The Biological Oxidant and Life Detection (BOLD) Mission: An outline for a new mission to Mars

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ABSTRACT

The Viking mission was the only mission to date that conducted life detection experiments. It revealed ambiguous and still controversial results. New findings and hypotheses urge a re-evaluation of the Viking results and a re-evaluation of the evidence for the possible presence of life on Mars in general. Recent findings of abundant water ice on Mars, the presence of liquid contemporary water on the Martian surface, and the detection of methane in the Martian atmosphere further support this possibility. Current missions to be launched focus on habitability considerations (e.g., NASA Phoenix, NASA Mars Science Laboratory), but shy away from directly testing for life on Mars, with the potential exception of the ESA ExoMars mission. If these currently planned missions collect positive evidence toward habitability and the possible existence of extraterrestrial (microbial) life on Mars, it would be timely to propose a new mission to Mars with a strong life detection component. We propose such a mission called BOLD: Biological Oxidant and Life Detection Mission**.** The BOLD mission objective would be to quantify the amount of hydrogen peroxide existing in the Martian soil and to test for processes typically associated with life. Six landing packages are projected to land on Mars that include a limited power supply, a set of oxidant and life detection experiments, and a transmitter, which is able to transmit information via an existing Mars orbiter back to Earth.

Keywords: BOLD, mission, Viking, life detection, oxidant, Mars, microbial life, Phoenix, Mars Science Laboratory, ESA Mars Express, ESA ExoMars, water

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1. INTRODUCTION

The Viking mission was the only mission so far that conducted life detection experiments on another planetary body. After the Viking mission the consensus view was that Viking did not detect life, but reactive soil chemistry based on (1) the evolution of O_2 upon wetting the soil, (2) the apparent absence of organic molecules in the soil, and (3) the weakly positive result of the single control test in the Pyrolytic Release experiment[1]. However, recent findings exert doubts on these interpretations. For example, a recent re-analysis indicated that the sensitivity of the Viking gas-chromatograph mass spectrometer was much less than originally thought due to interference with minerals in the Martian soil and other factors[2]. Further, Houtkooper and Schulze-Makuch[3] devised a logically consistent biological explanation that proposes an adapted biochemistry to Martian environmental conditions (hydrogen peroxide-water hypothesis) and would, at least in part, explain the ambiguous Viking lander results. A comparison of the explanation of the H_2O_2-H_2O hypothesis with traditional chemical explanations is provided in Table 1. Since Viking, Mars missions have focused on studying the geology and the environmental conditions on Mars. Key missions were the NASA Pathfinder and the on-going Mars Exploration Rover (MER) missions. The next mission to Mars is the NASA Phoenix lander mission to be launched in August 2007. It will attempt to answer the question whether Mars possessed an aqueous geochemical environment capable of supporting microbial life. This mission is followed by the NASA Mars Science Laboratory to be launched in 2009 to test the habitability of Mars, both in regard to possible future colonization attempts from Earth and endemic Martian life. A more aggressive approach is taken by the European Space Agency with the ExoMars mission, which is currently in the planning and design phase, to more directly detect biological activity. Most recent insights into the environmental conditions suggest at least the possibility of life on Mars. They include the presence of large amounts of water ice on Mars[4-7], evidence of contemporary liquid water on Mars[8], and the detection of methane and possibly formaldehyde in the Martian atmosphere[9-11]. Indications for hydrothermal activity on Mars are also accumulating[12]. Thus we feel that it is timely to propose a bold new mission that directly addresses the presence of the "mysterious" oxidizer on the Martian surface and any biological activity. In the following we detail such a mission called *BOLD: Biological Oxidant and Life Detection Mission*.

