Microbial Detection Array (MDA), a Novel Instrument for Unambiguous Detection of Microbial Metabolic Activity in Astrobiology Applications

Alexander Hoehn, Kennda L. Lynch, James Clawson, Jacob B. Freeman
BioServe Space Technologies, Aerospace Engineering Sciences, University of Colorado

Jason Kapit, Suzanne M.M. Young, Samuel P. Kounaves
In-Situ Planetary Chemical Analysis Laboratory, Department of Chemistry, Tufts University

Igor I. Brown
NASA Johnson Space Center

ABSTRACT

MDA is designed as a test bed for an astrobiology field instrument to detect microbial metabolic activity in terrestrial or extraterrestrial geological soil samples. MDA employs electrochemical sensors in a unique differential chamber configuration, able to detect minute changes in the chemical composition between the two otherwise identical chambers. Both chambers are filled with identical autoclave-sterilized, sample-water mixtures. Only one of the chambers receives an additional minute, non-sterilized inoculation sample. Under the minimal assumptions that the geological sample contained nutrients (energy), organisms, and required water to initiate growth, the differential electrochemical measurements would now allow detection of metabolic activity, in addition to the electrochemical characterization of the soil samples in both chambers.

INTRODUCTION

The MDA prototype instrument is designed for autonomous operation in the field with minimal mass, power and energy consumption, testing key technologies for a future spaceflight instrument while validating the underlying biological and geochemical sciences. Functionally, the MDA instrument has the capability to receive two equal mass, homogenized geological samples, provide sterilization through autoclaving, then mix the sterilized samples with sterile, metered water and observe, through arrays of electrochemical sensors, the chemical composition of each of the two chamber contents over time. Accurate temperature control for the two chambers allows incubation at user-selectable temperatures from +4°C to +45°C, depending on the native habitat of the expected organisms. After initial chemical stabilization of the sample-water slurry, one of the two identical chambers will receive a minute, non-sterilized inoculation sample, and electrochemical observation will continue over weeks of incubation. Under the minimal assumptions that the obtained geological samples contain all the nutrients (energy), organisms, and required water to initiate growth, the differential measurements would now allow detection of metabolic activity, in addition to the geochemical characterization of the samples in both chambers based on electrochemical sensor readings. Samples may be from the actual weathered surface (i.e. desert soils, lava soils, etc.), or from the unweathered subsurface.

The actively controlled incubator can replicate temperatures of the native environments associated with various terrestrial extremophiles ranging between +4°C and +45C°. The autoclave allows dry and steam autoclaving up to +148°C. The reaction and autoclave chambers can both be optionally pressurized to a desired pressure and atmosphere composition. The reaction chambers each accommodate 60 ccm of inoculation volume, while the autoclaves are designed for 10 ccm geological samples each. The reaction chambers allow sample withdrawal and minimal LED illumination. The wetted components can be sterilized for multiple uses. This paper concentrates on the engineering design challenges, trades, capabilities and initial qualification test results from the instrument.

SCIENCE OBJECTIVE

The question of whether or not life exists on Mars or elsewhere in the universe has been an increasing topic of focus among the space science community. The original debate, initiated prior to the Viking missions, was

fueled by the ambiguous results returned from the 1976 Viking Biological Experiments and the failure of the Viking gas chromatograph-mass spectrometer (GCMS) experiment to detect organics on the Martian Surface (Klein, 1978; Mancinelli, 1998; Glavin et al., 2001). This debate was exacerbated by the report of potential fossilized microorganism in the Allen Hills Meteorite, ALH84001 (McKay et al., 1996). The general consensus is that 20th century life detection technology failed to provide unambiguous results and new techniques need to be developed for future missions (Steele and Toporski, 2002; Mancinelli, 1998; Glavin et al., 2001).

To detect extraterrestrial organisms in a form similar to microbial life on Earth, the ideal detection methodology should be based on absolutely minimal assumptions about the nature of such an organism. Most characteristics may be significantly different from terrestrial ones, and to such an extent, that they cannot be accurately predicted. On the other hand, it is impossible not to make any assumptions at all. However, there are three properties of microbial life that we assume have a very high probability of being universal beyond Earth. These include; (1) the ability to reproduce, (2) the ability to transport and metabolize chemical species as needed, and (3) that it requires water (Kounaves et al., 2002). During the process of reproduction, the organism's metabolism, mediated by its membrane processes will, by necessity, change the surrounding aqueous environment, both chemically and physically. Given all of our assumptions, an appropriate detection methodology and instrument must be capable of detecting such changes with a high degree of sensitivity and most importantly, free of extraneous or non-biogenic interferences.

The presence of growing terrestrial microbes in culture media can be monitored through changes in the environment. These changes can be measured electrically (conductivity, pH, capacitance) or optically (turbidity, absorbance, reflectance or fluorescence). Although never flown, several of these methods have also been proposed for detection of extraterrestrial microbial life (Lunine, 2002). Of the above techniques, optical methods do not appear viable due to the presence of suspended sample particles in the aqueous solution of geological samples, which could lead to a false positive result. Biologically-induced fluorescence, or fluorescent biomarkers, requires assumption with respect to known terrestrial organisms which may not be valid for extra-terrestrial organisms. Additionally, mineral luminescence may interfere with weak biological fluorescence signals.

