

Methane and Life on Mars

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ABSTRACT

Mumma *et al.*¹ have confirmed earlier detections of methane in the Martian atmosphere, finding it localized and correlated with atmospheric water vapor. They determined that, because of the short half-life of methane, a continual replenishment is required to account for its presence. They also conclude that the dynamics of methane on Mars require a methane sink in the soil. It is suggested here that both phenomenon could be accounted for by an ecology of methane-producing and methane-consuming microorganisms. Such ecologies exist on Earth, where, generally, anaerobic methanogens live at depth and aerobic methanotrophs live at or near the surface. On Mars, with its essentially anaerobic atmosphere, both types of microorganisms could co-exist at or near the surface. It is possible that the Viking Labeled Release (LR) experiment detected methanogens in addition to other microorganisms evolving carbon dioxide since the LR instrumentation would detect methane, carbon dioxide, or any other carbon gas derived from one of the LR substrates. A simple modification of the LR experiment that could resolve the life on Mars issue is discussed.

Introduction

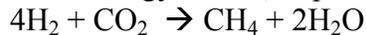
The Labeled Release Life Detection Experiment (LR) on board the 1976 Viking Mission to Mars sought to detect and monitor microbial metabolism by radiorespirometry². The results of that experiment were strongly positive^{3,4}. Although all the data, including varied controls, were indicative of, or consistent with, microbial life, the possibility was raised that a putative non-biological strong oxidant in the Martian surface material was mimicking life. With the 1996 discovery of Martian meteorites containing possible life forms⁵, with increasing evidence that liquid water could exist on Mars at least transitorily^{6,7}, and with many findings of terrestrial extremophiles in harsh Mars-like habitats, Levin (LR Principal Experimenter) concluded⁸ that the LR had indeed detected Martian microbial life. This conclusion was later supported by LR Co-Experimenter Straat⁹ based on data from continuing Mars missions, especially orbital images suggesting liquid water at shallow depths beneath the Martian surface¹⁰. Nonetheless, the life interpretation has remained controversial over the years despite mounting evidence against strong oxidants on the Martian surface¹¹. Now, the repeated and recent detection of methane plumes in the Martian atmosphere, and the requirement for a surface methane sink¹², coupled with continued evidence of liquid water^{13,14,15,16}, lends further credence to the life interpretation of the LR results. Such an interpretation is consistent with and harmonizes with all the relevant data on the crucial issue of life on Mars.

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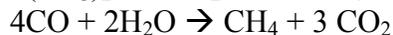
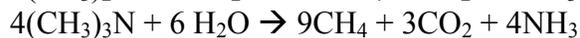
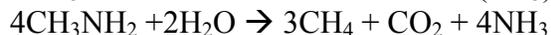
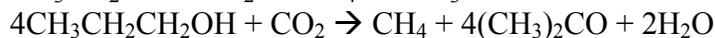
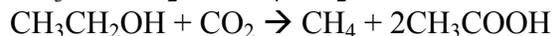
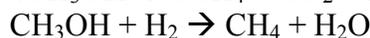
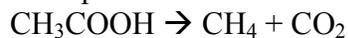
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Methane on Earth

On Earth, most atmospheric methane is of biological origin, originating from the activity of microbial methanogens in anaerobic environments. Methanogens are a diverse group of single cell microbes from the Kingdom Euryarchaeota, with over 40 species now described^{17, 18}. The most common metabolic reaction utilizes carbon dioxide and hydrogen, as its sole energy source, to produce methane according to the reaction:



The hydrogen for this reaction is often produced by other forms of anaerobic respiration. Hydrogen can also be obtained from the thermal and radioactive breakdown of water even in deep rocks. In addition, some genera can utilize acetate, formate, methanol, ethanol, 2-propanol, methylamine, dimethylamine, trimethylamine, dimethylsulfide, or carbon monoxide to produce methane as follows:



Most methanogens, including genera *Methanobacterium*, *Methanococcus*, *Methanobrevibacter*, *Methanopyrus*, and *Methanosarcina* utilize carbon dioxide and hydrogen to produce methane. However, the above additional substrates are not utilized by all genera, many being limited to just one or two of these substrates¹⁹:

Acetate (*Methanosarcina*, *Methanosaeta*)

Formate (*Methanobacterium*, *Methanococcus*, *Methanogenium*, ,
Methanocorpusculum)

Methanol + H₂ (*Methanosarcina*, *Methanohalophilus*)

Methanol (*Methanosphaera*, *Methanomicrococcus*)

Ethanol (*Methanospirillum*)

Propanol ((*Methanospirillum*, *Methanobacterium*, *Methanocorpusculum*)

Methylamine (*Methanosarcina*, *Methanococcoides*, *Methanolobus*)

Dimethylamine (*Methanosarcina*, *Methanococcoides*, *Methanolobus*)

Trimethylamine (*Methanosarcina*, *Methanococcoides*, *Methanolobus*)

Dimethylsulfide (*Methanomethylovorans*, *Methanohalophilus*, *Methanosalsum*)

Carbon Monoxide (*Methanobacterium*, *Methanosarcina*)

The two main groups of methanogens are the H₂/CO₂ utilizers and the acetate consumers. Of particular interest, some of the H₂/CO₂ utilizers are also capable of utilizing formate,

one of the LR substrates, to produce methane, whereas acetate consumers cannot utilize formate.

Methanogenic archaea live in a wide range of environments, many of them extreme²⁰. They are found inside intestinal tracts and in the bottom of lakes, ponds, and peat bogs. They have been found in freshwater and in marine environments, including oceans, and a few are known to be halophilic. They have been found at temperatures ranging from 0° C to 100° C, buried under glacial ice, and in hot dry desert soil. Most have a pH optima around 6.0, although some have been found at pH 3.0 to 9.2. Moran *et al.* reported²¹ detection of methane, apparently of biological origin, from deep (70 – 155 cm) samples taken from an arid Utah desert, in “...partial analogy to Martian conditions.”

While methanogens are strict anaerobes and reportedly cannot metabolize in aerobic environments, they can survive under certain conditions in oxygen-containing environments^{22, 23}. Methane producers can be found in low numbers in aerable soils; when placed in anaerobic environments, these populations begin methane production after a long lag phase^{24, 25}. Kato, Field and Lettinger^{26, 27} showed that methanogens in granular sludge have a high tolerance for oxygen; this was attributed to oxygen uptake by facultative bacteria as they degrade various adjacent substrates, thereby creating anaerobic microenvironments that protect methanogens. Even in the absence of oxygen respiration by other microorganisms, methanogens in sludge still showed some oxygen tolerance.