2. SCIENCE QUESTIONS, JUSTIFICATION, AND OVERALL MISSION DESIGN

The scientific questions to be answered by the BOLD mission are (1) to determine the unknown oxidant in the Martian soil, which was suggested to exist after the Viking mission[13], and (2) to probe whether there is life near the Martian surface. In contrast to the Viking mission with its underlying assumptions of heterotrophic life with a global distribution pattern[14], the BOLD mission is geared toward a more comprehensive search including lithoautrophic and photosynthetic microbes, and a variety of biomarkers. The proposed BOLD mission consists of a carrier vehicle with 6 probes attached. No orbiter is assumed. Instead the probes are taking advantage of an existing Mars communication orbiter (NASA Mars Reconnaissance Orbiter, ESA Mars Express, or some other spacecraft in orbit around Mars at that time). The number of probes is intended to provide a certain degree of mission redundancy in case some of them fail. Given sufficient propulsion capability of the landing system, a landing precision of 10 m is projected. To accomplish this accuracy, the BOLD mission will use an optical guidance system and will provide a live telemetry stream during the descent of the probes. The probes are light-weight with a science payload of less than 10 kg. The lander system uses a crushable shell behind the heat shield instead of landing gear. The landing probes will be powered by batteries. The mission duration for each landing probe is anticipated to be 10 Sols (Martian days). The BOLD mission design not only affords redundancy but in situ analysis of prime targets of elevated life potential otherwise difficult if not impossible to reach on Mars through current mission designs.

3. ANALYSIS OF BASIC MISSION PARAMETERS

A preliminary analysis of the basic design parameters has been conducted to determine the feasibility of the proposed mission. The study involved various aspects of the overall baseline mission including propulsion and orbital analysis, heating and load analysis during the entry phase to characterize the shielding and structural probe characteristics and communication capabilities of the payload. The overall approach is based on conducting first order analyses to understand the impact of a baseline mission profile on mass and power of the landing probe. Focusing on a single probe of the overall cluster of 6, the baseline mission consists of a sequence of distinct phases:

a) Probe deployment from orbit around Mars

b) Probe orbit change and insertion into a entry path
c) Guided descent via optical guidance system and live telemetry link
d) Ground delivery of probe
e) Initiation of probe science phase.

Table 1. Explanations for some remaining questions after Viking (modified from Houtkooper and Schulze-Makuch[3])

Question	Chemical Explanation	H_2O_2-H_2O hypothesis
Lack of identified organic molecules	The organics have been oxidized to nonvolatile salts of benzenecarboxylic acids, and perhaps oxalic and acetic acid[15].	Upon death of the organisms, the organics are spontaneously oxidized by the previously intracellularly bound H_2O_2 with no or very little organic residue. Non-biology bound organic molecules are oxidized chemically[15] and/or consumed by organisms. The release of 50-700 ppm of CO_2 by the Viking GC-MS may indicate that oxidation of organic material took place[2].
Lack of identified oxidant	There is some yet unidentified mechanism producing H_2O_2 or other oxidants. The oxidant might be present in form of a compound that has no analog on Earth. Suggested inorganic oxidants include metal oxides such as Fe- and Ti-oxides[16] and superoxide ions[17].	The H_2O_2 in the H_2O_2-H_2O mixture is part of the biochemistry of the putative Martian organisms. It would explain the oxidizing potential observed in the Viking results. However, some soil chemistry reactions certainly play a role in the response to the Viking lander experiments as well.
Release and Partial Resorption of O_2, CO_2, and N_2 in the GEx experiment	Evolution of O_2 upon humidification was suggested to involve one or more reactive species such as ozonides, superoxides, and peroxides[18]. CO_2 production in the wet mode can be interpreted to be related to the oxidation of nutrient organic compounds[19], and N_2 release can be interpreted to be related to an initial N_2 desorption from soil by water vapor and subsequent resorption in liquid water[19].	The release of O_2 (and possibly CO_2 to a lesser degree) can be interpreted as the result of an energy-producing metabolism. Upon humidification it could point also to the decomposition of dying Martian biota, as could the increase of N_2. The decrease of N_2 can be understood as biological fixation if it exceeded the amount due to physical sorption, a possibility also entertained by Oyama et al.[19].
Synthesis of organic material in PR experiment	No consistent explanation has been provided, but attempts to explain the observations include instrument malfunction, incorporation of ^{14}CO into carbon suboxide polymer preformed on the Martian surface, and reduction of ^{14}CO by H_2O_2 in the surface material[20].	Some of the putative organisms were able to metabolize and synthesize organic compounds before they died being overwhelmed by water.
Responses in the Labeled Release experiment	Laboratory tests on Earth using inorganic oxidants and clay minerals simulated many of the key findings, but not the decrease of responses after storage at elevated temperatures[1].	Limited metabolism[21-22] before the organisms died due to hyperhydration, osmotic pressure, and heat shock.