An instrument was proposed to detect microbial life forms with minimal assumptions (Kounaves et al., 2002). The proposed Microbial Detection Array (MDA) is based on previous work with the MECA electrochemical analysis suite originally developed and flight qualified for the cancelled MSP 2001 Lander. Electrochemical sensors for geological sample analysis without life detection capability are part of the 2007 Phoenix Mars

Scout Mission (Kounaves et al., 2003). Based on successful laboratory feasibility studies using similar electrochemical sensors, the MDA project proposed to design, build, and demonstrate a prototype field instrument, using electrochemical sensors to detect microbial life in aqueous solutions. The sensor array would be designed to provide a response to such biologically induced minimal chemical and physical changes occurring in only one of two identical growth chambers via differentially monitored electrochemical sensor arrays. Minimal metabolism will alter the physico-chemical steady state in one chamber such that a difference between the sensor arrays will result in a signal. Detecting changes in an organism's chemical environment with the MDA requires no prior knowledge of the microorganism. The only assumption is that microbial metabolism creates chemical disequilibrium in the local environment that can be detected and distinguished from non-biogenic chemical processes.

In addition to the use of an array of metabolism-sensitive sensors in a controlled environment, advanced sample handling systems are required for sample processing, and long-term monitoring under spaceflight constraint conditions (reliable, robust, low-mass, low-power).

MDA INSTRUMENT FUNCTIONAL SEQUENCE

At this prototype design stage it is assumed that a geological sample is processed and homogenized, metered and then delivered to the MDA instrument with known mass, or less ideally, with known volume (Fig. 1).
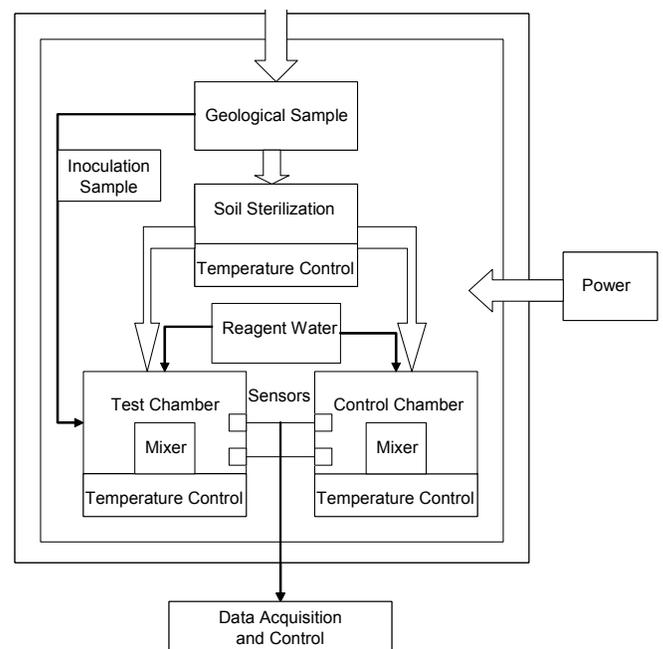

Figure 1. Functional Block Diagram for the MDA instrument.

In one design solution with two autoclaves, the homogenized sample is split into two equal mass samples, and automatically sterilized in the miniature autoclaves based on terrestrial sterilization processes of steam sterilization, with an option for dry sterilization as

2

well. After sterilization, the samples are transported, through a valve, to the two identical reaction chambers (Fig. 2) and mixed with sterile process water. The chambers are temperature controlled within an incubator volume, and are fitted with a mixer and identical, redundant electrochemical sensor arrays. The electrochemical properties of the sample-slurry can be recorded as voltage signals from the sensors for the two chambers. After stabilization and an initial baseline measurement, a small, unsterilized inoculation sample is added to one of the two chambers only. Assuming a sufficient concentration of organisms in the non-sterilized, relatively small inoculation sample, and sufficient nutrients from the native geological material, electrochemical changes should occur due to metabolic activity only, leading to relative signal changes between the otherwise identical chambers. The two chambers need not be at electro-chemical equilibrium, as the signals are measured differentially between the inoculated and non-inoculated chamber. Continuous recording times of 2-4 weeks are expected before typical extremophiles with the desired relative small inoculation sample would show sufficient and detectable growth and subsequent electrochemical changes in their bulk environment.
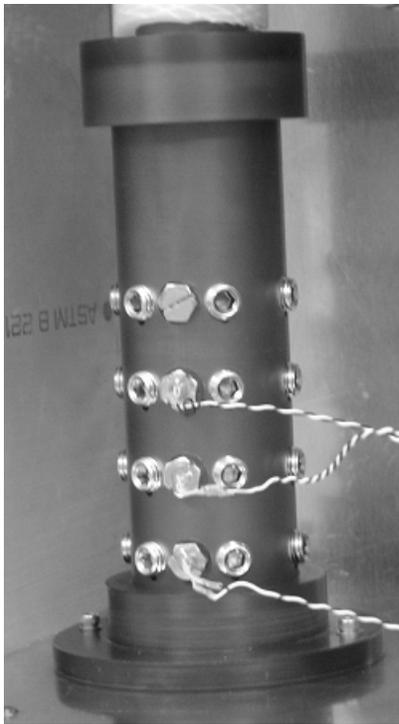

Figure 2. Reaction chamber with ISE sensor ports (plugged) and thermistor sensor ports. All ISE sensor ports can also be sealed with septa or used as multi-purpose ports (LED illumination, fluid sampling).

Initial laboratory characterization is conducted with known and well-characterized species of microorganisms such as cyanobacteria as well as extremophiles, terrestrial organisms adapted to extreme environments, such as high altitude desserts or Antarctic environments, with relevance to potential Mars micro-organisms.