Methanotrophs are often found in close proximity to methanogens. Methanotrophs, which are methane-oxidizing Proteobacteria, utilize methane as sole carbon and energy sources, combining methane with oxygen to form formaldehyde that is then converted into organic compounds. Using a coring technique on peat, Edwards *et al.*²⁸ showed that methanotrophs subsisted throughout 30 cm cores with the methane-oxidizing potential highest in the upper 10 cm. Methanogens were restricted to the lower zones and were absent in the top 5 cm. In other studies, methane produced deep within anoxic wetlands is oxidized in the upper levels or around roots of wetland plants where oxygen is available. Reeburgh²⁹ estimated that 30% of the methane produced in high-latitude wetlands was oxidized by mosses and plants. Epp and Chanton³⁰ observed rapid methane oxidation by roots of several plant species from the Florida Everglades.

Methanotrophs are widespread and constitute an extremely diverse and complex group. Aerobic methanotrophs are classified into three groups based on the assimilation pathway of formaldehyde along with various physiological and morphological features^{31, 32}. Type I and Type X methanotrophs use a ribulose monophosphate pathway for formaldehyde assimilation, whereas Type II methanotrophs employ a serine pathway. Type X grows at higher temperatures than Types I and II, and Type X DNA has a higher percentage G + C content than most Type I methanotrophs. Type I methanotrophs grow at low methane concentrations whereas growth of Type II methanotrophs is favored by high methane concentrations along with low levels of nitrogen and oxygen. In addition to these common methanotrophs, Horz *et al.*³³ have isolated some novel methanotrophs from California upland grassy soils, and Dedysh *et al.*³⁴ isolated some new acidophilic

methane-oxidizing bacteria from northern peat wetlands. Although methanotrophs have long been considered obligate, using methane as a sole carbon and energy source, it has recently been shown that some methanotrophs are facultative and can grow on a number of multicarbon substrates^{35, 36}.

In addition to aerobic obligate and facultative methanotrophs, anaerobic methanotrophs have also been described. The anaerobic oxidation of methane (AOM) was first discovered in 1976 in anoxic zones of marine sediments³⁷, and later found to be coupled to the reduction of sulfate to hydrogen sulfide³⁸. According to Birgel *et al.*³⁹, AOM is at least 300 million years old. The process, essentially a reversal of methanogenesis⁴⁰, appears to be mediated by a consortium of methanogens and sulfate-reducing bacteria surrounding methanotrophic archaea^{41, 42}. In anoxic regions of the Black Sea, these consortia create large reef-like structures above methane seeps⁴³. Similarly, Raghoebarsing *et al.*⁴⁴ recently described AOM in anoxic sediments within freshwater habitats coupled to denitrification of nitrate rather than to reduction of sulfate. Efforts to isolate the responsible anaerobic methanotrophs, however, have failed, possibly because the individual microbes do not function outside of the consortium⁴⁵.

Methanotrophs limit the amount of methane released to the atmosphere by methanogenic activity in flooded soils, wetlands and other wet environments⁴⁶. Boetius⁴⁷ has estimated that 90% of all methane evolved from marine sediments is anaerobically oxidized by consortia within those sediments before reaching the atmosphere. As reviewed by Hanson and Hanson⁴⁸, the amount of methane released to the atmosphere in any given ecosystem is the difference between that evolved from methanogens (and other non-biological sources) and that consumed by methanotrophs and anaerobic methane oxidizing bacteria. Because methane is 20-25 times more effective per molecule than carbon dioxide as a greenhouse gas^{49, 50}, methanotrophs have lately become of considerable interest as mitigating factors in global warming.

Once methane is in the atmosphere, its most effective sink is the troposphere where methane reacts with hydroxyl radicals to form water and carbon dioxide. However, methanotrophs in forest soils also provide an effective methane sink. Type II methanotrophs (functioning at high methane concentrations) act as sinks for methane produced within soils, whereas Type I methanotrophs (functioning at low methane concentrations) consume atmospheric methane. Although Type II methanotrophs have been cultured and identified, Type I methanotrophs are poorly understood⁵¹. The most important factor determining whether a forest soil acts as a sink or source of methane is water content. Where water levels are well beneath the surface, the growth of surface methanotrophs is favored; if the soil becomes waterlogged, the balance shifts to methanogens, and the soil becomes a methane source.

Of particular interest are studies conducted in permafrost and other low-temperature environments. Many species of methanogens have been isolated from low-temperature environments, including both Antarctica and the Arctic⁵². Wagner *et al.*⁵³ reported that the abundance and composition of methanogenic microbes in permafrost environments are similar to those of temperate ecosystems. In 2005, Tung *et al.*⁵⁴ reported

methanogens deep under glacial ice in Greenland, and provided evidence for in situ methanogenic activity. Additional studies have shown that methanotrophs are abundant and active under extremely cold environments⁵⁵; viable methane oxidizers have even been detected in deep Siberian permafrost sediments⁵⁶. Wagner and Liebner⁵⁷ recently reported highly active and abundant methanogens and methanotrophs in permafrost soils, and that the permafrost environment contains species not yet detected in temperate climates.

In summary of terrestrial methane metabolism, the microorganisms involved in methane production and oxidation are many and diverse. Methanogens can use several carbon sources and are found in a variety of habitats, many of them extreme. Although primarily associated with anaerobic environments, many can tolerate oxygen. Methanotrophs constitute a diverse and complex group, well-adapted to a wide variety of environments, including extreme environments. Methanotrophs are widespread in aerobic environments, although the anaerobic oxidation of methane has been described^{58, 59, 60} in both marine and freshwater sediments coupled with sulfate and nitrate reduction, respectively. It is also noteworthy that methanotrophs are frequently found in close proximity to methanogens, with the interplay between them dependent on several environmental factors, especially water.

Methane on Mars

Because of the photochemical dissociation of atmospheric methane in the troposphere, methane has a lifetime there of only several hundred years. Hitchcock and Lovelace⁶¹ were first to point out that, because of its short lifetime, the presence of atmospheric methane is indicative of constant replenishment. This replenishment could originate either from biological metabolism or from non-biological processes (volcanoes, etc.). Martian atmospheric methane as an indicator of life was sought as early as 1971 in the Infrared Spectroscopy (IRIS) experiment on board the Mariner 9 Mission to Mars⁶². However, it was not detected at that time within the limits of the IRIS sensitivity.

