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Can Martian Life Exist Under Dry Conditions?

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CAN MARTIAN LIFE EXIST UNDER DRY CONDITIONS?

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Abstract:

Recently, we have begun to hear more and more about news coming from Mars. With the recent confirmation of evidence of liquid water in Mars' past, evidence indicating liquid water in Mars' present, and as many missions planned for this decade and the next than have successfully reached the planet in the last 40 years, life on Mars is beginning to become a more relevant issue in our study of the planet.

Although there have been no indications of multicellular life on Mars, there may be a possibility of microbial life in the subsurface. Since it appears that all of the necessary elements for life on Mars exist, it is instrumental for scientists to analyze terrestrial ecosystems that might be analogous to those on Mars.

Life on Earth has shown a great amount of diversity in ability to survive extreme environments utilizing a stunning array of energy sources, electron donors, electron acceptors, salinity, pressures, and temperature ranges. From the study of Mars-like terrestrial ecosystems, some scientists have suggested that the methanogens would be a possible candidate to survive Mars' subsurface conditions.

There are various Mars' conditions that could be tested on Earth to confirm or deny the methanogens' ability to survive on the planet. Since the survival of methanogens depends on the presence of liquid water, which would probably be seasonal at best on Mars, their ability to survive desiccation will be key in their potential to inhabit Mars' subsurface.

In this research, the methanogens Methanobacterium formicicum, Methanothermobacter wolfeii, and Methanosarcina barkeri were grown on JSC Mars-1 soil simulant and exposed to varying times of desiccation. Since the byproduct of the methanogens' growth is methane, methane was measured in the head gas of the samples upon rehydration and incubation. These measurements were used to determine survival.

The methanogens studied were capable of survival and subsequently methane production upon rehydration after all of the periods of desiccation tested. M. formicicum was tested over periods of 10 and 27 days, while M. wolfeii and M. barkeri were tested over periods of 10 days.

These results support the hypothesis that methanogens could survive in Mars' subsurface if liquid water were present. The methanogens could be a key ingredient in the terraforming of Mars, and at this point remain an increasingly possible candidate for past, present, or future Martian life.

Introduction:

Mars:

For decades man has looked to the stars and wondered if we are alone- While we have not received any indication of "intelligent life" beyond the Earth, much research is being put into the possibility of past or present life on other planets. In particular, Mars has been the subject of considerable research for extraterrestrial life as we strive to uncover the mysteries of the red planet and its potential to house life.

Even though Mars is considerably different than the planet that we live on, it still seems similar enough to be a potential reservoir for past, present, or future life. In Appendix A, you can find a chart listing some interesting characteristics of Mars and how they compare to Earth (17). Mars' atmosphere is made up of 95.32%CO_2, 2.7% N_2, 1.6% ^{40}Ar, 0. 13% O_2, and less than I% of CO, H_20, $^{36+38}$ Ar, Ne, Kr, Xe, and O_3 (38). Its surface has been theorized to consist mainly of basalt, hematite, crystalline iron oxides, 21 clay materials, Fe^{2+}, carbonate, sulfate or bisulfate, and scapulite through spectroscopic data (44). It has been theorized that Mars soil houses a potent oxidant that rapidly converts Martian organic molecules into carbon dioxide (1), thus making organic nutrients scarce if not completely absent in Martian soil. Using electron paramagnetic resonance spectroscopy, it has been shown that superoxide radical ions form directly on Mars analog mineral surfaces exposed to ultraviolet radiation under a simulated Martian atmosphere that could accomplish this oxidation (50). It also appears that all of the water that we know of on the surface is frozen into two pronounced polar ice caps and sheets of ice distributed on the planets subsurface (45). This belief is the subject of much controversy and will be discussed later. These ice caps cycle continuously between their solid form with sublimation directly to gaseous water and carbon dioxide (8).

Because of the harsh conditions on Mars' surface and failed attempts to visualize any extraterrestrial life, it is believed that the most likely location for Martian organisms is beneath the surface. While we have found no indication of any life forms on Mars to this point, there has been considerable research regarding Mars evolutionary past and potential present subsurface microorganisms. This search for extraterrestrial life may be facilitated if ecosystems can be found on Earth that exist under conditions analogous to those present on other planets or moons. It has been proposed, on the basis of geochemical and thermodynamic considerations, that geologically derived hydrogen might support subsurface microbial communities on Mars in which methanogens form the base of the ecosystem (6). These organisms are the focus of this research.

If the methanogens, or any known Earth organisms, are able to grow on Mars, they will have to come into contact with liquid water. The existence of liquid water on Mars remains a critical issue to past or present life on Mars.