Sensor Array

The initial electrochemical sensors are being developed by Tufts University, In-Situ Planetary Chemical Analysis Lab (Kounaves, 2003). Ion-selective electrodes (ISE) for Sodium, Calcium and Potassium, electric conductivity and ISE pH sensors are used for the initial characterization, with new sensors being developed for the MDA project (Fig. 3). These sensors are partially derived from sensors used in the MECA instrument suite, planned for the Phoenix Mars Scout mission, to be launched in 2007 (Kounaves et al., 2006, 2003a, 2003b; Lukow and Kounaves, 2005). The ISE sensor voltages are measured relative to a common ISE reference electrode in each chamber, where the two reference electrodes are connected to each other. Sensor signals are buffered by ultra-low input current amplifiers, conditioned (low-pass filter, amplification) and recorded by the MDA data acquisition system. In addition, temperature sensors and pressure sensors characterize the incubator environmental conditions. A mixer in each chamber keeps the geological sample in suspension and maintains a uniform concentration with minimal boundary layers and concentration gradients.

The reaction chamber (Fig. 2) interfaces with the Tufts University electrochemical sensors (Fig. 3) as shown below in Figure 3. Sensors are replaceable, and seal against the chamber using their PVC membrane surface. Sensors can only be exposed to temperatures of less than 60°C, and therefore cannot be autoclaved. However, the sensors can be frozen for transport to Mars, as has been validated for the Phoenix mission. Sensors are typically alcohol-sterilized.
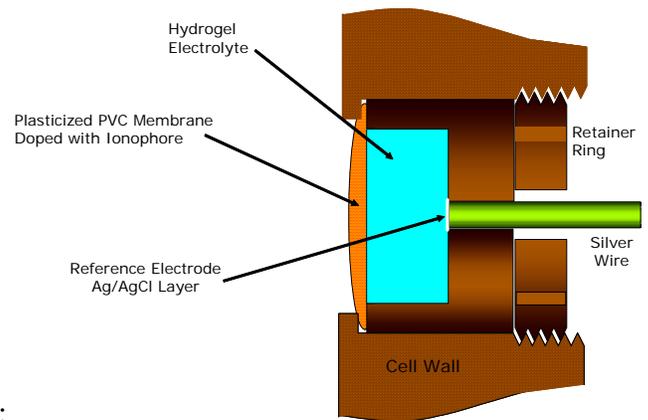

Figure 3. Concept of the Tufts University ISE sensors. The MDA reaction chambers have removable ISE sensor interfaces to allow for sterilization and sensor replacement.

Expected Data Analysis

Initial tests validated the functionality of the approach. Several calibrations have been performed on the sensors while differentially monitoring their signals, and all results indicate that reading a differential signal is a perfectly valid way to determine any change in the

readings of two identical sensors. However, these calibrations were done over short time periods, and further work needs to be conducted to determine if a differential signal will be subject to drifting effects over the longer time lengths required by the MDA experiments.

Future research beyond the baseline cyanobacteria feasibility study is planned to characterize growth responses for a variety of candidate organisms, and to correlate the growth rates to the electrochemical changes measured by the ion-sensitive, pH and electric conductivity electrodes. Additionally, the potential impact of inoculation sample size on the electrochemical signal will be characterized. The relative large growth rates required for a discernable signal change should ensure a positive correlation to biological vs. geochemical origin of change. The instrument therefore should be immune to false positives (life detected, but no organism present). However, inoculation below a minimal threshold, lack of appropriate growth conditions, or lack of nutrients may lead to false negatives (no growth detected with instruments, but microorganism actually present). Laboratory testing under way will define the sensitivity, i.e. the minimum inoculation concentration necessary for successful life detection (Broun, 2007).

MDA PROTOTYPE DESIGN REQUIREMENTS

The project tasks included requirements definition, design trades, and to design, build, test and qualify a portable, proof of concept field instrument, capable of providing a test environment for microbial life-detecting sensor arrays. The prototype instrument requirements still provide flexibility for a range of experimental and environmental conditions. Key design parameters are shown below and in Figure 4. Future flight prototypes would be optimized based on test results with the field instrument with respect to environmental and geometric parameters. A flight instrument can be further optimized and simplified for the Martian operational environment.

Reaction Chambers: minimum 60 mL volume, temperature control between +4º and +45ºC ±1ºC, up to 6 ISE ports per level, with up to 4 height levels for redundancy and/or variable volume. Stirred for minimal gradients, boundary layers and sample suspension. Additional sampling ports for external sample analysis. Potential for illumination / photosynthetic organisms. Inoculation sample of ≤1 mL.

Autoclaves: accommodate ≤25mL geological sample, plus ≥5mL water (for steam autoclave), minimum +121ºC / 202 kPa pressure capable, safety features.

Sample Transport: Provide aseptic transport between environments with minimal losses during transport through adherence to walls.

Data Acquisition and Control: autonomous operation with process control and data storage of 30 environmental sensors (field instrument) and up to 48 ISE sensor signals (multiplexed).

General: reusable after disassembly and sterilization, low mass for field use with external power supply, biocompatible and geo-chemically inert. Low power, designed to operate on ≤30W (expected typical Mars lander power availability for the experiment).

Field vs. Flight Prototype: flight-capable design features are included where possible and affordable for proof of concept, but design focus is on field-usable instrument for science validation and proof of concept in a relevant environment.

The different environments between Mars (very cold, ≈1 kPa) and terrestrial environments (cold to hot, ≤101 kPa) would require different design solutions (example: Mars: heat only, vs. Earth: heat or cool) that need to be addressed analytically for flight feasibility assessment.

