TESTING THE HYDROGEN PEROXIDE - WATER HYPOTHESIS OF LIFE ON MARS USING THE DIFFERENTIAL SCANNING CALORIMETER AS AN ANALOG FOR THE TEGA INSTRUMENT ON THE MARS PHOENIX LANDER

BY

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Abstract

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In the time since the Viking life detection experiments were conducted on Mars, many missions have enhanced our knowledge about the environmental conditions on the Red Planet. However, the martian surface chemistry and the Viking lander results remain puzzling. Non-biological explanations that favor a strong inorganic oxidant are currently favored (e.g., Mancinelli, 1989; Plumb et al.,1989; Quinn and Zent, 1999; Klein, 1999, Yen et al., 2000), but problems remain regarding the lifetime, source, and abundance of that oxidant to account for the Viking observations (Zent and McKay, 1994). Alternatively, a hypothesis that favors the biological origin of a strong oxidizer has recently been advanced (Houtkooper and Schulze-Makuch, 2007). Here, we report about laboratory experiments that simulate the experiments to be conducted by the Thermal and Evolved Gas Analyzer (TEGA) instrument of the Phoenix lander, which is to descend on Mars in May 2008. We also report on laboratory experiments involving water activity and hydrogen peroxide solutions. Our experiments provide a baseline for unbiased tests for chemical versus biological responses, which can be applied to the Phoenix mission and future Mars missions.

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Dedication

This thesis is dedicated to my mother, father and husband

who provided unfailing support and faith.

CHAPTER ONE: MARS, VIKING, PHOENIX AND H_2O_2

INTRODUCTION

As we explore the planets in our solar system, we continue to find evidence that many planets and their satellites may have been able to support life in the past and may even be capable of supporting life in the present. From Mars with its wind-swept plains, to the subsurface oceans of Europa, the possible habitats for life are numerous. The search for life on any planet must begin with a consideration of the basic requirements of life. Some of these requirements include a nutrition source, energy source and water or other suitable solvent availability.

Before moving our attention to other solar bodies we must first consider the limits within which life is found on our own planet. On Earth, microorganisms have been found in a wide variety of environments and indeed seem to define the very limits of biological life. Psychrophiles have been found actively growing at -20°C (Rivkina, Friedmann, McKay, & Gilichinsky, 2000) and thermophiles and hyperthermophiles have been found at temperature of up to 121°C (Kashefi & Lovley, 2003). Most microorganisms tolerate an external pH range from about 5 to 9 pH units. However, there are some acidophiles that tolerate pH values below 2 and some alkaliphiles that tolerate external pH values of up to 11 (Konhauser, 2007). Microorganisms can also tolerate a wide range of pressures from deep subsurface to interstellar space, a wide variety of aqueous habitats from acid mine drainage to alkaline lakes and even extreme desiccation (Rothschild & Mancinelli,

2001). Given these fairly broad constraints for life, it is possible to consider the environments of other solar bodies as refuges for life.

As one of our nearest neighbors in space, Mars has always been an object of fascination and interest. The martian surface boasts a canyon system larger than the Grand Canyon and peaks higher than Mt. Everest. The atmospheric pressure is about 7 mbar with a composition of mostly CO_2 (95%), N_2 (2%) and Argon (1%) with temperature ranges from -125°C in the poles to about 20°C near the equator (NASA Mars, 2009). Dust storms can sometimes range over the entire planet and the atmosphere, though thin by Earth standards, is enough to support clouds and weather systems.

The Viking mission to Mars began an era of investigation and hoped to answer the age-old question, are we alone? The two Viking Landers, Viking 1 and Viking 2 landed on Mars between July and September of 1976. Viking 1 landed on the western slopes of Chryse Planitia on July 20, 1976 and the Viking 2 landed in Utopia Planitia on September 3, 1976 (NASA Viking, 2009). Both spacecraft contained three main life detection experiments as shown in Figure 1: the Pyrolitic Release experiment (PR or Carbon Assimilation), the Gas Exchange experiment (GEx) and the Labeled Release (LR) experiment (Klein, 1976). A gas chromatograph-mass spectrometer (GCMS) was also on board the spacecraft as well as a seismograph (Klein, 1976).

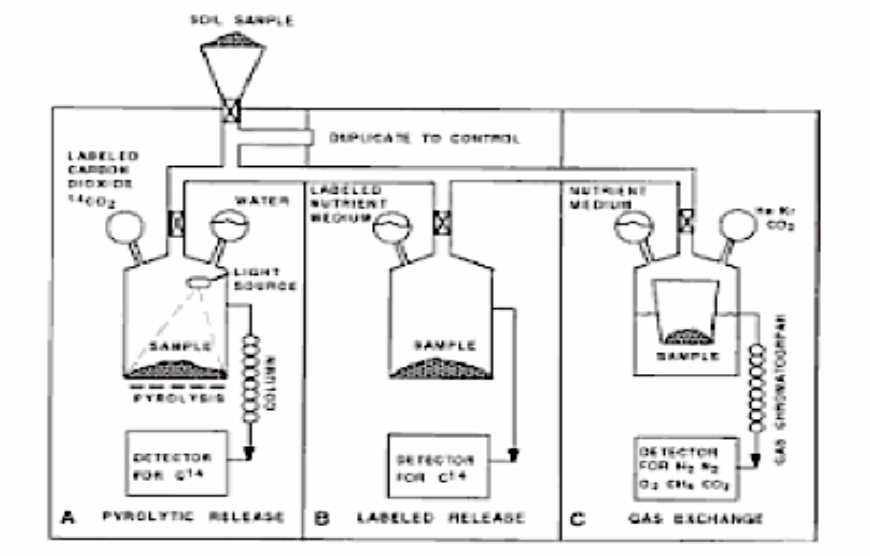


Figure 1: Diagram of the three life detection experiments on the Viking mission (from McFadden, 2007).

The Carbon Assimilation or Pyrolitic Release (PR) experiment tested the idea that potential martian microorganisms could utilize labeled $^{14}CO_2$ or ^{14}CO gases during either light or dark metabolism reactions. In the experiment, 0.25g of martian regolith was enclosed in a 4ml sample chamber and exposed to the radiolabeled gases, with or without added water vapor. The light source was a simulated martian spectrum (335-1000nm) and the reactions were allowed to run for 120 hours at temperatures between 8 ad 26°C (pressure was 10 mbar). The control samples were treated to all the same conditions except they were first heated to either 90°C or 175°C, with the later temperature considered a "sterilization" temperature (Klein et al., 1976). After the incubation period the samples vented and then pyrolyzed at 625°C to release any organic compounds and run through a gas chromatography column. The $^{14}CO_2$ and ^{14}CO gases were fixed in small but significant amounts: 7 pmole of CO or 26 pmole of CO_2. Additionally, the

reactions were not inhibited by water vapor or heating to 90°C, were enhanced by the simulated martian light source and reduced by 90% when heated to 175°C (Klein, 1976).

While the pyrolitic release results could be interpreted as consistent with microbial life on Mars there are several arguments against the biological explanation. Klein (1978) stated that since heating the samples to 90°C had no inhibiting effect and heating to 175°C only partially inhibited the release, then a non-biological explanation is supported. Horrowitz et al. (1977) argued against the biological explanation due to the fact that water vapor tended to inhibit or have no effect on the reactions, even though water would be predicted to enhance a biological reaction. Furthermore, the gas chromatography mass spectrometer (GCMS) onboard the Viking landers did not detect any soil organics (Biemann et al., 1976). While all these arguments are indeed compelling it is important to remember that: 1) extremophilic bacteria on Earth can withstand and even grow in temperatures up to 121°C (Kashefi & Lovley, 2003) and down to -2°C (Junge, Eicken, & Deming, 2004), 2) the GCMS used on the Viking mission failed to detect organic molecules in the Antarctic soil even though such organics were known to exist in the samples (Levin and Straat, 1981) and 3) the addition of water to a sample of martian regolith unused to such high amounts of the solvent could well have been detrimental to any microbes in the sample.

The objective of the Gas Exchange experiment (GEx) was to detect the evolution of gases resulting from microbial activity in hydrated regolith. The GEx could detect the following gases in both “humid” and “wet” mode: hydrogen, nitrogen, oxygen, carbon monoxide, nitrous oxide, methane, carbon dioxide, nitric oxide, hydrogen sulfide, neon, argon and krypton. The humid mode allowed the martian regolith to be exposed to water

vapor and the wet mode exposed the sample to an aqueous mixture of metabolites (amino acids, vitamins, salts and organic compounds) (Brown et al., 1978 and Oyama and Berdahal, 1977). All samples were tested at a total of 200 mbar (7 mbar of martian atmosphere plus added carbon dioxide, helium and krypton) with temperatures between 8°C and 15°C (Klein et al., 1976). Almost immediately after a 1 ml sample of regolith was humidified a large increase in O_2 was observed along with a smaller increase in CO_2. After 2.5 hours the CO_2 had increased 5-fold while the O_2 content had increased over 200-fold (Oyama and Berdahl, 1977). In the wet mode however, the amount of CO_2 and O_2 slowly decreased over several martian sols. The aqueous solution of nutrients was added to the samples several times and after each addition the absorption of CO_2 slowed down. This result was expected, however, since the same phenomena was observed in pre-launch tests using sterile terrestrial soil (Oyama et al., 1976).

The rapid release of oxygen after addition of water vapor was a complete surprise to the Viking team (Klein, 1978), as earlier experiments on Earth with terrestrial or lunar soils yielded no such result (Oyama et al., 1976). One chemical explanation for this rapid release of oxygen is that a peroxide or superoxide released the O_2 upon addition of the water vapor and that any carbonates or metal oxide ions the soil reacted with the water to create a slightly basic solution which could then re-adsorb the CO_2 (Klein 1978 and Klein et al., 1976).

The final life detection experiment, the Labeled Release (LR) experiment, was the only experiment aboard the Viking mission not to use copious amounts of water in the sample reaction. The experiment used ^{14}C-labeled carbohydrates reacting with the martian regolith to produce $^{14}CO_2$ evolved gas. Both the Krebs cycle in aerobic

metabolism and the Embden-Meyerhof pathway in anaerobic metabolism can make highly efficient use of ^{14}C-labeled metabolites and both can then produce ^{14}C-labeled carbon dioxide (Levin et al., 1964). Briefly, about 0.5 g of regolith was placed in a closed reaction chamber with 7 mbar of the ambient atmosphere. The chamber was then brought to 10°C and a series of helium and nutrient (radiolabeled carbohydrates) solution injections proceeded. The injections were about 115µl each and the pressure was about 92 mbar after the first injection and 116 mbar after the second injection. Evolved $^{14}CO_2$ gas was monitored for several sols after each injection (Levin and Straat, 1979a, 1979b).

Levin (1963) had previously shown that the Labeled Release experiment was sensitive in detecting metabolic activity from a wide range of aerobic and anaerobic microorganisms including *Bascillus subtilis, Micrococcus* spp., *Staphylococcus epidermidis* and *Pseudomonas* spp. In fact, as little as 1-2µCi.ml of ^{14}C-labeled nutrient solution was optimum for detecting 10-12 viable bacteria per ml within 1-3 hours of nutrient addition. Also, the increase in evolved carbon dioxide linearly corresponded to cell density so that each doubling of cell count resulted in doubling of the counts-per-minute (cpm) detected from the ^{14}C-labeled carbon dioxide (Levin et al., 1964). Figure 2 shows the LR results from Viking Lander-1 from two samples of non-heat treated regolith and one sample of heat-treated (160°C, 3 hours) regolith. The two active or non-heat treated samples show the classical biological response as seen by Levin (1964) and were taken to be evidence of biological activity in the martian regolith (Levin and Straat, 1979a, 1979b).

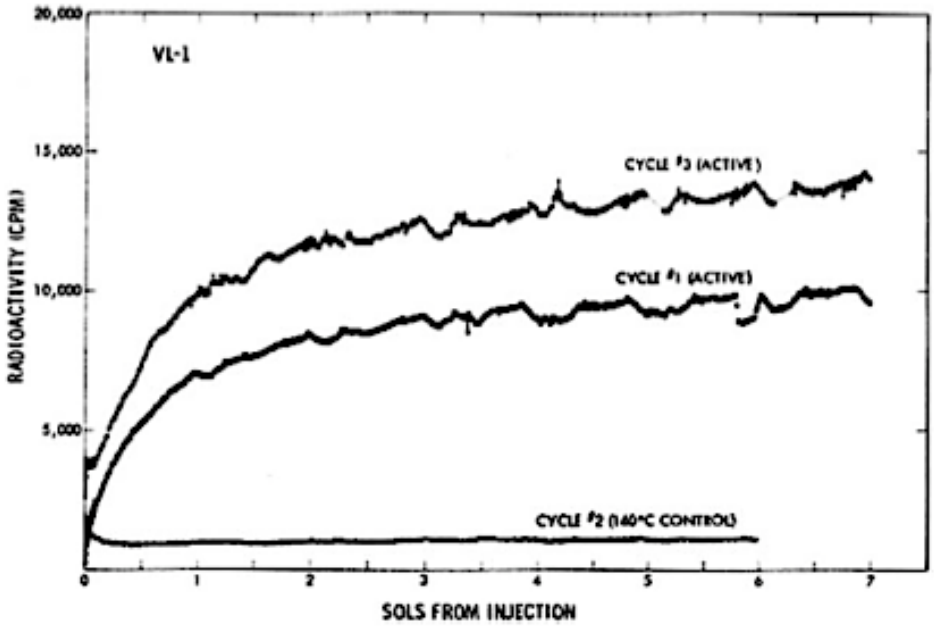


Figure 2: Viking Lander-1 data from the Labeled Release experiment. Cycles 1 and 3 were from non-heat treated sampled while cycle 2 was heat-sterilized for 3 hours at 160°C before the assay (Levin and Straat, 1979a).

While these results were seen as the strongest support for biological activity in the martian regolith they were also the most controversial. The objections are based on two key points: 1) following a second injection of the labeled nutrient solution the radioactivity decreased instead of increasing and 2) no organics were found with the GCMS aboard the Viking (Biemann et al., 1976). Levin and Straat (1979b) responded to the criticism involving the second nutrient injection by showing that this could have been caused by a chemical equilibrium reaction between carbon dioxide, water and regolith in the sample cell. As for the negative results in the GCMS organic molecule data, the same instrument used on the Viking mission failed to detect organics in the Antarctic soil when

those organics were known to be present (Levin and Straat, 1981). Still today, the LR results are considered unverified by most of the Astrobiological community.

On the whole, many questions were left unanswered and many controversies remained after the Viking mission to Mars. One such question relates to the nature of the oxidant in the martian regolith (Zent and McKay, 1994; Quinn and Zent, 1999; Yen et al., 2000; Mancinelli, 1989; Klein, 1999; Benner et al., 2000; Hurowitz et al., 2007; Houtkooper and Schulze-Makuch, 2007). The common explanation is that H_2O_2 and other strong oxidizing compounds oxidized the organic material near the surface. Based on the reactivity of the surface measured by the Viking Gas Exchange experiment (GEx), the amount of H_2O_2 on the martian surface was estimated to be between 1 ppm (Zent and McKay, 1994) and 250 ppm (Mancinelli, 1989). Yet, photochemical processes generate H_2O_2 in the atmosphere at a much lower rate, specifically in the parts per billion range. Atmospheric H_2O_2 abundances vary between 20 and 40 ppb by volume over the planet (Encrenaz et al. 2004), which appears to be a maximum concentration that occurs during favorable weather conditions (Atreya and Gu, 1994). Several hypotheses have been proposed to deal with this discrepancy and Nussinov et al. (1978) argued that, instead of H_2O_2, oxygen gas physically trapped in soil micropores might have been responsible for the Viking observations. Alternatively, Plumb et al. (1989) pointed out that a large number of diverse features of the GEx and Labeled Release experiment could be reproduced with ultraviolet-irradiated potassium nitrate. Possible oxidant reactions and their environmental problems on Mars were summarized by Zent and McKay (1994), who concluded that none of the hypotheses presented in the literature was free of serious troubles, many of which have to do with the instability of putative oxidants in the

presence of heat, light, or atmospheric carbon dioxide. Other objections included the problem that the suggested hypotheses would require elaborate formation mechanisms for which there is no evidence. Instead, Zent and McKay suggested that the results obtained by Viking could be best explained by some kind of heterogeneous surface chemistry that yields one or more types of oxidizing surfaces on the Martian regolith particles. Quinn and Zent (1994) proposed that hydrogen peroxide chemisorbed on titanium dioxide may have been responsible for the chemical reactivity seen in the Viking life detection experiments while Yen et al. (2000) suggested superoxide ions instead of H_2O_2.

Alternatively, Levin and Straat (1981) and Levin (2007) argued for a biological explanation but struggled to explain (1) the evolution of O_2 upon wetting the soil, (2) the apparent absence of organic molecules in the soil, and (3) the weakly positive results of the single control test in the Pyrolytic Release experiment. Using a different approach, Houtkooper and Schulze-Makuch (2007) suggested that putative martian organisms might employ a novel biochemistry; specifically, that they could utilize a water-hydrogen peroxide (H_2O-H_2O_2) mixture rather than water alone as an intracellular liquid. This adaptation would have the particular advantages of providing a low freezing point, a source of oxygen, and hygroscopicity in the martian environment, which would allow organisms to scavenge water molecules directly from the atmosphere, and address many of the puzzling Viking findings. H_2O_2-H_2O solutions are mostly known as disinfectants and sterilizing agents on Earth, but some microbial organisms produce hydrogen peroxide (e.g., certain *Streptococcus* and *Lactobacillus sp*.; Eschenbach et al., 1989), while other microbes utilize H_2O_2 (e.g., *Neisseria sicca*, *Haemophilus segnis;* Ryan and Kleinberg, 1995). Sensitivity to H_2O_2 varies drastically among microorganisms (Anders et al., 1970; Alcorn et al., 1994; Stewart

et al., 2000). Reported microbial survival rates range from greater than 80 % to less than 0.001 % after exposure to 30 mM hydrogen peroxide (Alcorn et al., 1994), and at least one organism, the microbe *Acetobacter peroxidans*, uses H_2O_2 in its metabolism (overall reaction H_2O_2 (aq) + H_2 (aq) ↔ $2H_2O$; Tanenbaum, 1956). Mixtures of H_2O_2 and H_2O freeze at temperatures significantly below the freezing point of water. Indeed, The lower eutectic point lies at -56.5°C for a mixture with 61.2 weight % H_2O_2 (Foley and Giguère, 1951). Also, mixtures with a high H_2O_2 concentration tend to super cool, which sometimes results in the formation of glasses, down to liquid-air temperatures (Giguère, and Secco, 1954).

On Earth, microorganisms commonly use a salt-water mixture as an antifreeze. Salts are common and readily available as they are highly soluble in the abundant water present on Earth. Thus, organisms on Earth learned to adapt to higher salt concentrations and use salts as antifreeze in cold environments such as in high mountain regions and the Arctic. Bacteria have been found surviving and thriving in the salt brine pockets of frozen wintertime sea ice in the Arctic at temperatures down to -20°C (Junge, Eicken and Deming, 2004). On Mars, however, liquid water was never as abundant as on Earth. Even during the wetter early martian history, liquid water bodies may not have remained permanently on the surface. Hydrogen peroxide, however, is naturally present on Mars at much higher concentrations than on Earth, today and probably in the past. These inorganic concentrations are still low, much too low to explain the Viking results, but its presence should have been sufficient to warrant biochemical adaptations. Putative martian microorganisms might have learned to use the properties of hydrogen peroxide to their advantage, especially as the planet dried up and became colder during its history. A

comparable process occurred on Earth more than 2 billions years ago, when microbes developed the ability to live with free oxygen and then thrive by using it in their favour (Brocks et al., 1999, Raymond and Segre, 2006; Catling and Buick, 2006). Thus, the antifreeze properties of hydrogen peroxide and its hygroscopic properties would become a great advantage for making a living on the dry and cold Mars. Also, adaptation to H_2O_2 would require adaptation to oxidation stress (e.g., protection from protein oxidation), which would have likely conveyed resistance to radioactivity as well (Daly et al. 2007), another trait useful to have in the martian near-surface environment.

The NASA Mars Phoenix Mission, which finished its mission on Mars in February of 2009, provides the unique possibility to test various hypotheses to explain the Viking results. The Phoenix lander includes the Thermal Evolved Gas Analyzer (TEGA) instrument, which is a combination of a high-temperature furnace and a mass spectrometer that will be used to analyze martian ice and soil samples. Once a sample is successfully received and sealed in the TEGA oven, temperature is slowly increased at a constant rate up to 1000°C, and the power required for heating is carefully and continuously monitored. This process, called scanning calorimetry, shows the transitions from solid to liquid to gas of the different materials in the sample. We used a complimentary technique, differential scanning calorimetry (DSC), to simulate the results of the TEGA instrument with various solutions of hydrogen peroxide, water, martian soil simulants and bacterial species *Pseudomonas* spp. and *Lactococcus lactus* (a hydrogen peroxide producing organism).

MATERIALS AND METHODS

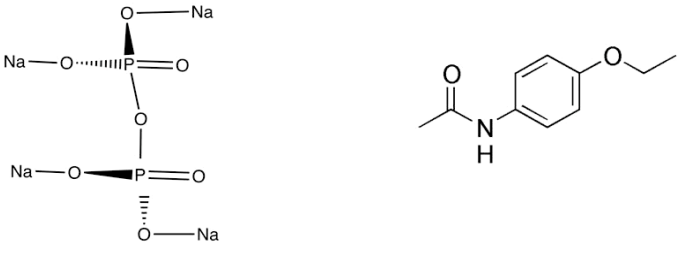
Both a brief description and detailed description of the materials and methods are included below.

BRIEF DESCRIPTION

We used differential scanning calorimetry to analyze phase transitions and thermodynamic properties of the oxidant compounds investigated. Two thermal cells were employed, one cell holding the reference capsule, the other the sample. A computer control system measured the amount of heat required to increase the temperature of each cell. If the temperature in one cell was not rising as fast as the temperature in the other cell, the instrument sent more energy (heat) to the heating coils in that cell to maintain the same temperature in each cell. The computer then recorded the difference between the energy requirements for each cell. The resulting graph (thermogram) of temperature versus energy difference between the two cells displays a peak whenever a phase transition occurred. The area under a positive peak (peak area) represents the energy required for the transition (enthalpy of the reaction, ΔH), thus any positive peaks are representative of endothermic reactions, while negative peaks are representative of exothermic reactions. The onset of a peak usually corresponds to the melting or evaporation temperature of a tested substance. If the weight of the sample is known, then

the Differential Scanning Calorimeter (DSC) can calculate the energy required per gram of sample (J/g) for the transition. In an exothermic process, less heat would be required by the sample than by the reference cell to keep a steady change in temperature. In this case, the resulting peak on the thermogram is a negative peak. The heat of the phase change can be adsorbed or released depending on the change in specific heat characteristics of each phase.

Sample compounds investigated included millipure water, 17.5% and 35 % hydrogen peroxide solution, 99.9% pure Fe_2O_3, 99.9% pure TiO_2, tetrasodium pyrophosphate ($Na_4P_2O_7$), phenacetin ($C_{10}H_{13}NO_2$), quartz sand, JSC-1 martian regolith simulant soil, and combinations thereof. $Na_4P_2O_7$ and phenacetin are stabilizers of H_2O_2 (Fig. 3) and were included in the test set because, if the martian H_2O_2 is mostly of biological nature, a chemical stabilizer has to be invoked to control the reactivity of the hydrogen peroxide.



a) Sodium Pyrophosphate b) Phenacetin

Figure 3: Structural formula of chemical stabilizers of hydrogen peroxide, a. sodium pyrophosphate, b. phenacetin .

Other potential chemical stabilizers for H_2O_2 solutions include sodium silicate (Na_2SiO_3), poly(α)hydroxyacrylic acid, phytate, citrate, and malonate (Charron et al., 2006; Watts et al., 2007). Tetrasodium pyrophosphate was chosen here due to its simplicity, efficacy (e.g., common use in commercial applications), and its similarity to ATP. Phenacetin was chosen due to its demonstrated long-term (> 3 months) effectiveness to keep hydrogen peroxide stable (Madanská et al., 2004). The martian regolith simulant is the <1mm fraction of weathered volcanic ash from Pu'u Nene, a cinder cone on the island of Hawaii, and was provided by the Johnson Space Center. We also tested two bacterial cultures: *Lactococcus lactis subsp. lactis*, which has been shown to produce hydrogen peroxide concentrations up to a level of about 350 ppm (Ito et al., 2003), and *Pseudomonas sp.*, a non-hydrogen peroxide producing bacterium. *Lactococcus lactis* subsp. *lactis* bacteria were obtained from the American Type Culture Collection (ATCC #11454, Manassas, VA) and were propagated according to standard procedures. Briefly, the freeze-dried pellet was rehydrated in 1.0mL of ATCC#17 broth and then added to 5.0mL of ATCC#17 broth in a 50mL conical tube. The resulting mixture was incubated at 37°C with shaking for 24 hours. *Pseudomonas sp.*, cultured from a single colony from an agar plate, was resuspended in 5mL of sterile Tryptic Soy Broth (TSB, Difco #211825) in a sterile 50mL conical tube and incubated at 37°C with shaking for 24 hours.