The following sub-sections summarize the basic preliminary analyses of a few critical aspects of the mission (details of the analyses will be provided elsewhere).

3.1 Propulsion System and Orbital Analysis

The carrier vehicle with the landing probes is propelled into a circular orbit around Mars (h = 400km). Each probe is assumed to be equipped with a small solid rocket to provide the delta-v required to insert the spacecraft in an entry trajectory that can safely land the payload on the Martian surface. Assuming a specific impulse of 290 sec and a structural mass fraction equal to 10% of the propellant mass, the Tsiolkovsky equation[23] was used to compute the propellant mass as a function of the desired delta-v with the payload mass as a parameter. Our preliminary calculations indicate that more than sufficient delta-v can be achieved.

3.2 Heat and Load Analysis during Entry

The propellant mass is clearly connected to the capability of the propulsion system to slow down the orbital speed and put the probe on a descent trajectory for ground delivery. A first order analysis has been applied to determine the maximum deceleration experienced by the probe during entry and to compute the total heat absorbed by the flight vehicle during the entry phase. The analysis consisted of determining the simplified equation of motion for a probe during ballistic entry[24]. We assumed a shallow entry flight-path and (atmospheric) no-lift conditions. The exponential atmospheric model was employed assuming a Martian height scale equal to 11.3 km. First order analytical solutions[25] were implemented in MATLAB to determine the maximum (absolute) deceleration as a function of the propellant mass (with the flight angle as a parameter). Heat load analysis was connected to the ballistic trajectory and the basic hypersonic flow characteristics[26] to determine the total heat load experienced by the probe as a function of the propellant mass used to insert the system into a descent trajectory. The total heat absorbed is on the order of 10^6 J while the maximum heating rate is estimated to be 26 kW/m^2. Both calculations show that both shielding via ablation and heat rejection are possible without substantial weight increase of the entry probe. The maximum rejection rate for a molybdenum shield is estimated to be 4435 kW/m^2 (blackbody radiator), which is enough to reject the occurring radiation. In case of ablation shielding, graphite high heat of evaporation (10^7 J/kg) is such that only a few grams of the shield evaporate during entry.

3.3 Payload Communication Analysis

A link budget design study has been performed to evaluate how much power is required to efficiently transmit data from the landing package to the orbiter. Assuming a target Bit Rate Error (BER) of 10^{-5}, the value of the Signal-to-Noise Ratio is estimated to be 4.10 dB. It is apparent that 1 W of power is sufficient to transmit data at rates up to 100 kbits/s. The carrier link margin (i.e. the power margin of the carrier required to provide adequate power for transmission) is also sufficient for efficient transmission

4. LANDING PACKAGE AND EXPERIMENTS

As with all space missions, payload mass, size, and power consumption remain the primary concerns and constraints. Therefore, to maintain the size and cost constraints of the proposed BOLD mission, the individual systems that make up the carrier spacecraft and in particular the individual lander probes ideally must themselves be miniaturized, if the overall mission payload is to remain within the mission parameters. In fact, given the overall weight constraints for each probe, the miniaturization requirement has to extend down to the component/sensor level, potentially warranting the use of *Micro Electro Mechanical Systems (MEMS)* as described in Fink et al.[27] MEMS devices, due to their inherently low mass, size, and power, are ideal for both space and sensor network applications. A number of MEMS devices have been specifically developed for the purpose of robotic planetary exploration.[28-29]

The landing package will include environmental sensors, a sampler, and four major experiments geared towards life-detection. Here, we focus on the description of the experiments to be conducted near the Martian surface:

4.1 Multispectral Microscopic Imager Experiment

The question whether Mars can and did support life is a major driving force behind planned Mars missions. A microscopic imager (MI) should therefore be designed to support the in-situ recognition and detection of life on Mars. It should enable the investigation of biosignatures by imaging rock and mineral particles showing evidence for the presence of water and biogenic material. It should provide sufficient resolution (1 to 2 μm) to find microfossils (i.e., well-preserved micro-organisms) in sedimentary and non-sedimentary rocks, or, in the absence of such markers, identify biominerals as a sign for biological activity.