Up to this point, scientists have studied fluvial features on the surface of Mars from Surveyor images to theorize about the presence of surface liquid water in its past (33), but confirmation of evidence of past surface liquid water detected by the Opportunity Rover was released by NASA on March 2, 2004 (19). The history and size of the water reservoirs on early Mars can be constrained using isotopic ratios of deuterium to hydrogen. With laboratory measurements of the ultraviolet cross-sections of water and its isotopomers, and modeling calculations in support of a photo-induced fractionation effect, it has been theorized that Mars had an early warm atmosphere and has lost at least a 50m global layer of water (7).

Although the presence of liquid water in the past has seemingly been confirmed, the presence of liquid water on Mars continues to be important to the existence of life on Mars today. A variety of experiments and calculations have been done that theorize the possibility of the presence of liquid water on and beneath Mars' surface. Information released on June 22, 2000 confirmed gullies visualized by Mars Global Surveyor that could've been caused by present day running water (20). Experiments done in Mars-like conditions demonstrated that transient melting of ice on Mars' surface may occur in depressions and gullies nearly anywhere on the planet where thin ice is illuminated by normal-incidence insolation. This suggests that cold trapping of winter condensation could concentrate a sufficient amount of ice to allow seasonal melting in gullies (16). Another experiment done exploring the constraints of the abundance of atmospheric water vapor, escape fluxes of hydrogen and deuterium, D/H ratios in the atmosphere and in hydrous minerals found in one Martian meteorite, alteration of minerals in other meteorites, and fluvial features on the Martian surface were consonant with the visual evidence obtained by the Mars Global Surveyor satellite (11). Other experiments, done after the discovery of hydrogen below the surface of Mars' polar regions by Mars Odyssey, used Mars like conditions to confirm that liquid water could be stable for extended periods of time on the Martian surface under present-day conditions (26). Another experiment combining Viking pressure and temperature data with Mars Orbital Laser Altimeter topography data has computed the fraction of the Martian year during which pressure and temperature allow for liquid water to be stable on the Martian surface. The experiment found that in certain geographical regions correlating to the distribution of valley networks, water could be stable in liquid form during up to 34% of the year (28).

Another important aspect of the search for life on Mars is the religious one. When we look at history, there have been a variety of scientific topics that have challenged the constraints of religion. The discovery that the Earth was not in fact the center of the universe, the discovery of scientific laws that seem to imply that the existence of a creator God would be unnecessary, cloning, and evolution are all examples of historically controversial scientific topics. The search for life on other planets is of course no different.

What would it mean if we found that we are not alone in this universe? For Christianity, the Bible does not mention the creation of life on other planets, and if there were life on other planets, would it be necessary for a Jesus Christ to be martyred on every inhabitable planet? If not, why was he sent to this planet, and how should we consider the religious rights of other intelligent life forms (23)?

In an essay regarding science and religion, Pope John Paul 11 said, "truth cannot contradict truth". If science or religion propose theories that are mutually exclusive, it must be apparent that one or both could be wrong, and ultimately truth will prevail. Since the search for life on Mars (and incidentally the study of methanogens) poses some questions that could potentially challenge ones beliefs, this search could become important in the never-ending quest for truth.

Mars Missions and Technology:

Even though there has been much research on Mars' potential, there still lie questions that could only be answered by traveling to the planet itself.

With five missions still exploring Mars (two of which arrived in 2004), seven previous successful missions to Mars in the past forty years, and as many as four planned missions to take off for the red planet in the next ten to twelve years, there are almost more missions occurring in this decade and the next than have been successfully conducted in the past forty years! The new missions boast a stunning array of scientific technology that will revolutionize Mars exploration consisting of various instruments for detecting subsurface water, a rock abrasion toot that is used to study the surface composition and texture, a camera more powerful than any other camera used on a space exploration mission used to identify surface obstacles that might hinder future missions, an interplanetary Internet that will be the

first link in a communications bridge back to Earth, an experimental optical navigation camera that will serve as a high-precision interplanetary lighthouse to guide future incoming spacecraft as they near Mars, airplanes and balloons used to survey Mars from a different perspective, a robotic arm used to dig into arctic terrain to search for environments suitable for microbes, a probe that takes soil samples and mixes them with water to observe the possible behavior that would be displayed in wet Martian soil, a roving long-range/long-duration science laboratory for refined and extensive scientific testing, deep subsurface drills, and many other scientific instruments to observe surface, atmospheric, and ultraviolet conditions (18).

There are various arenas for researching the potential of life on Mars. Although this field is ever expanding, some promising areas of study will be discussed below.

Polar Exploration:

Polar exploration is an important field of research because it investigates an ecosystem on the Earth for life that could be analogous to a Mars ecosystem.

