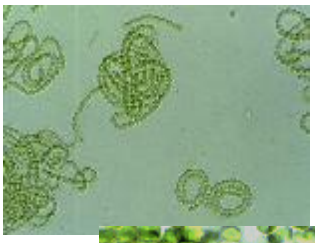
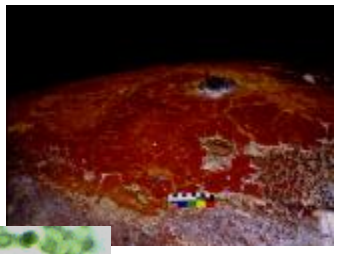
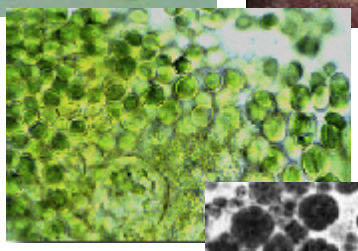
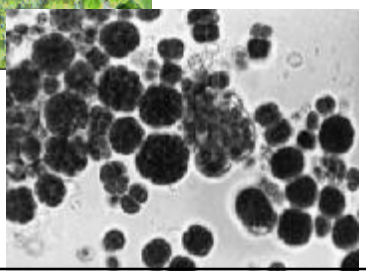
# Performance Data



# Requirements for Pioneer Martians

- Anaerobic
- UV resistant
- Low pressure
- Drought resistant
- Freeze resistant
- Phototrophic or lithotrophic
- Nitrogen fixing

C. McKay, 2004

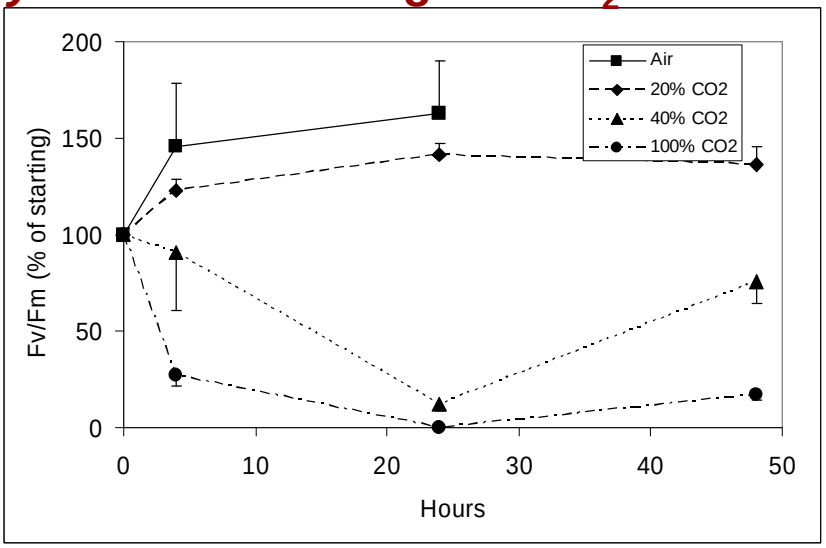
The Yungay-Paranal area of the Atacama desert, Chile is one of the most Mars-like places on Earth.



# *Chroococcidiopsis*

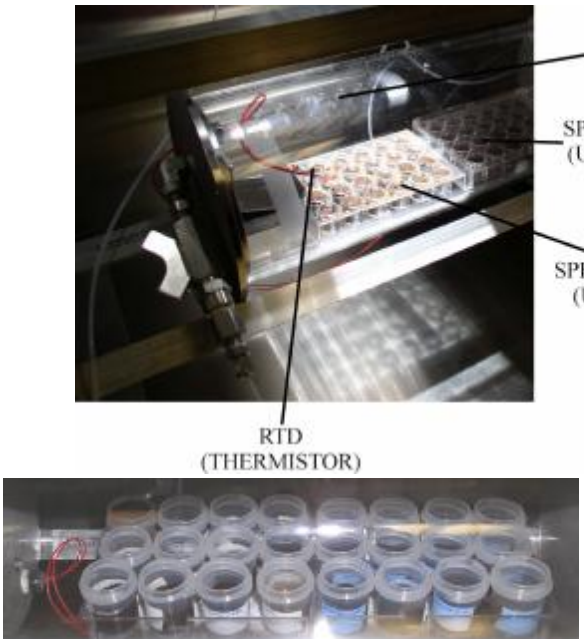
- Primitive cyanobacterial genus.
- Unicellular, multicellular.
- Capable of surviving in a large variety of extreme conditions: aridity, salinity, high and low temperature.
- Sole surviving organism in hostile environments.
- Often endolithic.