ENVISIONED OPERATION

As shown in Figure 4, a pre-processed, homogenized sample of known mass (volume), and grain size is received by the MDA field instrument and sterilized (steam, optional dry autoclave), then automatically transported to the control and experiment reaction chamber. Once the sterile water is added to each chamber, the resulting 'broth' can be characterized using the electrochemical sensors (Figure 3).
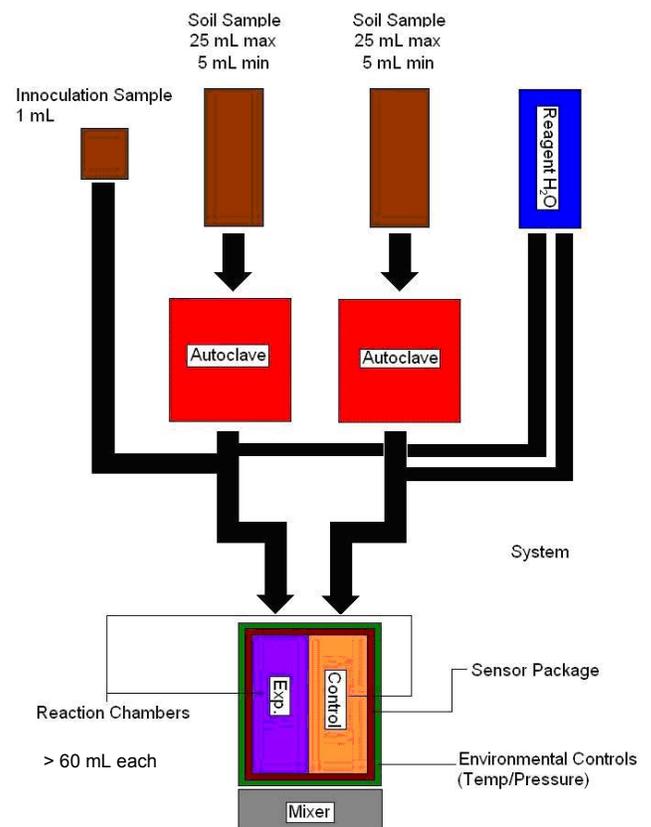

Figure 4. Design volumes for MDA prototype instrument.

4

After initial stabilization, a minute, non-sterilized inoculation sample is added to the experiment chamber only. Continued electrochemical observation over extended periods of time (weeks) would show a differential change between the sterile control and the non-sterile experiment chamber based on presumed metabolic activity if growth occurs. Both chambers may drift throughout the test phase, but a differential change between the chambers would be based on metabolic activities. The chambers are fitted with additional test ports to retrieve samples for independent verification of metabolic activity (Fig. 2). The field instrument can be re-used by sterilization of the reaction chambers and all wetted components. A flight instrument would be single use only. The field instrument may also accommodate flush operations. Alternative to the shown pathway in Figure 4, the non-sterile inoculation sample can also be loaded through the autoclave without actually operating the autoclave cycle, further simplifying sample routing.
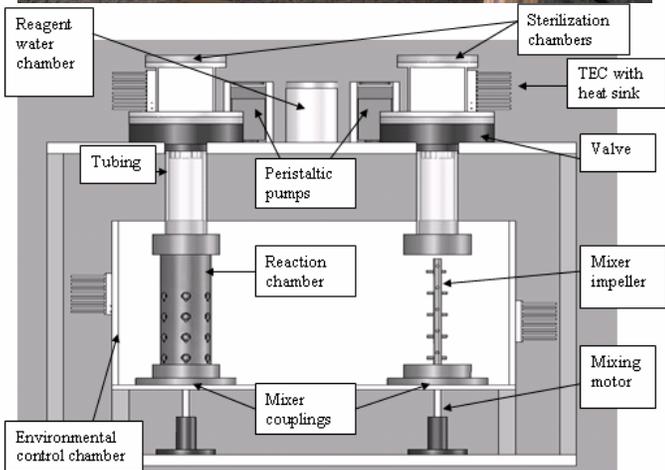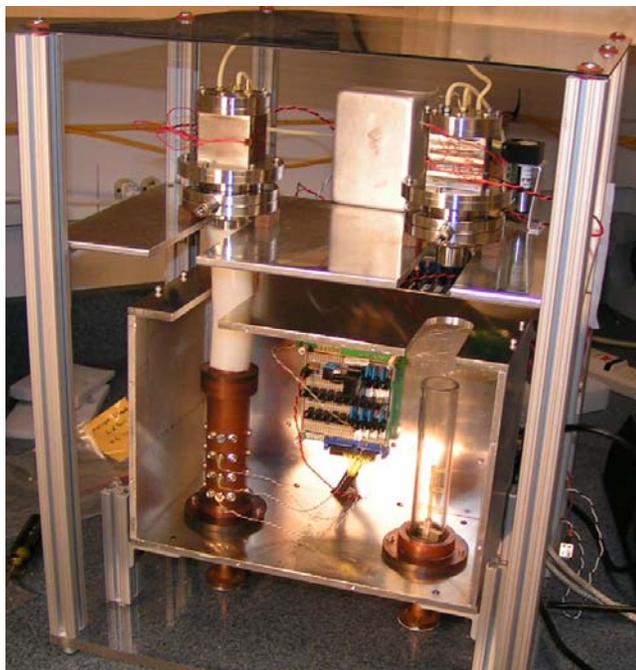

Figure 5. Prototype layout without insulation. The two parallel systems can be seen with autoclaves (top), and reaction chambers inside thermoelectrically controlled incubator (one chamber removed for mixing visualization).

All process control is automated due to the long operating conditions. A typical autoclave cycle consists of heating to +121°C in the sealed autoclave, holding for a minimum of 15 minutes, then cooling down. Self-imposed power limitations to typical power availability for Mars experiments require 1-2 hour heating times with high performance insulation. Tests with the non-optimized prototype autoclaves under stringent power limits (12W) required 5 hrs (Fig. 7). Autoclave cycles may be repeated .once every 24 hours for up to 3 cycles to ensure sterility with spore-forming organisms that could survive single autoclave cycles.