The debris-rich basal ice layers of a high Arctic glacier have been shown to contain metabolically diverse microbes that could be cultured oligotrophically at low temperatures (0.3 to 40C). These organisms included aerobic chemoheterotrophs and anaerobic nitrate reducers, sulfate reducers, and methanogens. When electron microscopy of thawed basal ice samples were studied, various cell morphologies, including dividing cells were revealed. These finding suggested that the subglacial environment beneath a polythermal glacier provides a viable habitat for life and that microbes may be widespread where the basal ice is temperate and water is present at the base of the glacier and where organic carbon from glacially overridden soils is present. This environment provides a model for viable habitats for life on Mars, since similar conditions may exist or may have existed in the basal sediments beneath the Martian north polar ice cap (43).

Other studies have confirmed that the permanent ice covers of Antarctic lakes in the McMurdo Dry Valleys develop liquid water inclusions in response to solar heating of internal aeolian-derived sediments which serve as nutrient (inorganic and organic) enriched microzones for the establishment of a physiologically and ecologically complex microbial consortium capable of contemporaneous photosynthesis, nitrogen fixation, and decomposition. This consortium could also provide a viable habitat for microbial pockets in Mars' ice caps (39).

Another application for polar exploration is to study polar regions on Mars to learn more about the history and possible previous existence of microbial life. The Martian polar regions have accumulated extensive mantles of ice and dust that cover individual areas of approximately 106 kM2 and total as much as three to four km thick, and are thought to be comparatively young from the scarcity of superposed craters on their surface. These regions preserve a record of the seasonal and climatic cycling of atmosphericC02, H20, and dust over the past approximately 105_ 108 years. Because of this cycling, the regions could serve as a Rosetta stone for understanding the geologic and climatic history of the planet including variations in insolation (due to quasiperiodic oscillations in the planet's obliquity and orbital elements), volatile mass balance, atmospheric composition, dust storm activity, volcanic eruptions, large impacts, catastrophic floods, solar luminosity, supernovae, and perhaps a record of microbial life (8).

Impact Excavation:

Because of the ubiquity of subsurface microbial life on Earth, examination of the subsurface of Mars could provide an answer to the question of whether microorganisms exist or ever existed on that planet (9).

Impact craters provide a natural mechanism for accessing the deep substrate of Mars and exploring its exobiological potential. Based on equations that relate impact crater diameters to excavation depth, the observed crater diameters that are required to prospect to given depths in the Martian subsurface have been estimated and related to observed microbiological phenomena in the Earth's subsurface. Simple craters can be used to examine material to a depth of approximately 270 meters. Complex craters can be used to reach greater depths, with craters of diameters greater than or equal to 300 km required to reach depths of 6 km or greater, which represent the limit of the terrestrial deep subsurface biosphere (9).

Also, several lines of evidence strongly support the exploration of large impact craters to study deposits important for astrobiology. The great depth of impact craters, up to several kilometers relative to the surrounding terrain, can allow the breaching of local aquifers, providing a source of water for lakes and hydrothermal systems. Craters can also be filled with water from outflow channels and valley networks to form large lakes with accompanying sedimentation. Impact melt and uplifted basement heat sources in craters greater than 50 km in diameter should be sufficient to drive substantial hydrothermal activity and keep crater lakes from freezing for thousands of years, even under cold climatic conditions. Fluid flow in hydrothermal systems is focused at the edges of large planar impact melt sheets, suggesting that the edge of the melt sheets will have experienced substantial hydrothermal alteration and mineral deposition. Hydrothermal deposits, fine-grained lacustrine sediments, and playa evaporite deposits may preserve evidence for biogeochemical processes that occurred in the aquifers and craters. Therefore, large craters may represent "giant petri dishes for culturing preexisting life on Mars and promoting biogeochemical processes" (37).

Methanogens:

Methanogens are methane-producing organisms of the domain Archaea. The archaea were once grouped among the

Eubacteria until 1974 when Carl Woese used DNA sequences to sort out parts of the bacterial family tree, and found that archaea were able to comprise their own major branch of the tree of life. Archaea share some genes with bacteria and eukaryotes, but they also contain a variety of genes that are unique (14). They are distinguishable from true bacteria by the possession of membrane lipids composed of isoprenoids (ether-linked to glycerol or other carbohydrates), a lack of peptidoglycan containing muramic acid, and distinctive 16S ribosomal RNA sequences (5).

Most methanogens grow by using H_2 as an electron source, while some can grow using a $C0_2$-reducing pathway using a series of four two-electron reductions to convert $C0_2$ or bicarbonate to methane (5). It has been reported that certain methanogens will consume H_2 down to partial pressure as low as 4 Pa ($4X10^{-5}$ atm) with $C0_2$ as the sole carbon source at a rate of 0.7ng H_2 per minute per microgram cell protein. This lower limit of pH_2 for growth of methanogens is based on the assumption that the pH_2 needs to be high enough for one ATP to be synthesized Per $C0_2$ reduced (25). In addition to being able to survive on H_2 and $C0_2$, some methanogens can grow either without or with sparse organic nutrients (5). K. H. Nealson (35) said, "The ability to grow at the expense of inorganic redox couples allows microbes to occupy niches not available to the more metabolically constrained eukaryotes." Following this principle, the properties described above are consistent with the known limits of Mars' atmospheric and surface components, thus making them potential Mars organisms (36).