Methane was discovered in the Martian atmosphere in 2003, and, to date, three separate groups have reported its detection^{63, 64, 65}. In a recent report, Mumma *et al.*⁶⁶ describe detection of methane in extended plumes in Northern Summer 2003 of a magnitude comparable to massive hydrocarbon seeps in Santa Barbara, California. Because the amount of methane decreases in gradients moving away from the origins, they hypothesized that the methane issues from discrete sources and is dispersed throughout the planet by atmospheric circulation, as opposed to being generated throughout the planet.

As to the origin of this Martian methane, Mumma *et al.*⁶⁷ considered both biological sources, such as production by methanogenic microorganisms, and geochemical sources. While not able to distinguish between these possibilities, they drew an analogy to methane evolved from deep bio-communities in the Witwatersrand Basin in South Africa where methane is produced by microbial reduction of carbon dioxide. It is assumed that hydrogen, essential to this process, is produced by radiolysis of water. These authors

propose that analogous microbes may have survived for eons on Mars at depths where liquid water may be available.

By comparing the amount of methane observed in 2003 with that observed in 2006, Mumma *et al.*⁶⁸ calculated a 50% decrease had occurred from the first to the second observation. This decrease is much faster than can be accounted for by photochemical destruction. They therefore propose the presence of a geochemical methane sink, suggesting that it might be the strong oxidant(s) long proposed in the Martian soil, and name several possibilities.

We note that a decrease of this magnitude also indicates that methane should completely disappear from the atmosphere within a few years. Thus, there must be episodic production of methane followed by its rapid destruction. Previous unsuccessful searches may have been conducted in between episodes. No thermal hot spots, indicating active volcanism, have been reported in Mars-wide searches⁶⁹. Yet the amount of methane recently detected on Mars exceeds the production of the Mauna Loa volcano⁷⁰. If the production of methane is not volcanic, biology remains an attractive alternative.

Alternative Hypothesis for Distribution of Methane on Mars

We hypothesize that the methane is evolved not just from a few northern sources, but, in addition, is generated throughout the planet by surface methanogens. Terrestrial methanogens are anaerobic, and therefore generally limited to deep underground environments and other habitats where oxygen is lacking, as discussed above. This limitation does not apply to Mars with its essentially anaerobic atmosphere; on Mars, methanogens could exist and produce methane throughout the surface soil. The Martian atmosphere contains about 0.1 percent oxygen; as discussed above, terrestrial methanogens are known to tolerate such small amounts of oxygen. However, should they require a completely anaerobic microenvironment, the “stickiness” of icy Martian soil recently encountered by the Phoenix Lander⁷¹ may provide it.

Methanogenic activity requires hydrogen according to the equation $4\text{H}_2 + \text{CO}_2 \rightarrow \text{CH}_4 + 2\text{H}_2\text{O}$, with H_2 provided by the splitting of water molecules. Mumma *et al.*⁷² commented that the hydrogen utilized by deep terrestrial bio-communities of Witwatersrand Basin resulted from radiolysis of water deep within. By analogy, perhaps hydrogen is similarly produced deep within the source of the Martian methane plumes. On the surface, hydrogen could result from photolysis of atmospheric water vapor. However, since levels of available water and water vapor are low, the limiting factor in the amount of methane produced by surface methanogens could be available hydrogen. Mumma *et al.*⁷³ observed a water vapor gradient distributed roughly in direct proportion to that of methane, decreasing with distance from the plumes, which supports this theory. The earlier finding reported by Formisano *et al.*⁷⁴, that methane concentrations detected in three equatorial regions were coincident with water vapor concentrations in the lower atmosphere further supports this theory. Since water vapor occurs at the ground surface over wide regions of Mars, at least seasonally and diurnally⁷⁵, this moisture could support methanogenic activity. However, methane levels produced may be below heretofore applied detection limits.

Alternative Hypotheses for Methane Sink on Mars

As stated above, the Mumma *et al.*⁷⁶ paper calls for a methane sink to account for the larger-than-expected disappearance of Martian methane than if atmospheric photolysis were its sole means of destruction. In proposing chemical oxidants on the surface of the planet as the methane sink, they offer the strong positive response obtained by the Viking LR experiment as evidence. This contrary use of the LR data is without direct supporting evidence. As previously stated, we have concluded that the LR results and all other relevant findings support the Mars LR response as biological. While many may still regard this conclusion as controversial, the evidence, summarized below, against any strong oxidant coating on Mars is no longer scientifically disputable:

1. The IRIS experiment aboard Mariner 9 found no evidence of H₂O₂ in the Martian atmosphere to an upper limit of several ppb.⁷⁷
2. No oxidant, or any other agents (other than living microorganisms) of the many proposed and tested, including the recent finding of perchlorates by Phoenix⁷⁸, has the required thermal sensitivity profile to reproduce the LR data obtained from the active agent on Mars⁷⁹.
3. The Viking Pyrolytic Release (PR) experiment demonstrated the formation of organic material in Martian soil⁸⁰, and the organic matter thus formed survived the seven days of the PR test run, which it would not have done in the presence of a strong oxidant in the soil.
4. Organic matter was shown to form in the same manner in PR laboratory experiments, just as it subsequently formed on Mars⁸¹.
5. The Viking Magnetic Properties experiment found much magnetic material in the Martian topsoil, evidence that it was not fully oxidized⁸².
6. Pathfinder found the Martian soil to be well below full oxidation of its metallic soil particles⁸³.
7. Two sensitive attempts to find H₂O₂ in the Martian atmosphere found none at the very low upper limits of the observations^{84, 85}.
8. A third more sensitive observation found H₂O₂, but only at levels far too low to have caused, directly or indirectly, the Viking LR responses⁸⁶.
9. The Rover Opportunity analyzed the oxidation of metals in the Martian soil and found them far below full oxidation^{87, 88}.

The alternative to a chemical sink for Mars methane is a biological sink. On Earth, a significant amount of biologically-produced methane is oxidized by aerobic soil methanotrophs. Terrestrial methanotrophs are frequently found in the same ecosystems as methanogens, and it would seem that, if methanogens are present on Mars, then methanotrophs are also likely to be present. It is possible that, once the limited amount of oxygen is consumed in oxidizing methane, methane oxidation by methanotrophs would cease, and methanogens would begin producing methane, pending availability of water vapor. A second possibility is that Mars supports anaerobic methane oxidation similar to terrestrial AOM, in which methanotrophic oxidation is coupled to sulfate or nitrate reduction. As discussed above, this type of oxidation, found in marine sediments in the Baltic Sea, is an extremely effective methane sink; as mentioned earlier, Boetius *et al.*⁸⁹ estimated that 90% of the methane generated in the marine sediments by

methanogens is anaerobically oxidized via sulfate reduction coupling before being released to the atmosphere.