Water is considered to be a pre-requisite for life, and therefore, the search for life in the solar system has been associated with the search for liquid water. Minerals that contain water or that can only precipitate from an aqueous solution can be potential markers for water. The microscopic imager (MI) is designed to support the *in-situ* recognition and detection of both extant and fossilized life (i.e., microfossils) in sedimentary and non-sedimentary rocks as well as biominerals as proxy for biological activity. The MI will be equipped with a 20x objective lens with a 30 mm working distance, LED light source illumination, CCD camera and spectral photometer. It operates in both reflected and transmitted light on different materials including individual mineral grains, thin sections, and fractured surfaces of rocks

and minerals. More importantly, the design of the system enables the identification of organic and inorganic material present as inclusions or as precipitates on rough surfaces and in the fractures of crystalline material. It is anticipated that different combinations of white and UV light will enable the distinction among evaporite, clay and carbonate minerals. The application of LED-UV light enables the identification of organic and inorganic components that autofluoresce. UV-induced fluorescence is crucial for the detection of biogenic traces. The resolution and detection limit of the MI is sufficient to resolve individual bacteria and image their content without the need of fluorescent dyes. The high optical resolution restricts the field of view to a few millimeters, so that contextual views of 10 to 20 mm have to be provided by the microscopic imager as well, though at a lower resolution. A unique advantage of this instrument is its enormous degree in maneuver freedom. It can be installed on an arm that can be positioned at any given point in 3D space. The spectral analyzer of the MI interfaces with the Fluorescent Stain Experiment and is described in Section 4.2

4.2 The Fluorescent Stain Experiment

The Fluorescent Stain (FS) Experiment consists of two types of experiments, both based on biochemistry as known from Earth organisms. The first experiment assumes the presence of DNA in putative organisms, the second the presence of esterases.

DNA is much more stable compared to other major biological components like proteins or lipids, and it can be amplified with the polymerase chain reaction (PCR). Using random primers, extremely small amounts of unknown DNA can rapidly be multiplied to a detectable amount. In addition, PCR can be performed on chips[30]. New developments such as real time PCR use fluorescent dyes to monitor the amplification during PCR by fluorescent resonance energy transfer (FRET). We will employ these techniques on a chip to detect DNA in the soil of Mars. Excitation will be achieved using a laser diode, and a photodiode to detect the emitted light.

One of the obstacles of extraterrestrial microscopic investigations is that it is extremely difficult to discriminate the potential organisms from inorganic material. It seems impossible to find an individual bacterium within millions of minerals grains of roughly the same size. However, new developments have lead to techniques that might overcome these problems. Many fluorescent dyes cannot pass biological membranes since they carry polar groups. Because of that, specific fluorescent dyes have been developed, where an acetate residue covers the polar residue of the fluorescent dye, turning it into a hydrophobic non-fluorescent molecule, able to pass the membrane passively. Once inside the cell, unspecific esterases cleave the acetate residue and convert the substance into a polar fluorescent dye that is now trapped within the cell. The cells become bright fluorescent. Esterases are ubiquitous enzymes found in all kinds of living organisms on Earth, since they control fundamental processes. Nowadays, this mechanism is widely used in biology as a live/dead cell detection system, since dead cells lack esterase activity (i.e., the dye will not become fluorescent) and membrane integrity.

We will combine this method with our microscopic imager. The laser diode will be used for excitation and the CCD chip for the detection and imaging of the fluorescent organisms. The spectral analyzer on the microscopic imager described above can be focused on any spot of the object under investigation with a resolution of 1 to 2 μm. The MI will have UV illumination and any future laser diode available as an excitation source. Only one wavelength of light is needed to access a wide spectrum of different components, which can then be separated by their emission spectra. This instrument can be used for both autofluorescent and fluorescent stain (FS) experiments. The spectral analyzer to be used in conjunction with the MI could be based on a commercially available grating spectrometer for a resolution better than 2 nm or on a linearly variable filter for resolutions larger than 2 nm.

