

Martian soil biosensors based on dielectric spectroscopy

John H. Miller, Jr., Jie Fang, David Warmflash, David S. McKay, Jeffrey A. Jones, and Fathi Karouia

ABSTRACT—This project is focused on measuring the electromagnetic responses of living organisms, and the potential of such measurements for biosensors in astrobiology and medicine. Dielectric spectroscopy at various temperatures can, for example, distinguish live organisms from nonliving complex macromolecules and may eventually be suitable for *in situ* astrobiology studies on the surface of Mars or in the liquid ocean beneath the ice of Europa. More recent experiments in our lab involve nonlinear (harmonic generation) responses of live organisms to sinusoidal electric fields. Our results indicate that active biological motors and other enzyme complexes generate harmonics over specific frequency ranges. Photosynthetic organisms and organelles, such as chloroplasts from plants, are especially suitable as model organisms because the physiologically active processes are light-activated. Finally, enzyme complexes in the mitochondrial inner membrane, which are involved in the production of adenosine triphosphate (ATP), are of great interest because of a growing body of evidence showing the role of mitochondrial dysfunction in age-related metabolic and degenerative diseases, including type-2 diabetes, heart disease, cancer, and Alzheimer's disease.

INTRODUCTION

Premise of the project. The possibility of extraterrestrial life on Mars, either in the past or at present,¹ has been of great interest for well over a century. The scientific implications of the discovery of life on Mars would be profound. The Viking program attempted, in 1976, to detect evidence for living or fossilized organisms in Martian soil, and yielded ambiguous results.² More recent studies³ of the Martian meteorite Allan Hills 84001 (ALH84001) suggest that microbial life existed on Mars about 4 billion years ago. Evidence includes magnetite (Fe_3O_4) crystals found in carbonate globules and their associated rims in the meteorite.⁴ About 25 percent of these tiny magnetites are nearly identical to those produced by magnetotactic bacteria on Earth, and are not likely to be produced by abiotic means. Thus, such Martian magnetite crystals could potentially be magnetofossils, which, if true, would constitute evidence of the oldest life forms known.⁵

Several studies suggest that subsurface Martian life could potentially survive even today.⁶ Geological evidence suggests that ice was once deposited in the regolith, where it may be present above mid-latitudes.⁷ Such ice, which could extend several kilometers below the surface, might be a source of liquid water near magmatic intrusions.⁸ Below the surface of the Earth, the biomass of subterranean organisms may even exceed that at the surface.⁹ Many such organisms can live in highly saline conditions at temperatures from 115°C to -20°C.^{10,11} Conditions such as these might prevail, for example, in a subsurface aquifer or hydrothermal system. Thus, there is considerable interest in developing new techniques for detecting subsurface life on Mars. Moreover, the likelihood that oceans of liquid water exist below the icy surfaces of Europa

and other moons make these exciting candidates for possible extraterrestrial life.

A key hypothesis for this project is that an oscillatory field induces proteins and other macromolecules to change conformation. The rate of conformational change depends on frequency and on each enzyme's charge distribution, structure, and state of activity. The resulting motion of charged macromolecules leads to a nonlinear response, and to the generation of higher harmonics, providing a powerful functional spectroscopy tool. This hypothesis is supported by several observations.

In particular, oscillatory fields induce ac components of transmembrane potentials that add to the intrinsic potentials.¹² A low-frequency electric field polarizes live cells or macromolecules,¹³ resulting in enormous dielectric responses, and also modulates each cell's membrane potential.¹⁴ Second, sinusoidal fields can induce membrane pumps to translocate cations^{15,16} and generate harmonics.¹⁷ Membrane proteins exhibit nonlinear behavior¹⁸ since domains with dipole moments interact with the induced transmembrane potential, driving them to change conformation. The combination of conformational changes and ion translocation creates a nonlinear response. For example, cation pumps such as P-type ATPases¹⁹ have been reported to generate harmonics.²⁰ We have developed a sensitive method,²¹ using superconducting quantum interference devices (SQUIDS), to measure the harmonics produced by such membrane pumps at low frequencies. Further support is provided by our recent harmonic generation spectroscopy measurements, which will be discussed in the Results and Discussion section.

Goal of the project. The goal of this project is to develop sensors capable of detecting signatures of living organisms,

METHODOLOGY

Our previously reported experiments on linear dielectric response involved variable temperature dielectric spectroscopy of live organisms and Martian soil simulants. These experiments utilized a Solartron Impedance Analyzer, which measures complex admittance at frequencies up to 32 MHz. Here, we report on harmonic generation spectroscopy measurements that appear to be capable of detecting physiologically active processes.

A four-electrode setup is employed in conjunction with a Stanford Research SR780 or SR785 signal analyzer, operated as a spectrum analyzer, for measurements at kilohertz frequencies. A function generator applies a sinusoidal signal to the outer electrodes, while the voltage difference between the inner electrodes is fed into Channel 1 of the SR780, which records the induced harmonics.