That some sulfates are present on Mars has been shown by several groups. The OMEGA imaging spectrometer obtained signatures attributed to calcium-rich sulfates in the dunes of Olympia Planitia in northern polar regions⁹⁰. Glotch and Bandfield⁹¹ reported sulfate-rich sedimentary rocks in Eagle Crater at the Meridiani Planum landing site for the Mars Rover Opportunity. More recently, Squyres *et al.*⁹² reported finding sulfate-rich sedimentary rocks in Victoria Crater at Meridiani Planum. When compared to their earlier studies at Eagle Crater⁹³ and Endurance Crater⁹⁴, which are a few miles away from Victoria Crater, the three craters showed similar compositions. They indicate that the sulfate salts were most likely produced by the interaction of basalt with acidic water, and suggest that the water-induced alterations at Meridiani Planum were regional in scope. The widespread presence of sulfate salts suggests that a biological coupling of anaerobic oxidation of methane with sulfate reduction on Mars is a feasible concept.

Implications for LR of Finding Methane on Mars

It is entirely plausible that methanogens were in the Martian soil sample contained in the LR test cell, and that they metabolized the added LR nutrient. Any methane produced from any of the labeled nutrients would have been detected by the LR, just as would carbon dioxide or any other carbon gas produced from the LR nutrients.

In summary of the LR experiment on Mars⁹⁵, 0.5 cc samples of the Martian soil were challenged with 0.115 ml of an aqueous nutrient solution. The liquid-to-soil ratio in the test cell was such that a moisture gradient was created in the soil from wet in the center to moist around the edges. Seven organic compounds, namely, formate, glycine, glycolic acid, D-lactate, L-lactate, D-alanine and L-alanine, were contained in the liquid nutrient. Each was present at a concentration of 2.5×10^{-4} M and each was uniformly labeled with C^{14} . There were a total of 17 carbon atoms present, each with the same specific radioactivity, namely $2 \mu\text{Ci/mole carbon}$. Based on the specific radioactivity of each carbon and the counting efficiency of the instrument, the μmoles of released carbon gas could readily be calculated.

On Mars, nutrient added to untreated “active” soil, resulted in a rapid evolution of radioactive counts^{96,97}. This “active” experiment was repeated four times, twice at each lander site, with similar results each time, namely, the rapid evolution of radioactivity, almost reaching a plateau of 10,000 – 15,000 cpm over an experimental period of approximately seven sols. This corresponded to total utilization of at least one of the 17 carbon atoms, or, alternatively, fractional amounts of one or more of the organic compounds.

The controls support the biological origin of the active response. When a duplicate sample of the active soil was heat-treated for three hours at 160° C, cooled, and the LR experiment then performed on it, this response was essentially eliminated. This large difference between active and control samples defined a positive response. An additional mild heat treatment control, in which the Martian soil was pre-treated at 50° C for three

hours, reduced the active response by approximately 65%. Since few, if any, chemicals that could oxidize any of the LR nutrients are sensitive to a 50° C treatment, this result was considered strong evidence that the positive LR response was a life response. Even stronger evidence against a chemical reaction was that, when nutrient was added to Martian soil samples stored in the distribution chamber at 10° C for three (Viking Lander-2) and five months (Viking Lander-1), the response from the formerly active soils was virtually eliminated⁹⁸.

Although any carbon-based gas produced from any of the LR substrates would have been detected by the LR experiment, our laboratory simulations⁹⁹ indicated that the gas was, at least in part, carbon dioxide. For each of the four active cycles on Mars, a second injection of nutrient was added after approximately eight days. In each case, a brief spike in evolved gas was observed followed immediately by an approximately 30% decrease in the radioactive gas present in the test cell. This was interpreted to indicate that much of the gas evolved was most likely carbon dioxide that, upon addition of more water, was absorbed into the Mars soil sample because of a changed carbon dioxide/water/soil equilibrium. This absorption of carbon dioxide indicated an alkaline pH. Neither carbon monoxide nor methane would be expected to be absorbed from the headspace upon addition of more water to the soil. The remaining 70% of the evolved gas could have been carbon dioxide, or a mixture of carbon dioxide and methane; each carbon gas would have been equally detected.

As noted above, one of the LR substrates was formate. Formate was selected for inclusion in the LR nutrient because it is relatively stable, is one of the Miller-Urey products, and is a substrate for a wide variety of terrestrial microorganisms, including primitive microorganisms, such as methanogens. As discovered by Sohngen¹⁰⁰, methanogens that produced methane from carbon dioxide and hydrogen are able to oxidize formate according to the equation: $4\text{HCOOH} \rightarrow \text{CH}_4 + 3\text{CO}_2 + 2\text{H}_2\text{O}$. If the formate available in the LR nutrient were utilized only by Martian methanogens, the gaseous end-product could, therefore, have been 25% methane and 75% carbon dioxide.

Discussion and Conclusions

Based on Darwinian principles of evolution, if methanogens are present on Mars, it would be expected that some biological mechanism would have evolved to oxidize methane in an anaerobic environment. Coupling the biological anaerobic oxidation of methane with the reduction of sulfate, similar to that reported for AOM in the Black Sea, seems a plausible scenario, especially since sulfates are readily available, at least at the dunes of Olympia Planitia¹⁰¹ and at Meridiani Planum¹⁰². We propose that it may be just such a biological sink that accounts for the larger-than-expected disappearance of methane observed by Mumma *et al.*¹⁰³. This proposal accommodates the long-popular theory of a strong oxidant on Mars, except that the oxidant would be biological rather than chemical.

If methanogen-type microorganisms are responsible for the methane plumes reported by Mumma *et al.*¹⁰⁴, and if they are distributed throughout the planet, then they would have been present on the surface at both Viking Lander sites. Methane was not detected at the

Viking landing sites¹⁰⁵, possibly because trace amounts were below the limits of detection. However, it is also possible that methanogens may have been present but inactive on the surface, and did not produce methane until inside the LR test cell and re-invigorated by the addition of the LR nutrient. With water and formate supplied, any methanogens present could then have produced both methane and carbon dioxide from the formate.