Besides their metabolic characteristics that give them unique abilities to survive in the Mars's subsurface, methanogens appear to have fewer salt bridges, less packed hydrophobic cores, and a reduction of proline residues in loop structures that confer the organism low temperature activity and included greater structural flexibility than other organisms (46). Also in order to grow and reproduce in high-salt, low-water activity environments, the halophilic archaea have made basic biochemical adaptations in their proteins, osmoregulation mechanisms, nucleic acids, and lipids that could help them in surviving Mars' subsurface (27).

With these things in mind, some scientists have theorized about the plausibility of either the present or past existence of methanogens on Mars.

Using geochemical and thermodynamic considerations, it has been proposed that geologically derived hydrogen might support subsurface microbial communities on Mars in which methanogens form the base of the ecosystem. More than 90% of the 16S ribosomal DNA sequences recovered from hydrothermal waters circulating through deeply buried igneous rocks in Idaho are related to hydrogen-using methanogenic microorganisms. Geochemical characterization indicates that geothermal hydrogen, not organic carbon, is the primary energy source for this methanogen-dominated microbial community. This information demonstrates that hydrogen-based methanogenic communities occur in Earth's subsurface, providing an analogue for possible subsurface microbial ecosystems on other planets (6).

Scientists studying the composition of the shergottite, nakhlite, and chassigny (SNC) meteorites' isotope composition for carbon and organic matter grew a pure *M. formicicum* culture in a mineral nutrient medium in an atmosphere of H_2 and $C0_2$ (4:1) and confirmed that the isotope composition could in fact have been explained by the action of the methanogens (21). These scientists then theorized that the delta ^{13}C value of calcite is accounted for by the microbial reaction $C0_2 + H_2 \rightarrow CH_4 + H_20$, as well as ^{12}C fractionation potentially performed by methanogens. The formation of the calcite of "SNC" meteorites was accomplished in an environment favorable for the activity methanogens, thus providing an even stronger argument for the existence of methanogens in the meteorites (22).

Following the logic that organisms possibly could exist on Mars in the subsurface, scientists have tried to determine whether abundant hydrothermal or atmospheric energy is present on Mars to supply a subsurface biological ecosystem. For hydrothermal energy models, host rock based upon the composition of Martian meteorites was reacted with one of three groundwater compositions at high temperatures and the Gibbs energy for reactions that are important for terrestrial chemosynthetic organisms and likely representatives for putative Martian microbes were calculated. The results indicated that substantial chemical energy could be present depending on host rock composition to support suitable environments for Martian life (47). It has been shown that Martian organisms could be supplied with a large energy flux from the oxidation of photochemically produced atmospheric H_2 and CO diffusing into the regolith, but surface abundance measurements of these gases demonstrate that no more than a few percent of this available flux is actually being consumed. This suggests that biological activity is limited in the top few hundred meters of the subsurface, and implies that the apparent scarcity of life on Mars is not attributable to lack of energy. Instead, the availability of liquid water may be a more important factor limiting biological activity because the photochemical energy flux can only penetrate to 100-1,000m depth, where most water is probably frozen (48).

Terraforming:

An interesting prospect for the use of methanogens is in the terraforming of Mars. While global warming, overpopulation, and pollution step more into the forefront of our daily lives, terraforming Mars has become an increasingly interesting topic of debate and research that is pertinent to the study of methanogens.

It has been suggested that with the use of supergreenhouse gases, Mars' surface could be heated to and maintained at Earth-like temperatures (15), and Mars' atmosphere could be thickened so that liquid water is stable on the surface. This process has been theorized to need approximately 100 years to occur. The thick

carbon dioxide atmosphere that would result could support many types of microorganisms, plants, and invertebrates. If these organisms converted CO_2 into O_2 with an average efficiency equal to that of Earth's, it would take more than 100,000 years to create Earth-like oxygen levels necessary to support human life (3 1). It has been proposed that organisms under study today could "provide the hardy stock of pioneering Martian organisms" that would be followed by other life forms such as plants to begin this process (32). Methanogens could be instrumental in this process because of their ability to produce methane (a greenhouse gas) and ability to survive extreme environments.

The ethics of this situation are of course important (especially if microbial life already exists there today) to the advancement of making Mars habitable, and should be considered in depth before any action is taken. Please refer to McKay et al. (31) for further discussion considering the ethics of terraforming and to Mancinelli (29) for ethical considerations for the search for life on Mars.

Desiccation:

As expressed above, the availability of liquid water may be an important factor limiting biological activity because the photochemical energy flux of atmosphere can only penetrate to 100-1,000m depth, where most water is probably frozen (48).