The scanning rate of the DSC was set at 10°C per minute, and the sample amount used was 10 to 20 mg to simulate the thermograms that will be obtained by the TEGA instrument on Mars. Samples were put in standard 20 µl aluminum sample pans with covers and sealed with the standard sample pan crimper press. Each pan was fully loaded and weighted before and after the addition of the sample. The sample preparation was completed in less than 5 minutes, since it was observed that the hydrogen peroxide started to decompose under atmospheric conditions within 40 min, which affected some of the thermograms by not showing an exothermic peak (Figure 4). All data from the 20 minute and 40 minute DSC run were still saved in the archive files. An indium standard was used to calibrate the DSC and a baseline run with the use of an empty aluminum pan was conducted prior to each sample run. The DSC was programmed to automatically subtract each baseline run from the sample run.

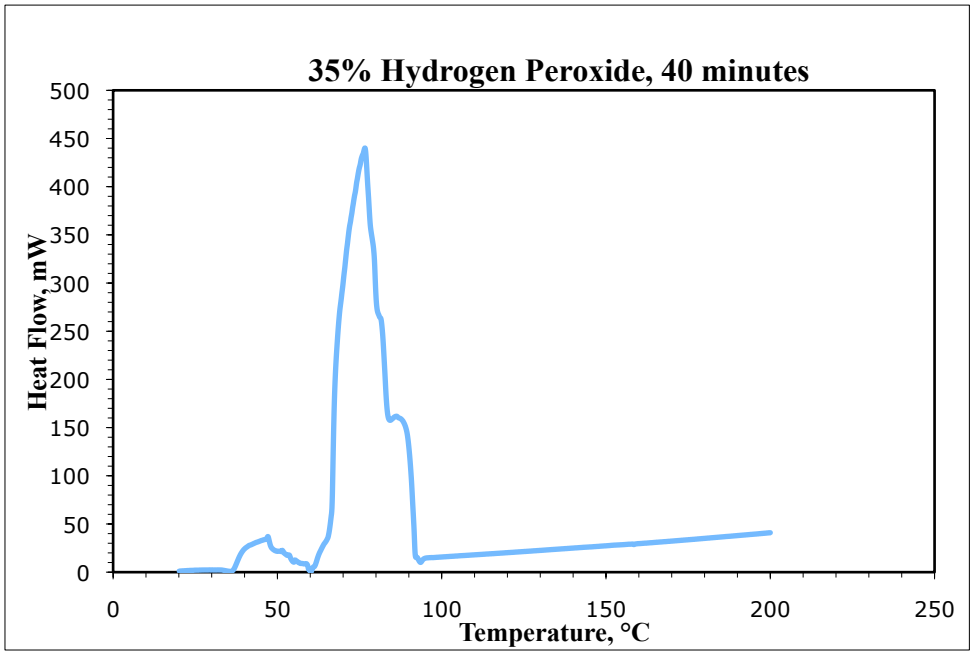


Figure 4: Example of 35% hydrogen peroxide thermogram exposed to the air for 40 minutes before commencing sample run.

MATERIALS AND METHODS

DETAILED DESCRIPTION

All DSC spectra were processed on a Perkin Elmer (Perkin Elmer, Waltham, MA USA) DSC 7 (Differential Scanning Calorimeter) instrument using the standard thermal analysis DSC 7 program. Two different types of sample pans were used in the analysis; the aluminum 20µl volatile two-piece pans (Perkin Elmer #02190062), which were sealed using the Perkin Elmer volatile sealer assembly (Perkin Elmer #02190061) and the standard aluminum sample pans with covers (Perkin Elmer #02190041) which were sealed using the standard sample pan crimper press (Perkin Elmer #02190048). Each pan was loaded up to the maximum amount of sample (20µl or 20mg) using a Labmate P20 pipette for the volatile samples and a stainless steel micro-spatula for the solid samples. Pans were weighed before and after the addition of sample so that a milligram weight could be determined for each sample. A baseline run using an empty aluminum pan of the appropriate type was used for each DSC run and an indium standard was used to calibrate the DSC 7 at the beginning of each day. All baseline files were automatically subtracted from the sample runs according to the parameters below. Independent replicates were run of each sample on separate days and samples were not re-used.

Reference Standards Parameters:

Each day before sample collection on the DSC 7, an indium standard was run and used to check the status of the instrument. Indium run parameters are shown below in

Table 1 and a standard indium reference peak of 157°C to 160°C was collected. The onset of the peak was 153.67°C (+/- 0.51°C) with a ΔH of 30.14 J/g (+/- 0.37 J/g) and a maximum peak of 158.71°C (+/- 1.34°C). Also, baselines for each type of sample pan used were collected and subtracted from the appropriate sample runs. Baseline parameters for each sample pan type are shown below in Table 2. The baseline file baseNwsh was used for the first batch of volatile aluminum sample pans, the baseline files bas2Nwsh was used for the second batch of volatile aluminum sample pans and the baseline file baseNws2 was used for the standard solid aluminum sample pans.

Table 1: Indium Standard DSC 7 Parameters

Final Temperature, ^{o}C	170
Start Temperature, ^{o}C	100
Scan Rate, in ^{o}C per minute	10
Y-Axis, heat flow in milliwats (mW)	100
Sample weight in milligrams	8.0mg
Baseline	Yes, baseNwsh, baseNws2 or bas2Nwsh
Multitasking (yes or no)	No

Table 2: Baseline DSC 7 Parameters (files, baseNwsh, bas2Nwsh or basNws2)

Final Temperature, ^{o}C	210
Start Temperature, ^{o}C	10
Scan Rate, in ^{o}C per minute	10
Y-Axis, heat flow in milliwats (mW)	300
Sample weight in milligrams	0mg
Baseline	No
Multitasking (yes or no)	No

Millipure Water samples (MPH2O):

Millipure water was obtained from a nanopure filtration device (Millipore Inc, USA) and stored in sterile brown glass 500mL media jars until use. Immediately before use, 5mL aliquots were transferred to 15mL glass sample jars with sealing polypropylene

lids. The 20µl portions were then transferred from the sample jar to the volatile aluminum sample pans using a P100 pipet. The pans were sealed using the volatile sealer assembly. The sample pan was then placed directly into the DSC 7 and run with the parameters below in Table 3. The water sample was exposed to the atmosphere for no more than 5 minutes during the preparation.

Table 3: MP H2O DSC 7 Parameters:

Final Temperature, ^{o}C	150
Start Temperature, ^{o}C	20
Scan Rate, in ^{o}C per minute	10
Y-Axis, heat flow in milliwats (mW)	320
Sample weight in milligrams	Varies per sample between 12-22mg
Baseline	Yes, file = baseNwsh, volatile pan
Multitasking (yes or no)	No

35% Hydrogen Peroxide samples (35% H2O2):

35% Hydrogen Peroxide, 500mL, was obtained from the Acros Organics Chemical Company (catalogue #202465000) and stored in a 500mL sterile brown glass media jar at +4^{o}C until use. Immediately before use, small aliquots (5-10mL) were transferred to 15mL glass sample jars with sealing polypropylene lids. Fresh samples were exposed to the atmosphere for no more than 5 minutes. For the samples reacted with the atmosphere, the 35% hydrogen peroxide was allowed to sit in the sterile glass sample jar with the lid removed for 20 or 40 minutes. The samples indicated as “fresh” were transferred from the 15mL glass sample jar to the volatile aluminum samples pans using a P100 pipet (20µl portions) and sealed using the volatile sealer assembly. The sample pans were then placed directly into the DSC 7 and run using the parameters below in Table 4. The 20 minute and 40 minute samples were prepared and run in the same manner except

they were allowed to sit in the glass sample jar with the lid off for either 20 or 40 minutes.

Table 4: 35% H2O2 DSC 7 Parameters:

Final Temperature, ^{o}C	200
Start Temperature, ^{o}C	20
Scan Rate, in ^{o}C per minute	10
Y-Axis, heat flow in milliwats (mW)	200
Sample weight in milligrams	Varies per sample between 12-22mg
Baseline	Yes, file = baseNwsh or bas2Nwsh, volatile pan
Multitasking (yes or no)	No

17.5% Hydrogen Peroxide (17.5% H2O2):

The 17.5% hydrogen peroxide solution was made by combining 4mL of the 35% hydrogen peroxide mentioned above with 4mL of the millipure water with mixing into q 15mL glass sample jar with sealing polypropylene lid. The solution was used immediately (fresh sample, up to 5 minutes to prepare) or was exposed to the atmosphere for 20 or 40 minutes. The samples indicated as "fresh" were transferred from the 15mL glass sample jar to the volatile aluminum samples pans using a P100 pipet (20µl portions) and sealed using the volatile sealer assembly. The sample pans were then placed directly into the DSC 7 and run using the parameters below in Table 5. The 20 minute and 40 minute samples were prepared and run in the same manner except they were allowed to sit in the glass sample jar with the lid off for either 20 or 40 minutes.

Table 5: 17.5% H2O2 DSC 7 Parameters:

Final Temperature, ^{o}C	200
Start Temperature, ^{o}C	20
Scan Rate, in ^{o}C per minute	10
Y-Axis, heat flow in milliwats (mW)	200
Sample weight in milligrams	Varies per sample between 12-22mg
Baseline	Yes, file = baseNwsh or bas2Nwsh, volatile pan
Multitasking (yes or no)	No

35% Hydrogen Peroxide with 10% (w/v) Tetrasodium Pyrophosphate, anhydrous (35% H2O2/10% NaPPi)

One gram of tetrasodium pyrophosphate or sodium pyrophosphate (anhydrous, NaPPi) was obtained from Sigma-Aldrich Chemical Company (catalogue #P-8010) and combined with 9.0mL of the 35% hydrogen peroxide with stirring to obtain a final concentration of 10% NaPPi in a sealed 15mL glass jar with sealing polypropylene lid. The samples indicated as "fresh" were transferred from the 15mL glass sample jar to the volatile aluminum samples pans using a P100 pipet (20µl portions) and sealed using the volatile sealer assembly. The sample pans were then placed directly into the DSC 7 and run using the parameters below in Table 6. The 20 minute and 40 minute samples were prepared and run in the same manner except they were allowed to sit in the glass sample jar with the lid off for either 20 or 40 minutes.

Table 6: 35% H2O2/10%NaPPi DSC 7 Parameters:

Final Temperature, ^{o}C	200
Start Temperature, ^{o}C	20
Scan Rate, in ^{o}C per minute	10
Y-Axis, heat flow in milliwats (mW)	200
Sample weight in milligrams	Varies per sample between 12-22mg
Baseline	Yes, file = baseNwsh or bas2Nwsh, volatile pan
Multitasking (yes or no)	No

JSC-1 Martian Analog Soil Samples

A 200 g aliquot of JSC-1 Martian regolith simulant was obtained from the NASA Johnson Space Center through Dr. Carlton C. Allen. JSC-1 samples were run with the soil alone, with 10% Millipure water, (from above) with 10%, 35% hydrogen peroxide (from above), with 10%, 17.5% hydrogen peroxide (above) or with 10% of the 35%

hydrogen peroxide/10% sodium pyrophosphate mixture (from above). The JSC-1 soil samples were run using the standard aluminum sample pans and covers, with a stainless steel micro-spatula used to transfer the soil from the glass sample jar to the sample pan. The standard aluminum sample pans were sealed using the standard pan crimper press and then placed immediately into the DSC-7 and run according to the parameters below. All mixtures were prepared as before in 15mL glass sample jars with sealing polypropylene lids. Mixing was achieved with a glass stir rod. All samples were used "fresh" and were allowed to react with the atmosphere for no more than 5 minutes. Samples were transferred from the 15mL glass sample jars to the standard solid aluminum sample pans using a stainless steel micro-spatula and were then sealed using the standard sample pan crimper press. The sample pans were placed immediately into the DSC-7 and run according to the parameters in table 7.

Table 7: JSC-1 Soil Simulant DSC-7 Parameters:

Final Temperature, ^{o}C	200
Start Temperature, ^{o}C	20
Scan Rate, in ^{o}C per minute	10
Y-Axis, heat flow in milliwats (mW)	200
Sample weight in milligrams	Varies per sample between 16-25mg
Baseline	Yes, file = baseNws2, solid pan
Multitasking (yes or no)	No

Quartz Samples

The crystalline silica (quartz) used for the quartz samples was obtained from Quikrete ® (Quikrete Play Sand, Premium 22.7kg, catalogue #1113). Quartz samples were run with the quartz alone, with 10% Millipure water, (from above) with 10%, 35% hydrogen peroxide (from above), with 10%, 17.5% hydrogen peroxide (above) or with 10% of the 35% hydrogen peroxide/10% sodium pyrophosphate mixture (from above).

The quartz samples were run using the standard aluminum sample pans and covers, with a stainless steel micro-spatula used to transfer the quartz from the glass sample jar to the sample pan. The standard aluminum sample pans were sealed using the standard pan crimper press and then placed immediately into the DSC-7 and run according to the parameters below. All mixtures were prepared as before in 15mL glass sample jars with sealing polypropylene lids. Mixing was achieved with a glass stir rod. All samples were used "fresh" and were allowed to react with the atmosphere for no more than 5 minutes. Samples were transferred from the 15mL glass sample jars to the standard solid aluminum sample pans using a stainless steel micro-spatula and were then sealed using the standard sample pan crimper press. The sample pans were placed immediately into the DSC-7 and run according to the parameters in table 8.

Table 8: Quartz Sand DSC-7 Parameters

Final Temperature, ^{o}C	200
Start Temperature, ^{o}C	20
Scan Rate, in ^{o}C per minute	10
Y-Axis, heat flow in milliwats (mW)	200
Sample weight in milligrams	Varies per sample between 19-25mg
Baseline	Yes, file = baseNws2, solid pan
Multitasking (yes or no)	No

Iron (III) Oxide Samples

The iron (III) oxide (anhydrous) was obtained from Fisher Chemicals (catalogue #I116-500). Iron oxide samples were run with the chemical alone, with 10% Millipure water, (from above) with 10%, 35% hydrogen peroxide (from above), with 10%, 17.5% hydrogen peroxide (above) or with 10% of the 35% hydrogen peroxide/10% sodium pyrophosphate mixture (from above). The iron oxide samples were run using the standard aluminum sample pans and covers, with a stainless steel micro-spatula used to transfer

the iron oxide from the glass sample jar to the sample pan. The standard aluminum sample pans were sealed using the standard pan crimper press and then placed immediately into the DSC-7 and run according to the parameters below. All mixtures were prepared as before in 15mL glass sample jars with sealing polypropylene lids. Mixing was achieved with a glass stir rod. All samples were used "fresh" and were allowed to react with the atmosphere for no more than 5 minutes. Samples were transferred from the 15mL glass sample jars to the standard solid aluminum sample pans using a stainless steel micro-spatula and were then sealed using the standard sample pan crimper press. The sample pans were placed immediately into the DSC-7 and run according to the parameters in table 9.

Table 9: Iron (III) Oxide DSC-7 Parameters

Final Temperature, ^{o}C	200
Start Temperature, ^{o}C	20
Scan Rate, in ^{o}C per minute	10
Y-Axis, heat flow in milliwats (mW)	200
Sample weight in milligrams	Varies per sample between 17-22mg
Baseline	Yes, file = baseNws2, solid pan
Multitasking (yes or no)	No

Titanium Oxide Samples

The titanium (IV) oxide (anhydrous) was obtained from Fisher Chemicals (catalogue #202465000). Titanium oxide samples were run with the chemical alone, with 10% Millipure water, (from above) with 10%, 35% hydrogen peroxide (from above), with 10%, 17.5% hydrogen peroxide (above) or with 10% of the 35% hydrogen peroxide/10% sodium pyrophosphate mixture (from above). The titanium oxide samples were run using the standard aluminum sample pans and covers, with a stainless steel micro-spatula used to transfer the titanium oxide from the glass sample jar to the sample

pan. The standard aluminum sample pans were sealed using the standard pan crimper press and then placed immediately into the DSC-7 and run according to the parameters below. All mixtures were prepared as before in 15mL glass sample jars with sealing polypropylene lids. Mixing was achieved with a glass stir rod. All samples were used "fresh" and were allowed to react with the atmosphere for no more than 5 minutes. Samples were transferred from the 15mL glass sample jars to the standard solid aluminum sample pans using a stainless steel micro-spatula and were then sealed using the standard sample pan crimper press. The sample pans were placed immediately into the DSC-7 and run according to the parameters in table 10.

Table 10: Titanium (IV) Oxide DSC-7 Parameters

Final Temperature, ^{o}C	200
Start Temperature, ^{o}C	20
Scan Rate, in ^{o}C per minute	10
Y-Axis, heat flow in milliwats (mW)	200
Sample weight in milligrams	Varies per sample between 14-20mg
Baseline	Yes, file = baseNws2, solid pan
Multitasking (yes or no)	No

Phenacetin Samples

The phenacetin was obtained from MP Biomedicals, LLC (catalogue #151817). Phenacetin samples were run with the chemical alone, as a 10% mixture in the 35% hydrogen peroxide from above, and as a 10% mixture with the hydrogen peroxide from above and exposure to the atmosphere for 20 minutes or 40 minutes. The phenacetin samples were run using the standard aluminum sample pans and covers, with a stainless steel micro-spatula used to transfer the solid from the glass sample jar to the sample pan. The standard aluminum sample pans were sealed using the standard pan crimper press and then placed immediately into the DSC-7 and run according to the parameters below.

All mixtures were prepared as before in 15mL glass sample jars with sealing polypropylene lids. Mixing was achieved with a glass stir rod. For the samples reacted with the atmosphere, the 35% hydrogen peroxide/10% phenacetin mixture was allowed to sit in the sterile glass sample jar with the lid removed for 20 or 40 minutes. The samples indicated as "fresh" were allowed to react with the atmosphere for no more than 5 minutes. All samples were transferred from the 15mL glass sample jar to the volatile aluminum samples pans using a P100 pipet (20µl portions) and sealed using the volatile sealer assembly. The sample pans were then placed directly into the DSC 7 and run using the parameters below in Table 11.

Table 11: Phenacetin DSC-7 Parameters

Final Temperature, ^{o}C	200
Start Temperature, ^{o}C	20
Scan Rate, in ^{o}C per minute	10
Y-Axis, heat flow in milliwats (mW)	200
Sample weight in milligrams	Varies per sample between 16-22mg
Baseline	Yes, file = base2Nwsh, volatile pan
Multitasking (yes or no)	No

Bacterial Samples

We also tested two bacterial cultures: *Lactococcus lactis subsp. lactis*, which has been shown to produce hydrogen peroxide concentrations up to a level of about 350 ppm (Ito et al., 2003), and *Pseudomonas sp.*, a non-hydrogen peroxide producing bacterium. *Lactococcus lactis* subsp. *lactis* bacteria were obtained from the American Type Culture Collection (ATCC #11454, Manassas, VA) and were propagated according to standard procedures. Briefly, the freeze-dried pellet was rehydrated in 1.0mL of ATCC#17 broth and then added to 5.0mL of ATCC#17 broth in a 50mL conical tube. The resulting mixture was incubated at 37°C with shaking for 24 hours. *Pseudomonas sp.*, cultured

from a single colony from an agar plate, was resuspended in 5mL of sterile Tryptic Soy Broth (TSB, Difco #211825) in a sterile 50mL conical tube and incubated at 37°C with shaking for 24 hours.

All samples used were allowed to react with the atmosphere for no more than 5 minutes. Samples were transferred from the 50mL conical tube to the standard solid aluminum sample pans using a Labmate P20 (20µl) and were then sealed using the standard sample pan crimper press. The sample pans were placed immediately into the DSC-7 and run according to the parameters in table 12.

Table 12: Bacterial Sample DSC-7 Parameters

Final Temperature, ^{o}C	200
Start Temperature, ^{o}C	20
Scan Rate, in ^{o}C per minute	10
Y-Axis, heat flow in milliwats (mW)	200
Sample weight in milligrams	Varies per sample between 16-21mg
Baseline	Yes, file = baseNws2, solid pan
Multitasking (yes or no)	No

Data Analysis

All sample data files were saved on the Perkin Elmer DSC-7 as text files and then transferred to Microsoft Excel and converted into Excel files and all paper copies of the run parameters were saved in a three-ring binder. As discussed above, all baseline files were automatically subtracted from the sample runs, however, the heat flow raw data from the text files must be manually corrected due to a "shift" that occurs when the raw data is saved on disk from the DSC-7 and then imported to Excel. The manual correction involves recording up to seven temperature and associated heat flow (value = Y) values from the DSC-7 sample run. These values are recorded in the Excel spreadsheet along with the Excel heat flow (value = Y1) values corresponding to the recorded DSC-7

temperature values. The correction value is calculated by dividing the Y values (DSC-7 heat flow values) by the Y1 values (Excel heat flow values). These corrected heat flow (mW) values are then averaged together. The Excel heat flow values are then divided by the average heat flow value (corrected). This results in a corrected heat flow (mW) value that can them be graphed with the corresponding temperature (°C) values. All values for the heat of decomposition were taken directly from the areas of the curves (or negative curves) on the DSC-7 read-out. Readings were recorded as J/g and then converted to kJ/mol for comparison to theoretical values.

RESULTS

The thermograms of various solutions of different concentrations of hydrogen peroxide are shown in Figure 5. This group of thermograms was used to determine the limit of detection of hydrogen peroxide using the DSC-7. The graph shows that a range of concentrations was successfully detected from 70 ppm (.007%) to 438 ppm (.04%) but the sharpest thermogram peak was observed at the 368 ppm (about .04%) concentration.

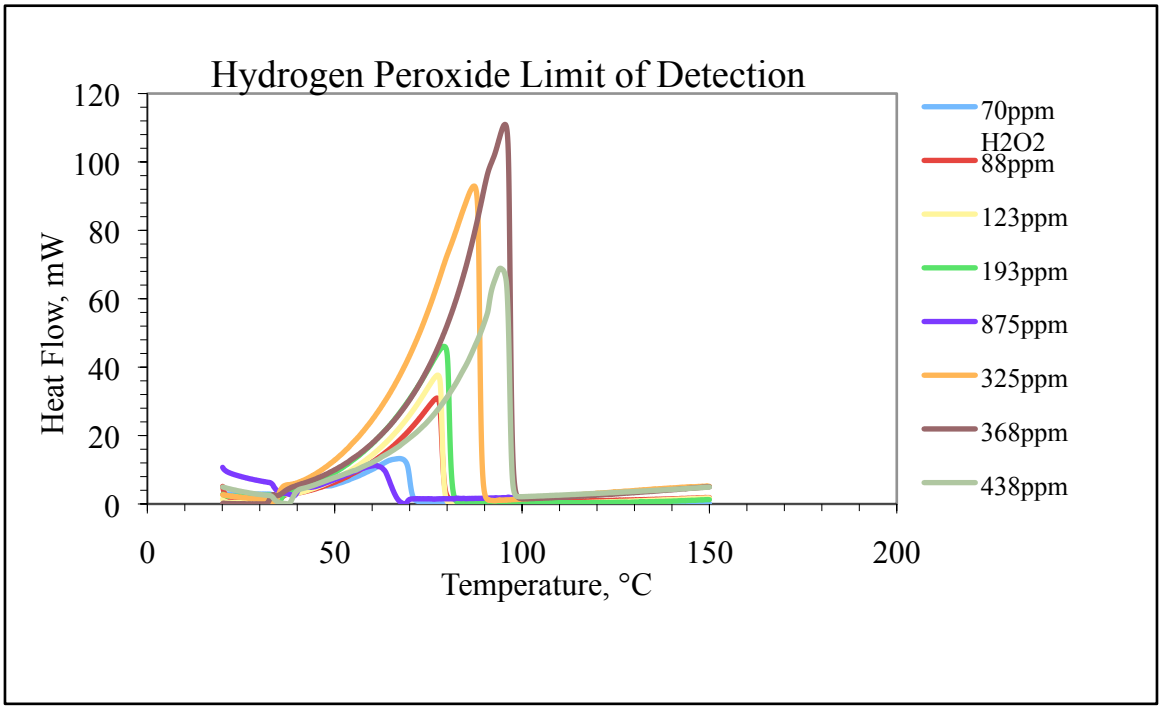


Figure 5: Various concentrations of hydrogen peroxide (ppm) used to determine the limit of detection of hydrogen peroxide solutions on the DSC-7.

The thermograms of the various compounds and solutions of hydrogen peroxide with and without the chemical stabilizers are shown in Figures 6 through 11 below. Any

differences observed in peaks, ranges, and energies in duplicate runs (Fig. 6-11) were a function of the total amount of solutes used in the DSC and due to instrument variation.