Reinforced by our suggested interpretation of the methane discovery, we strongly believe that the search for life on Mars should be pursued. Based on our hypothesis of a Martian biological methane cycle, we recommend returning to either of the Viking landing sites (Chryse Planitia or Utopia Planitia), where positive LR responses were obtained, and conducting LR-like tests enhanced as described below. Abundant sulfur, approximately 100 times the average on Earth, was detected in the Martian soil at both Viking landing sites¹⁰⁶. The soil composition at both landing sites, 4000 miles apart, was similar, with the sulfur content of the duricrust higher than that of the fines. Both sulfur dioxide¹⁰⁷ and sulfur trioxide¹⁰⁸ were detected, and it was speculated that the duricrust is cemented by magnesium sulfate, although sulfate values were not reported. Alternatively, since the LR positive response appears widespread, sulfate-containing regions such as Olympia Planitia and Meridiani Planum could be considered in a search for methanogens.

A simple variation of the LR experiment^{109, 110} is suggested. All seven of the LR substrates would be tested individually with the Martian soil. Heat-treated controls would be conducted in duplicate samples of each soil. Adding formate alone, and analyzing the composition of the gaseous end product(s) would test the methanogen hypothesis. Coupled with the control, the result would also test the chemical oxidant theory, since a non-biological oxidant would convert formate to carbon dioxide and survive the control regimen. Especially important is the separate and individual application of the respective chiral isomers of DL-lactate and DL-alanine. Terrestrial organisms show preferences for L-sugars and for D-amino acids to the exclusion of the other respective isomer, whereas non-biological reactions show no such preferences. If Martian organisms showed a preference for one isomer over the other, most astrobiologists would accept that as conclusive evidence for microbial life on Mars. And, if the preference were for opposite isomers than preferred by terrestrial organisms, that would indicate a fundamentally different type of life.

References

1. Mumma, M.J., G.L. Villanueva, R.E. Novak, T. Hewagama, B.P. Bonev, M.A. DiSanti, A. M. Mandell, and J.D. Smith, "Strong Release of Methane on Mars in Northern Summer 2003", *Science*, **323**, 1041-1045, 2009.
2. Levin, G.V. and P.A. Straat, "Labeled Release – An Experiment in Radiorespirometry," *Origins of Life*, **7**, 293-311, 1976.
3. Levin, G.V. and P.A. Straat, "Viking Labeled Release Biology Experiment: Interim Results," *Science*, **194**, 1322-1329, 1976.
4. Levin, G.V. and P.A. Straat, "Life on Mars? The Viking Labeled Release Experiment," *BioSystems*, **9**, 165-174, 1977.

5. McKay, D.S., E.K. Gibson, Jr., K.L. Thomas-Keptra, H. Vali, S., Romanek, S.J. Clemett, X.D.F. Chillier, C.R. Macechling, and R.N. Zare, "Search for Past Life on Mars: Possible Relic Biogenic Activity in Martian Meteorite ALH 84001," *Science*, **273**, 924–930, 1996.
6. Moore, H.J., R.E. Hutton, R.F. Scott, C.R. Spitzer, R.W. Shorthill, "Surface Materials of the Viking Landing Sites," *J. Geophys. Res.*, **82(28)**, 4497-4523, 1977.
7. Carr, M.H., "Water on Mars," figure 2, p.10, Oxford University Press. New York. 1996.
8. Levin, G.V., "The Viking Labeled Release Experiment and Life on Mars," *Instruments, Methods, and Missions for the Investigation of Extraterrestrial Microorganisms*, SPIE Proc., **3111**, 146-171, 1997.
9. Straat, P.A., Personal communication to G.V. Levin, 2000.
10. Malin, M.C. and K.S. Edgett, "Evidence for Recent Groundwater Seepage and Surface Runoff on Mars," *Science*, **288(5475)**, 2330-2335, 2000.
11. Levin, G.V., "The Oxides of Mars," *Instruments, Methods, and Missions for Astrobiology*, SPIE Proceedings, **4495**, 131-135, 2001.
12. Op. cit. 1.
13. Mohlmann, D.T.F., "The Influence of van der Waals Forces on the State of Water in the Shallow Subsurface of Mars," *Icarus*, **195**, 131-139, 2008.
14. Hudson, T.L., A. Zent, M.H. Hecht, S. Wood, and D. Cobos, "Near-Surface Humidity at the Phoenix Landing Site as Measured by the Thermal and Electrical Conductivity Probe (TECP)," Presented at the Lunar and Planetary Science Conference, Houston, March 23, 2009.
15. Renno, N.O., B.J. Bos, D. Catling, B.C. Clark, L. Drube., D. Fisher, W. Goetz, S.F. Hviid, H. Keller, J.F. Kok, S.P. Kounaves, K. Leer, M. Lemmon, M. B. Madsen, W. Markiewicz, J. Marshall, C. McKay, M. Mehta, M. Smith, M.P. Zorzano, P.H. Smith, C. Stoker, and S.M.M. Young, "Physical and Thermodynamical Evidence for Liquid Water on Mars," Presented at the Lunar and Planetary Science Conference, Houston, March 23-27, 2009.
16. Sizemore, H.G., M.T. Mellon, M.L. Searls, A.P. Zent, T.L. Heet, R.E. Arvidson, and the Phoenix Science Team, "In Situ Analysis of Ice Table Depth Variability Under a Rock at the Phoenix Landing Site, Mars," Presented at the Lunar and Planetary Science Conference, Houston, March 23-27, 2009.
17. Miyamoto, K., ed. "Renewable Biological Systems for Alternate Sustainable Energy Production," Food and Agriculture Organization of the United Nations Services Bulletin #128. 1997.
18. Chaban, B., S.Y.M. Ng, and K.F. Jarrall, "Archaean Habitats – from the Extreme to the Ordinary," *Can. J. Microbiol.*, **52**, 73-116, 2006.
19. Ibid.
20. Ibid.
21. Moran, M., J.D. Miller, T. Kral, and D. Scott, "Desert Methane: Implications for Life Detection on Mars," *Icarus*, **178**, 277-280, 2005.
22. Fetzer, S., F. Bak, and R. Conrad, "Sensitivity of Methanogenic Bacteria from Paddy Soil to Oxygen and Dessication," *FEMS Microbiol. Ecol.*, **12**, 107-115. 2003.
23. Kiener, A., and T. Leisinger, "Oxygen Sensitivity of Methanogenic Bacteria," *Syst. Appl. Microbiol.*, **4**, 305-312, 1983.