Since it appears at this point that there is no liquid water on the surface of Mars, we can only hypothesize that liquid water exists beneath the surface. Even though there has been much scientific evidence pointing to liquid waters' presence on Mars, we do not know its amount beneath the surface nor do we know the depths beneath the surface that it would exist. Warmth gained through geothermal heating could theoretically melt ice layers in the subsurface, but this liquid water could be seasonal (as stated above with liquid water only being available some places 34% of the year on the surface) or too far from the surface to have access to the necessary energy for microbial growth. As a result of depth constraints and water changes, it appears that any organism that needs water to grow (all known Earth organisms) would have to be able to adapt and respond to desiccation (drying).

Since there hasn't been much literature on the ability of methanogens to survive desiccation, information from other organisms that have undergone significant desiccation studies will be of use.

Desiccation leads to dramatic lipid phase changes whereas carbohydrates, proteins and nucleic acids initially suffer spontaneous, reversible low activation energy Maillard reactions thus forming products that more slowly re-arrange, cross-link, etc. to give nonnative states. While initial products spontaneously may reverse to native states by raising water activity, later products only do so through energy consumption and enzymatic activity. Yet, native states of lipid membranes and associated enzymes are required to generate energy. Consequently, good reserves of high-energy compounds like ATP and of membrane stabilizers like trehalose may be expected to enhance survival following drying and rehydration (10). It has also been noted that the inactivation of the anhydrobiotic organisms *Bacillus subtilis* (spores) and *Deinococcus radiodurans* during long-term exposure of up to several weeks to extreme dryness (especially vacuum) is correlated with an increase in the number of DNA-strand breaks and other DNA lesions (12). These DNA lesions will continue to accumulate if an organism is not given intermittent periods of activity to repair them. Unfortunately, it appears that if brief rehydration does not occur to reactivate an organism during prolonged desiccation, survival might be reduced to only a couple of decades (13). The survival of *B. subtilis* and *D. radiodurans* appears to depend on their repair of DNA damage (12). The removal of water through air-drying is lethal to the majority of organisms, yet some vegetative cells of bacteria and Cyanobacteria survive extreme dryness (2).

The Cyanobacteria have undergone quite a bit of scientific research for their abilities to survive extreme dryness, and could be useful in discovering mechanisms that would help with adapting other organisms to survive prolonged desiccation. Desiccation studies have shown that DNA damages seem to be reduced by the presence of the trehalose disaccharide mentioned above (41), as well as novel water-stress proteins with a protective function on a structural level (40).

Methanogens have shown signs of surviving periods of prolonged desiccation. Studies on sediment samples in methanogenic reservoirs in Australia have shown that the methanogens consistently recovered upon rewetting of the sediments (34) as well as survival and rapid reactivation after prolonged drying (3). In another study conducted on methanogens, their survival and potential CH_4 production increased dramatically in presence of pyrite (FeS_2) grains, while as much as 10% of the initial methanogenic population survived oxic desiccation. This information is in relatively good agreement with observations that methanogens in rice fields survive the periods when the paddy soil is dry and oxic (42).

If methanogens are to be able to exist on Mars' surface or subsurface, the existence of liquid water is potentially the most important rate-limiting factor. Since the existence of liquid water hypothetically seems to be sporadic on the surface at best and seasonally present in the subsurface (at least at depths shallow enough to receive any atmospheric energy), it appears that the methanogens' ability to survive on Mars revolves around their ability to survive periods of desiccation. This research was designed to test the ability of methanogens to survive desiccation.

Materials and Methods:

Organisms and Media:

Stock cultures of three methanogens obtained from David Boone (Portland State University) were grown on 3 different types of media: standard medium (MS) for *Methanosarcina barkeri* (4), standard medium with sodium formate added (MSF)

for *M. formicicum* (4), and standard medium without the organic materials for *Methanothermobacterium woffeii* (MM; 49). The anaerobically prepared media were added to test tubes inside of a Coy anaerobic chamber. The tubes were then sealed with butyl rubber stoppers and crimped with aluminum caps. The tubes were then removed from the chamber and pressurized to 200kPa with pure H_2 gas using a gassing manifold. When necessary, tubes were repressurized to 200kPa with a 75% H_2/25% $C0_2$ mix. One hour before the media were inoculated, 0. 15ml of sterile sodium sulfide solution (2.5%) was added using a sterile 3ml syringe to eliminate any residual oxygen (4).

Data Collection:

Since the by-product of the growth of methanogens is methane, their growth was monitored by testing for the presence and amount of methane in the head gas of the culture tube. Readings were taken by removing a 1 ml sample of the head gas of the tube of interest with a 3ml sterile syringe and injecting it into a Hewlett Packard 5890 Series 11 Gas Chromatograph. The GC had a thermoconductivity detector, oven set at 45∫ C, and argon used as the carrier gas. The GC was set to measure the percent of H_2, N_2, CH_4, and $C0_2$ in the sample.