Once the autoclave cycles are complete, the samples are moved to the reaction chambers below, and mixed with process water. Continuous mechanical mixing is designed to maintain uniform electrochemical composition throughout the chamber (Fig. 5). After electrochemical stabilization (days), the inoculation sample is added and may require incubation periods of weeks (≤4) for successful growth detection, under controlled and/or uniform thermal conditions. Mixing is required to ensure a homogeneous electrochemical composition and minimal boundary layers near the electrochemical sensors, but it may not be necessary, nor possible, to keep all components of the geological sample in suspension. However, the liquid phase should be as uniformly mixed as possible throughout the chambers for the sensors to adequately detect changes in the electrochemical composition.

The instrument can be 'deployed' in the field for the intended operational time. Data is stored on-board, or can be transmitted, comparable to the spacecraft application, to a ground support equipment (GSE) computer for display and manual commanding of the instrument.

STERILIZATION METHOD SELECTION

Prior to use, the instrument surfaces and volumes in contact with the sample materials, the initial geological samples and the reagent water must be sterile for successful instrument operation. During instrument assembly, all components must be handled in a sterile environment to avoid contamination of the interior volumes with microorganisms. In select locations within the autoclave system, sterilization will be possible during instrument operation, while other volumes such as the reaction chamber, the wetted sensors and interconnecting plumbing require sterilization of individual parts and sterile assembly and sterile containment until use of the instrument. Several sterilization methods were investigated, including heat (dry and steam sterilization, flame), chemical sterilization (ethylene oxide, Deoxycholate, alcohols), and radiation sterilization (gamma, UV). Responses of Earth organisms to these investigated sterilization methods are fairly well understood, while their effectiveness with potential Martian organisms cannot be known with certainty.

It is assumed that any life form-containing geological samples on Mars may be sterilized by similar means as here on Earth. For the field instrument design, sterilization methods for any Earth-based microorganisms, including extremophiles and spore-forming organisms, were required. The sterilization process must be compatible with spaceflight limitations (low power, low mass), materials, available resources, and compatible with the fundamental operating constraints of the electro-chemical sensors (< 60°C). At the same time, chemical alterations of the geological sample during sterilization should be minimized.

For the geological sample sterilization, steam sterilization was selected as the most suitable process. Multiple cycles would allow sterilization of spore-forming organisms. Dry heat sterilization can be accomplished with the same design by simply not adding the water during the sterilization and increasing the heat exposure times and/or the temperature for the same results, depending on species and sample.

The 316 stainless steel prototype autoclaves (Fig. 6) have an internal cylindrical volume to accommodate ≤25 mL of geological sample and 5 mL of water for steam sterilization. The outer geometry is square to initially accommodate film heaters and high temperature thermoelectric coolers (not shown) for active cooling after sterilization. Heat losses may be sufficient to provide adequate cooling rates (Fig. 7), but low temperature spore germination between autoclave cycles for spore-forming organisms can not be actively controlled without the thermoelectric coolers.
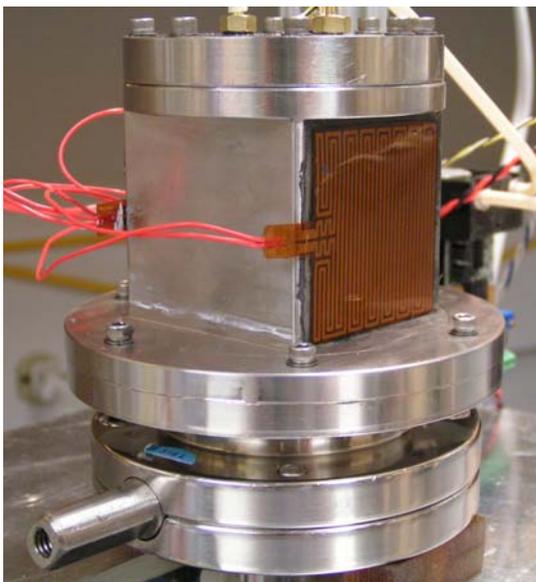

Figure 6. Prototype autoclave with film heaters and valve to reaction chamber (insulation not shown).

Thermal analysis has shown general feasibility under the power limits, but challenges remain to accommodate co-location of the autoclave (121°C) with the temperature-sensitive reaction chamber (<60°C). Currently, the autoclave and reaction chamber are isolated by 10 cm of low thermal conductivity tubing to minimize heat losses

in the autoclave (Fig. 5). However, this resulted in a larger than desired design, and partial loss of sample material due to adherence to the walls during transfer from the autoclave to the reaction chambers.

The current insulation material is a Melamine foam (0.036W/m/K) due to its high temperature compatibility to +148ºC. Pyrogel (0.0155 W/m/K) aerogel high performance insulation for the autoclave can reduce power requirements further, but increases the time for cool-down after autoclaving. Currently, the two autoclaves cannot be operated simultaneously under the 30W overall instrument power constraint. Instead, the autoclaves are operated sequentially to stay within the imposed power limits, resulting in heating times of 5 hours (Fig. 7). The performance depends on the thermal mass of the system and the sample.
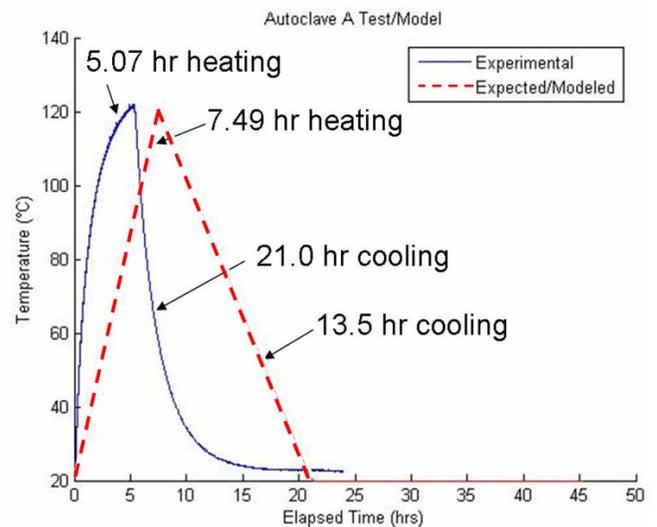