# *Synechococcus* High % $CO_2$ tolerance

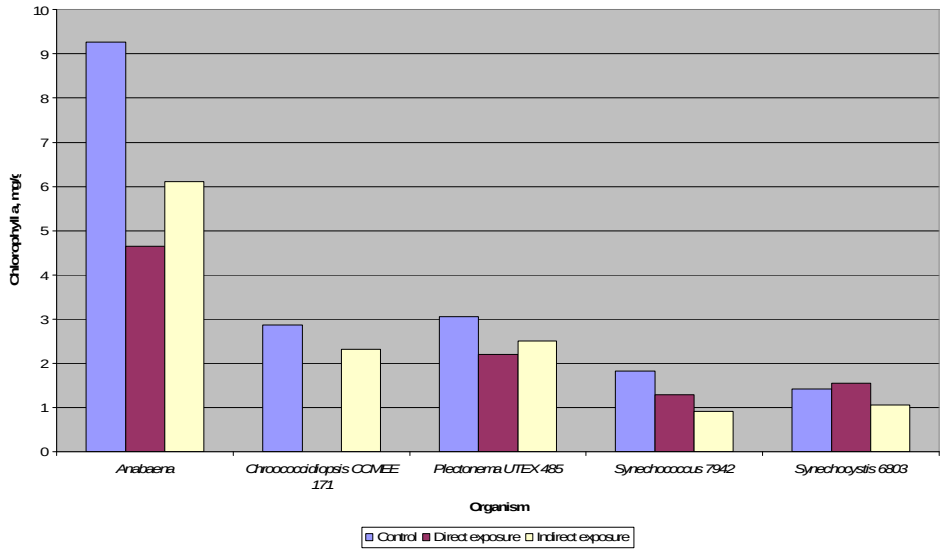



**$CO_2$ effects on *Synechococcus*.** Responds to high $CO_2$ similarly to *Anabaena*. PS-II activity increases at 20% $CO_2$, but is inhibited at 40-100% $CO_2$. At 100%, the photosystems do not recover after 24 hours in air (n = 4, bars = s.d.). David Thomas.

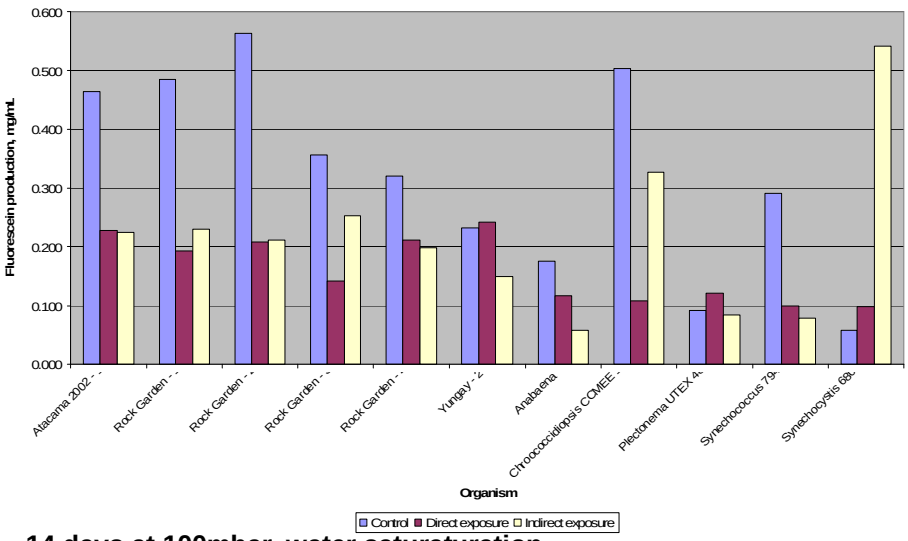
# Experiments with Microbial Specimens




# 14-day trial, chlorophyll *a*



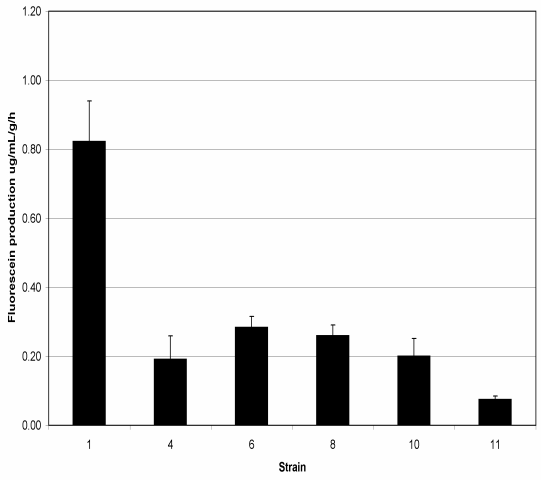
# Preliminary Results:Microbial Experiments




- **14 days at 100mbar, water saturaturation**
- **Cyanobacteria (5 strains), Atacama desert bacteria (6 isolates)**
- **Esterase activity, FDA assay**

# Preliminary Results:Microbial Experiments

- **5 weeks at 100mbar, water saturaturation**
- **Cave and desert varnish bacteria (6 strains)**
- **Cultures from agar plates mixed with BG-11 amended JSC Mars-1 in polypropylene containers in direct light**
- **Esterase activity, FDA assay**
- **No. 1 identified as *Pedomicrobium manganicum* isolated from desert varnish**



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