Desiccation Experiment:

For the desiccation experiment, the organism to be tested was transferred into its respective medium and allowed to grow for one week in optimal growth conditions. After a week's worth of growth, 4ml of the media/organism were transferred via a 5ml sterile syringe to 5g of JSC Mars- I soil simulant, pressurized to 200kPa with H_2 gas, and allowed to grow in optimal growth conditions for approximately one week. After the growth process, the tubes were numbered and initial methane readings were recorded for each tube. If no significant methane readings were present in the majority of the tubes, the organisms were allowed more time for growth in the soil simulant. Once the organism growth was significant, the tubes were transferred into a Coy anaerobic chamber where they were uncrimped and unstoppered. Their contents were scraped into individually numbered (corresponding to the number of the tube whose contents they held) 15ml plastic beakers. The beakers containing the "mud" mixture were left sitting out in the open in the anaerobic chamber until all of the samples appeared dry to the eye. Once all of the mud was dry, the beakers were set inside a Nalgene Desiccator with Drierite under the platform. From this point, the beakers would remain in the desiccator until a predetermined time interval for removal. Once removed, the contents of the beaker would be scraped into a correspondingly labeled test tube and hydrated with 4ml of carbonate buffer. The tube would then be stoppered, crimped, subjected to 0.15ml of sodium sulfide (2.5%), pressurized to 200kPa with the $C0_2/H_2$ mix, and placed in the appropriate incubator for the organism. Methane readings were taken on each tube 24 hours, 1 week, 2 weeks, 3 weeks, 1 month, and then monthly after rehydration to monitor the organisms' growth.

Preliminary Desiccation Procedure:

Two of the earlier experiments were performed without a desiccator. These experiments both used *M. formicicum.* The results for these experiments are shown in Figures 1 and 2. This procedure is virtually identical to the procedure discussed above except petri dishes were used in place of 15ml beakers. The mud in the petri dishes was set out in the anaerobic chamber until all of the petri dish's mud had been uniformly dried. The "dust" was then scraped back into tubes at predetermined intervals, hydrated, stoppered, etc.

Results:

The results from the experiment can be seen in Figures 1-11. Generally, methane was absent 24 hours after rehydration, and the amount of methane grew to greater than 1% in around 80% of all samples after a month of incubation following desiccation (except in experiments seen in Figures 1, 2, and 3). Methane increased to greater than 10% in 78% of all samples after two months of growth (except in the experiment seen in Figure 1), and was present in 80% of all samples desiccated at the completion of the project (except in the experiments seen in Figures 8 and 11 that have yet to be completed). Methane was present in 86% of all samples desiccated at the completion of the project excluding the experiment seen in Figure 1. No observable trends were apparent in samples with absence of measured methane increase. Higher methane was seen in Figures 6 and 7, which is consistent with other *M. woffieii* data regarding growth rates.

In Figure 1, methane occurrence was only observed in samples desiccated one and eight days.

The results for these experiments, as well as initial methane readings taken before desiccation, can be seen in chart form in Appendix B.

(Editor's note: Space precludes publication of figures 2-11 and the tables of Appendix B. (Contact the author or his mentor for this information.)

Discussion:

It appears that the methanogens studied are capable of surviving at least some periods of desiccation. Even though *M. wolfeii* tended to produce methane earlier and faster than the other organisms, longer range studies would lead to the belief that all of the organisms will produce significant methane with time.

All of the organisms displayed an ability to survive all of the periods of desiccation tested. *M. formicicum* survived ten days of desiccation and had methane levels above 3 0% in a sample desiccated for over 26 days. *M. woffieii* and *M. barkeri* both were able to survive periods of desiccation spanning ten days.

There were no observable trends for the lack of methane production in some samples, except for an occurrence of methane absence in samples desiccated for seven to nine days (usually eight) in Figures 2, 3, 4, 5, 6, 7, 9, and 10. No explanation for this phenomenon has been proposed as growth has occurred on samples dried seven to nine days in some experiments, as well as unexplained variations for growth on samples desiccated for eight days in Figures 1 and 2. There also seems to be no correlation between post-desiccation methane production, and pre-desiccation methane measurements shown in Tables 2, 4, 5, 6, 7, 8, 9, 10, and 11 in Appendix B.

No proposed explanation has been formulated for the non-congruity of Figure 1 with the rest of the results. This was the first experiment conducted by the researcher, so experimental error seems likely.

Many of these experiments were conducted simultaneously, so cross-contamination is a possibility, even though the growth conditions and non-relatedness of the results of the experiments would hold this unlikely. Unfortunately, experimenter error is also always a possibility.