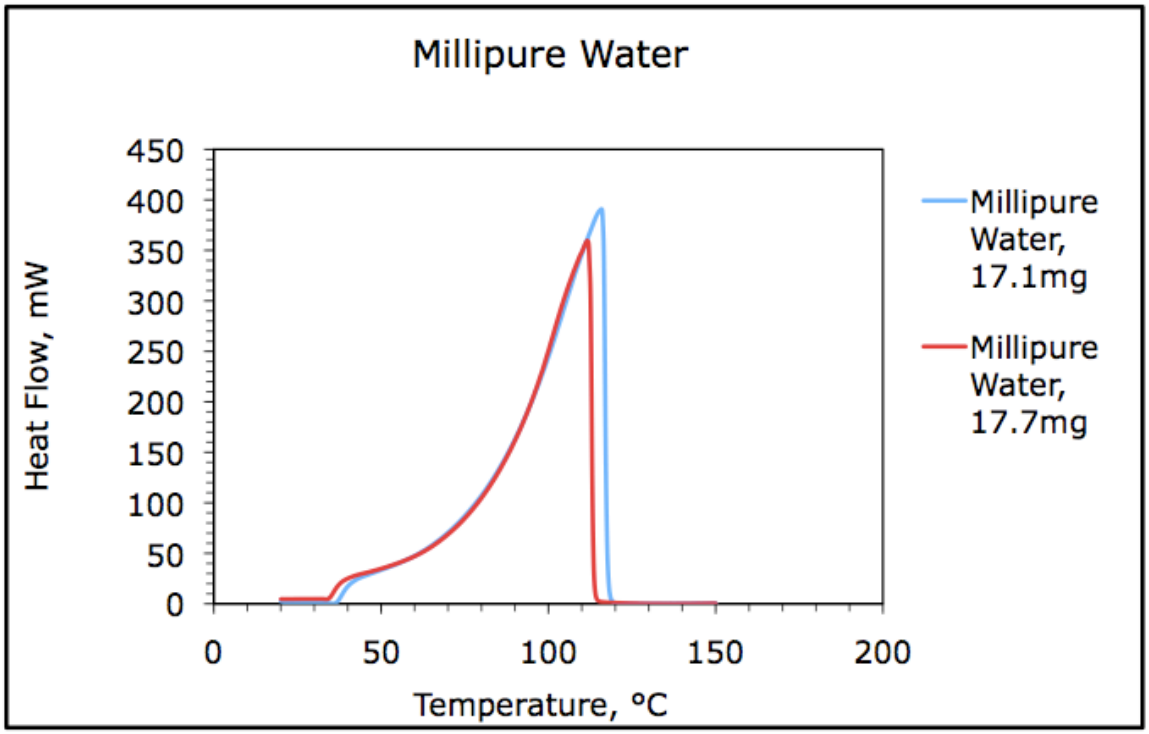


Figure 6: Millipure water thermogram with a peak value of 114°C (+/- 3°C). The peak ranged from 80°C (+/- 1°C) to 117°C (+/- 3°C) and the peak area was 1403 J/g (+/- 105 J/g).

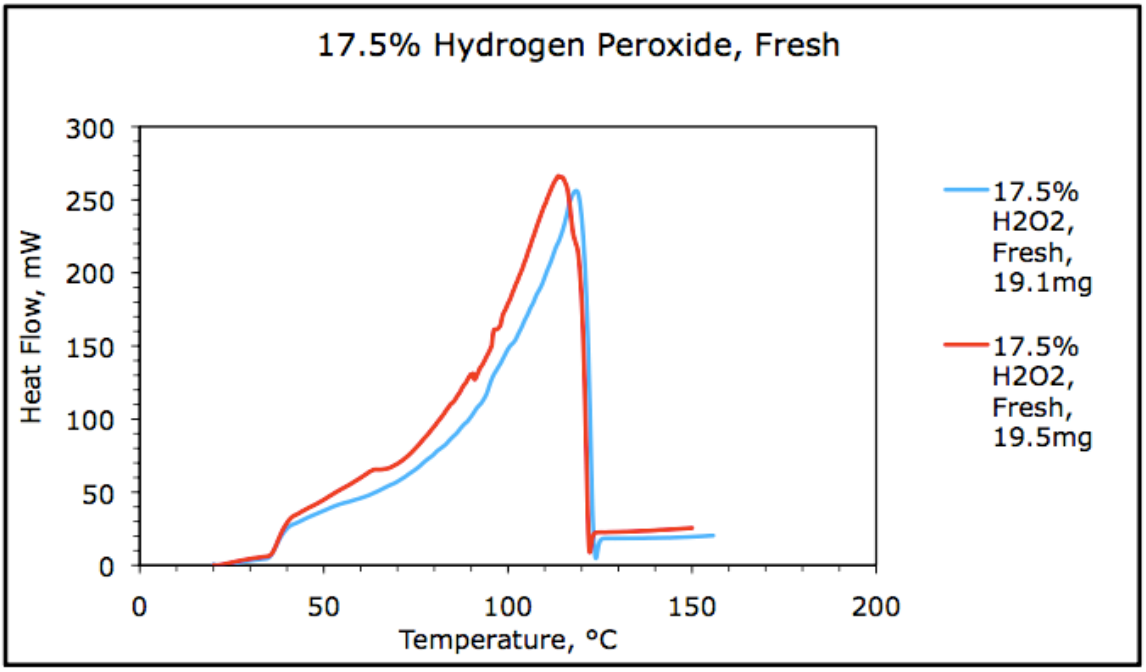


Figure 7: 17.5% Hydrogen peroxide solution thermogram with a peak value of 117°C (+/- 3°C). The peak area was 1270 J/g (+/- 0.2 J/g) with the peak ranging from 68°C (+/- 4) to 123°C (+/- 2°C). The solution also exhibited a small negative peak at 123°C (+/- 1°C) with an area of –7 J/g (+/- 0.3°C).

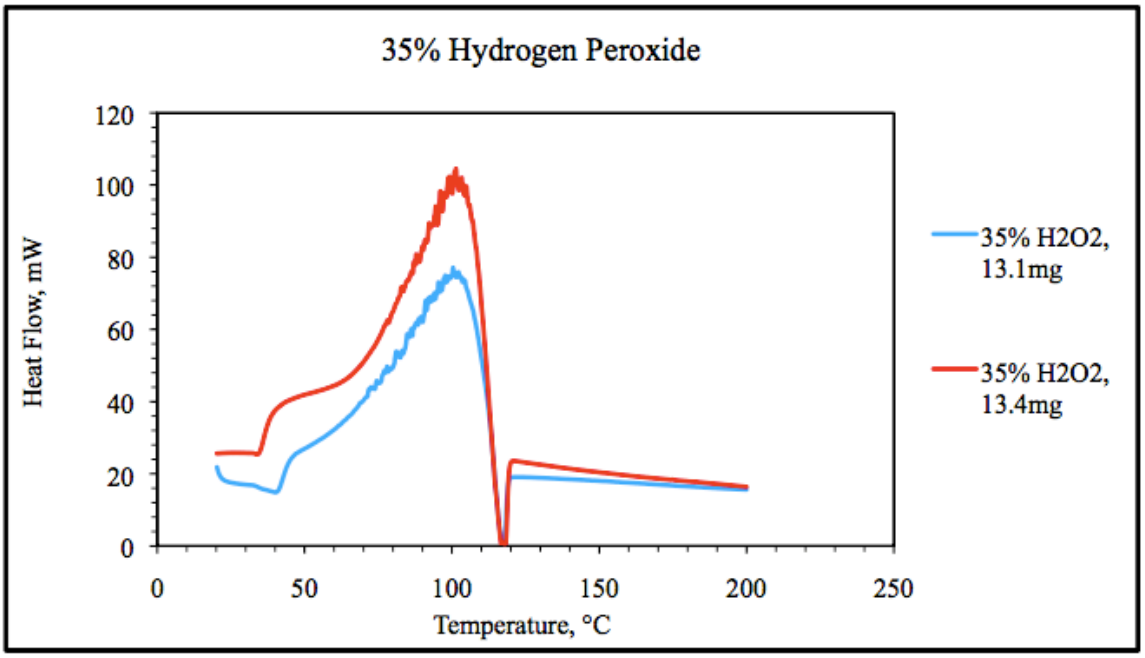


Figure 8: 35% Hydrogen peroxide solution thermogram with a peak value of 101°C (+/- 1°C). The peak ranged from 52°C (+/-1) to 117°C +/-1). The negative peak is at 117°C (+/-1°C) with an area of -27J/g (+/- 5J/g).

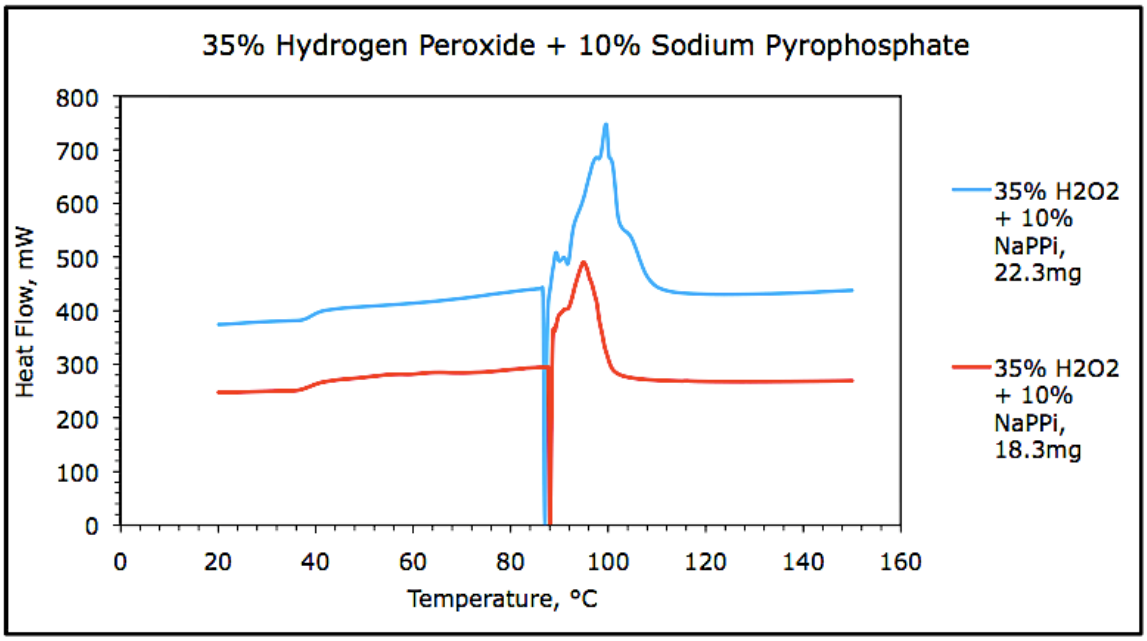


Figure 9: 35% Hydrogen peroxide solution thermogram with sodium pyrophosphate at a ratio of 9 to 1. The thermogram revealed a large exothermic peak at about 86°C with an area of –103 J/g (+/- 71 J/g). The main peak had a value of 97°C (+/- 3°C), a range from 87°C (+/- 0.5°C) to 107°C (+/- 6°C) and an area of 874 J/g (+/- 570 J/g).

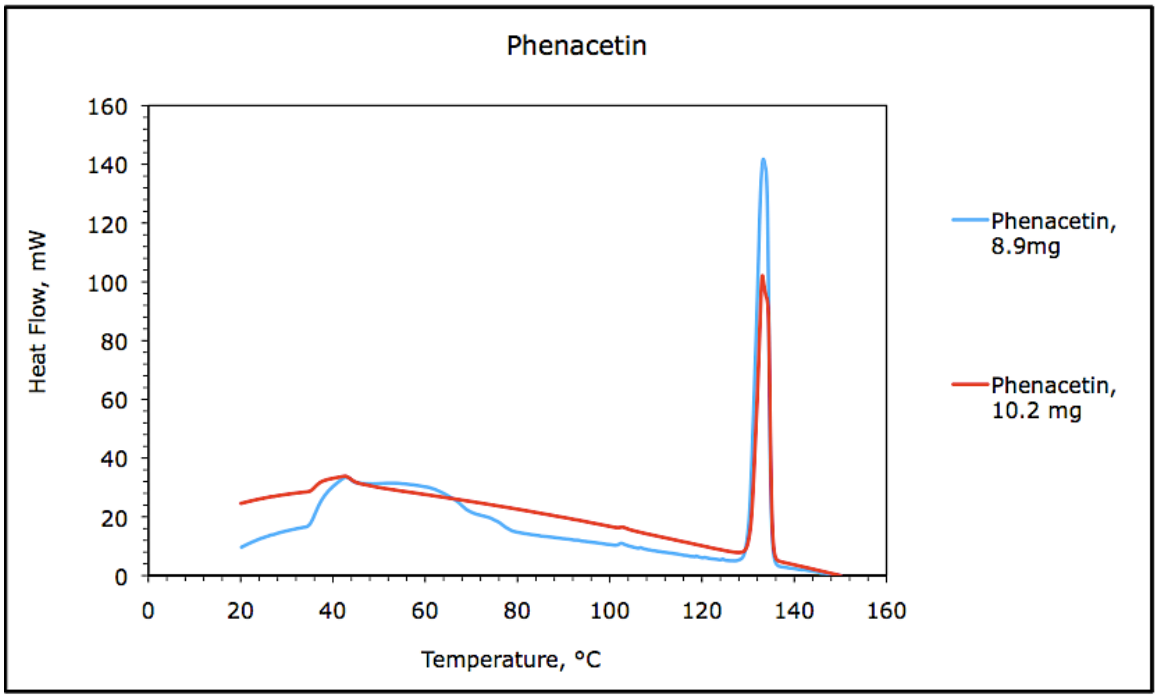


Figure 10: The phenacetin thermogram revealed a single peak at 133°C (+/- 1°C) with an area under the curve of 173 J/g (+/- 8 J/g).

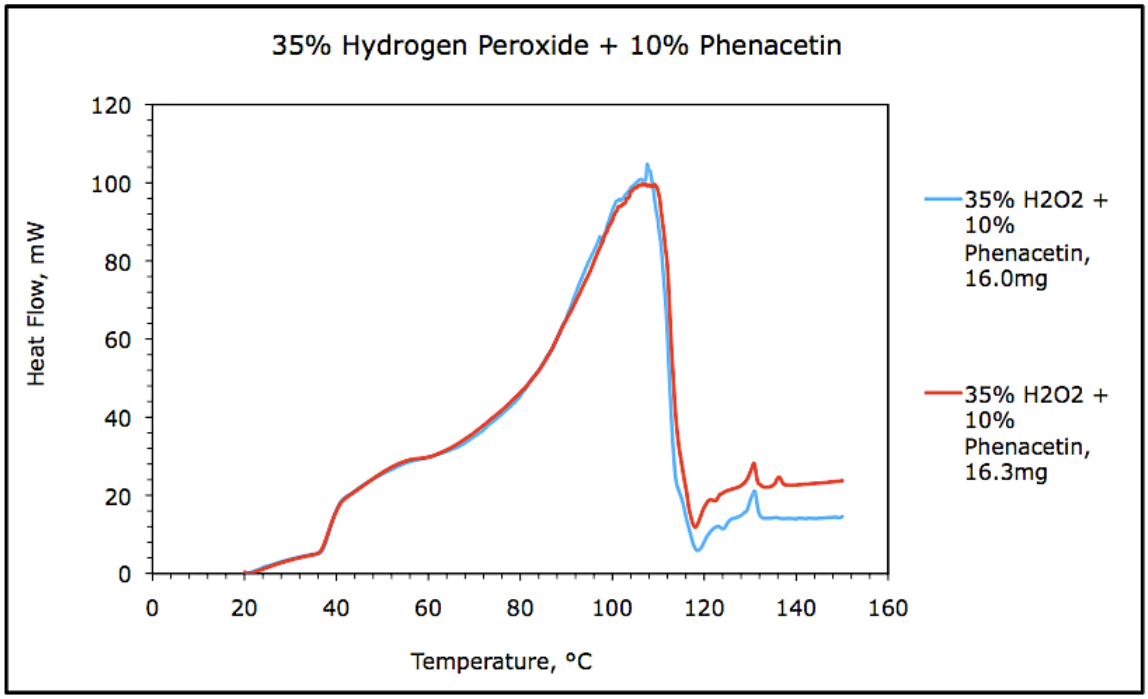


Figure 11: 35 % hydrogen peroxide solution with 10 % phenacetin thermogram. The peak was at 108°C (+/- 1°C) with a range of 61°C (+/- 3°C) to 118°C (+/- 1°C) and an area under the curve of 785 J/g (+/- 37 J/g). The phenacetin peak appears as a minor peak at a value of 131°C (+/- 1°C) with an area of 4 J/g (+/- 1 J/g). The solution also displayed again a small negative peak at 118°C (+/- 1°C) characteristic for the exothermic reaction of H_2O_2 with an area under the curve of –8 J/g (+/- 1 J/g).

The millipure water (Fig. 6) revealed a behavior characteristic of the phase transition from liquid water to water vapor with a peak at about 110°C. The 17.5 % hydrogen peroxide solution revealed a very similar behavior to pure water, but also exhibited a small negative peak at 123°C (Fig. 7). This negative peak was more pronounced when using a 35 % hydrogen peroxide solution (Fig. 8). The area under the large positive (endothermic) peak of the 35 % hydrogen peroxide solution is 1.096 kJ/g +/- 0.31 kJ/g. A solution of 35% hydrogen peroxide with tetra sodium pyrophosphate at a

ratio of 9:1 revealed a very different pattern (Fig. 9). It produced a strong exothermic response at about 80°C and a large endothermic peak close to 100°C. The thermogram of the chemical stabilizer phenacetin reveals a characteristic single peak at about 132°C (Fig. 10), which is revealed as well in the thermogram of the 35 % hydrogen peroxide solution when phenacetin was added at a 9:1 mass ratio of 35% H_2O_2 to phenacetin (Fig. 11). The phenacetin peak appears at a value of 131°C for the hydrogen peroxide solution, while the main peak correlates to 108°C. The thermogram displays again a small negative peak at 118°C. Thermograms at slower scan rates were also produce (Figure 12).

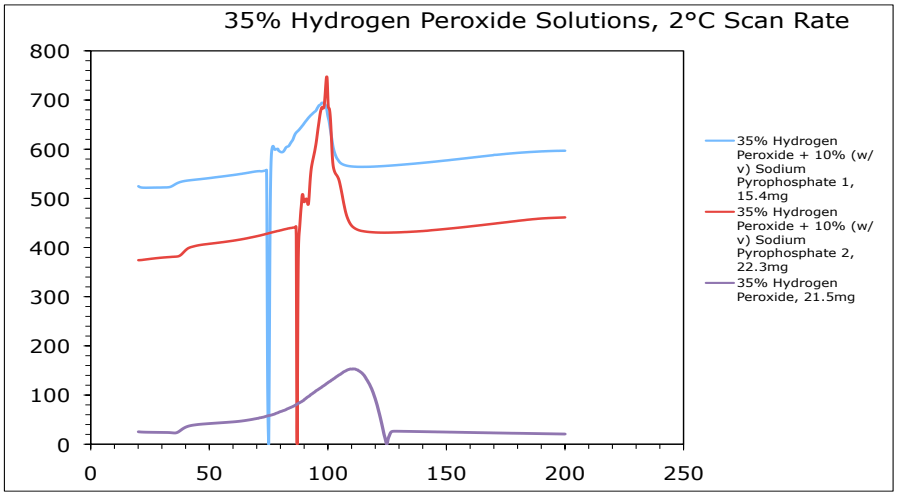


Figure 12: 35% hydrogen peroxide and 35% hydrogen peroxide with 10% tetra sodium pyrophosphate run at a scan rate of 2°C per minute. The sodium pryophosphate solutions had negative peaks at 74°C and 86°C respectively, while the 35% hydrogen peroxide gave a negative peak at 123°C.

In the next set of sampling runs, various soil media and metal oxides were exposed to the previously tested solutions (Figures 13-16 below). The soil media included JSC-1 Mars analog soil and quartz sand while the metal oxides used were iron (III) oxide and titanium (IV) oxide.

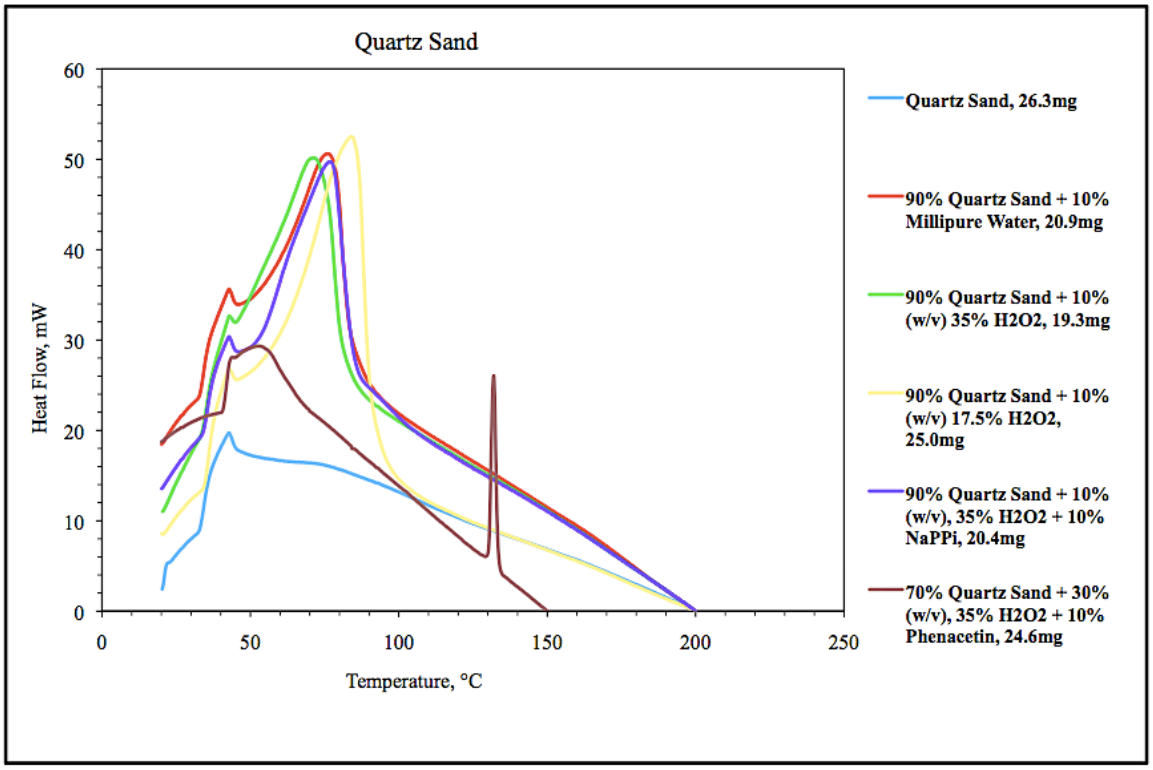


Figure 13: Thermogram of quartz sand and quartz sand solutions. The first peaks appear at a value of 44°C (+/- 4°C) with a range of 35°C (+/- 6°C) to 50°C (+/- 9°C) and a peak area of 8 J/g (+/- 1 J/g). The second, larger peaks have a value of 77°C (+/- 5°C) with a range from 45°C (+/- 1°C) to 91°C (+/- 6°C) and a peak area of 117 J/g (+/- 21 J/g). A sharp phenacetin peak is observed at 132°C for the hydrogen peroxide solution with 10 % phenacetin.

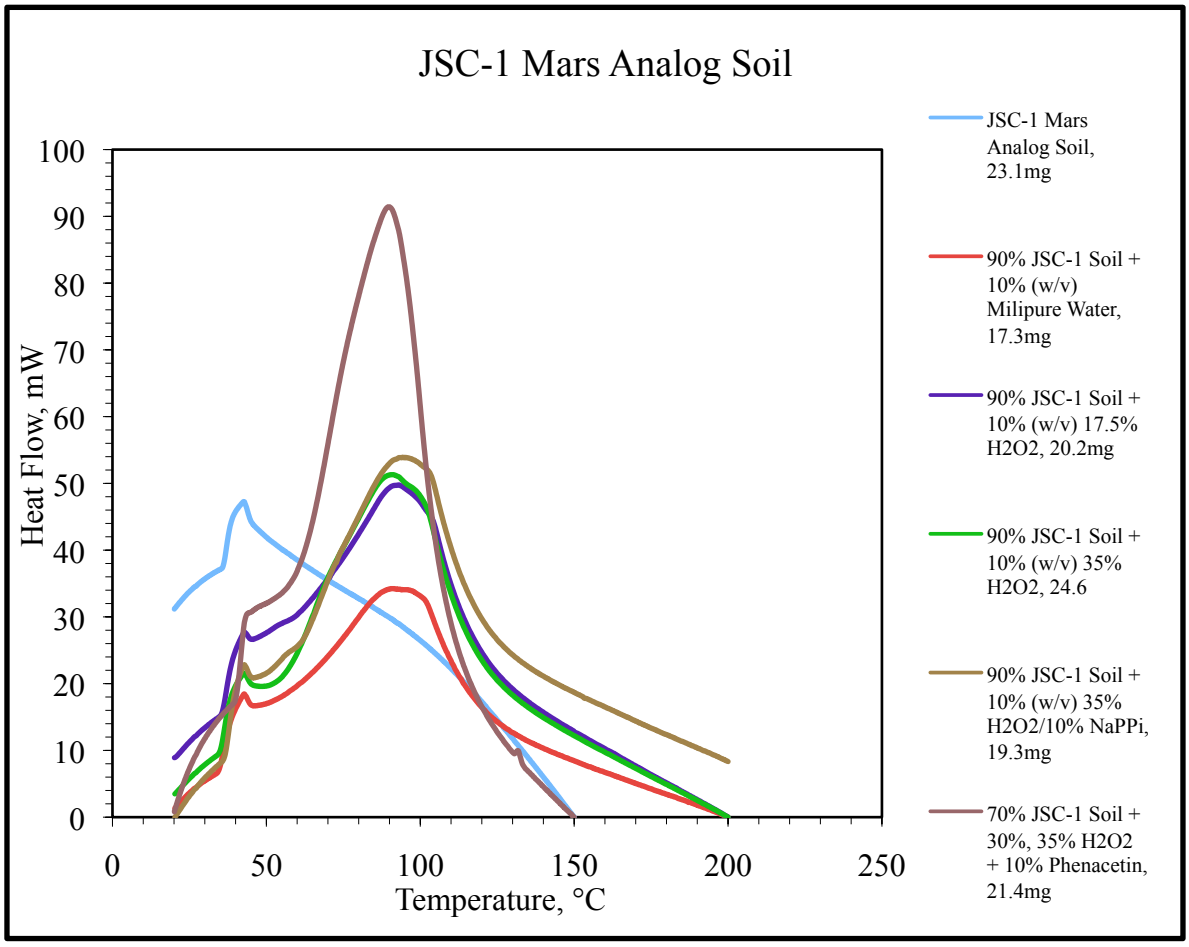


Figure 14: JSC-1 Mars stimulant soil and solutions thermogram. The first peaks appear at a value of 41°C (+/- 1°C) with a range from 35°C (+/- 1°C) to 46°C (+/- 1°C) and a peak area of 9 J/g (+/- 2 J/g). The second, larger peaks average at a value of 92°C (+/- 2°C) with a range from 55°C (+/- 6°C) to 120°C (+/- 8°C) and a peak area of 224 J/g (+/- 47 J/g). The phenacetin peak is again observed at a value of 132°C, but smaller in magnitude.