24. Mayer, H.P., and R. Conrad, "Factors Influencing the Populations of Methanogenic Bacteria and the Initiation of Methane Production upon Flooding of Paddy Soil," *FEMS Microbiol. Ecol.*, **73**, 103-112, 1990.
25. Peters, J., and R. Conrad, "Methanogenic and other Strictly Anaerobic Bacteria in Desert Soil and other Oxidic Soils," *Appl. Environ. Microbiol.*, **61(4)**, 1673-1676, 1995.
26. Kato, M.T., J.A. Field, and J. Lettinga, "High Tolerance of Methanogens in Granular Sludge to Oxygen," *Biotechnol. Bioeng.*, **42**, 1360-1366, 1993.
27. Kato, M.T., J.A. Field, and J. Lettinga, "Anaerobic Tolerance to Oxygen and the Potentials of Anaerobic and Aerobic Cocultures for Wastewater Treatment," *Braz. J. Chem. Eng.*, **14(4)**, ISSN 0104-6632, Dec. 1997.
28. Edwards, C., B.A. Hales, G.H. Hall, I.R. McDonald, J.C. Murrell, R. Pickup, D.A. Ritchie, J.R. Saunders, B.M. Simon, B.M., and M. Upton, "Microbiological Processes in the Terrestrial Carbon Cycle: Methane Cycling in Peat," *Atmos. Environ.*, **32(19)**, 3247-3255, 1998.
29. Reeburgh, W.S., S.C. Whalen, and M.J. Alperin, "The role of methylotrophy in the global methane budget," p. 1-14 in J.C. Murrell and D.P. Kelley (ed.), *Microbial growth on C1 Compounds*. Intercept Press, Ltd., Andover, United Kingdom. 1993.
30. Epp, M.A., and J.P. Chanton, "Rhizospheric Methane Oxidation determined via the Methylfluoride Inhibition Technique," *J. Geophys. Res.*, **98D**, 18413-18422, 1993.
31. Hansen, R.R. and T.E. Hanson, "Methanotrophic Bacteria," *Microbiol. Rev.*, **60 (2)**, 439-471, 1996.
32. Op. cit. 18.
33. Horz, H.P., V. Rich, S. Avrahami, and B.J.M. Bohannon, "Methane-Oxidizing Bacteria in a California Upland Grassland Soil: Diversity and Response to Simulated Global Change," *Appl. Environ. Microbiol.*, **71(5)**, 2642-2652, 2005.
34. Dedysh, S.N., N.S. Panikov, W. Liesack, R. Groskopf, J. Zhou, and J.M. Tiedje, "Isolation of Acidophilic Methane-Oxidizing Bacteria from Northern Peat Wetlands," *Science*, **282**, 281-281, 1998.
35. Theisen, A.R. and J.C. Murrell, "Facultative Methanotrophs Revisited," *J. Bacteriol.*, **187**, 4303-4305, 2005.
36. Dedysh, S.N., C. Knief, and P.F. Dunfield, "Methylocella Species are Facultatively Methanotrophic," *J. Bacteriol.*, **187**, 4665-4670, 2005.
37. Reeburgh, W.S., "Methane Consumption in Cariaco Trench waters and Sediments," *Earth Planet Sci. Lett.*, **28**, 33-344, 1976.
38. Alperin, M. and W.S. Reeburgh, "Inhibition Experiments on Anaerobic Methane Oxidation," *Appl. Environ. Microbiol.*, **50 (4)**, 940-945, 1985.
39. Birgel, D., T. Himmler, A. Freiwald, A., and J. Peckman, "A New Constraint on the Antiquity of Anaerobic Oxidation of Methane: Late Pennsylvania Seep Limestone from Southern Namibia," *Geology*, **36(7)**, 543-546, 2008.
40. Op. cit. 18.
41. Hoehler, T.M., M.J. Alperin, D.B. Albert, and C.S. Martens, "Field and Laboratory Studies of Methane Oxidation in an Anoxic Marine Sediment: Evidence for Methanogen-Sulphate Reducer Consortium," *Global Biochem. Cycles*, **8 (4)**, 451-463, 1994.
42. Boetius, A., K. Ravenschlag, C.J. Schubert, D. Rickert, F. Widdel, A. Giesecke, R. Amann, B.B. Joergensen, U. Witte, and O. Pfannkuche, "A Marine mMicrobial