Discovering a time limit of desiccation upon which the organism would not survive upon rehydration would seem critical. Since the hypothesis of liquid water availability at this point indicates that dryness would be a norm, periods of desiccation longer than ten days to a month seem more than likely. And since it has been theorized that survival for longer than a couple decades without intermittent rehydration is not likely with known organisms, it would be interesting to know if this were true for methanogens. It is interesting to note that the viability and methane production of methanogens tend to do better with rapid desiccation (42), the recovery of dried bacteria after desiccation increases with slow rehydration (24), and that desiccation procedures conducted at sub-room-temperature conditions (like those on Mars) might help preserve the organism and accommodate desiccation tolerance. Research regarding desiccation and rehydration rates could also provide a better understanding of the ability of methanogens to survive on Mars.

There have been some studies that indicate that the mechanisms necessary to survive desiccation are also useful for surviving ionizing radiation (30). If it became apparent that the methanogens are fit to survive prolonged desiccation, implications for ionizing radiation survival seem important. Since Mars' ecosystems experience a greater flux of radiation from the sun than those of the Earth, radiation survival would also be important for potential Mars organisms.

There could be a possibility that the JSC Mars-I soil simulant might confer higher desiccation tolerance. Experiments using -non-desiccation tolerant prokaryotes with the desiccation procedure used here could provide further insight into the implications of this research and the characteristics of the soil simulant.

Conclusions:

I. *Methanobacterium formicicum, Methanothermobacter wolfeii*, and *Methanosarcina barkeri*, under the conditions tested here, survived desiccation for a period of at least ten days.

II. *Methanobacterium formicicum* survived desiccation for at least one month.

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	Mars	Earth
Average Distance from Sun	142 million miles	93 million miles
Average Speed in Orbiting Sun	14.5 miles per second	18.5 miles per second
Diameter	4,220 miles	7,926 miles
Tilt of Axis	25 degrees	23.5 degrees
Length of Year	687 Earth Days	365.25 Days
Length of Day	24 hours 37 minutes	23 hours 56 minutes
Gravity	.375 that of Earth	2.66 times that of Mars
Temperature	Average -81 degrees F	Average 57 degrees F
Atmosphere	mostly carbon dioxide some water vapor	nitrogen, oxygen, argon, others
# of Moons	2	1

Appendix A

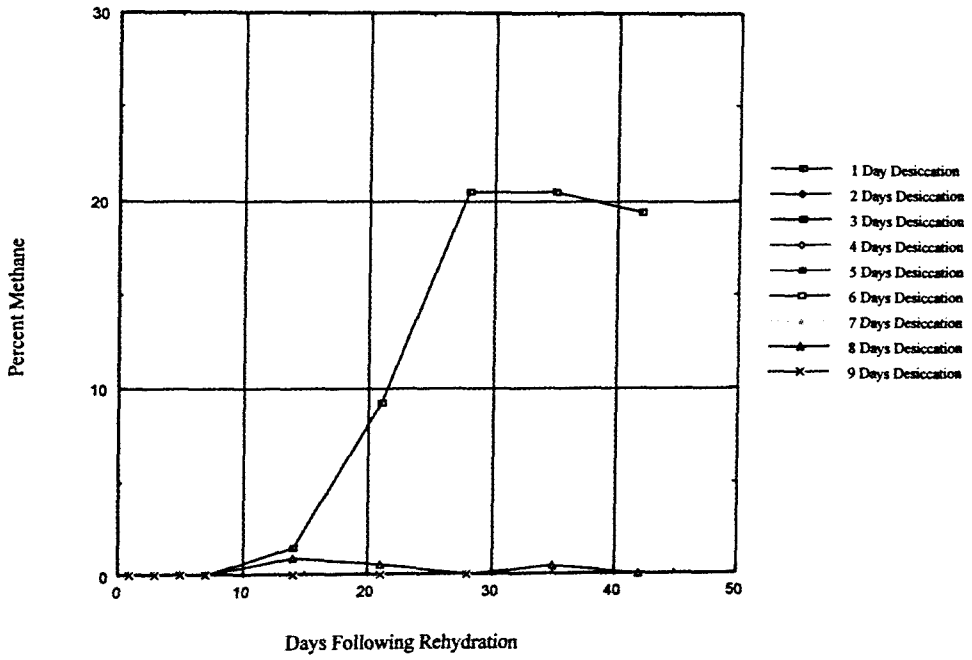

Figure 1: Methane production by *Methanobacterium formicicum* following desiccation in petri dishes for the first experiment on *M. formicicum.*