Figure 7. Autoclave testing (without hold period) to verify heat losses / insulation properties and to determine required heating and cooling times under the power constraints imposed.

THERMAL CONTROL

Two thermal control zones are implemented – the autoclaves for sterilization (Fig. 6), and the incubator around the reaction chambers for biological evaluation (Fig. 5). The long autoclave exposure times at high temperature (+121°C) impose challenging requirements on thermal insulation and control of the reaction chamber, which cannot be exposed above +60°C (sensor limit). For simplicity in the field instrument, this challenge may be overcome by larger geometric separation, while a flight instrument may utilize higher performance, but more expensive materials choices. Simultaneous thermal control of autoclave and reaction chamber to their respective temperature setpoints may not be feasible due to self-imposed flight instrument power limits.

Due to the intended use of the field instrument in remote sites and/or with extremophiles of various environments, accurate thermal control of the reaction chamber to

specified values are required for science evaluation. To include tests with microorganisms native to low temperature environments, a thermo-electrically controlled incubator was designed around the reaction chambers of the prototype instrument. This design feature is heavy and would not be required for operation in a very cold environment such as on Mars or in Antarctica, where much simpler film heaters can be used directly on the reaction chambers. The large clearance around the reaction chambers within the incubator on the other hand facilitates sensor installation and access to the instrumentation in the laboratory.

MATERIAL SELECTION

The wetted materials require biocompatibility and chemical inertness, while also providing compatibility with the working environments including autoclave temperatures (nominal 121°C) and pressures 202 kPa (29.8 psia). While Epoxy resins had been used in the past on the MECA instrument for the Phoenix 2007 mission, Ultem (Polyetherimid) was selected for MDA due to previous use and experience for spaceflight biotechnology instruments (Hoehn et al., 2006). Ultem can be autoclaved repeatedly. Citric acid passivated 316 stainless steel is used for structural and wetted components under pressure, such as the autoclave. Due to the imposed power and mass limitations to prove feasibility in a spaceflight setting, a low mass and low heat capacity is desired for the system. For prototype testing and evaluation of long-term and multi-use effects, the initial reaction chamber was designed in Ultem1000, with a 316 stainless steel autoclave. The field instrument is currently designed with autoclavable and removable Pharmed tubing and miniature peristaltic metering pumps (Instech) for sterile water delivery. While previously used for spaceflight applications (Hoehn et al., 2006), their suitability for a Martian environment is still under investigation.
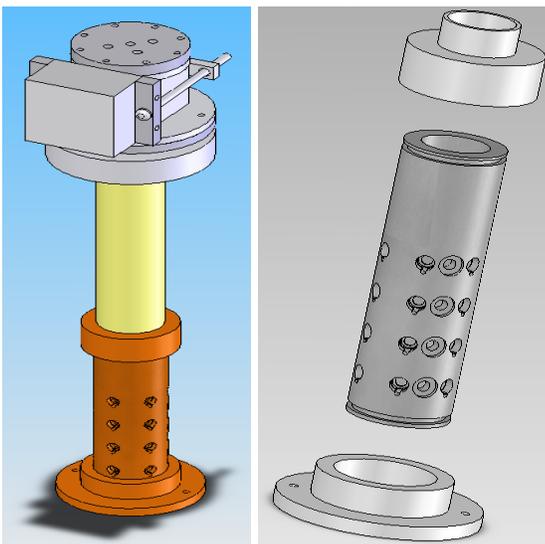

Figure 8. Prototype autoclave (top) and high-temperature valve (top), thermally insulated by 10 cm tubing from the Ultem reaction chamber with 24 sensor ports on 4 levels (bottom).

SYSTEM MASS FLOW

The prototype autoclave requires externally processed, pre-metered manual geological sample addition. An automated valve / drawer interface concept has been developed, but not implemented for the prototype. After the lid is sealed manually, the automated process software adds water, heats and autoclaves for multiple cycles if desired (adjustable hold temperatures and times). After completion of the autoclave cycle, the transfer valve is opened manually (motorized version possible), and the sample is transferred to the reaction chamber. Pre-sterilized water is added through the autoclave and aids in additional transport of sample material trapped on the internal surfaces. Water transport is through miniature peristaltic metering pumps and gravity. All components with the exception of the autoclave require disassembly, sterilization and re-assembly under sterile condition in a controlled environment between operations. The sterile process water and interconnection tubing are also sterilized. The autoclave interior will be automatically autoclaved during sample autoclaving. All parts with the exception of the ISE sensors can be steam autoclaved. The sensors must be alcohol sterilized.