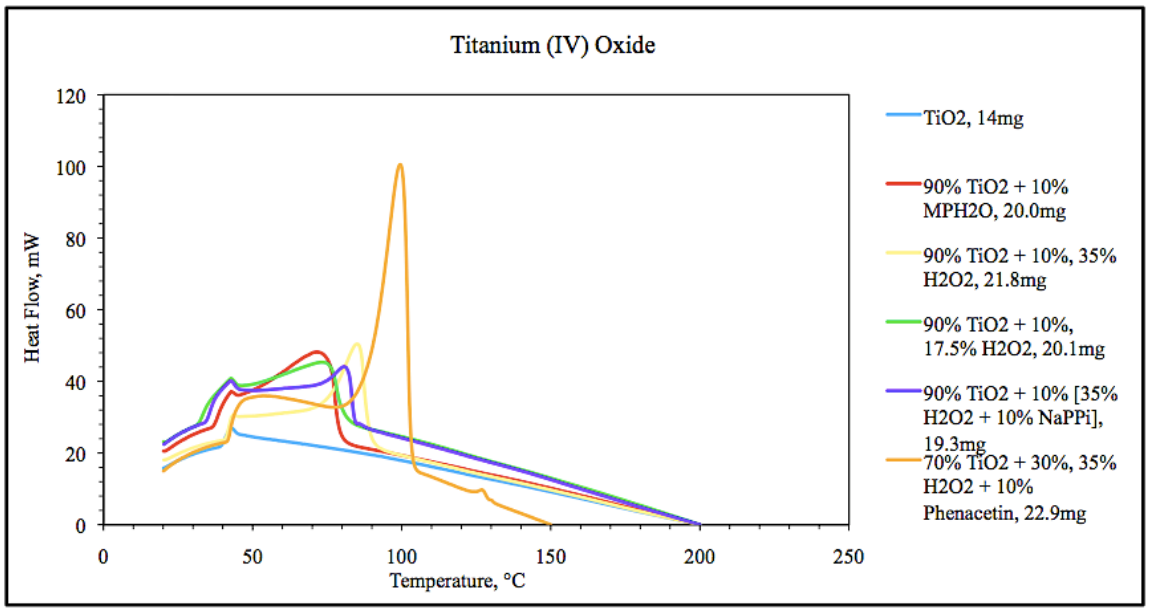


Figure 15: Titanium (IV) oxide and solutions thermogram. The first peaks have a value of 42°C (+/- 1°C) with a range from 35°C (+/- 4°C) to 46°C (+/- 1°C) and a peak area of 6 J/g (+/- 3 J/g). The second, larger peaks show much variation with a range from 62°C (+/- 16°C) to 90°C (+/- 9°C) and an average peak area of 69 J/g (+/- 41 J/g). The phenacetin peak is observed again at a value of 127°C.

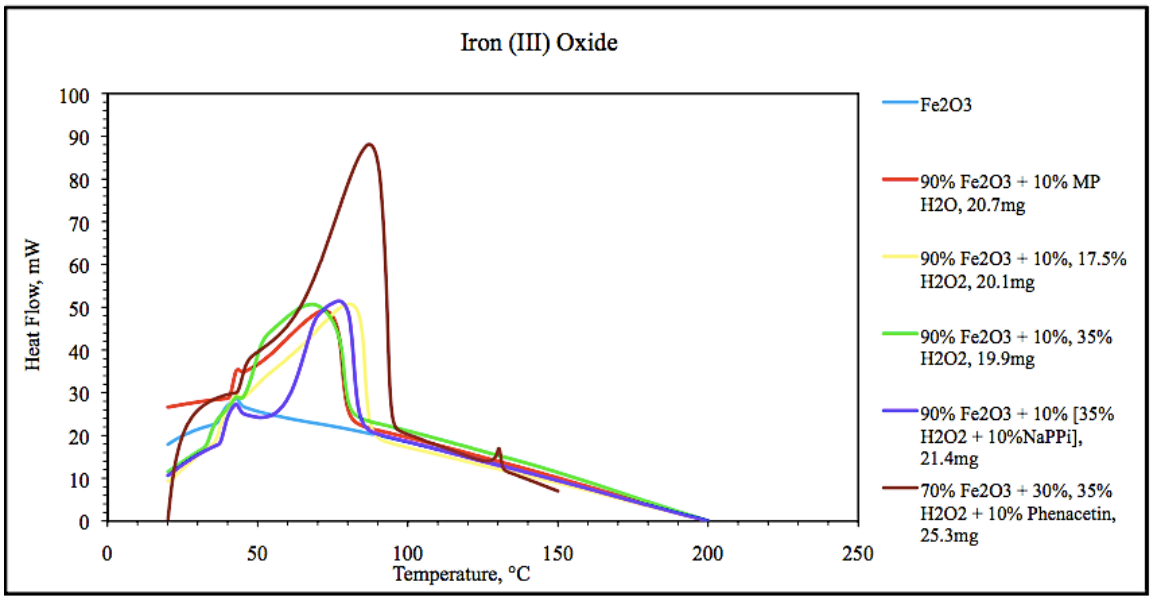


Figure 16: Iron (III) oxide and solutions thermogram. The first peaks have a value of 41°C (+/- 3°C) with a range from 37°C (+/- 4°C) to 47°C (+/- 3°C) and a peak area of 7 J/g (+/- 4 J/g). The second, larger peaks vary largely with a range from 49°C (+/- 6°C) to 85°C (+/- 11°C) and an average peak area of 146 J/g (+/- 49 J/g). The phenacetin peak is observed at a value of 130°C.

When the mass ratio of the phenacetin spiked hydrogen peroxide solution was lowered to 10 % of the mass fraction of the soil (phenacetin ~ 1 % of the total mass of the soil), the signature of the phenacetin was too small to be unambiguously identified in the JSC-1 soil and the metal oxides tested (Figure 17).

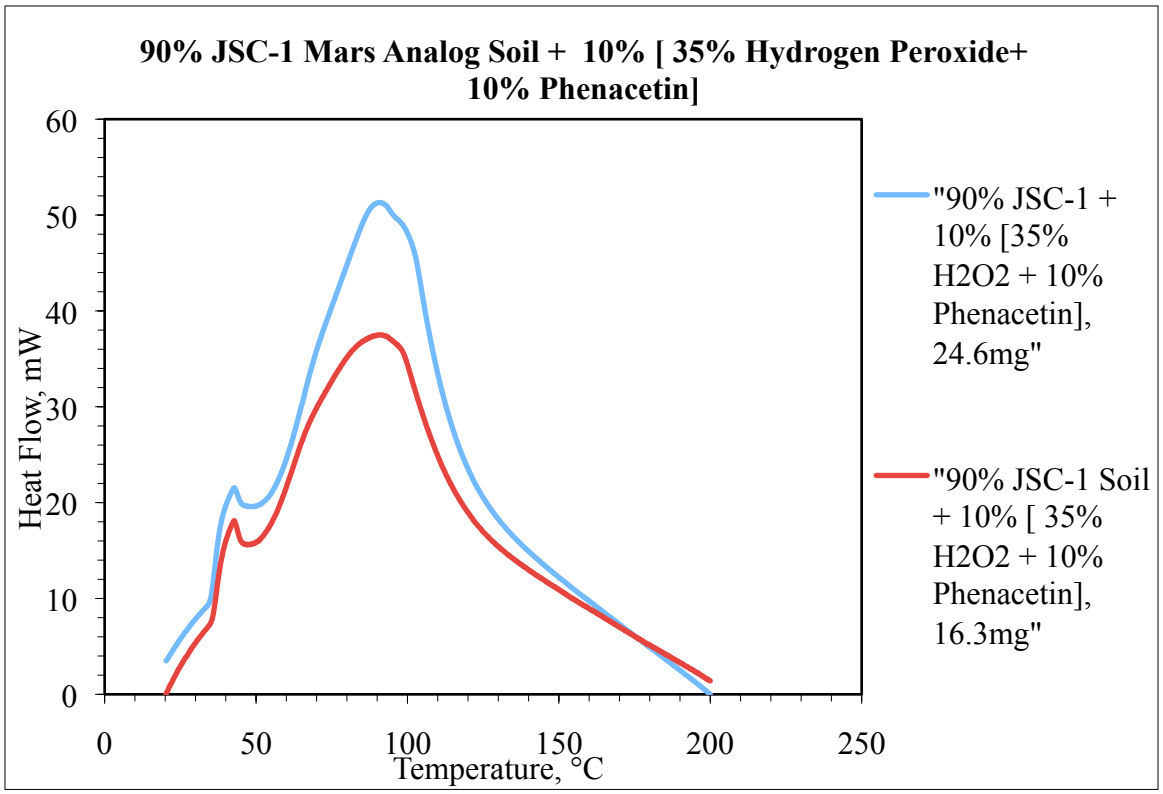


Figure 17: Thermograms with phenacetin at 10% the mass fraction of the media tested. JSC-1 soil + 10% (35% hydrogen peroxide +10% sodium pyrophosphate), peak = 93.13°C (+/- 1°C).

The two bacterial cultures tested generated two distinctly different thermograms (Fig. 18). The hydrogen peroxide producing *Lactococcus* strain thermogram displays a larger peak area with a peak that is significantly shifted toward a higher temperature, while the *Psedomonas* strain displays a peak shifted towards a lower temperature.

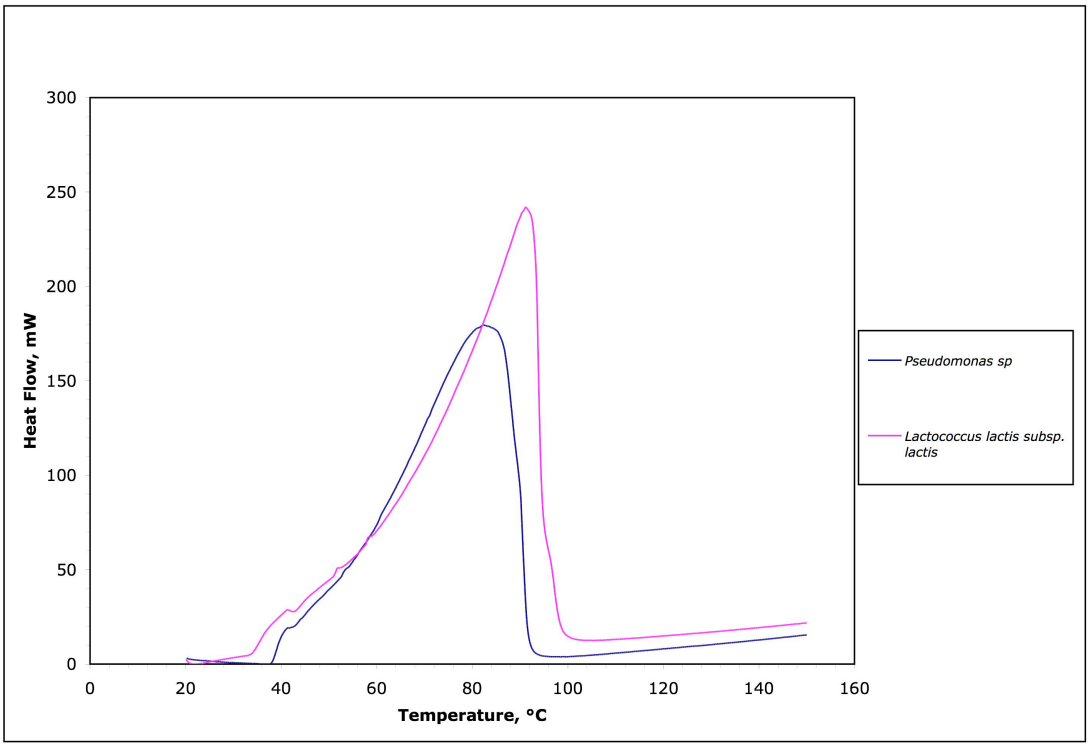


Figure 18: Thermograms of hydrogen peroxide producing *Lactococcus lactis* subsp. *lactis* and *Pseudomonas sp.* (non-hydrogen peroxide producing). The thermograms shown are the result of four independent DSC runs for each culture. The average peak area is 1,871 J/g (+/-61 J/g) for *Lactococcus lactis* and 1,488 J/g (+/-169 J/g) for *Pseudomonas sp*. The *Lactococcus lactis* subsp. *lactis* peak has an average peak temperature of 93°C (+/- 3°C), while the *Pseudomonas sp.* peak has an average peak temperature of 85°C (+/- 3°C).

Additional thermograms, including individual sample runs, can be found in Appendix B. These thermograms were used to create the grouped Figures discussed above.

DISCUSSION

A range of concentrations of hydrogen peroxide was successfully detected in Figure 5 from 70 ppm (.007%) to 438 ppm (.04%). However, the sharpest thermogram peak was observed at the 368 ppm (about .04%) concentration. There was a bell-curve type relationship apparent between the concentration of hydrogen peroxide and the sharpness of the peak observed. The lowest (70 ppm) and highest (875 ppb) concentrations of hydrogen peroxide gave the smallest, broadest peaks, while the mid-range concentrations (i.e. 368 ppm) showed sharp well-defined peaks. With concentrations of hydrogen peroxide on the martian surface varying from 1 ppm (Zent and McKay, 1994) to 250 ppm (Mancinelli, 1998), the TEGA instrument on the Mars Phoenix lander should be able to detect most of the hydrogen peroxide on the surface through calorimetry.

The thermograms for milllipure water (Figure 6) show a peak value of 114°C (+/- 3°C), which differ from the theoretical boiling point of pure water (100°C). This difference is most likely due to the range of temperatures seen in the indium standard runs used when calibrating the DSC instrument. While the theoretical values for the indium peak are between 157°C and 160°C, the instrument gave a peak value of 158.71°C (+/- 1.34°C) with an onset of the peak at 153.67°C (+/- 0.51°C). This slight shift from the theoretical values would explain the shift in the millipure water peak so that it was not exactly 100°C.

The 17.5 % hydrogen peroxide solution revealed a very similar behavior to pure water, but also exhibited a small negative peak at 123°C (Fig. 7). The negative peak appeared when almost all of the water had evaporated and represents the heat given

off by the H_2O_2 as it decomposed exothermally. This negative peak was more pronounced when using a 35 % hydrogen peroxide solution (Fig. 8). The area under the large positive (endothermic) peak of the 35 % hydrogen peroxide solution is 1.096 kJ/g +/- 0.31 kJ/g. The characteristic thermogram of a 35 % H_2O_2 solution was clearly identifiable at concentrations down below 450 ppm or about 9 ng (Fig. 1). A solution of 35% hydrogen peroxide with tetra sodium pyrophosphate at a ratio of 9:1 revealed a very different pattern (Fig. 9). It produced a strong exothermic response at about 80^{o}C, which was likely due to the hydrolysis of the pyrophosphate ion, and a large endothermic peak close to 100^{o}C. The stabilizing effect of the pyrophosphate must have ceased after all the pyrophosphate ions had been hydrolyzed. It should be noted that the overall energy required for the phase transition was greatly increased compared to millipure water and the hydrogen peroxide solutions (scale on y-axis of Fig. 9). The thermogram of the chemical stabilizer phenacetin reveals a characteristic single peak at about 132°C (Fig. 10), which is revealed as well in the thermogram of the 35 % hydrogen peroxide solution when phenacetin was added at a 9:1 mass ratio of 35% H_2O_2 to phenacetin (Fig. 11). The phenacetin peak appears at a value of 131°C for the hydrogen peroxide solution, while the main peak correlates to 108°C. The thermogram displays again a small negative peak at 118°C, which is characteristic for the exothermic decomposition of hydrogen peroxide.

To confirm that the negative peaks seen in the thermograms of hydrogen peroxide and hydrogen peroxide/tetra sodium pyrophosphate solutions were not an artifact of the DSC instrument, several sample runs were completed at a much lower scan rate of 2°C per minute. As seen in Figure 12, the negative peaks are still seen in the hydrogen peroxide solutions at the lower scan rate. After these confirmatory

thermograms, the scan rate was again increased to 10°C to mimic the scan rate used on the Mars Phoenix lander.

The thermograms of quartz sand (Fig. 13) display a characteristic peak at about 44°C, which, for thermograms of JSC-1 Mars simulant soil (Fig. 14), appears more pronounced. The second, larger peak reflects the evaporation of water. The addition of the chemical stabilizers $Na_4P_2O_7$ and phenacetin resulted in a shift of the peaks toward higher temperatures. In addition, the phenacetin peak is clearly identifiable for all media when phenacetin was used at a mass fraction of 3 % (Figs. 13-16). However, when the mass ratio of the phenacetin spiked hydrogen peroxide solution was lowered to 10 % of the mass fraction of the soil (phenacetin ~ 1 % of the total mass of the soil), the signature peak of the phenacetin was too small to be unambiguously identified in the JSC-1 soil and the metal oxides tested (Figure 17).

The negative peak of the decomposition of hydrogen peroxide is not discernable when using soil media and metal oxides. When using the JSC-1 martian simulant soil, which contains many metal oxides, a larger amount of endothermic energy was needed to evaporate the water. The peak energy was reached at significantly higher temperatures compared to quartz sand (Figs. 13,14). The same pattern is revealed when using titanium- and iron oxides as a medium (Figs. 15 and 16, respectively). However, the second, larger peak varies much more for the different solutions within metal oxides as a medium. For example, when using TiO_2 as a medium, a plateau is displayed for water and most hydrogen peroxide solutions between the two peaks. A prominent peak, however, appears if phenacetin was added to the solutions and if the tested medium contained metal oxides (JSC-1 soil and metal oxides). In the metal-oxide containing media, the phenacetin peak

at about 132°C is not as strong as in quartz sand, but still discernable at the concentrations tested.

The thermograms for the JSC-1 simulant soil and the metal oxides are very similar, which indicates that the thermogram of JSC-1 soil is dominated by the response of its metal oxide composition. The thermograms are very sensitive to moisture and allow an easy detection of water. The exothermic decomposition of H_2O_2 is detectable down to concentrations of at least 368 ppm and below, but more difficult to detect within soil media. Further compounding the difficulty of H_2O_2 detection was that laboratory runs under atmospheric conditions indicated that H_2O_2 had its characteristic signature decay. This is also the case under martian atmospheric conditions, because light, especially UV irradiation, destabilizes hydrogen peroxide. The average lifetime of H_2O_2 is on the order of only 2 days (Atreya et al. 2006). The Phoenix lander was equipped with a soil sampler, however, which ensured that the tested samples were not exposed for too long a time to martian atmospheric conditions. We can infer from our testing that H_2O_2 would be easier to detect by TEGA within liquid water rather than within a soil matrix.

The addition of a chemical stabilizer to a hydrogen peroxide solution can be identified in the thermogram. More endothermic energy is required during the heating process, which shifts the peak energy toward higher temperatures. In addition, phenacetin generates a characteristic peak at about 132°C. The detection of the chemical stabilizer is more challenging at lower concentrations within a soil matrix. The phenacetin was clearly identifiable in all four tested media at a tested mass fraction of about 3 % (Figs. 13-14) but the peak generated at a tested mass fraction of about 1 % is not significant

(Figure 17). Concentrations of $Na_4P_2O_7$ have to be even higher in concentration to be clearly discernable in the thermograms.

The two bacterial cultures tested generated two distinctly different thermograms (Fig. 18). The hydrogen peroxide producing *Lactococcus* strain thermogram displays a larger peak area with a peak that is significantly shifted toward a higher temperature. However, hydrogen peroxide concentrations within the *Lactococcus* strain were not high enough to display its characteristic negative peak and neither phenacetin nor tetrasodium pyrophosphate were identified. However, the presence of a chemical stabilizer can be inferred from the characteristic shift toward higher peak temperatures (Figure 18). Lactic acid bacteria exhibit an inducible oxidative stress response when exposed to sublethal levels of H_2O_2 (Condon, 1987), which can be at least as high as 350 ppm (Ito et al., 2003), and would also be expected to employ a chemical stabilizer to control the reactivity of H_2O_2.

If the H_2O_2-H_2O hypothesis of martian life (Houtkooper and Schulze-Makuch, 2007) is applied, then an organic stabilizer such as phenacetin could be understood as a more sophisticated evolutionary adaptation of life to martian conditions than an inorganic stabilizer such as $Na_4P_2O_7$. Both, however, would serve as a biomarker for possible life on Mars. The advantage of phenacetin is that its biosignature can be more easily detected in thermograms (via the signature peak) and there is no plausible way of an inorganic production of this organic compound. Alternatively, the H_2O_2 in the martian soil may be due to inorganic processes (Hurowitz et al., 2007), in which case no stabilizer and no evidence for a stabilizer would be present.

Obviously, the idea that the Viking lander observations, which implied a strong oxidizing agent for the observed reactions (Klein, 1999), were a result of biology are highly speculative, but so is nearly any conjecture in astrobiology. The main distinction here is that the biological and the chemical hypotheses presented are testable, which is rare in this field of study. The experiments reported here were conducted to simulate the analyses of the TEGA instrument of the Phoenix lander and future Mars lander instruments as well. The results of the Phoenix lander have been decidedly mixed. While the mission did confirm the presence of water ice just below the surface and found evidence of perchlorates and calcium carbonates(Smith et al, 2009; Hecht et al, 2009 and Boynton et al., 2009), the TEGA results have been inconclusive and prone to instrument error (ice clogging the sample doors for instance). New hope is being given, however, to the Mars Science Laboratory (MSL) mission and its Sample Analysis at Mars (SAM) suite.

The Mars Science Laboratory (MSL) is a new rover/lander that is scheduled to launch in the fall of 2011 and land on Mars in 2012. The whole purpose of the MSL is to assess the habitability of Mars. In other words, to find out if Mars can support microbial life in the present or has ever supported microbial life in the past (NASA JPL, 2009). The lander will carry a variety of instruments including cameras, spectrophotometers, radiation detectors and environmental sensors (Atreya et al., 2006). The instrument suite specific to our discussion, however, is the SAM (sample analysis at Mars suite). This suite carries many of the basic functions of chemistry laboratory here on Earth. Specifically, the SAM will search for carbon compounds (including methane, hydrogen, oxygen and nitrogen) using a gas chromatograph (GC), mass spectrometer (MS) and

tunable laser spectrometer (TLS) (Mahaffy, 2007). The three SAM instruments are complimented by a sample manipulation system (SMS) and a Chemical Separation and Processing Laboratory (CSPL). The CSPL includes micro valves, gas manifolds (with heaters and monitors), chemical and mechanical pumps, pressure monitors and pyrolysis ovens (Mahaffy, 2007). The results of the SAM instrument, and the pyrolysis oven in particular, should be comparable to our results with the DSC here on Earth. Any chemical stabilizers should show up as a shift in the thermogram peaks or as a signature peak. Also, hydrogen peroxide itself should be readily visible in the graphs with its characteristic broad endothermic peak followed by the sharp negative peak (allowing for scan rates between 2°C and 10°C per minute).

With either the TEGA instrument on the Mars Phoenix lander or the pyrolysis oven on the Mars Science Laboratory, the detection of a sufficient amount of H_2O_2 by itself would not provide evidence for the H_2O_2-H_2O hypothesis. However, the H_2O_2-H_2O hypothesis for life on Mars would be supported by the following observations: The detection of hydrogen peroxide plus (1) the detection of fragments of organic molecules by the mass spectrometer (MS) of TEGA, (2) the production of excess heat (exothermic signature) and the detection of the gaseous decomposition products of organic molecules such as CO_2, H_2O, O_2, and N_2 (the decomposition product of H_2O_2 would only be H_2O and O_2), and (3) a measured distinct change in isotope fractionation ratios of $^{13}C/^{12}C$ and $^{18}O/^{16}O$ toward lighter isotopes at temperatures at which organic molecules decompose. In addition, the identification of the characteristic signatures of the chemical stabilizers(such as phenacetin or tetrasodium pyrophosphate) or the characteristic shift produced by a chemical stabilizer in the thermograms, would add another line of

evidence. Thus, by combining the experimental results of the DSC, the TEGA results of the Mars Phoenix lander and the SAM results of the MSL rover, a better understanding of the surface of Mars can be reached as well as a broadening of our search for life on other planets.