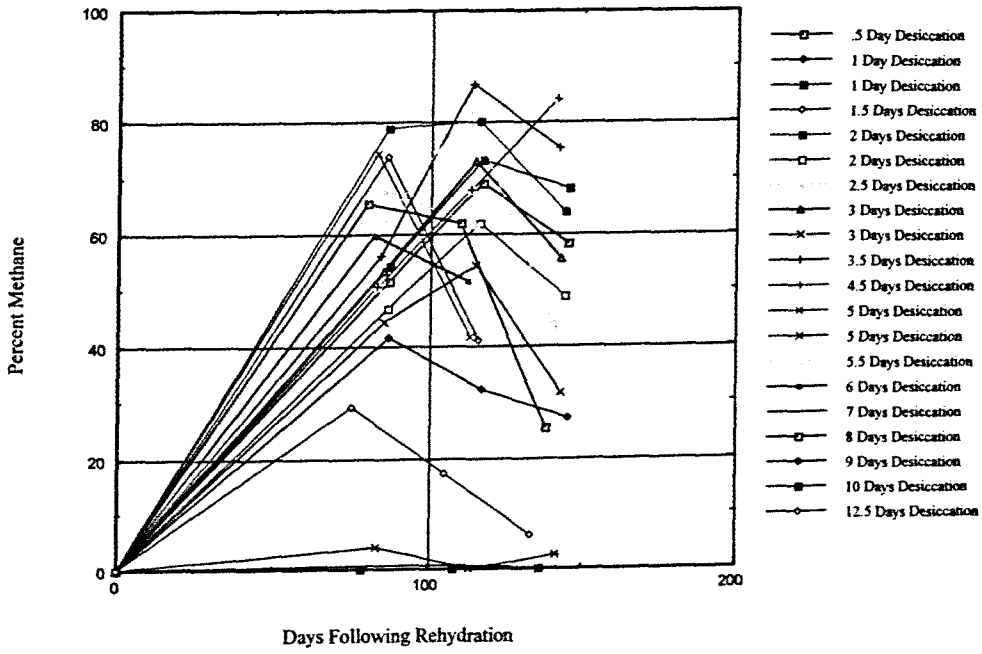

Figure 2: Methane production by *Methanobacterium formicicum* following desiccation in petri dishes for the second experiment on *M. formicicum.*

Faculty comment:

Mr. Kendrick's mentor, Dr. Timothy Kral, is very complimenty about his student's work. He said,

> Throughout recorded history, humankind has been fascinated by the heavens above. I am not referring to a religious heaven, but rather to the star-filled universe. From the time that the lights in the sky were identified as stars and planets, humans have wondered about the possibility of life out there. Today there is great excitement in the air because we are closer than ever to discovering the answer to the question: Are we alone? Unfortunately, our exploration has been limited to the inner reaches of our solar system where we are fairly certain that other intelligent life does not exist. Nonetheless, discovery of the simplest microbe on another planetary body would have tremendous significance.
>
> The target planet is Mars. Early in its history, Mars was an earthlike planet, with liquid water on its surface. It was warmer and had a thicker atmosphere than it does today. It would have been very conducive to life as we know it. Today, the surface is barren. Conditions would suggest that life as we know it could not exist. The surface is extremely cold (600∫ C average) and dry; the atmospheric pressure is less than one-hundredth that of Earth's; and it is constantly bombarded by lethal radiation.
>
> Below the surface is another matter. Conditions are probably warmer with a higher pressure, and the rock above would protect from the radiation. If liquid water is present below the surface, and there is no reason to believe that it is not present, conditions would be conducive to an Earth organism known as a methanogen. Methanogens are microorganisms in the domain Archaea that live below the surface of the Earth and deep within our oceans. They may indeed be the predominate life form on planet Earth.
>
> During the 12 years that I have been working in this area, we have been studying methanogens as life forms that potentially could survive and thrive below the surface of Mars. We have been exposing methanogens to conditions that approach those known to exist on Mars including lower pressure, lower temperature, dry conditions, radiation, and exposure to oxygen. (Methanogens are poisoned by molecular oxygen, as are most life forms on planet Earth.)
>
> This year I am mentoring five honors students who are working on one of the conditions mentioned above. This letter is in support of Michael Kendrick who is competing for one of the undergraduate research awards. Michael approached me early last spring semester about doing research in my lab. He began his research last spring and has been heavily involved in it ever since. His project involves the effects of drying on methanogens. We know that methanogens would do well growing on a Mars soil simulant under reduced pressures until they dry out. Michael grew methanogens in a standard medium, added them to a Mars soil simulant, and then dried them for varying lengths of time. His initial experiments were performed in our anaerobic chamber in petri dishes. His later experiments utilized newly purchased desiccation jars. Michael discovered that the methanogens that we work with could survive drying for much longer periods of time than we imagined. *Why is this research critical?* As mentioned previously, for life to exist below the surface of Mars, liquid water would have to be present. Depending on the depth of that liquid water, seasonal surface changes (Mars has seasons just as Earth does) could result in water freezing or drying up for various periods of time. It is crucial to know how long methanogens can survive under those potential dry periods. How long liquid water is present could be the limiting factor in determining whether life can exist below the surface of Mars. It is that important.