PROCESS CONTROL

The prototype is controlled from a MOPSlcd6 PC104 Pentium computer (Kontron, http://us.kontron.com), with a Diamond-MM32 data acquisition and control PC104 board (32 analog channels at 16 bit A/D, 24 digital control lines, 4 12-bit analog outputs for proportional control, see http://www.diamondsystems.com/). The computer operating system currently resides on a 60GB 2.5" hard disk, but is normally operated from a 4 GB compact flash (CF) solid state drive for flight operations. The prototype software runs under Windows XP and is a Labview virtual instrument for laboratory testing. Once the hardware design has been fully tested and qualified, the software is implemented under the Linux operating system in C++ code on the compact flash drive. For laboratory use, the PC104 computer connects to a VGA monitor, keyboard and mouse. For field use, the system can operate without a user interfaces autonomously, or through a terminal interface (Ethernet connection from laptop computer).

The control system currently uses 12 power switches (on/off: thermoelectric controllers, heaters, motors, metering water pumps, lights), 4 proportional controls (2 temperature2, 2 motor mixing speed), and 32 analog sensors (temperature, pressure, current draws, system and ISE sensor voltages). Of the 30 engineering sensors, only the incubator temperature and the autoclave temperatures are used for feedback control. The autoclave pressures are used as redundant safety sensors in case of a run-away oven. All other sensors are used for instrument characterization and process evaluation.

SAFETY CONSIDERATIONS

The small autoclaves create high temperatures (+121ºC nominal, +150ºC max) and high pressure steam (202 kPa). In addition to the computer-based control algorithms, passive bimetallic switches limit the thermal excursion in case of malfunctions, and the autoclave is fitted with pressure relief valves at 303 kPa to avoid structural damage. Foam insulation prevents accidental contact with hot surfaces during operation, and process control indicators warn of high temperature / pressure conditions.

PROTOTYPE FIELD VS. FLIGHT INSTRUMENT

Several design implementation are currently unique to the prototype design, and would be configured differently for laboratory vs. field vs. flight use as listed below.

Power: The instrument components all operate from 12VDC, with an optional 220/110VAC to 12VDC power supply for laboratory use. Instead, 12VDC batteries can be used (autonomous field use), or a 28VDC to 12VDC DCDC converter (flight).

Thermal: To facilitate a variety of environmental conditions, the field instrument is fitted with thermoelectric coolers for heating or cooling relative to ambient condition, and the instrument is built for use in an atmosphere (fan cooled). The flight instruments in a cold environment would only require heating, which is a more efficient and less complex design. Power use for the field instrument therefore over-estimates power requirements of a flight instrument. The current thermal control system of the prototype relies on convective cooling of powered components in the field and laboratory, and is not configured for the low pressure / low convective heat transfer environment of Mars.

Pressurized gas: The reaction chambers and autoclave are capable of supporting a 101 kPa differential pressure if necessary, but the field instrument currently has no pressure source to create a pressurized growth environment for operation in a low pressure environment like Mars. This could be added through existing multi-use ports.

Data acquisition and control: The PC104 form-factor computer has been used for spaceflight on ISS only and is not designed for radiation environments such as those found on Mars. For ease of user familiarity, the software is currently implemented in Labview on a Windows XP operating system. For field testing, the computer can be operated in a pre-programmed autonomous mode, or in the lab with manual control using keyboard, mouse and computer screen. The flight software would need to be implemented in a robust, high reliability operating system and control software.

EDUCATION AND PUBLIC OUTREACH

A subset of the MDA project was also proposed to the Aerospace Engineering Science (ASEN) department of the University of Colorado (CU) as a project for the Senior Design Lab (SDL) capstone design class (1 year, 5 credit hours, 7-8 students per group). MDA was accepted by the department and a group of 8 senior students started design work on the MDA prototype. The department co-funded the project with $4,000. The project followed standard aerospace project milestones of PDR (October 2006), CDR (December 2006), TRR (April 2007), with final working, tested and analyzed product delivery in May 2007. As part of the final project evaluation, students had to present their designs to a project advisory board for evaluation, as well as at a public design exposition at the Integrated Teaching and Learning Laboratory (ITLL), located at the university http://www.colorado.edu/ASEN/SrProjects/.

SUMMARY

Many important design considerations have been taken into account in the development of the MDA prototype device. The prototype design provides automated capabilities for sample sterilization, delivery into two identical chambers, mixing with water, thermal control and process and sensor variable recording over extended periods of time. The prototype field instrument was designed for a variety of potential growth environments, specifically a wide range of sample sizes and thermal environment. A future flight instrument would be more mass- and size-optimized for a specific target environment. However, many sub-components have been selected for MDA with regard to flight optimization of minimal energy, power, size, and mass. Material selections were driven by biochemical and process compatibility. The prototype instrument has been functionally tested and is awaiting fully integrated biological testing in the laboratory and relevant field environments.

For the human-tended field work, several operations such as sample preparation, splitting, metering and delivery of the homogenized samples to the autoclaves are currently manually operated for cost reasons, but concept designs have been started for fully automatic sample processing in the future.

CONCLUSION

Initial engineering tests in the laboratory and engineering demonstrations of a prototype field instrument support the potential of using electrochemical sensors in a differential chamber setup for both future electrochemical characterization of geological / soil samples and potential microbial life detection in aqueous solutions. Detailed biochemical characterization in the developed and engineering-validated prototype instrument is under way during the summer of 2007 to

supplement the initial life detection data collected under controlled laboratory conditions.