CHAPTER TWO: WATER ACTIVITY AND HYDROGEN PEROXIDE

INTRODUCTION

Water is not only essential to life on Earth but it is also a major component of living organisms. While the meaning of the term water content is usually familiar, the term water activity is mainly used in food handling and processing and not in general use. Water content refers to the amount water present in a sample and is usually determined by assessing the wet weight versus dry weight of a sample (Duckworth, 1975). Even as water content is crucial in meeting labeling requirements of food, it is a poor indicator in predicting microbial responses in solutions (Prior, 1979). To predict microbial behavior a new measurement, called water activity, is needed. Water activity (a_w) is a measurement of how tightly water is bound (either structurally or chemically) in a sample and is indicative of the energy status of the water in a sample (Troller and Christian, 1978). Water activity is measured as the relative humidity of a sample in a sealed instrument chamber; in fact the liquid phase of the water in the sample is equilibrated with the vapor phase of the water in the headspace of the chamber (Prior, 1979). Water activity is closely related to a property of thermodynamics called water potential (also called chemical potential or μ). This water potential is the change in Gibbs free energy (G) when water concentration changes and equilibrium in a system is achieved when the μ is the same everywhere in the system (pawkit manual, 2008). Thus, when there is equilibrium between the liquid and vapor phases of water in a sample, the μ is same in both phases. It is this property of water potential that allows us to measure the water activity of a sample. The water activity measurement is key

in determining the perishability of a substance and the relative activity of microorganisms in the substance.

As a standard baseline, the water activity of pure water is 1.0, while the water activity of a saturated NaCl solution is 0.75 and a saturated $MgCl_2$ solution is 0.3 (Ha and Chan, 1999). The lowest water activity in which bacteria will grow or spores will germinate is around 0.6-0.7 and no activity is recorded at 0.5 (Duckworth, 1975 and Mugnier and Jung, 1985). Honey, for example, with its water activity of 0.6 does not support the growth of microorganisms but often contains endospores of the bacterium *Clostridium botulinum*, which can cause illness in young children (Shapiro, 1998). As a reference the water activities of several common food items are given below in table 13.

Table 13: Water Activities of Common Food Items (adapted from Duckworth, 1975)

Food Item	Water Activity, a_w
Milk	0.97
Bacon, cooked	~0.85
Orange Juice	0.97
Dried Fruits	0.5-0.6

While humans can survive almost two weeks without food, the human body can only last two days without water. On Mars, however, liquid water was never as abundant as on Earth. Even during the wetter early martian history, liquid water bodies may not have remained permanently on the surface. Hydrogen peroxide, however, is naturally present on Mars at much higher concentrations than on Earth, today and probably in the past. These inorganic concentrations are still low but its presence should have been

sufficient to warrant biochemical adaptations. Putative martian microorganisms might have learned to use the properties of hydrogen peroxide to their advantage, especially as the planet dried up and became colder during its history. A comparable process occurred on Earth more than 2 billions years ago, when microbes developed the ability to live with free oxygen and then thrive by using it in their favour (Brocks et al., 1999, Raymond and Segre, 2006; Catling and Buick, 2006). Thus, the antifreeze properties of hydrogen peroxide and its hygroscopic properties would become a great advantage for making a living on the dry and cold Mars.

The hygroscopic properties of hydrogen peroxide are of particular interest to our hydrogen peroxide-water hypothesis of life on Mars. If putative microorganisms on Mars do indeed use a hydrogen peroxide-water mixture as a type of antifreeze and water gathering, then the hygroscopic properties of such a mixture are of particular interest. Fortunately, water activity can be measured readily using a hardy portable device. Here we use the Pawkit water activity meter (Decagon Devices) to measure the water activities of various hydrogen peroxide-water solutions with added glucose, salt and perchloric acid. Samples from the pitch or asphalt lakes in Trinidad (Trinidad and Tobego) are also measured for comparison purposes. The hygroscopic and water-scavenging properties of these solutions are then evaluated.

MATERIALS AND METHODS

Hydrogen Peroxide/Water Solutions:

35% hydrogen peroxide, 500mL, was obtained from the Acros Organics Chemical Company (catalogue #202465000) and stored in a 500mL sterile brown glass media jar at +4^{o}C until use. Immediately before use, small aliquots (5-10mL) were transferred to 15mL glass sample jars with sealing polypropylene lids. Fresh samples were exposed to the atmosphere for no more than 5 minutes and the remaining was stored at +4°C for up to one hour. The 17.5% hydrogen peroxide solution was made by combining 4mL of the 35% hydrogen peroxide mentioned above with 4mL of the millipure water with mixing into q 15mL glass sample jar with sealing polypropylene lid. The solution was used immediately (fresh sample, up to 5 minutes to prepare) or stored at +4°C and tested up to one hour later. Sterile Millipore water was stored in brown glass jars (500mL) and aliquoted into sterile 50mL conical tubes 5 minutes before use.

Salt Solutions:

Sodium Chloride (NaCl) was obtained from Acros Organics (catalogue #AC42429-0250) and stored at room temperature. A 10% NaCl (w/v) solution was made by dissolving 1 gram of solid NaCl in 9 ml of 35% or 17.5% hydrogen peroxide (above). The solutions were then immediately tested for water activity.

Glucose Solutions:

Glucose powder was obtained from Acros Organics (catalogue #AC41095-5000) and stored in a brown glass jar at room temperature protected from light. A 10% glucose solution was made by dissolving 1 gram of glucose powder in either 35% or 17.5% hydrogen peroxide (above). The solutions were used immediately and tested for water activity.

Perchloric Acid Solutions:

Perchloric acid (60%) was obtained from Acros Organics (catalogue #A2286) and stored in a clear glass jar protected from light. A 10% solution of perchloric acid was made by combining 1.7ml of 60% perchloric acid with 8.3ml of either 35% or 17.5% hydrogen peroxide (above). The solutions were used immediately and tested for water activity.

Water Activity Measurements:

Water activity measurements were taken with the Pawkit water activity meter (Decagon Devices, Pullman, WA) using individual disposable plastic sample cups (Decagon) and between 2 and 3 grams of the appropriate solution above. Measurements were taken over a five minute interval. The initial and final a_w readings were taken for all hydrogen peroxide solutions and the final a_w was taken for the pure water. The Pawkit was calibrated every week with 6.0 molal NaCl (a_w 0.760) and 13.41 molal LiCl (a_w 0.250). All calibration solutions were supplied by Decagon Devices (Pullman, WA) and the error of the instrument is 0.003 a_w.

Trinidad Lake Samples:

Samples of pitch from the asphalt lakes of Trinidad (Trinidad and Tobego) were collected by hand by Dirk Schulze-Makuch and placed into 100ml plastic sample containers with screw top lids. 2-3 gram samples were then scooped into the Decagon Devices disposable plastic sample cups using a metal spatula. The water activity was taken using the Pawkit water activity meter (Decagon Devices).

RESULTS

The water activity measurements of pure Millipore water are shown in Figure21. The recorded value of 0.98 (+/- 0.01) falls near the theoretical value of a_w=1.0 for pure water.

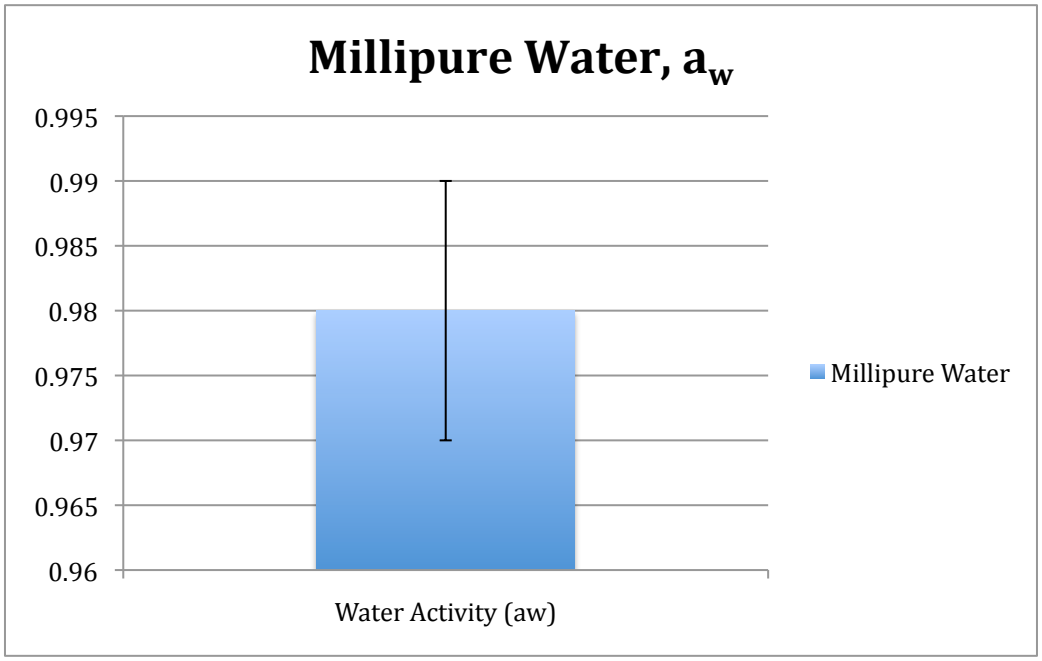


Figure 19: Graph of Millipore water: water activity averaged over 10 samples was 0.98 (+/- 0.01), weight = 2.9 g (+/- 0.2 g), temperature = 21.5°C (+/- 1.5°C).

Figure 22 shows the water activity of 35% hydrogen peroxide at both room temperature (20.7°C +/- 1.3°C) and at +4°C (+/- 0.9°C). At room temperature, the water activity starts at an initial a_w of 0.75 (+/- .04) and increases to 1.00 (+/- .02) after five minutes. At +4°C the a_w starts at .75 (+/- .04) and increases to .00 (+/- .02) after five minutes.

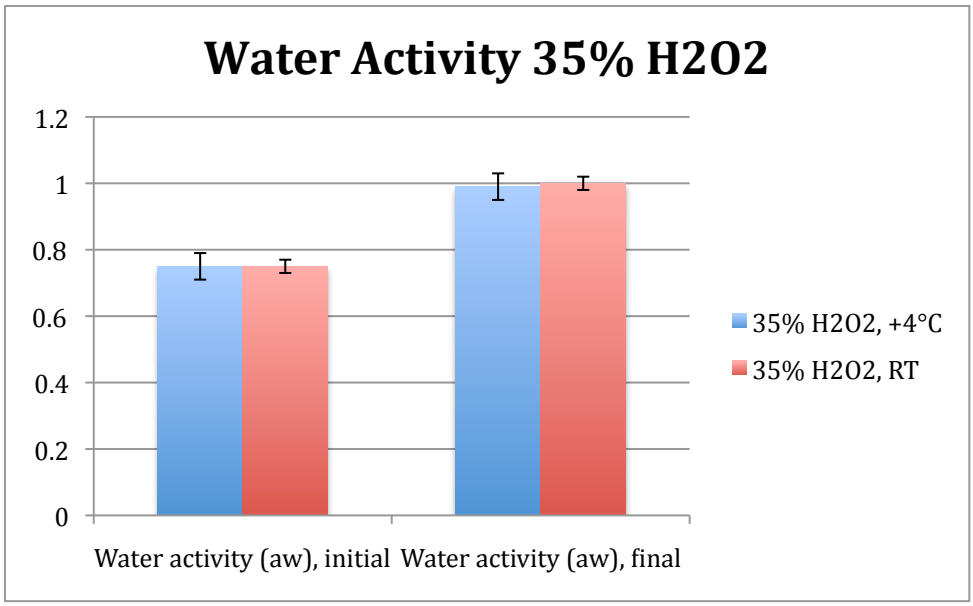


Figure 20: Water activity of 35% hydrogen peroxide at various temperatures. At room temperature, 20.7°C (+/- 1.3°C), the a_w began at .75 (+/- .04) and increased to 1.00 (+/- .02) after five minutes with a sample weight of 3.31 g (+/- 1.2 g). At cooler temperatures, 4.6°C (+/- .3°C), the a_w began at .75 (+/- .04) and increased to .99 (+/- .02) after five minutes with a sample size of 2.96 g (+/- .5 g). Measurements averaged over ten samples each.

With a decreased hydrogen peroxide content of 17.5%, the water activities remained significantly unchanged from initial reading to five minutes later. In Figure 23 the initial water activity readings for both room temperature and 4°C are 0.99 (+/- .01) and both readings end at 1.01 (+/- .01) after five minutes. The temperature for the room temperature sample was 20.7°C (+/- .8°C) while the cooler temperature was 4.7°C (+/- .2°C).

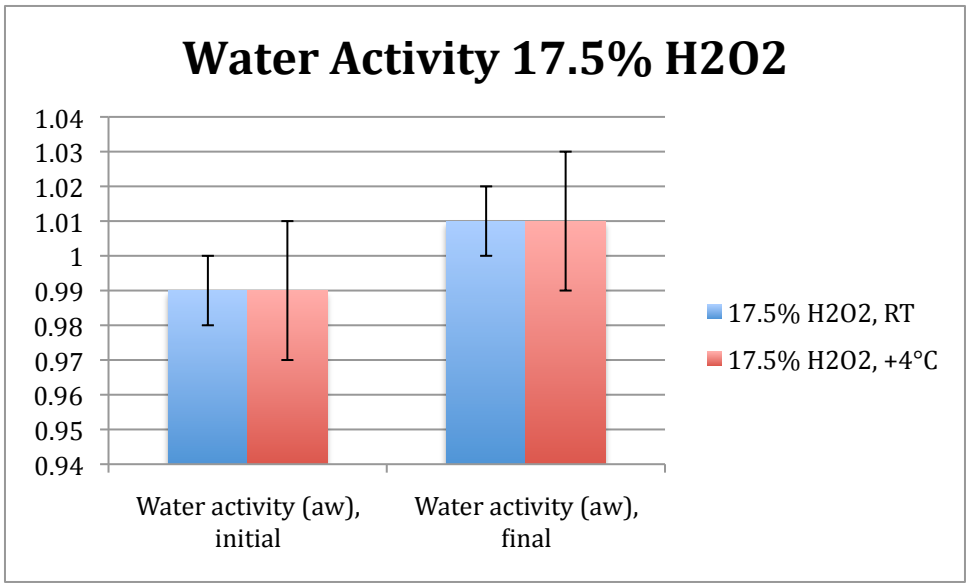


Figure 21: Water activity of 17.5%% hydrogen peroxide at various temperatures. At room temperature, 20.7°C (+/- .8°C), the a_w began at .99 (+/- .01) and increased to 1.01 (+/- .01) after five minutes with a sample weight of 3.4 g (+/- .2 g). At cooler temperatures, 4.7°C (+/- .2°C), the a_w began at .99 (+/- .01) and increased to 1.01 (+/- .01) after five minutes with a sample size of 3.2 g (+/- .2 g). Measurements averaged over ten samples each treatment.

The water activity of several different 35% hydrogen peroxide solutions was then tested at room temperature in Figure 24. Initially the 35% H_2O_2 sample started with a water activity of 0.75 (+/- .4) and increased to 1.00 (+/- .02) after five minutes. The 35% H_2O_2 solutions plus 10% NaCl, 10% glucose or 10% perchloric acid started with a_w values of 0.87 (+/- .01), 0.89 (+/- .02) and 0.89 (+/- .02) respectively and ended after five minutes with a_w values of 0.98 (+/- .02), 0.99 (+/- .02) and 0.99 (+/- .02) respectively. Each increase was statistically significant throughout the samples.

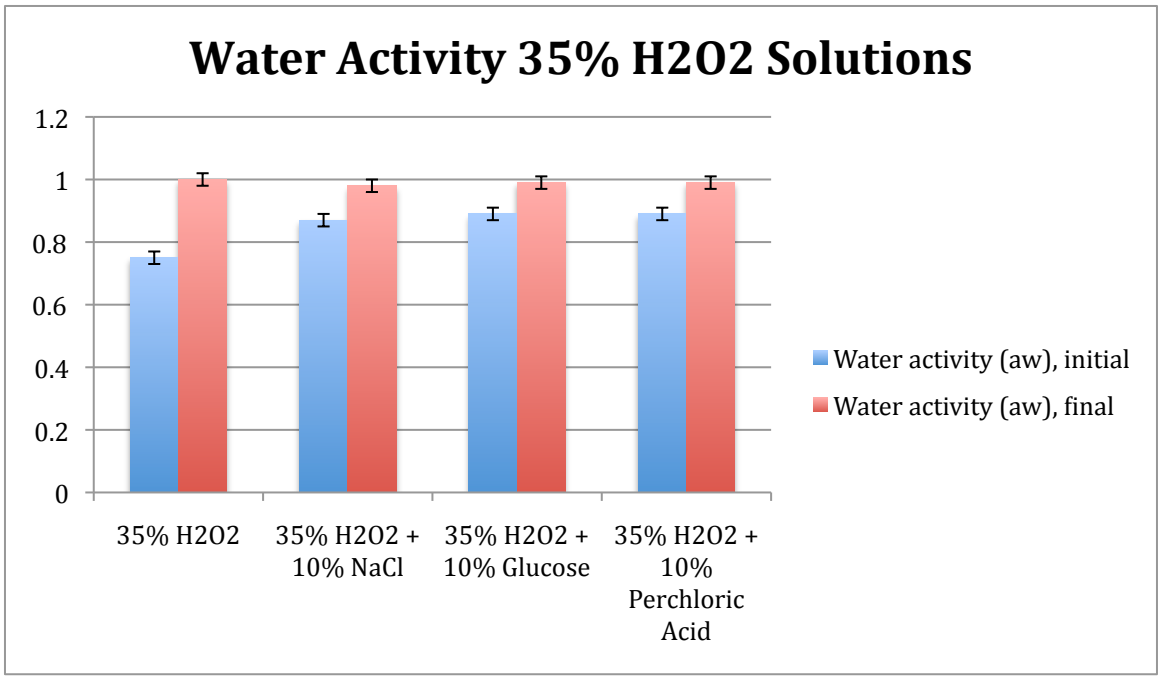


Figure 22: Water activity of various 35% hydrogen peroxide solutions. The 35% hydrogen peroxide solution of 3.3 g (+/- 1.2 g) started with a_w=0.75 (+/- .04) and ended after five minutes with a_w=1.00 (+/- 0.2) with a temperature of 20.7°C (+/- 1.3°C). The 35% hydrogen peroxide + 10% NaCl solution of 2.9 g (+/-.1 g) started with a_w=0.87 (+/- .01) and ended after five minutes with a_w=0.98)+/- .02) with a temperature of 20.8°C (+/-.6°C). The 35% hydrogen peroxide + 10% glucose solution of 3.0 g (+/-.1 g) started with a_w=0.89 (+/- .02) and ended after five minutes with a_w=0.99)+/- .02) with a temperature of 21.0°C (+/-.9°C). The 35% hydrogen peroxide + 10% perchloric acid solution of 3.0 g (+/-.1 g) started with a_w=0.89 (+/- .02) and ended after five minutes with a_w=0.99 (+/- .02) with a temperature of 20.6°C (+/-.7°C). Measurements averaged over ten samples each treatment.

The Trinidad lake samples (Figure 25) showed water activity levels that were constant between the initial reading and final reading after five minutes. The water activity

measurement was 0.78 (+/- .04) with a temperature of 26.2°C (+/- .3°C) and a weight of 2.7 g (+/- .6 g). The Trinidad samples were compared with the Millipore water samples for graphical purposes.

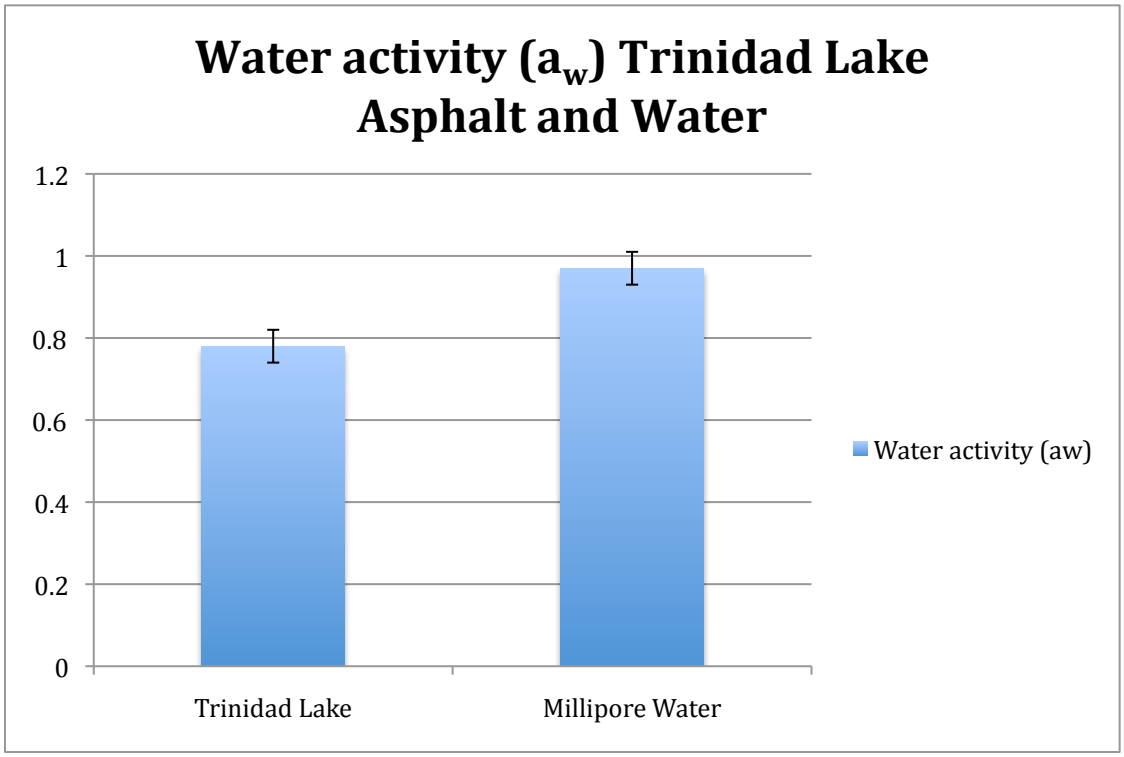


Figure 23: Water activity of Trinidad asphalt lake samples and Millipore water. Water activity for Millipore water was 0.98 (+/- 0.01), weight = 2.9 g (+/- 0.2 g), temperature = 21.5°C (+/- 1.5°C). Trinidad asphalt lake samples had a_w=0.78 (+/- .04) with a temperature of 26.2°C (+/- .3°C) and a weight of 2.7 g (+/- .6g). Measurements were averaged over ten samples in each treatment.

DISCUSSION

Overall the water activity measurements yielded values not out of the expected ranges for bacterial growth, a_w= >0.6 (Duckworth, 1975). The millipore water values (Fig. 21) were all significantly close to the theoretical value of aw= 1.0. The water activity of 17.5% hydrogen peroxide at either room temperature or a cooler temperature showed no significant increase in value (Fig. 23). This could be due to the fact that the hydrogen peroxide content was not high enough to reveal the scavenging effects of the solution. However, when we consider the 35% hydrogen peroxide solution at both room temperature and 4°C, the initial water activity values were all significantly lower than the final values (Fig. 22). At room temperature, 20.7°C (+/- 1.3°C), the a_w began at .75 (+/- .04) and increased to 1.00 (+/- .02) after five minutes with a sample weight of 3.31 g (+/- 1.2 g). At cooler temperatures, 4.6°C (+/- .3°C), the a_w began at .75 (+/- .04) and increased to .99 (+/- .02) after five minutes with a sample size of 2.96 g (+/- .5 g). This increase demonstrated the tendency of the 35% hydrogen peroxide solutions to scavenge water from their surroundings.

The scavenging effect was also seen in the 35% hydrogen peroxide solutions containing 10% NaCl, 10% glucose and 10% perchloric acid. The 35% hydrogen peroxide + 10% NaCl solution of 2.9 g (+/-.1 g) started with a_w=0.87 (+/- .01) and ended after five minutes with a_w=0.98)+/- .02) with a temperature of 20.8°C (+/-.6°C). The 35% hydrogen peroxide + 10% glucose solution of 3.0 g (+/-.1 g) started with a_w=0.89 (+/- .02) and ended after five minutes with a_w=0.99)+/- .02) with a temperature of 21.0°C (+/-.9°C). The 35% hydrogen peroxide + 10% perchloric acid solution of 3.0 g (+/-.1 g) started with a_w=0.89 (+/- .02) and ended after five minutes with a_w=0.99 (+/- .02) with a temperature of 20.6°C

(+/-.7°C). The salt and glucose solutions mimic the osmotic balance solutions of bacteria here on Earth and could offer a possible alternative for putative martiam microorganisms.

The perchloric acid water activity results are particularly interesting due recent finding by the Mars Phoenix mission. Perchlorates are strong oxidants and used in many industrial processes here on Earth from dry cleaning agents to rocket fuel additives (Sellers et al., 2006). Perchlorates, even though they are chiefly man-made, can be utilized by several anaerobic bacteria (*Shewanella* spp., *Acetobacterium* spp and *Pseudomonas* spp.) as an energy source, thus showing the ability of microorganisms to adapt to their environment and the metabolites available (Denis et al., 2003). Recently, the Mars Phoenix lander found evidence of perchlorates on the surface of Mars along with abundant subsurface water ice (NASA Phoenix, 2009). A perchlorate-hydrogen peroxide mixture might allow potential martian microorganisms to both scavenge water and create energy for the cell.