- Consortium Apparently Mediating Anaerobic Oxidation of Methane,” *Nature*, **407**, 623-626, 2000.
43. Michaelis, W., R. Seifert, K. Nauhas, T. Treude, V. Thiel, M. Blumenberg, K. Knittel, A. Gieseke, K. Peterknecht, T. Pape, A. Boetius, R. Amann, B.B. Joergensen, F. Widdel, J. Peckmann, N.V. Pimenov, and M.B. Gulin, “Microbial Reefs in the Black Sea Fueled by Anaerobic Oxidation of Methane,” *Science*, **297**, 1013-1015, 2002.
 44. Raghoebarsing, A.A., A. Pol, K.T. van de Pas-Schoonen, J.J. Smolders, K.F. Ettwig, W.I. Riipstra, S. Schouten, J.S. Damste, H.J. Op den Camp, M.S. Jetten, and M. Strous, “A Microbial Consortium Couples Anaerobic Methane Oxidation to Denitrification,” *Nature*, **440**, 918-921, 2006.
 45. Op. cit. 18.
 46. Conrad, R., “Soil Microorganisms as Controller of Atmospheric Trace Gases (H₂, CO, CH₄, OCS, NO₂, and NO),” *Microbiol. Rev.*, **60**, 609-640, 1996.
 47. Op. cit. 42.
 48. Op. cit. 31.
 49. Blake, D.R. and F.S. Rowland, “Continuing Worldwide Increase in Tropospheric Methane 1978-1987,” *Science*, **239**, 1129-1131, 1988.
 50. Rodhe, H., “A Comparison of the Contribution of Various Gases to the Greenhouse Effect,” *Science*, **248**, 1217-1219, 1990.
 51. Reay, D.S., C.N. Hewitt, K.A. Smith, and J. Grace, eds., “Greenhouse Gas Sinks,” CABI Publishing, 320 p. 2007.
 52. Op. cit. 18.
 53. Wagner, D., A. Lipski, A. Embacher, and A. Gattinger, “Methane Fluxes in Extreme Permafrost Habitats of the Lena Delta: Effects of Microbial Community Structure and Organic Matter Quality,” *Environ. Microbiol.*, **7**(10), 1582 – 1592 (2005).
 54. Tung, H.C., N.E. Bramall, and P.B. Price, “Microbial Origin of Excess Methane in Glacial Ice and Implications for Life on Mars,” *Proc. Natl. Acad. Sci.*, **102**, 18292 – 18296, 2005.
 55. Trotsenko, Y.A., and V.N. Khmelenina, “Aerobic Methanotrophic Bacteria of Cold Ecosystems,” *FEMS Microbiol. Ecol.*, **53**, 15-26, 2005.
 56. Khmelenina, V.N., V.A. Makutina, M.G. Kaluzhnaya, E.M. Rivkina, D.A. Gilichinsky, and Y.A. Trotsenko, “Discovery of Viable Methanotrophic Bacteria in Permafrost Sediments of northeast Siberia,” *Dokl. Biol. Sci.*, **384**, 235-237, 2001.
 57. Wagner, D., and S. Liebner, “Global Warming and Carbon Dynamics in Permafrost Soils: Methane Production and Oxidation,” In R. Margesin (ed.), *Permafrost Soils. Soil Biology*, **16**, Springer Berlin, pp. 219-236. (2009).
 58. Op. cit. 41.
 59. Op. cit. 42.
 60. Op. cit. 44.
 61. Hitchcock, D.R. and J.E. Lovelace, “Life Detection by Atmospheric Analysis,” *Icarus*, **7**, 149-159, 1967.
 62. Hanel, R., B. Conrath, W. Hovis, V. Kunde, P. Lowman, W. Maguire, J. Pearl, J. Pirraglia, C. Phabhakara, B. Schlachman, G.V. Levin, P.A. Straat, and T. Burke, “Investigation of the Martian Environment by Infrared Spectroscopy on Mariner 9,” *Icarus*, **17**, 423-442, 1972.

63. Mumma, M.J., R.E. Novak, M.A. DiSanti, and B.P. Bonev, "A Sensitive Search for Methane on Mars," *Bull. Am. Astron. Soc.*, **35**, 937 (2003).
64. Formisano, V., S. Atreya, T. Encrenaz, N. Ignatiev, and M. Giuranna, "Detection of Methane in the Atmosphere of Mars," *Science*, **306**, 1758-1761, 2004.
65. Krasnopolsky, V.A., J.P. Maillard, and T.C. Owen, "Detection of Methane in the Martian Atmosphere: Evidence of life?," *Icarus*, **172**, 537-547, 2004.
66. *Op. cit.* 1.
67. *Ibid.*
68. *Ibid.*
69. Kerr, R., "Life or Volcanic Belching on Mars?," *Science*, **303**, 1953, 2004.
70. Ryan, S., E.J., Dlugokencky, P.P. Tams, M.E. Trudeau, "Mauna Loa Volcano is not a Methane Source: Implications for Mars," *Geophys. Res. Lett.*, **33(12)**, 22 June 2006.
71. Kerr, R.A., "Phoenix's Water May be Gumming up the Works," *Science*, **321**, 758, 2008.
72. *Op. cit.* 1.
73. *Ibid.*
74. *Op. cit.* 64.
75. Clancy, R.T., A.W. Grossman, M.J. Wolff, P.B. James, D.J. Rudy, Y.N. Billawala, B.J. Sandor, s.W. Lee and D.O. Muhleman, "Water Vapor Saturation at Low Altitudes around Mars Aphelion: A Key to Mars Climate?," *Icarus*, **122**, 36-62, 1996.
76. *Op. cit.* 1.
77. Hanel, R. and W. Maguire, Mariner 9 IRIS Team, personal communications, 1980.
78. Hecht, M.H., D.C. Catling, B.C. Clark, L. DeFlores, K. Gospodinova, J. Kapit, S.P. Kouvaves, D.W. Ming, R.C. Quinn, S.J. West, S.M.M. Young, and the Phoenix Team, "Perchlorate in Martian Soil: Evidence and Implications," Presented at the Lunar and Planetary Science Conference, Houston, March 23-27, 2009.
79. Levin, G.V. and P.A. Straat, "Possible Evidence for Panspermia: The Labeled Release Experiment," *Internat. J. Astrobiol.*, **6(2)**, 95-108, 2007.
80. Horowitz, N.H., G.L. Hobby, and J.S. Hubbard, "Viking on Mars: The Carbon Assimilation Experiments," *J. Geophys. Res.*, **82**, 4659-4662, 1977.
81. Hubbard, J.S., J.P. Hardy, G.E. Voecks, and E.E. Golub, "Photocatalytic Synthesis of Organic Compounds from CO and Water: Involvement of Surfaces in the Formation and Stabilization of Products," *J. Mol. Evol.*, **2**, 149-166, 1973.
82. Hargraves, R.B., D.W. Collinson, R.E. Arvidson, and C.R. Spitzer, "The Viking Magnetic Properties Experiment: Primary Mission Results," *J. Geophys. Res.*, **82**, 4547-4558, 1977.
83. Hviid, S.F., M.B. Madsen, H.P. Gunnlaugsson, W. Goetz, J.M. Knudsen, R.B. Hargraves, P. Smith, D. Britt, A.R. Dinesen, C.T. Mogensen, M. Olsen, C.T. Pedersen and L. Vistisen, "Magnetic Properties Experiments on the Mars Pathfinder Lander: Preliminary Results," *Science*, **278** (5344), 1768-1770, 1997.
84. Krasnopolsky, V.A., "Photochemistry of the Martian Atmosphere (mean conditions)," *Icarus*, **101**, 313-332, 1993
85. Krasnopolsky, V., G.G. Bjoraker, J.J. Mumma and D.E. Jennings, "High-Resolution Spectroscopy of Mars at 3.7 and 8.4 μm : A Sensitive Search for H_2O_2 , H_2CO , HCl and CH_4 , and Detection of HDO ," *J. Geophys. Res.*, **102**, 6525-6534, 1997.