4.3 The Hydrogen Peroxide Detection Experiment

The Mass Chromatograph Mass Spectrometer (MC-MS) onboard the 1976 Viking Landers indicated the absence of organic molecules in the parts per billion range[31-32], although this is controversial[2, 15]. One likely explanation for this is the presence of a potent oxidizing agent in the soil, but its nature remains enigmatic. The Gas Exchange (GEx) and Labeled Release (LR) experiments, also onboard Viking, determined that the Martian soil contains the unknown compound, which is of a highly oxidizing nature[19,33]. The GEx experiments showed that the humidification of 1 cm^3 of Martian soil sample produced as much as 790 nmol of O_2 gas[19]. In the LR experiment, the addition of a radioactive ^{14}C labeled nutrient solution to soil samples resulted in the production of $^{14}CO_2$ due to the breakdown of the organic species introduced[33]. The MER missions have provided evidence that intense oxidative chemistry was frequent early in the history of Mars, when water was stable on its surface. The discovery of sulfate-rich layered deposits with hematite spherules in Meridiani Planum has been interpreted as the result of mineral deposition in an aqueous, acidic solution, following the near-surface, aqueous oxidation of pyrite[34]. Therefore, organic biomarkers may only be found below the

oxidation layer defined by the diffusivity of atmospheric oxidants in the soil. Previous models have constrained the depth of this layer to less than 3 m[35].

While photochemically produced oxidants may be partially responsible for the oxidation of the Martian soil, a putative photochemical origin for oxidants in aqueous solutions seems more problematic. It is widely accepted that the most likely oxidant species is hydrogen peroxide (H_2O_2), produced photochemically in the Martian atmosphere, which later diffuses into the soil[35-36]. The same origin is assumed for the oxidant agents in past aqueous environments[34]. However, H_2O_2 is unstable under the environmental conditions near the surface of Mars, as in the acidic Martian environments[37]. It decomposes to[38]

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + \bullet OH + OH^-$$
$$Fe^{3+} + H_2O_2 \rightarrow Fe^{2+} + HO_2\bullet + H^+$$

Under these conditions, the short lifetime of H_2O_2 makes it extremely difficult to detect its presence, but there are some methods. A promising approach is the leuco crystal violet (LCV) method originally developed by Mottola et al.[39]. This methodology was recently improved for H_2O_2 detection and quantification in the μM to several hundred nM range, in iron-containing solutions with varying pH[40]. The LCV method involves oxidation of 4,4',4"-methylidynetris in the presence of H_2O_2 and horseradish peroxidase (HRP) to form the crystal violet ion, CV^+, which absorbs at 590 nm. Calibration curves for pH and the presence of iron have been developed, as the LCV method is strongly affected by pH, with an optimal pH range of 3.6–4.2[40]. Importantly, the colored CV^+ is stable for days, which makes this method independent from immediate access to a spectrophotometer, and thus especially appropriate for Martian in situ analyses.

4.4 The Chiral Detection Experiment

Nearly all biologically formed chiral compounds are synthesized as one or the other enantiomer. For example, all amino acids which are used by organisms on Earth are left handed chiral molecules, while all of the sugars in nucleic acids have the opposite spatial relationship. Recently, the National Research Council suggested the use of chirality as a biomarker[41]. The Viking Labeled Release Experiment contained a variety of organic molecules, but mission constraints prevented the inclusion of any chirality measurements. Thus, some of the same labeled substances (e.g., alanine and lactate) plus some other chiral compounds (e.g., cysteine) will be included in the BOLD mission. If labeled gas would evolve exclusively or predominantly from soil injected with one of the isomers, as opposed to its enantiomer, this would be strong evidence for a biological response. No utilization, or the use of both stereoisomers would be an indication for the absence of a biological response. The experiment could be conducted very similar to the original Labeled Release experiment on Viking [42], or in a modified version.

5. CONCLUSIONS

The time has clearly come for a more ambitious, bold mission to Mars, with the objective to either detect life or to resolve what the nature of the "mysterious" oxidizer is that puzzled investigators since the Viking mission. Here, we take the first steps toward such a mission and outline some of its major features.

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