In conclusion, when considering the possible refuges for life on other planets and satellites, we must first consider the energetic and environmental requirements of such life. While it has been challenging to even agree upon a definition of life here on out own planet, most would agree that life needs, at the least, an energy source and a solvent (Schulze-Makuch and Irwin, 2006). Here on Earth water is, of course, the solvent but organisms also obtain energy through a variety of mechanisms including aerobic respiration, iron reduction, sulfate reduction and even methanogenesis (Konhauser, 2007). Environmental factors are also important when considering the constraints of life. The study of life in extreme environments gives us some basic constraints for thinking about life on other worlds. For example, the study of psycrophiles yielded evidence of bacteria surviving temperatures of -2°C (Junge, Eicken, & Deming, 2004) and

thermophiles have been found living at 121°C (Kashefi & Lovley, 2003). Thus it should be evident that life adapts to its environment and when considering life on other worlds we should keep this principle in mind when looking for potential energy sources and solvents.

APPENDIX A: COPY OF THE PUBLISHED PAPER

BASED ON THE DSC RESULTS

Reference: Schulze-Makuch, D., Turse, C., Houtkooper, J.P. and McKay, C.P. Testing the H_2O_2-H_2O Hypothesis of Life on Mars with the TEGA Instrument on the Phoenix Lander. *Astrobiology*. 2008 April 8 (2):205-14.

Testing the H_2O_2-H_2O Hypothesis of Life on Mars with the TEGA Instrument on the Phoenix Lander.

Schulze-Makuch, D., Turse, C., Houtkooper, J.P. and McKay, C.P.

INTRODUCTION

The Viking mission left open many questions; particularly the nature of the oxidant in the martian soil remains enigmatic (Zent and McKay, 1994; Quinn and Zent, 1999; Yen et al., 2000; Mancinelli, 1989; Klein, 1999; Benner et al., 2000; Hurowitz et al., 2007; Houtkooper and Schulze-Makuch, 2007). The common explanation is that all the organic material near the surface was oxidized by H_2O_2 and other strong oxidizing compounds. Based on the reactivity of the surface measured by the Viking Gas Exchange experiment (GEx), the amount of H_2O_2 on the martian surface was estimated to be between 1 ppm (Zent and McKay, 1994) and 250 ppm (Mancinelli, 1989). Yet, photochemical processes generate H_2O_2 in the atmosphere at a much lower rate in the parts per billion range. Atmospheric H_2O_2 abundances vary between 20 and 40 ppb by volume over the planet (Encrenaz et al. 2004), which appears to be a maximum concentration that occurs during favorable weather conditions (Atreya and Gu, 1994). Nussinov et al. (1978) argued

that, instead of H_2O_2, oxygen gas physically trapped in soil micropores may have been responsible for the Viking observations. Plumb et al. (1989) pointed out that a large number of diverse features of the GEx and Labeled Release experiment can be reproduced with ultraviolet-irradiated potassium nitrate. Possible oxidant reactions and their environmental problems on Mars were summarized by Zent and McKay (1994), who concluded that none of the hypotheses presented in the literature was free of serious objections, many of which have to do with the instability of putative oxidants in the presence of heat, light, or atmospheric carbon dioxide. Or the suggested hypotheses would require elaborate formation mechanisms for which there is no evidence. Instead, Zent and McKay suggested that the results obtained by Viking could be best explained by some kind of heterogeneous surface chemistry that yields one or more types of oxidizing surfaces on the martian regolith particles. Quinn and Zent (1994) proposed that hydrogen peroxide chemisorbed on titanium dioxide may have been responsible for the chemical reactivity seen in the Viking life detection experiments. Yen et al. (2000) suggested superoxide ions instead of H_2O_2.

Alternatively, Levin and Straat (1981) and Levin (2007) argued for a biological explanation but struggled to explain (1) the evolution of O_2 upon wetting the soil, (2) the apparent absence of organic molecules in the soil, and (3) the weakly positive results of the single control test in the Pyrolytic Release experiment. Using a different approach, Houtkooper and Schulze-Makuch (2007) suggested that putative martian organisms might employ a novel biochemistry; in particular, they could utilize a water-hydrogen peroxide (H_2O-H_2O_2) mixture rather than water as an intracellular liquid. This adaptation would have the particular advantages of providing a low freezing point, a source of oxygen, and

hygroscopicity in the martian environment, which would allow organisms to scavenge water molecules directly from the atmosphere, and address many of the puzzling Viking findings. H_2O_2-H_2O solutions are mostly known as disinfectants and sterilizing agents on Earth, but some microbial organisms produce hydrogen peroxide (e.g., certain *Streptococcus* and *Lactobacillus sp*.; Eschenbach et al., 1989), while other microbes utilize H_2O_2 (e.g., *Neisseria sicca*, *Haemophilus segnis;* Ryan and Kleinberg, 1995). Sensitivity to H_2O_2 varies drastically (Anders et al., 1970; Alcorn et al., 1994; Stewart et al., 2000). Reported microbial survival rates range from greater than 80 % to less than 0.001 % after exposure to 30 mM hydrogen peroxide (Alcorn et al., 1994), and at least one organism, the microbe *Acetobacter peroxidans*, uses H_2O_2 in its metabolism (overall reaction $H_2O_2(aq) + H_2(aq) \leftrightarrow 2H_2O$; Tanenbaum, 1956).

Mixtures of H_2O_2 and H_2O freeze at temperatures significantly below the freezing point of water. The lower eutectic point lies at -56.5°C for a mixture with 61.2 weight % H_2O_2 (Foley and Giguère, 1951). Also, mixtures with a high H_2O_2 concentration tend to super cool, which sometimes results in the formation of glasses, down to liquid-air temperatures (Giguère, and Secco, 1954). On Earth, microorganisms commonly use salts as antifreeze. Salts are common and readily available as they are highly soluble in abundant water present on Earth. Thus, organisms on Earth learned to adapt to higher salt concentrations and use salts as antifreeze in cold environments such as in high mountain regions and the Arctic. On Mars, however, liquid water was never as abundant as on Earth. Even during the wetter early martian history, liquid water bodies may not have remained permanently on the surface. Hydrogen peroxide, however, is naturally present on Mars at much higher concentrations than on Earth, today and probably in the past.

These inorganic concentrations are still low, much too low to explain the Viking results, but its presence should have been sufficient to warrant biochemical adaptations. Putative martian microorganisms might have learned to use the properties of hydrogen peroxide to their advantage, especially as the planet dried up and became colder during its history. A comparable process occurred on Earth more than 2 billions years ago, when microbes developed the ability to live with free oxygen and then thrive by using it in their favour (Brocks et al., 1999, Raymond and Segre, 2006; Catling and Buick, 2006). Thus, the antifreeze properties of hydrogen peroxide and its hygroscopic properties would become a great advantage for making a living on the dry and cold Mars. Also, adaptation to H_2O_2 would require adaptation to oxidation stress (e.g., protection from protein oxidation), which would have likely conveyed microbial radioresistance as well (Daly et al. 2007), another trait useful to have in the martian near-surface environment.

The NASA Mars Phoenix Mission, which is currently on its way to Mars, provides the unique possibility to test various hypotheses to explain the Viking results. The Phoenix lander includes the TEGA instrument, which is a combination of a high-temperature furnace and a mass spectrometer that will be used to analyze martian ice and soil samples. Once a sample is successfully received and sealed in the TEGA oven, temperature is slowly increased at a constant rate up to 1000°C, and the power required for heating is carefully and continuously monitored. This process, called scanning calorimetry, shows the transitions from solid to liquid to gas of the different materials in the sample.

MATERIALS AND METHODS

We used differential scanning calorimetry to analyze phase transitions and thermodynamic properties of the oxidant compounds investigated. Two thermal cells were employed, one cell holding the reference capsule, the other the sample. A computer control system measured the amount of heat required to increase the temperature of each cell. If the temperature in one cell was not rising as fast as the temperature in the other cell, the instrument sent more energy (heat) to the heating coils in that cell to maintain the same temperature in each cell. The computer then recorded the difference between the energy requirements for each cell. The resulting graph (thermogram) of temperature versus energy difference between the two cells displays a peak whenever a phase transition occurred. The area under a positive peak (peak area) represents the energy required for the transition (enthalpy of the reaction, ΔH), thus any positive peaks are representative of endothermic reactions, while negative peaks are representative of exothermic reactions. The onset of a peak usually corresponds to the melting or evaporation temperature of a tested substance. If the weight of the sample is known, then the Differential Scanning Calorimeter (DSC) can calculate the energy required per gram of sample (J/g) for the transition. In an exothermic process, less heat would be required by the sample than by the reference cell to keep a steady change in temperature. In this case, the resulting peak on the thermogram is a negative peak. The heat of the phase change can be adsorbed or released depending on the change in specific heat characteristics of each phase.

Sample compounds investigated included millipure water, 17.5% and 35 % hydrogen peroxide solution, 99.9% pure Fe_2O_3, 99.9% pure TiO_2, tetrasodium pyrophosphate ($Na_4P_2O_7$), phenacetin ($C_{10}H_{13}NO_2$), quartz sand, JSC-1 martian regolith simulant soil, and combinations thereof. $Na_4P_2O_7$ and phenacetin are stabilizers of H_2O_2 (Fig. 1) and were included in the test set because, if the martian H_2O_2 is mostly of biological nature, a chemical stabilizer has to be invoked to control the reactivity of the hydrogen peroxide. Other potential chemical stabilizers for H_2O_2 solutions include sodium silicate (Na_2SiO_3), poly(α)hydroxyacrylic acid, phytate, citrate, and malonate (Charron et al., 2006; Watts et al., 2007). Tetrasodium pyrophosphate was chosen here due to its simplicity, efficacy (e.g., common use in commercial applications), and its similarity to ATP. Phenacetin was chosen due to its demonstrated long-term (> 3 months) effectiveness to keep hydrogen peroxide stable (Madanská et al., 2004). The martian regolith simulant is the <1mm fraction of weathered volcanic ash from Pu'u Nene, a cinder cone on the island of Hawaii, and was provided by the Johnson Space Center. We also tested two bacterial cultures: *Lactococcus lactis subsp. lactis*, which has been shown to produce hydrogen peroxide concentrations up to a level of about 350 ppm (Ito et al., 2003), and *Pseudomonas sp.*, a non-hydrogen peroxide producing bacterium. *Lactococcus lactis* subsp. *lactis* bacteria were obtained from the American Type Culture Collection (ATCC #11454, Manassas, VA) and were propagated according to standard procedures. Briefly, the freeze-dried pellet was rehydrated in 1.0mL of ATCC#17 broth and then added to 5.0mL of ATCC#17 broth in a 50mL conical tube. The resulting mixture was incubated at 37°C with shaking for 24 hours. *Pseudomonas sp.*, cultured from a single colony from an agar plate, was resuspended in 5mL of sterile Tryptic Soy

Broth (TSB, Difco #211825) in a sterile 50mL conical tube and incubated at 37°C with shaking for 24 hours.

The scanning rate of the DSC was set at 10^{o}C per minute, and the sample amount used was 10 to 20 mg to simulate the thermograms that will be obtained by the TEGA instrument on Mars. Samples were put in standard 20 µl aluminum sample pans with covers and sealed with the standard sample pan crimper press. Each pan was fully loaded and weighted before and after the addition of the sample. The sample preparation was completed in less than 5 minutes, since it was observed that the hydrogen peroxide started to decompose under atmospheric conditions within 40 min, which affected some of the thermograms (Figs. not shown). An indium standard was used to calibrate the DSC and a baseline run with the use of an empty aluminum pan was conducted prior to each sample run. The DSC was programmed to subtract automatically each baseline run from the sample run.

RESULTS

The thermograms of the various compounds and solutions are shown in Fig. 2. The millipure water revealed a behavior characteristic of the phase transition from liquid water to water vapor with a peak at about 110^{o}C. Any differences observed in peaks, ranges, and energies in duplicate runs (Fig. 2a-f) were a function of the total amount of solutes used in the DSC and due to instrument variation. The 17.5 % hydrogen peroxide solution revealed a very similar behavior to pure water, but also exhibited a small negative peak at 123°C (Fig. 2b). The negative peak appeared when almost all of the water had evaporated and represents the heat given off by the H_2O_2 as it decomposed exothermally. This negative peak was more pronounced when using a 35 % hydrogen peroxide solution (Fig. 2c). The area under the large positive (endothermic) peak of the 35 % hydrogen peroxide solution is 1.096 kJ/g +/- 0.31 kJ/g. The characteristic thermogram of a 35 % H_2O_2 solution was clearly identifiable at concentrations down to about 450 ppm or about 9 ng (not shown). A solution of 35% hydrogen peroxide with tetra sodium pyrophosphate at a ratio of 9:1 revealed a very different pattern (Fig. 2d). It produced a strong exothermic response at about 80^{o}C, which was likely due to the hydrolysis of the pyrophosphate ion, and a large endothermic peak close to 100^{o}C. The stabilizing effect of the pyrophosphate must have ceased after all the pyrophosphate ions had been hydrolyzed. It should be noted that the overall energy required for the phase transition greatly increased compared to millipure water and the hydrogen peroxide solutions (scale on y-axis of Fig. 2d). The thermogram of the chemical stabilizer phenacetin reveals a characteristic single peak at about 132°C (Fig. 2e), which is revealed as well in the thermogram of the 35 % hydrogen peroxide solution when phenacetin was

added at a 9:1 mass ratio of 35% H_2O_2 to phenacetin (Fig. 2f). The phenacetin peak appears at a value of 131°C for the hydrogen peroxide solution, while the main peak correlates to 108°C. The thermogram displays again a small negative peak at 118°C, which is characteristic for the exothermic decomposition of hydrogen peroxide.

In the next set of sampling runs, various soil media and metal oxides were exposed to the previously tested solutions (Fig. 3a-d). Thermograms of quartz sand display a characteristic peak at about 44°C, which, for thermograms of JSC-1 Mars simulant soil, appears more pronounced. The second, larger peak reflects the evaporation of water. The addition of the chemical stabilizers $Na_4P_2O_7$ and phenacetin resulted in a shift of the peaks toward higher temperatures. In addition, the phenacetin peak is clearly identifiable for all media when phenacetin was used at a mass fraction of 3 % (Fig 3a-d). However, when the mass ratio of the phenacetin spiked hydrogen peroxide solution was lowered to 10 % of the mass fraction of the soil (phenacetin ~ 1 % of the total mass of the soil), the signature of the phenacetin was too small to be unambiguously identified in the JSC-1 soil and the metal oxides tested (Fig. not shown). The negative peak of the decomposition of hydrogen peroxide is not discernable when using soil media and metal oxides. When using the JSC-1 martian simulant soil, which contains many metal oxides, a larger amount of endothermic energy was needed to evaporate the water. The peak energy was reached at significantly higher temperatures compared to quartz sand (Fig. 3a,b). The same pattern is revealed when using titanium- and iron oxides as a medium (Fig. 3c and 3d, respectively). However, the second, larger peak varies much more for the different solutions within metal oxides as a medium. For example, when using TiO_2 as a medium, a plateau is displayed for water and most hydrogen peroxide solutions between

the two peaks. A prominent peak, however, appears if phenacetin was added to the solutions and if the tested medium contained metal oxides (JSC-1 soil and metal oxides). In the metal-oxide containing media, the phenacetin peak at about 132^{o}C is not as strong as in quartz sand, but still discernable at the concentrations tested.

The two bacterial cultures tested generated two distinctly different thermograms (Fig. 4). The hydrogen peroxide producing *Lactococcus* strain thermogram displays a larger peak area with a peak that is significantly shifted toward a higher temperature, which indicates the possible presence of a chemical stabilizer. However, hydrogen peroxide concentrations within the *Lactococcus* strain were not high enough to display its characteristic negative peak and neither phenacetin nor tetrasodium pyrophosphate were identified.

CONCLUSIONS

The thermograms for the JSC-1 simulant soil and the metal oxides are very similar, which indicates that the thermogram of JSC-1 soil is dominated by the response of its metal oxide composition. The thermograms are very sensitive to moisture and allow an easy detection of water. The exothermic decomposition of H_2O_2 is detectable down to concentrations of at least 450 ppm, but more difficult to detect within soil media. Further compounding the difficulty of H_2O_2 detection was that laboratory runs under atmospheric conditions indicated that H_2O_2 and its characteristic signature decay. This will also be the case under martian atmospheric conditions, because light, especially UV irradiation, destabilizes hydrogen peroxide. The average life time of H_2O_2 is on the order of only 2 days (Atreya et al. 2006). The Phoenix lander is equipped with a soil sampler, however, which will ensure that the tested samples will not be exposed for too long a time to martian atmospheric conditions. We can infer from our testing that H_2O_2 would be easier to detect by TEGA within liquid water rather than within a soil matrix. Thus, if an ice sample can be obtained from the Phoenix landing site, H_2O_2 is more likely to be detected, if present.

The addition of a chemical stabilizer to a hydrogen peroxide solution can be identified in the thermogram. More endothermic energy is required during the heating process, which shifts the peak energy toward higher temperatures. In addition, phenacetin generates a characteristic peak at about 132^{o}C. The detection of the chemical stabilizer is more challenging at lower concentrations within a soil matrix. The phenacetin was

clearly identifiable in all four tested media at a tested mass fraction of about 3 % (Fig 3a-d), but the peak generated at a tested mass fraction of about 1 % is not significant. Concentrations of $Na_4P_2O_7$ have to be even higher in concentration to be clearly discernable in the thermograms. A chemical stabilizer was not detected in *Lactococcus lactis* subsp. *lactis,* but the presence of a chemical stabilizer can be inferred from its characteristic shift toward higher peak temperatures (Fig. 4). Lactic acid bacteria exhibit an inducible oxidative stress response when exposed to sublethal levels of H_2O_2 (Condon, 1987), which can be at least as high as 350 ppm (Ito et al., 2003), and would also be expected to employ a chemical stabilizer to control the reactivity of H_2O_2.

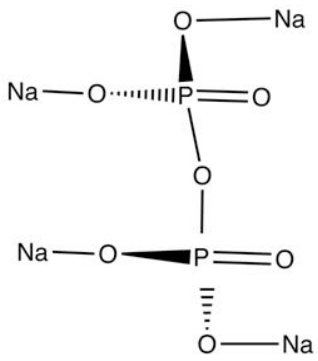
If the H_2O_2-H_2O hypothesis of martian life (Houtkooper and Schulze-Makuch, 2007) is considered, then an organic stabilizer such as phenacetin could be understood as a more sophisticated evolutionary adaptation of life to martian conditions than an inorganic stabilizer such as $Na_4P_2O_7$. Both, however, would serve as a biomarker for possible life on Mars. The advantage of phenacetin is that its biosignature can be more easily detected in thermograms and there is no plausible way of an inorganic production of this organic compound. Alternatively, the H_2O_2 in the martian soil may be due to inorganic processes (Hurowitz et al., 2007), in which case no stabilizer and no evidence for a stabilizer would be present.

Thus, the detection of a sufficient amount of H_2O_2 by itself would not provide evidence for the H_2O_2-H_2O hypothesis. However, the H_2O_2-H_2O hypothesis for life on Mars would be supported by the following observations: The detection of hydrogen peroxide plus (1) the detection of fragments of organic molecules by the mass spectrometer (MS) of TEGA (the TEGA MS cannot detect compounds above 140 daltons

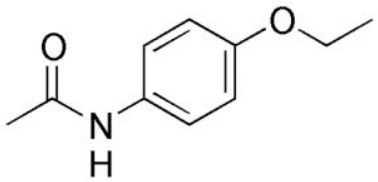
and, for example, would not be able to detect phenacetin directly, given its molecular weight of about 195 daltons), (2) the production of excess heat (exothermic signature) and the detection of the gaseous decomposition products of organic molecules such as CO_2, H_2O, O_2, and N_2 (the decomposition product of H_2O_2 would only be H_2O and O_2), and (3) a measured distinct change in isotope fractionation ratios of $^{13}C/^{12}C$ and $^{18}O/^{16}O$ toward lighter isotopes at temperatures at which organic molecules decompose. In addition, the identification of the characteristic signatures of the chemical stabilizers phenacetin or tetrasodium pyrophosphate, or the characteristic shift produced by a chemical stabilizer in the thermograms, would add another line of evidence.

Obviously, the idea that the Viking lander observations, which implied a strong oxidizing agent for the observed reactions (Klein, 1999), were a result of biology are highly speculative, but so is nearly any conjecture in astrobiology. The main distinction here is that the biological and the chemical hypotheses presented are testable, which is rare in this field of study. The experiments reported here were conducted to simulate the analyses of the TEGA instrument on Mars. Thus, a heating rate of 10^oC per minute and the same sampling volume was used. However, even if the Phoenix mission scientists in some later meeting would decide to reduce the agreed upon heating rate (which is technically possible), our work suggests that the thermograms would only be smoothened, but the general trends and shapes of the thermograms would remain the same. The results obtained from the TEGA instrument of the Phoenix lander will thus provide an unbiased test of the nature of the oxidant on Mars and aid in the interpretation of the results of the Sample Analysis at Mars Instrument Suite (SAM) of the Mars Science Laboratory (MSL) mission.

FIGURES



a) Sodium Pyrophosphate



b) Phenacetin

Figure 1 (Thesis Fig. 24): Structural formula of chemical stabilizers of hydrogen peroxide, a. sodium pyrophosphate, b. phenacetin

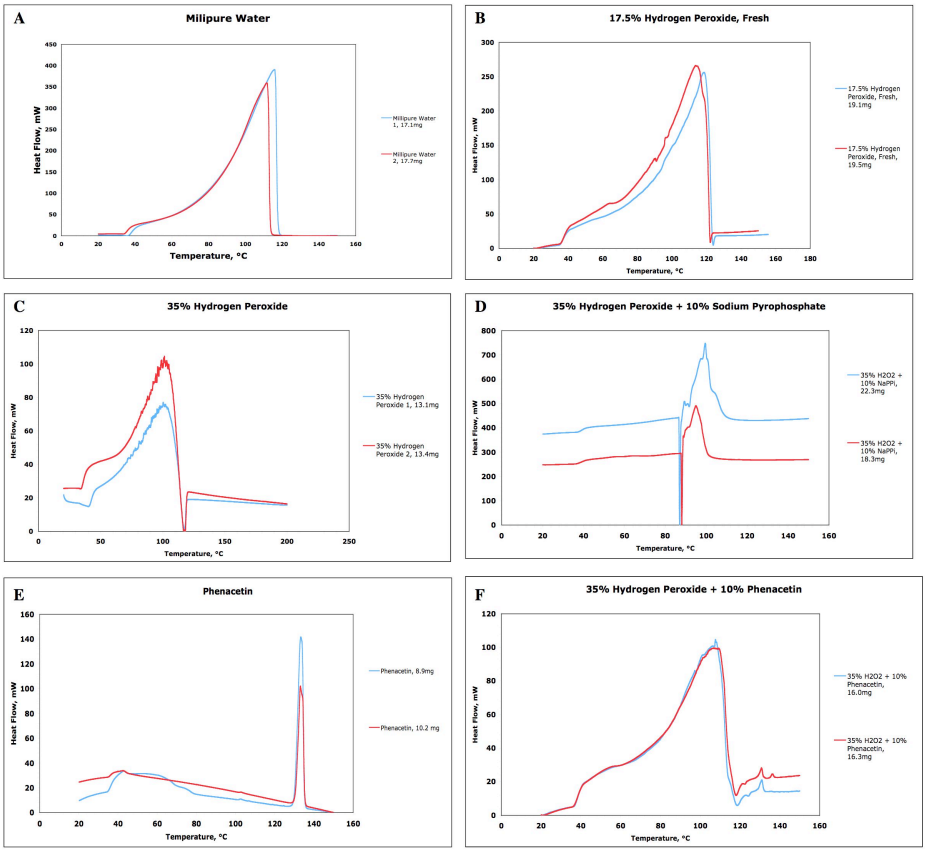


Figure 2 (Thesis Fig. 25): Thermograms of various solutions. A. Millipure water with a peak value of 114°C (+/- 3°C). The peak ranged from 80°C (+/- 1°C) to 117°C (+/- 3°C) and the peak area was 1403 J/g (+/- 105 J/g). B. 17.5% hydrogen peroxide solution with a peak value of 117°C (+/- 3°C). The peak area was 1270 J/g (+/- 0.2 J/g) with the peak ranging from 68°C (+/- 4) to 123°C (+/- 2°C). The solution also exhibited a small negative peak at 123°C (+/- 1°C) with an area of –7 J/g (+/- 0.3°C). C. 35% H_2O_2 solution with a peak value of 101°C (+/-1°C). The peak ranged from 52°C (+/-1) to 117°C +/-1). The negative peak is at 117°C (+/-1°C) with an area of -27J/g (+/- 5J/g). D. 35% hydrogen peroxide solution with sodium pyrophosphate at a ratio of 9 to 1. The thermogram revealed a large exothermic peak at about 86°C with an area of –103 J/g (+/-

71 J/g). The main peak had a value of 97°C (+/- 3°C), a range from 87°C (+/- 0.5°C) to 107°C (+/- 6°C) and an area of 874 J/g (+/- 570 J/g). E. Phenacetin revealed a single peak at 133°C (+/- 1°C) with an area under the curve of 173 J/g (+/- 8 J/g). F. 35 % hydrogen peroxide solution with 10 % phenacetin. The peak was at 108°C (+/- 1°C) with a range of 61°C (+/- 3°C) to 118°C (+/- 1°C) and an area under the curve of 785 J/g (+/- 37 J/g). The phenacetin peak appears as a minor peak at a value of 131°C (+/- 1°C) with an area of 4 J/g (+/- 1 J/g). The solution also displayed again a small negative peak at 118°C (+/- 1°C) characteristic for the exothermic reaction of H_2O_2 with an area under the curve of –8 J/g (+/- 1 J/g).