86. Encrenaz, Th., B. Bezard, T.K. Greathouse, M.J. Richter, J.H. Lacy, S.K. Atreya, A.S. Wong, S. Lebonnois, F. Lefevre and F. Forget, "Hydrogen peroxide on Mars: Evidence for Spatial and Seasonal Variations," *Icarus*, **170**, 424-429, 2004.
87. Klingelhofer, G., "Mossbauer In Situ Studies of the Surface of Mars," *Hyperfine Interactions*, **158**, 117-124, 2004.
88. Rieder, R., B. Gellert, R.C. Anderson, J. Bruckner, B.C. Clark, G. Dreibus, T. Economou, G. Klingelhofer, G.W. Lugmair, D.W. Ming, S.W. Squyres, C. d'Uston, H. Wanke, A. Yen, and J. Zipfel, "Chemistry of rocks and soils at Meridiani Planum from the alpha particle x-ray spectrometer," *Science*, **306**, 1746- 1749, 2004.
89. Op. cit. 42.
90. Langevin, Y, F. Poulet, J-P. Bibring, and B. Gondet, "Sulfates in the North Polar Region of Mars Detected by OMEGA/Mars Express," *Science*, **307**, 1584-1586, 2005.
91. Glotch, T.D. and J.L. Bandfield, "Determination and Interpretation of Surface and Atmospheric Miniature Thermal Emission Spectrometer Spectral End-Members at the Meridiani Planum Landing Site," *J. Geophys. Res.* **111**, E12S06, 2006.
92. Squyres, S.W., A.H. Knoll, R.E. Arvidson, J.W. Ashley, J.F. Bell, III, W.M. Calvin, P.R. Christensen, B.C. Clark, B.A. Cohen, P.A. de Souza, Jr., L. Edgar, W.H. Farrand, I. Fleischer, R. Gellert, M.P. Golombek, J. Grant, J. Grotzinger, A. Hayes, K.E. Herkenhoff, J.R. Johnson, B. Jolliff, G. Klingelhofer, A. Knudson, R. Li, T.J. McCoy, S.M. McLennan, D.W. Ming, D.W. Mittlefehldt, R.V. Morris, J.W. Rice, Jr., C. Schroder, R.J. Sullivan, A. Yen, and R.A. Yingst, "Exploration of Victoria Crater by the Mars Rover Opportunity," *Science*, **324 (5930)**, 1058-1061, 2009.
93. Squyres, S.W., R.E. Arvidson, J.F. Bell, III, J. Bruckner, N.A. Cabroi, W. Calvin, M.H. Carr, P.R. Christensen, B.C. Clark, L. Crumpler, D.J. Des Marals, C. d'Uston, T. Economou, J. Farmer, W. Farrand, W. Folkner, M. Golombek, S. Gorevan, J.A. Grant, R. Greeley, J. Grotzinger, L. Haskin, K.E. Herkenhoff, S. Hviid, J. Johnson, G. Klingelhofer, A.H. Knoll, G. Landis, M. Lemmon, R. Li, M.B. Madsen, M.C. Malin, S.M. McLennan, H.Y. McSween, D.W. Ming, J. Moersch, R.V. Morris, T. Parker, J.W. Rice, Jr., L. Richter, R. Rieder, M. Sims, M. Smith, P. Smith, L.A. Soderblom, R. Sullivan, H. Wanke, T. Wdowlak, M. Woiff, and A. Yen, "The Opportunity Rover's Athena Science Investigation at Meridiani Planum, Mars," *Science*, **306 (5702)**, 1698-1703, 2004.
94. Squyres, S.W., A.H. Knoll, R.E. Arvidson, B.C. Clark, J.P. Grotzinger, B.L. Joliff, S.M. McLennan, N. Tosca, J.F. Bell, III, W.M. Calvin, W.H. Farrand, T.D. Glotch, M.P. Golombek, K.E. Herkenhoff, J.R. Johnson, G. Klingehofer, H.Y. McSween, and A.S. Yen, "Two Years at Meridiani Planum: Results from the Opportunity Rover," *Science*, **313(5792)**, 1403-1407, 2006.
95. Op. cit. 2.
96. Op. cit. 3.
97. Op. cit. 4.
98. Levin, G.V., and P.A. Straat, "Completion of the Viking Labeled Release Experiment on Mars," *J. Mol. Evol.*, **14**, 157-183, 1979a.
99. Levin, G.V., and P.A. Straat, "Laboratory Simulations of the Viking Labeled Release Experiment: Kinetics following Second Nutrient Injection and the Nature of the Gaseous End Product," *J. Mol. Evol.*, **14**, 185-197, 1979b.

100. Sohngen, N.L., "Sur le Role du Methane dan la Vie Organique," *Rec. Trav. Chim. Pays Bas*, **29**, 238, 1910.
101. Op. cit. 88.
102. Op. cit. 90.
103. Op. cit. 1.
104. Ibid.
105. Owen, T, K. Biemann, D.R. Rushneck, J.E. Biller, D.W. Howarth, and A.L. Lafleur, "The Composition of the Atmosphere at the Surface of Mars," *J. Geophys. Res.*, **82(28)**, 4635-4639, 1977.
106. Clarke, B.C.III, A.K. Baird, H.J. Rose, P. Toulmin III, R.P. Christian, W.C. Kelleher, A.J. Castro, C.D. Rowe, K. Keil and G.R. Huss, "The Viking X Ray Fluorescence Experiment: Analytical Methods and Early Results," *J. Geophys. Res.*, **82(28)**, 4577-4594, 1977.
107. Baird, A.K., A.J. Castro, B.C. Clarke, P. Toulmin III, H. Rose, Jr., K. Keil and J.L. Gooding, "The Viking X Ray Fluorescence Sampling Strategies and Laboratory Simulations," *J. Geophys. Res.*, **82(28)**, 4595-4624, 1977.
108. Toulmin III, P., A.K. Baird, B.C. Clarke, K. Keil, H.J. Rose, R.P. Christian, P.H. Evans and W.C. Kelleher, "Geochemical and Mineralogical Interpretation of the Viking Inorganic Chemical Results," *J. Geophys. Res.*, **82(28)**, 4625-4634, 1977.
109. Levin, G.V., J.D. Miller, P.A. Straat, and R.B. Hoover, "A Sterile Robotic Mars Soil Analyzer," *Instruments, Methods, and Missions for Astrobiology*, SPIE Proceedings, **4859**, 78-86, 2002.
110. Levin, G.V., J.D. Miller, P.A. Straat, R.A. Lodder, and R.B. Hoover, "Detecting Life and Biology-Related Parameters on Mars," *Spectrum*, **3(10)**, 1-15, 2007.