REFERENCES

1. Brown (aka Broun), I.I. (2007). Personal communication. NASA Johnson Space Center, JSC-ESCG, 2101 NASA Parkway, Houston, TX 77058, USA. igor.i.broun@nasa.gov.
2. Glavin, D.P., et al. (2001): "Detecting Pyrolysis Products from Bacteria on Mars." Earth and Planetary Science Letters, 2001. 185(1-2): p. 1.
3. Hoehn, A., Freeman, J.B., Koenig, P., Stodieck, L.S., Vellone, M., Williams, S., deLuis, J., Feenstra, W., Parrish, J.C., Pretorius, S., Sun, L., Dyble, S., Searby, N., Vandendriesche, D. (2006): "Single Loop for Cell Culture (SLCC) – Development and Spaceflight Qualification of a Perfusion Cell Culture System." *SAE-paper 2006-01-2212*.
4. Klein, H.P. (1978): "The Viking Biological Experiments on Mars." *Icarus*, 1978. 34(3): p. 666.
5. Kounaves, S.P., Lynch, K.L., Broun, I.I., Hoehn, A., McKay, D.S., and Young S.M.M. (2006): "Microbial Detection Array." *Astrobiology*, Mar 2006, Vol. 6(1), p. 282.
6. Kounaves, S.P., Lukow, S.R., Comeau, B.P., Hecht, M.H., Grannan-Feldman, S.M., Manatt, K., West, S. J., Wen, X., Frant, M. and Gillette, T. (2003): "Mars Surveyor Program '01 Mars Environmental Compatibility Assessment Wet Chemistry Lab: A Sensor Array for Chemical Analysis of the Martian Soil." *J. Geophys. Res.*, Vol.108 (E7), pp.5077-5088.
7. Kounaves, S.P. (2003): "Electrochemical Approaches for Chemical and Biological Analysis on Mars." *ChemPhysChem*, 2003, Vol. 4, pp. 162-168.
8. Kounaves, S.P., Noll, R.A., Buehler, M.G., Hecht, M.H., Lankford, K. and West, S.J. (2002): "Microbial Life Detection with Minimal Assumptions." Society of Photo-Optical Instrumentation Engineers (SPIE), Proc., *SPIE 4495* (Instruments, Methods, and Missions for Astrobiology IV), Vol.137, pp. 137-144.
9. Lukow, S. R. and Kounaves, S.P. (2005): "Analysis of Simulated Martian Regolith Using an Array of Ion Selective Electrodes." *Electroanalysis*, 17, pp. 1441-1449.
10. Lunine, J. (2002): "Signs of Life: Report of a NRC Workshop on Life Detection." In: 34th COSPAR Scientific Assembly. 2nd World Space Congress, held 10-19th October, 2002 in Houston, Texas.
11. Mancinelli, R.L., (1998): "Prospects for the Evolution of Life on Mars: Viking 20 years later." *Advances in Space Research*, 1998. 22(3): p. 471.
12. McKay, D.S., Gibson Jr., E.K., Thomas-Keprta, K.L., Vali, H., Romanek, C.S., Clemett, S.J., Chillier, X.D.F., Maechling, C.R., and Zare, R.N. (1996): "Search for Past Life on Mars: Possible Relic Biogenic Activity in Martian Meteorite ALH84001." *Science*, 1996. Vol.273(5277): pp. 924-930.
13. Steele, A. and Toporski, J. (2002): "Astro-biotechnology." In: *ESA SP-518,* 2nd European Exo- and Astrobiology Workshop, Graz, Austria, 16–19th Sept. 2002, pp 235-238.

CONTACT

Dr. Alex Hoehn is the associate director of engineering of BioServe Space Technologies, a NASA Research Partnership Center, located at the University of Colorado, Aerospace Engineering Sciences. He can be reached at: BioServe Space Technologies - University of Colorado, 429 UCB, Boulder, CO 80309-0429, USA. Tel. +1-303-492-5875, hoehn@colorado.edu.

Dr. Sam Kounaves is the principle investigator for the NASA ASTIID grant. He can be reached at the *In-Situ Planetary Chemical Analysis Laboratory*, Department of Chemistry, Tufts University, Medford, Massachusetts, 02155, USA. http://planetary.chem.tufts.edu/.

DEFINITIONS, ACRONYMS, ABBREVIATIONS

A	Area
A/D	Analog to Digital conversion
aka	also known as
ASEN	Aerospace Engineering Sciences at University of Colorado at Boulder
ASTID	Astrobiology Science and Technology Instrument Development

ccm, cm^3 cubic centimeter, 1 ccm = 1 mL
cm, cm^2 centimeter, centimeter squared
CF Compact flash (memory)
COTS Commercial Off-The-Shelf
CU University of Colorado
g Earth gravity, 9.81 ms^{-2}
GB Giga-Byte, 10^9 Bytes.
GCMS Gas chromatograph-mass spectrometer
GSE Ground support equipment
H2O Water
ISE Ion-Selective (Specific) Electrode
k kilo, 10^3
K Kelvin, 1°C = 271.15°K = 32°F
kg Kilogram
kPa kilo Pascal, 101.325 kPa = 14.7 psia.
LED Light-emitting Diode
MDA Microbial Detection Array, sometimes MiDa or MiDAS
MECA Microscopy, Electrochemistry, and Conductivity Analyzer, a wet chemistry laboratory aboard Phoenix (2007)
Min Minute
mL milli-Liter. 1mL = 1 ccm^3
MSP Mars Surveyor Program
NASA National Aeronautics and Space Administration
Pa Pascal; 101,325 Pa = 14.7 psi = 1 atm.
pH Potential of Hydrogen, measure of acidity
Psi, psia Pounds per square inch, 101,325 Pa = 14.7 psi = 1 atmosphere (atm.)
PEI Polyetherimide, trade name Ultem
PVC Polyvinyl Chloride.
rH Relative humidity [%]
SDL Senior Design Laboratory
T Temperature
TRL Technology Readiness Level
ULTEM® Polyetherimide, PEI
UV Ultraviolet (light)
VDC Voltage Direct Current
W Watt