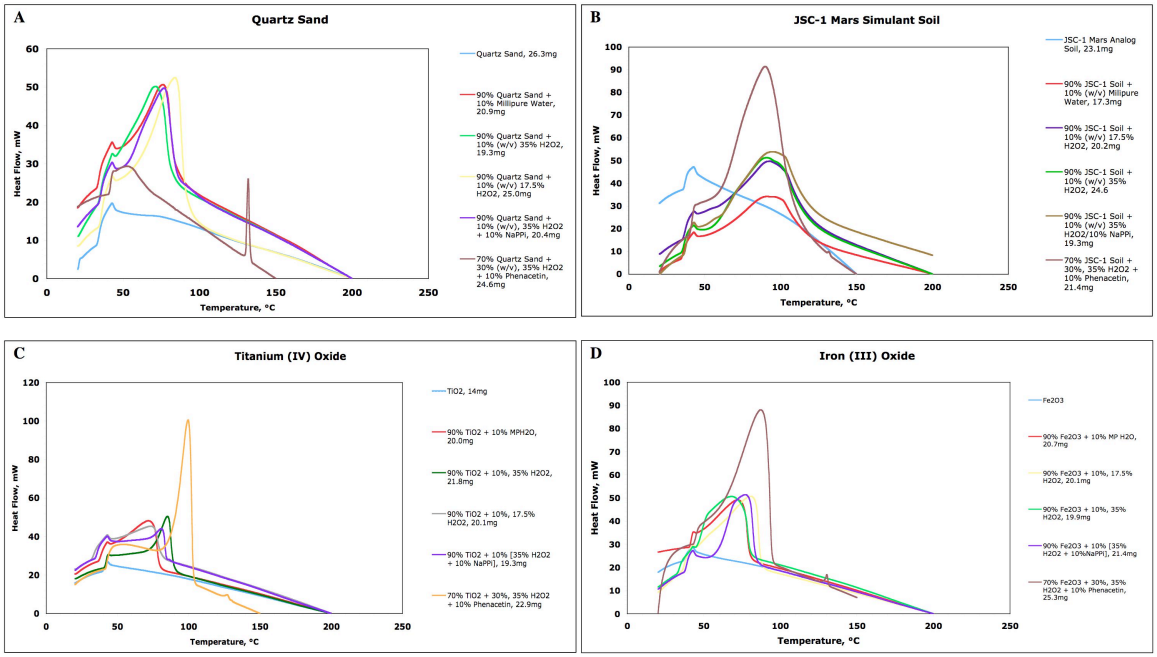


Figure 3 (Thesis Fig. 26): Thermograms with various media and solutions. A. Quartz sand. The first peaks appear at a value of 44°C (+/- 4°C) with a range of 35°C (+/- 6°C) to 50°C (+/- 9°C) and a peak area of 8 J/g (+/- 1 J/g). The second, larger peaks have a value of 77°C (+/- 5°C) with a range from 45°C (+/- 1°C) to 91°C (+/- 6°C) and a peak area of 117 J/g (+/- 21 J/g). A sharp phenacetin peak is observed at 132°C for the hydrogen peroxide solution with 10 % phenacetin. B. JSC-1 Mars stimulant soil. The first peaks appear at a value of 41°C (+/- 1°C) with a range from 35°C (+/- 1°C) to 46°C (+/- 1°C) and a peak area of 9 J/g (+/- 2 J/g). The second, larger peaks average at a value of 92°C (+/- 2°C) with a range from 55°C (+/- 6°C) to 120°C (+/- 8°C) and a peak area of 224 J/g (+/- 47 J/g). The phenacetin peak is again observed at a value of 132°C, but smaller in magnitude. C. Titanium (IV)

oxide. The first peaks have a value of 42°C (+/- 1°C) with a range from 35°C (+/- 4°C) to 46°C (+/- 1°C) and a peak area of 6 J/g (+/- 3 J/g). The second, larger peaks show much variation with a range from 62°C (+/- 16°C) to 90°C (+/- 9°C) and an average peak area of 69 J/g (+/- 41 J/g). The phenacetin peak is observed again at a value of 127°C. D. Iron (III) oxide. The first peaks have a value of 41°C (+/- 3°C) with a range from 37°C (+/- 4°C) to 47°C (+/- 3°C) and a peak area of 7 J/g (+/- 4 J/g). The second, larger peaks vary largely with a range from 49°C (+/- 6°C) to 85°C (+/- 11°C) and an average peak area of 146 J/g (+/- 49 J/g). The phenacetin peak is observed at a value of 130°C.

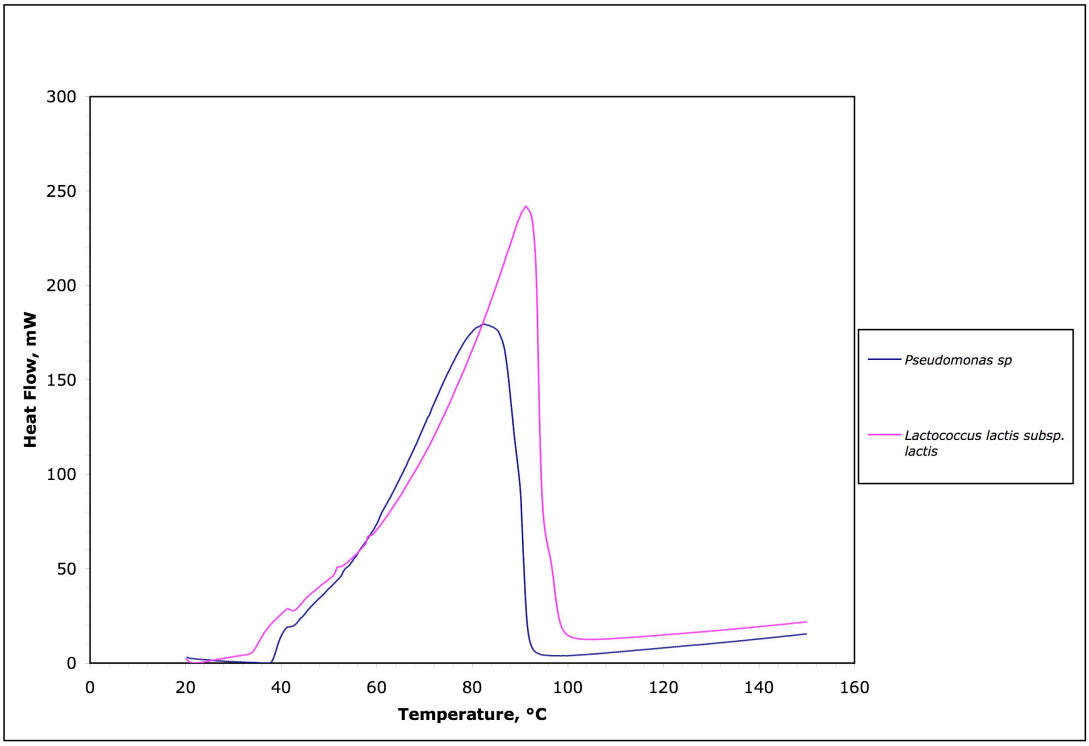


Figure 27: Thermograms of hydrogen peroxide producing *Lactococcus lactis* subsp. *lactis* and *Pseudomonas sp.* (non-hydrogen peroxide producing). The thermograms shown are the result of four independent DSC runs for each culture. The average peak area is 1,871 J/g (+/-61 J/g) for *Lactococcus lactis* and 1,488 J/g (+/-169 J/g) for *Pseudomonas sp*. The *Lactococcus lactis* subsp. *lactis* peak has an average peak temperature of 93°C (+/- 3°C), while the *Pseudomonas sp.* peak has an average peak temperature of 85°C (+/- 3°C).

APPENDIX B: SUPPLEMENTARY THERMOGRAMS

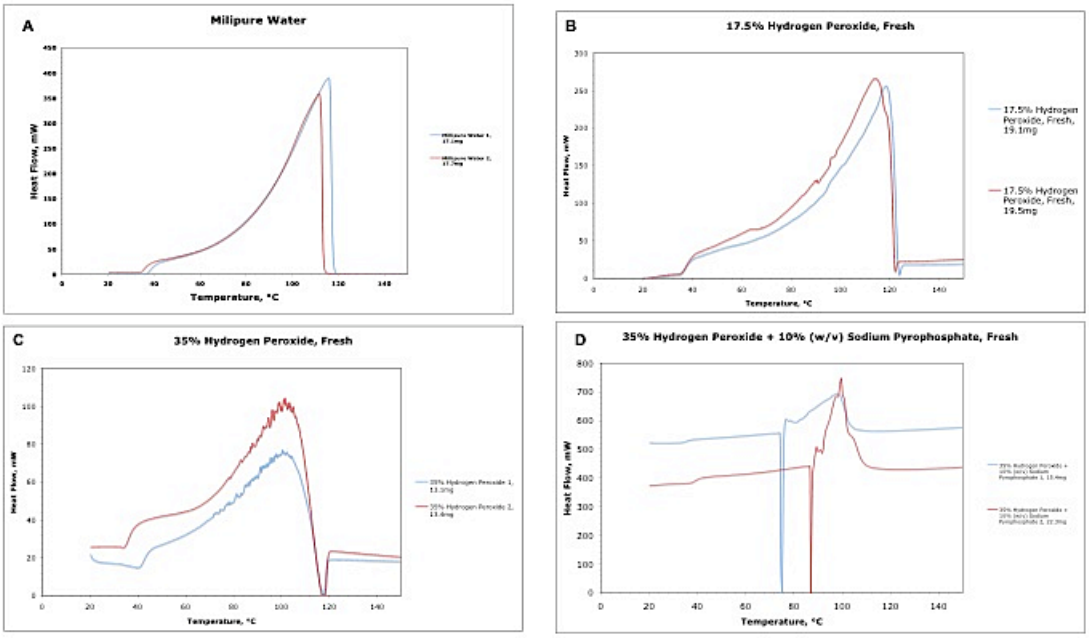


Figure 28: A) Millipore water, peak = 113.63°C (average), B) 17.5% hydrogen peroxide, peak = 116.63°C (average), C) 35% hydrogen peroxide, peak = 100.49°C (average), D) 35% hydrogen peroxide with 10% sodium pyrophosphate, peak = 98.40°C (average)

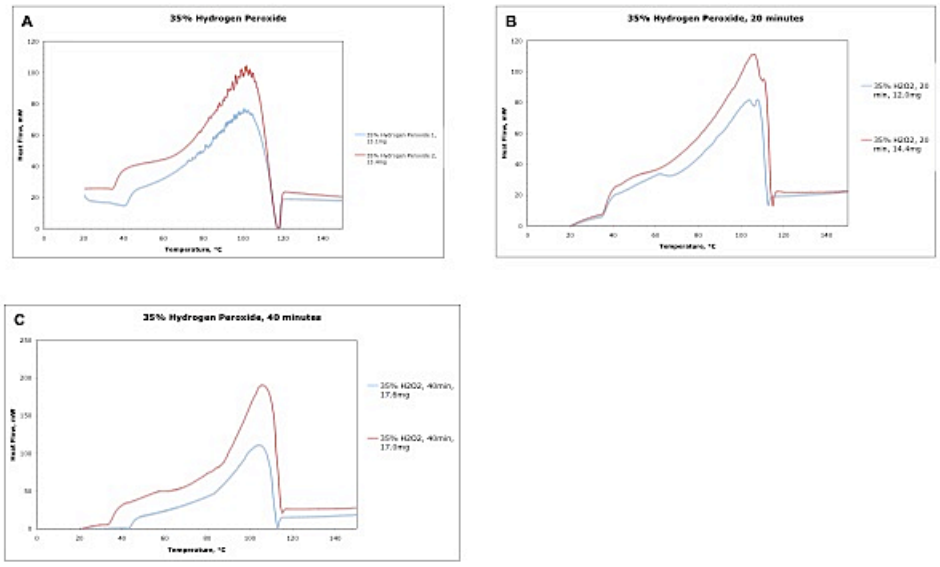


Figure 29: A) 35% hydrogen peroxide. Fresh, peak = 100.49°C (average), B) 35% hydrogen peroxide, 20 minute reaction with the atmosphere, peak = 106.60°C (average), C) 35% hydrogen peroxide, 40 minute reaction with the atmosphere, peak = 105.66 °C

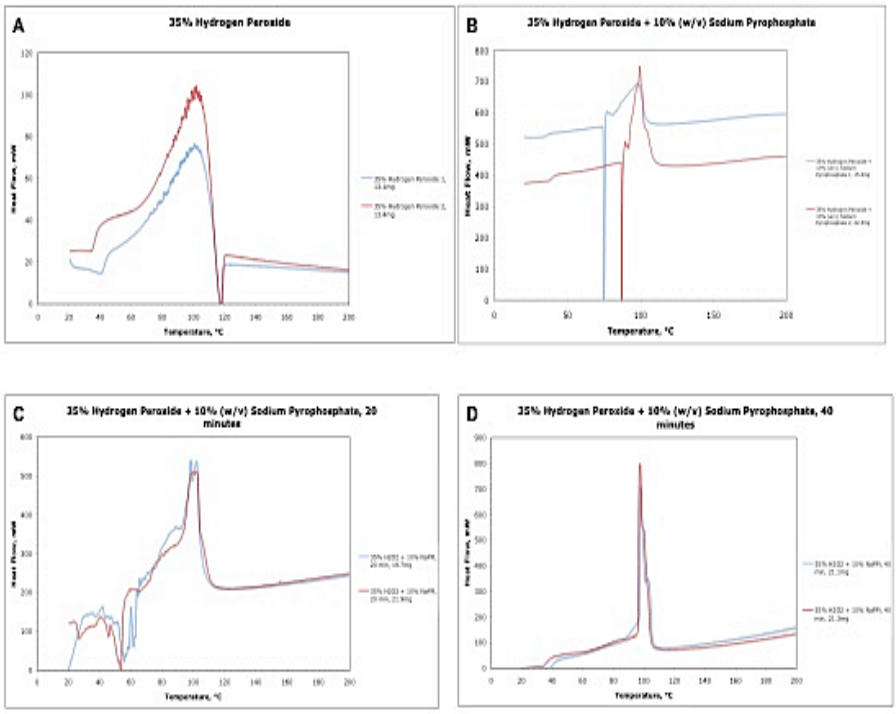


Figure 30: : A) 35% hydrogen peroxide, fresh, peak = 100.49°C (average), B) 35% hydrogen peroxide + 10% (w.v) sodium pyrophosphate, fresh, peak = 98.40°C (average), C) 35% hydrogen peroxide + 10% (w/v) sodium pyrophosphate, 20 minute reaction with the atmosphere, peak = 99.68°C (average), D) 35% hydrogen peroxide + 10% (w/v) sodium pyrophosphate, 40 minute reaction with the atmosphere, peak = 97.29°C (average).

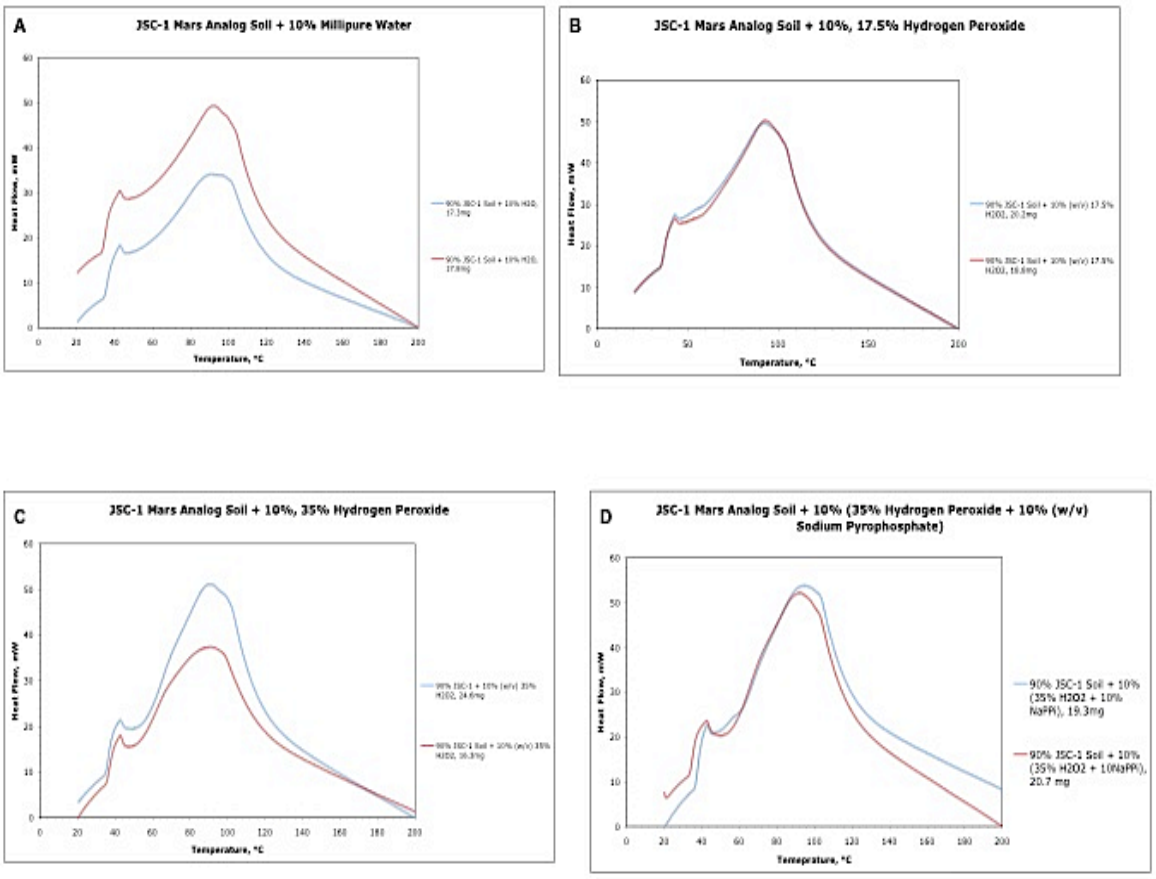


Figure 31: All the JSC-1 Martian analog soil sample mixtures had a small first peak at 41.92°C (average). A) JSC-1 soil + 10% Millipure water, peak = 92.53°C (average), B) JSC-1 soil + 10%, 17.5% hydrogen peroxide, peak = 92.78°C (average), C) JSC-1 soil + 10%, 35% hydrogen peroxide, peak = 90.31 (average), D) JSC-1 soil + 10% (35% hydrogen peroxide +10% sodium pyrophosphate), peak = 93.13°C (average).

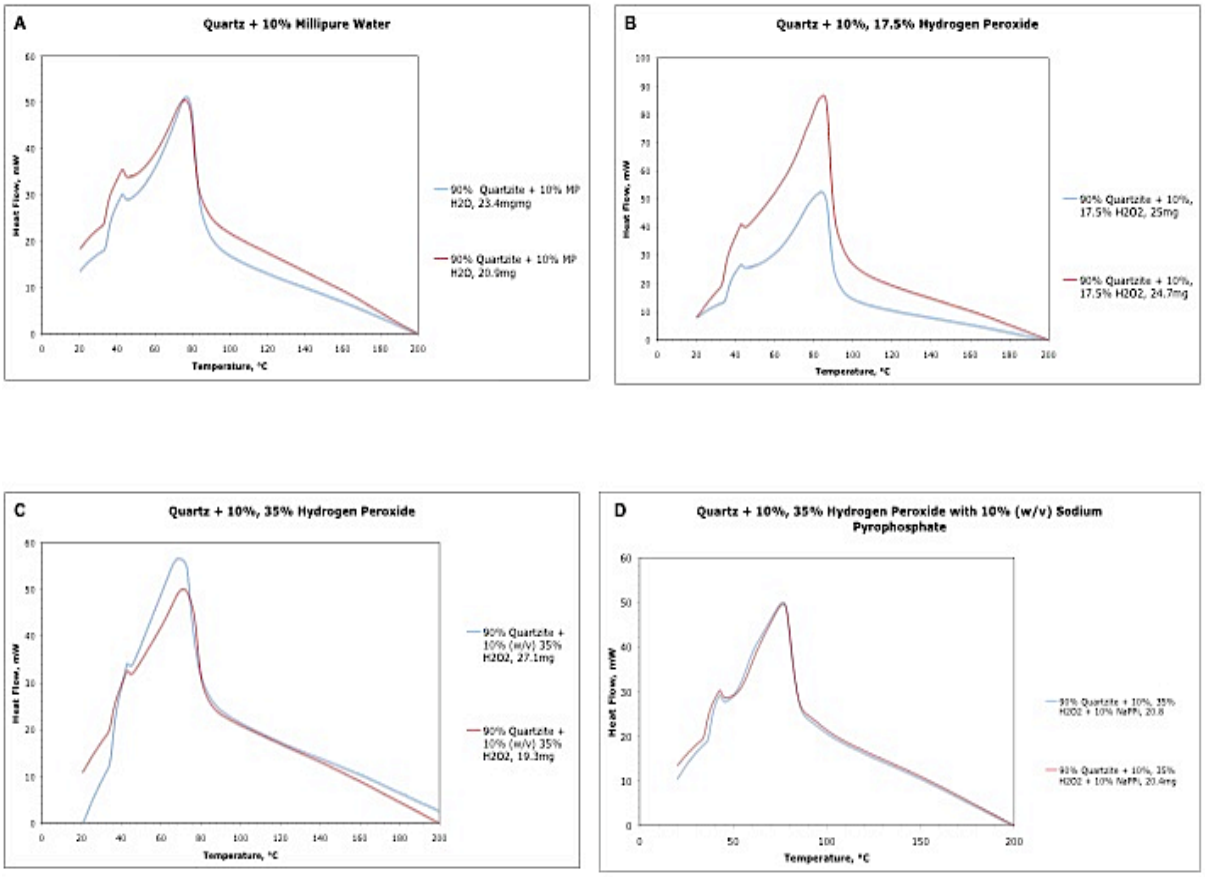


Figure 32: Quartz mixture sample series. A) Quartz + 10% millipure water, peak = 76.42°C (average), B) Quartz + 10%, 17.5% hydrogen peroxide, peak = 84.33°C (average), C) Quartz + 10%, 35% hydrogen peroxide, peak = 69.81°C (average), D) Quartz + 10% (35% hydrogen peroxide + 10% sodium pyrophosphate), peak = 76.39°C (average).

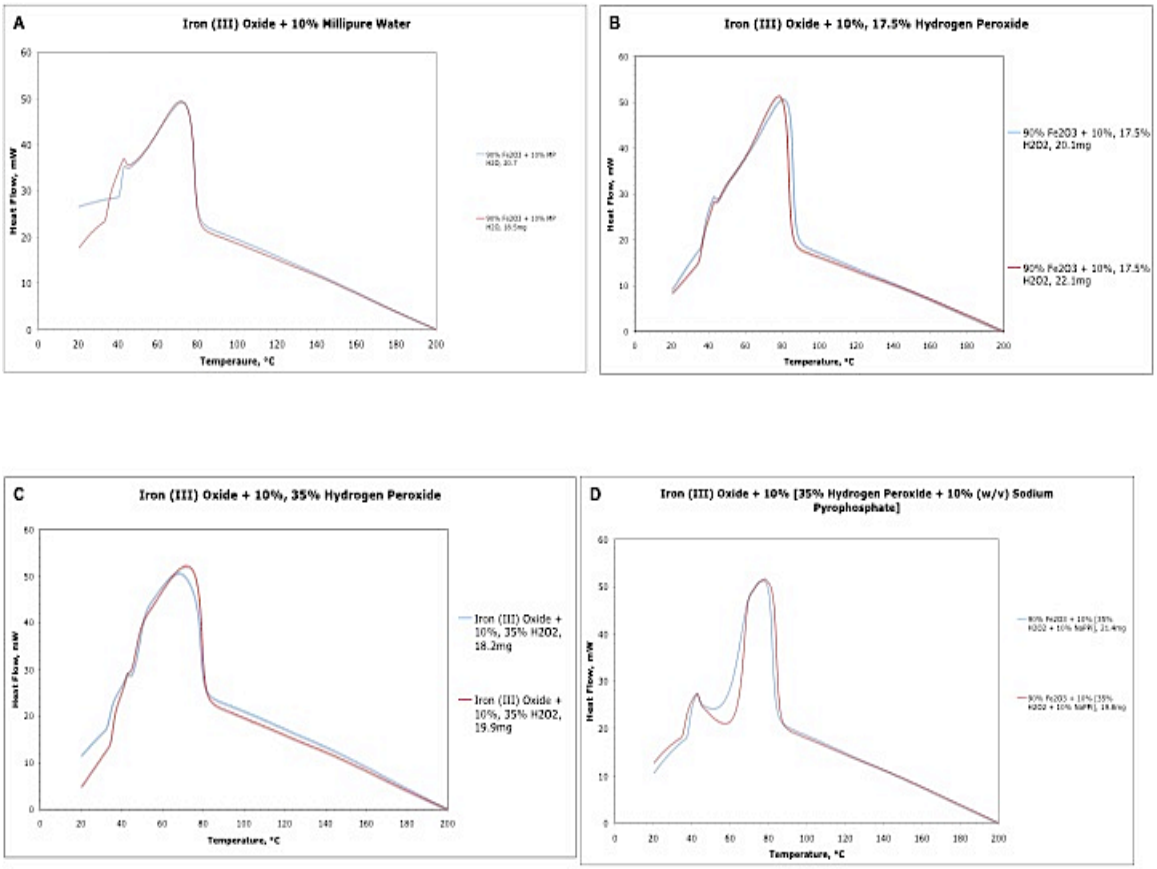


Figure 33: Iron (III) oxide sample mixture series. A) Iron oxide + 10% Millipure water, peak = 74.38°C (average), B) Iron oxide + 10%, 17.5% hydrogen peroxide, peak = 84.33°C (average), C) Iron oxide + 10%, 35% hydrogen peroxide, peak = 69.81 °C (average), D) Iron oxide + 10% (35% hydrogen peroxide + 10% sodium pyrophosphate), peak = 77.37°C (average).

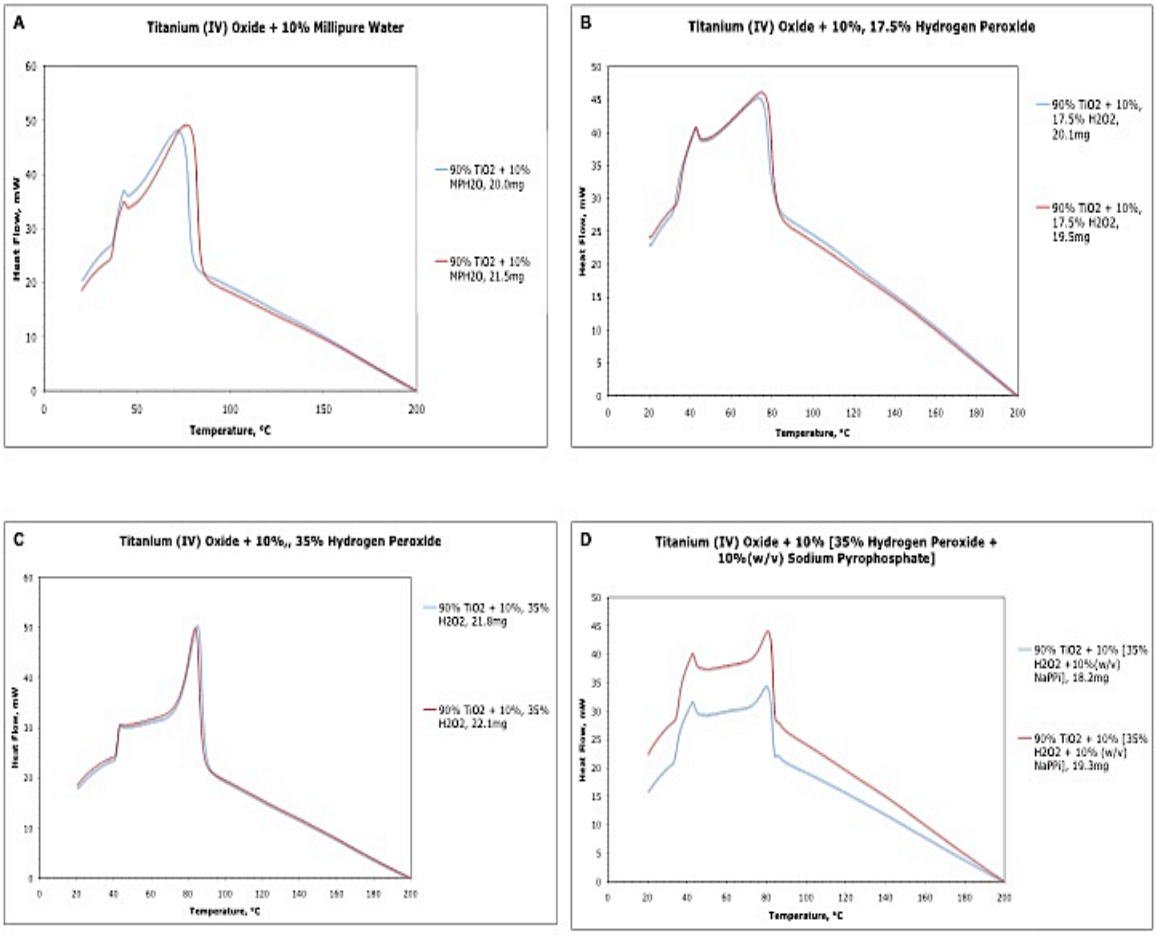


Figure 34: Titanium (IV) oxide sample mixture series. A) Titanium oxide + 10% Millipure water, peak = 75.04°C (average), B) Titanium oxide +10%, 17.5% hydrogen peroxide, peak = 74.95°C (average), C) Titanium oxide + 10%, 35% hydrogen peroxide, peak = 84.33°C (average), D) Titanium oxide + 10% (35% hydrogen peroxide + 10% sodium pyrophosphate), peak = 80.58°C (average).

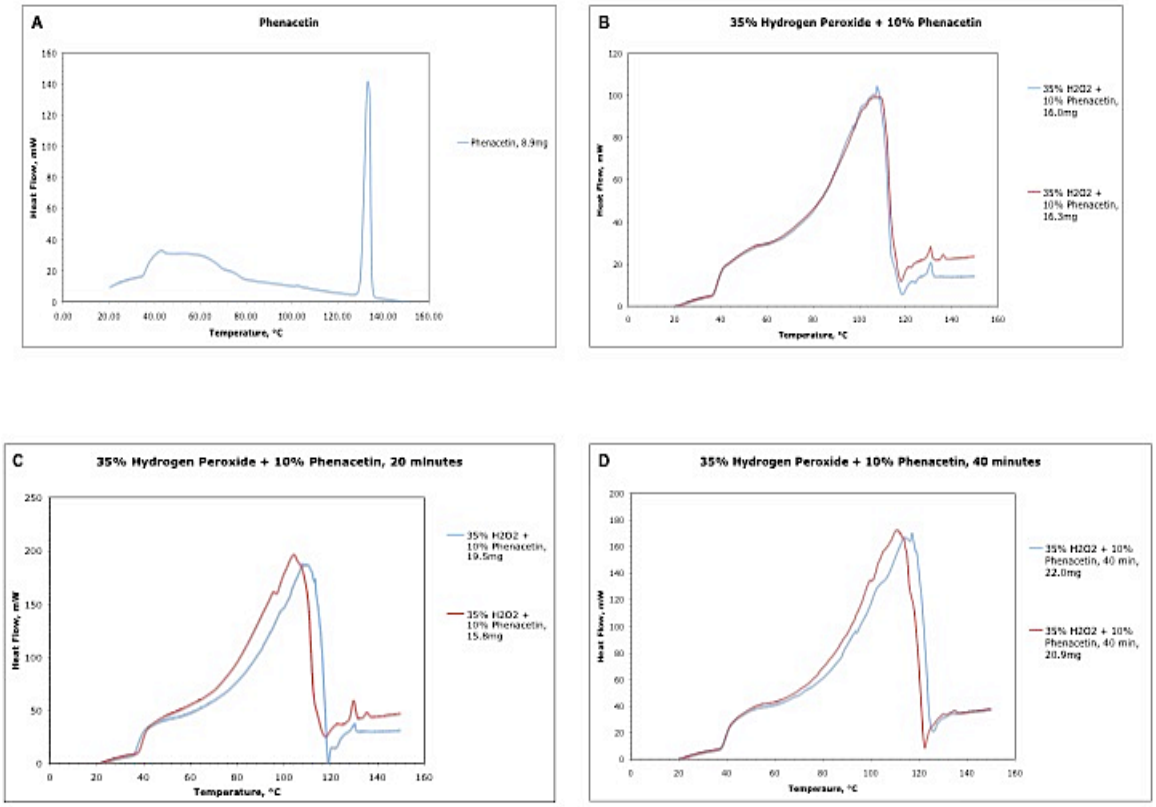


Figure 35: Hydrogen peroxide and Phenacetin sample mixture series. A) Phenacetin alone, peak = 133.02°C, B) 35% hydrogen peroxide + 10% phenacetin, peak = 108.24°C (average), B) 35% hydrogen peroxide + 10% phenacetin, 20 minute reaction with atmosphere, peak = 107.37°C (average), D) 35% hydrogen peroxide + 10% phenacetin, 40 minute reaction with the atmosphere, peak = 114.11°C (average).

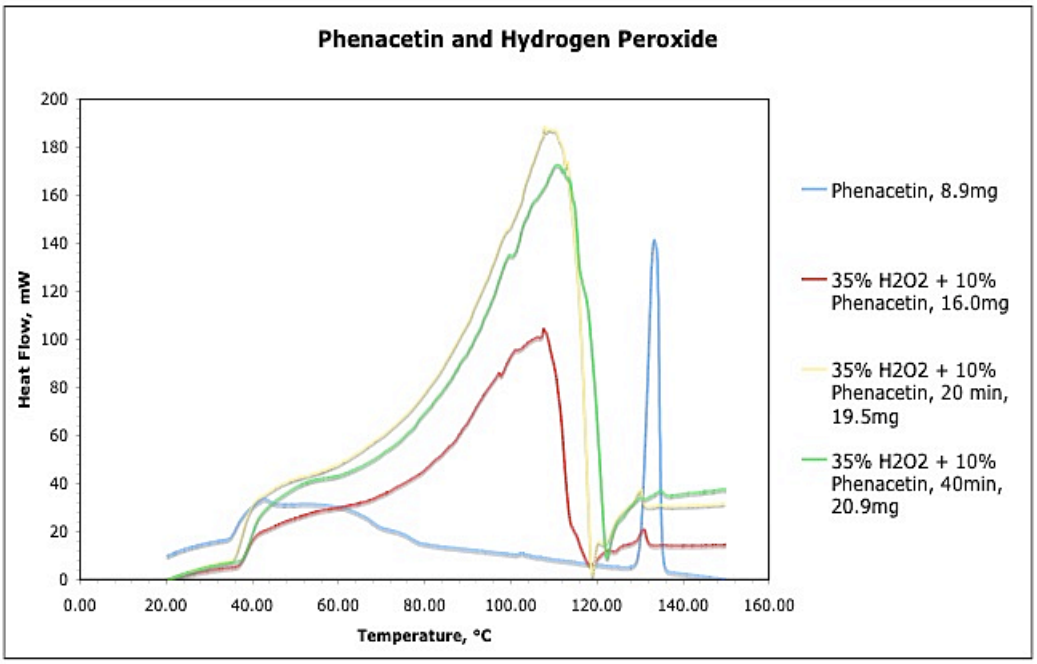


Figure 36: Phenacetin sample mixture combined series, including phenacetin alone. All phenacetin samples show a peak between 130°C - 134°C, near the flash point of .pure phenacetin (134°C).

REFERENCES

Alcorn, T.M., Zheng, H.-Y., Gunther, M.R., Hassett, D.J., and Cohen, M.S. (1994) Variation in hydrogen peroxide sensitivity between different strains of *Neisseria gonorrhoeae* is dependent on factors in addition to catalase activity. *Infection and Immunity* 62, 2138-2140.

Anders, R.F., Hogg, D.M., and Jago, G.R. (1970) Formation of hydrogen peroxide by group N streptococci and its effect on their growth and metabolism. *Applied Microbiology* 19, 608-612.

Atreya, S.K. and Gu, Z. (1994) Stability of the Martian atmosphere: Is heterogeneous catalysis essential? *J. Geophys. Res.* 99, 13133-13145.

Atreya, S.K., Mahaffy, P.R. and Wong, A.S. (2006) Methane and related trace species on Mars: origin, loss, implications for life and habitability. *Planetary and Space Sci.* 55(3) 358-369.

Atreya, S.K., Wong, A.-S., Renno, N.O., Farrell, W.M., Delory, G.T., Sentman, D.D., Cummer, S.A., Marshall, J.R., Rafkin, S.C.R., and Catling, D.C. (2006) Oxidant enhancement in Martian dust devils and storms: implications for life and habitability. Astrobiology 6, 429-450.

Benner, S.A., Devine, K.G., Matveeva, L.N., and Powell, D.H. (2000) The missing organic molecules on Mars. *Proc. Natl. Acad. Sci. USA*, 97, 2425-2430.

Biemann, K, Oro, J., Orgel, L.E., Nier, A.O., Anderson, D.M., Simmonds, P.G., Flory, D., Diaz, A.V., Rushneck, D.R., Biller, J.A. (1976) Search for Organic and Volatile Inorganic Compounds in Two Surface Samples from the Chryse Planitia Region of Mars. *Science* 194:72-76.

Boynton, W.V., Ming, D.W., Kounaves, S.P., Young, S.M.M., Arvidson, R.E., Hecht, M.H., Hoffman, J., Niles, P.B., Hamara, D.K., Quinn, R.C., Smith, P.H., Sutter, B., Catling, D.C. and Morris, R.V. Evidence for Calcium Carbonate at the Mars Phoenix Landing Site. *Science*. 3 July 2009:58-61.

Brocks, J.J., Logan, G.A., Buick, R., and Summons, R.E. (1999) Archaean molecular fossils and the early rise of eukaryotes. *Science* 285, 1033-1036.

Brown, F.S., Adelson, H.E., Chapman, M.C., Clausen, O.W., Cole, A.J., Cragin, J.T., Day, R.J., Debenham, C.H., Fortney, R.E., Gilje, R.I., Harvey, D.W., Kroop, J.L., Loer, S.J., Logan, J.L., Potter, W.D. Rosiak, G.T. (1978) The biology instrument for the Viking Mars mission. *Rev. Sci. Instrum.* 49:139-182.

Catling, D.C. and Buick, R. (2006) Oxygen and life in the Precambrian. *Geobiology* 4, 225-226.

Charron, I., Couvert, A., Laplanche, A., Renner, C., Patria, L., and Requieme, B. (2006) Treatment of odorous sulphur compounds by chemical scrubbing with hydrogen peroxide--stabilisation of the scrubbing solution. *Environmental Science and Technology* 40, 7881-7885.

Condon, S. (1987) Responses of lactic acid bacteria to oxygen. *FEMS Microbiology Letters* 46, 269-280.

Daly, M.J., Gaidamakova, E.K., Matrosova, V.Y., Vasilenko, A., Zhai, M., Leapman, R.D., Lai, B., Ravel, B., Li, S.-M.W., Kemmer, K.M., and Fredrickson, J.K. (2007) Protein oxidation implicated as the primary determinant of bacterial radioresistance. *PloS Biology* 5, 769-779.

Dennis, P.C., Sleep, B.E., Fulthorpe, R.R., Liss, S.N. (2003) Phylogenetic analysis of bacterial population in an anerobic microbial consortium capable of degrading saturation concentrations of tetrachloroethylene. *Can. J. Microbiol.* 49:15-27.

Duckworth, R. (1975). Water Relations of Foods. *Academic Press*, New York.

Encrenaz, Th., Bézard, B, Greathouse, T.K., Richter, M.J., Lacy, J.H., Atreya, S.K., Wong, A.S., Lebonnois, S., Lefèvre, F., and Forget, F. (2004) Hydrogen peroxide on Mars: evidence for spatial and seasonal variations. *Icarus* 170, 424-429.

Eschenbach, D.A., Davick, P.R., Williams, B.L., Klebanoff, S.J., Young-Smith, K., Critchlow, S.M., and Holmes, K.K. (1989) Prevalence of hydrogen peroxide-producing Lactobacillus species in normal women and women with bacterial vaginosis. *J. Clin. Microbiol.* 27, 251-256.

Foley, W.T., Giguère, P.A. (1951) Hydrogen peroxide and its analogues: II. Phase equilibrium in the system hydrogen-peroxidewater. *Can. J. Chemistry*, 29, 123-132.

Giguère, P.A., Secco, E.A. (1954) Hydrogen peroxide and its analogues: V. Phase equilibria in the system D_2O-D_2O_2 *Can. J. Chemistry*, 32, 550-556.

Ha, Z. and Chan, C.K. (1999). The water activities of $MgCl_2$, $Mg(NO_3)_2$, $MgSO_4$ and their mixtures. *Aerosol Science and Technology*. 31:154-169.

Hecht, M.H., Kounaves, S.P., Quinn, R.C., West, S.J., Young, S.M.M., Ming, D.W., Catling, D.C., Clark, B.C., Boynton, W.V., Hoffman, J., DeFlores, L.P., Gospodinova, K., Kapit, J. and Smith, P.H. Detection of Perchlorate and the Soluble Chemistry of the Martian Soil at the Phoenix Landing Site. *Science*. 3 July 2009:64-67.

Hurowitz, J.A., Tosca, N.J., McLennan, S.M., and Schoonen, M.A.A. (2007) Production of hydrogen peroxide in Martian and lunar soils. *Earth and Planetary Science Letters* 255, 41-52.

Horowitz, N.H., Hobby, G.L., Hubbard, J.S. (1977) Viking on Mars- the carbon assimilation experiments. *J. Geophys. Res.* 82:4659-4662.

Houtkooper, J.M. and Schulze-Makuch, D. (2007) A possible biogenic origin for hydrogen peroxide on Mars: the Viking results reinterpreted. *Int. J. of Astrobiology* 6, 147-152.

Ito, A., Sato, Y., Kudo, S., Sato, S., Nakajima, H., and Toba, T. (2003) The screening of hydrogen peroxide-producing lactic acid bacteria and their application to inactivating psychrotrophic food-borne pathogens. *Current Microbiology* 47, 231-236.

Junge, K., Eicken, H., & Deming, J. W. (2004). Bacterial Activity at -2 to -20°C in Artic Wintertime Sea Ice. *Applied and Environmental Microbiology*, *70*(1), 550-557.

Kashefi, K. & Lovley, D. R. (2003). Extending the upper temperature limit for life. *Science*, *301*, 934.

Klein, H.P. (1978) The Viking biological experiments on Mars. *Icarus* 34:666-674.

Klein, H.P. (1999) Did Viking discover life on Mars? *Orig. Life Evol. Biosph*. 29, 625-631.

Klein,H.P., Horowitz, NH; Levin, GV; Oyama, VI; Lederberg, J; Rich, A; Hubbard, JS; Hobby, GL; Straat, PA; Berdahl, BJ; Carle, GC; Brown, FS; Johnson, RD. (1976) The Viking Biological Investigation: Preliminary Results. *Science* 194(4260):99-105.

Konhauser, K. (2007). *Introduction to Geomicrobiology.* Blackwell Science Ltd.

Levin, G.V. (1963) Rapid Microbiological Determinations with Radioisotopes. *Adv. Appl. Microbiol.* 5:95-133.

Levin, G.V. (2007) Possible evidence for panspermia: the labeled release experiment. *Int. J. of Astrobiology* 6, 95-108.

Levin, G.V. and Straat, P.A. (1979a) Completion of the Viking Labeled Release Experiment on Mars. *J. Mol. Evol.* 14:167-183.

Levin, G.V. and Straat, P.A. (1979b) Laboratiry 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.

Levin, G.V. and Straat, P.A. (1981) A search for a nonbiological explanation of the Viking Labeled Release Life Detection Experiment. *Icarus* 45, 494-516.

Levin, G.V.; Heim, A.H.; Thompson, M.F.; Beem, D.R. (1964) "Gulliver", an experiment for extraterrestrial life detection and analysis. *Life Sci. Space Res.* 2:124-132

Madanská, J., Vitková, Z., and Capková, Z. (2004) Examination of the stability of hydrogen peroxide solutions. *Ceska Slov Farm*. 53, 261-263.

Mahaffy, P.R. (2007) Exploration of the Habitability of Mars: Development of Analytical Protocols for Measurement of Organic Carbon on the 2009 Mars Science Laboratory. *Space Sci. Rev.* 135:255-268.

Mancinelli, R.L. (1989) Peroxides and the survivability of microorganisms on the surface of Mars. *Adv. Space Res*. 9, 191-195.

McFadden Adams L.A.; Weisman P.R.; Johnson, T.V. (2007) Encyclopedia of the Solar System, Edition 2. *Academic Press*.

Mugnier, J. and Jung, G. (1985). Survival of bacteria and fungi in relation to waer activity and the solvent properties of water in biopolymer gels. *Appl. Env. Microbiol.* 50(1):108-114.

NASA JPL Mars Science Laboratory Homepage http://marsprogram.jpl.nasa.gov/msl/index.html downloaded July 21, 2009.

NASA Mars fact sheet: http://www.nasa.gov/worldbook/mars_worldbook.html, downloaded June 15, 2009.

NASA Mars Phoenix home page: http://phoenix.lpl.arizona.edu/. Downloaded July 1, 2009.

NASA Viking fact sheet: http://www.nasa.gov/mission_pages/viking/viking30_fs.html, downloaded June 10, 2009.

Nussinov, M.D., Chernyak, Y.B., and Ettinger, J.L. (1978) Model of the fine-grain component of martian soil based on Viking lander data. *Nature* 274, 859-861.

Oyama, V.I., Berdahl, B.J., Carle, G.C., Lehwalt, M.E., Ginoza, H.S. (1976) The search for life on Mars: Viking 1976 gas changes as indicators of biological activity. *Orig. Life* 7:313-333.

Oyama, V.I. and Berdhal, B.J. (1977) The Viking gas exchange experiment results from Chryse and Utopia surface samples. *J. Geophys. Res.* 82:4669-4676.

Pawkit Water Activity Meter Manual. (2008). Version 4, Decagon Devices, Pullman, WA.

Plum, R.C., Tantayanon, R., Libby, M., and W.W. Xu (1989) Chemical model for Viking biology experiments : implications for the composition of the martian regolith. *Nature* 338, 633-635.

Prior, B.A. (1979). Measurement of water activity in foods: A review. *Journal of Food Protection.* 42(8):668-674.

Quinn, R.C. and Zent, A.P. (1999) Peroxide-modified titanium dioxide: a chemical analog of putative Martian soil oxidants. *Orig. Life Evol. Biosph*. 29, 59-72.

Raymond, J. and Segre, D. (2006) The effect of oxygen on biochemical networks and the evolution of complex life. *Science* 311, 1764-1767.

Rivkina, E. M., Friedmann, E. I., McKay, C. P., & Gilichinsky, D. A. (2000). Metabolic activity of permafrost bacteria below the freezing point. *Applied and Environmental Microbiology*, *66*, 3230-3233.

Rothschild, L. J. & Mancinelli, R. L. (2001). Life in Extreme Environments. *Nature*, *409*, 1092-1101.

Ryan, C.S. and Kleinberg, I. (1995) Bacteria in human mouths involved in the production and utilization of hydrogen peroxide. *Arch. Oral. Biol*. 40, 753-763.

Schulze-Makuch, D. & Irwin, L. N. (2006). *Life in the Universe, Expectations and Contraints.* Springer.

Schuerger, AC and Benton, CC. (2008) Viking Biology Experiments: Lessons learned and the role of ecology in future Mars life-detection experiments. *Space Sci Rev.* 135:233-243.

Sellers, K, Weeks, K., Alsop, W., Clough, S.R., Hoyt, M., Pugh, B., Robb, J. (2006) Perchlorate: Environmental Problems and Solutions. *CRC Press,* New York.

Shapiro, R.L., Hatheway, C.,Swerdflow, D. (1998). Botulism in the United States: A Clinical and Epidemiologic Review. *Annals of Internal Medicine* 129 (3): 221.

P. H. Smith, L. K. Tamppari, R. E. Arvidson, D. Bass, D. Blaney, W. V. Boynton, A. Carswell, D. C. Catling, B. C. Clark, T. Duck, E. DeJong, D. Fisher, W. Goetz, H. P. Gunnlaugsson, M. H. Hecht, V. Hipkin, J. Hoffman, S. F. Hviid, H. U. Keller, S. P. Kounaves, C. F. Lange, M. T. Lemmon, M. B. Madsen, M. Malin, W. J. Markiewicz, J. Marshall, C. P. McKay, M. T. Mellon, D. W. Ming, R. V. Morris, W. T. Pike, N. Renno, U. Staufer, C. Stoker, P. Taylor, J. A. Whiteway, A. P. Zent. H_2O at the Phoenix Landing Site. *Science*. 3 July 2009: 58-61.

Stewart, P.S. Roe, F., Rayner, J., Elkins, J.G., Lewandowski, Z., Ochsner, U.A., and Hassett, D.J. (2000) Effect of catalase on hydrogen peroxide penetration into *Pseudomonas aeruginosa* biofilms. *Applied Environmental Microbiology* 66, 836-838.

Tanenbaum, S.W. (1956) The metabolism of *Acetobacter peroxidans*. I. Oxidative enzymes. *Biochim. Biophys. Acta* 21, 335-342.

Troller, J.A. and Christian, J.H.B. (1978). Water Activity and Food. *Academic Press*, New York.

Watts, R.J., Finn, D.D., Cutler, L.M., Schmidt, J.T., and Teel, A.L. (2007). Enhanced stability of hydrogen peroxide in the presence of subsurface solids. *J Contam Hydrol*. 91, 312-326.

Yen, A.S., Kim, S.S., Hecht, M.H., Frant, M.S., and Murray, B. (2000) Evidence that the reactivity of the Martian soil is due to superoxide ions. *Science* 289, 1909-1912.

Zent, A.P. and McKay, C.P. (1994) The chemical reactivity of the Martian soil and implications for future missions. *Icarus* 108, 146-157.