In some experiments, as described in our previous report, a reference spectrum is acquired using a supernatant, whose conductivity has been adjusted (with distilled water, to compensate for the volume fraction of the cells present in the sample) to be identical to that of the sample at the frequency of interest. The supernatant typically consisted of an aqueous solution of ~1-10 mM NaCl. Two different types of control files are used, depending upon whether the reference is to be logged using the same set of electrodes or a separate matched reference cell. In either case, the logging, windowing, and Fourier transform routines are identical and provide a power spectrum of the reference cell, which is also recorded as a data file in the computer. Finally, the sample power spectrum obtained from the sample (e.g., cell suspension or soil sample) of interest is divided by the reference power spectrum and also stored. The entire procedure is automated using LabVIEW data acquisition software. The power of this approach was to allow the effects of nonlinearities with the electrochemical system to become deconvolved from those due to the biological cells themselves.

Another approach is to expose the biological system to environmental factors (e.g., light, glucose, activators, inhibitors, electron acceptors, etc.) and to measure changes in harmonics as functions of time as the physiological state evolves. This is the approach used in the data presented in this report.

EQUIPMENT AND SPECIAL TECHNOLOGY

Experiments at kilohertz frequencies employ a 4-electrode configuration, discussed in our previous report, in which electrodes are immersed into a suspension of cells or organelles. A waveform generator applies a sinusoidal voltage of high spectral purity to the two outer electrodes, while the response across the inner electrodes is measured with a Stanford Research SR780 or SR785, which shows the generated higher harmonics. Typically, the second or third harmonic generated by the suspension is recorded vs. amplitude and frequency, and all measurements are automated with LabVIEW software.

We have carried out measurements on suspensions of whole cells, mitochondria, and chloroplasts, the latter of which provide validation and are of fundamental interest since the pho-

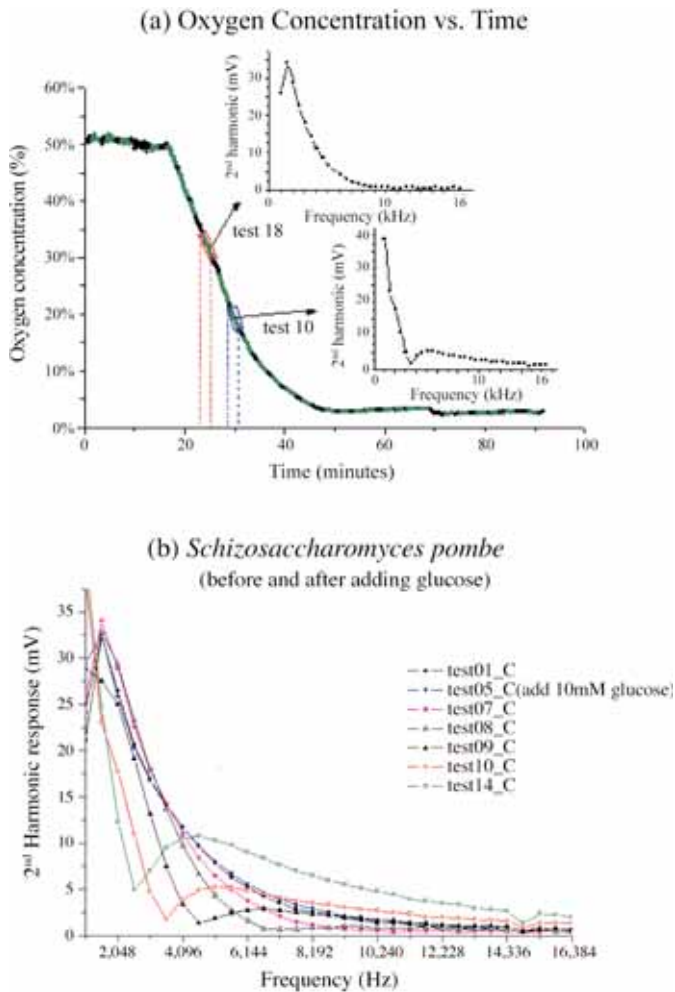


Figure 1. (a) Oxygen concentration vs. time and second harmonic response vs. applied frequency (inset) of *S. pombe* (fission yeast) at selected time intervals. Glucose was added at $t = 18$ min, at which time oxygen concentration began to drop due to oxygen consumption by the yeast cells. (b) Family of curves showing the generated second harmonic response vs. fundamental frequency (peak-to-peak amplitude = 8 V across outer two electrodes with 1 cm spacing) of the same *S. pombe* suspension at different test runs both before and after adding glucose.

which may not necessarily be based on DNA, for astrobiology applications such as the search for life in the Martian soil. We are mainly investigating the use of dielectric spectroscopy²² and related methods, including harmonic generation spectroscopy,²³ which can detect the activity of membrane-bound enzyme complexes that have electric dipole moments and undergo rapid changes in conformational state. Our project aims to elucidate possible signatures of active macromolecular complexes unique to living biological systems. Moreover, we believe that Earth-bound medical applications, such as the detection of mitochondrial dysfunction implicated in numerous age-related diseases, could emerge as important spin-off applications.

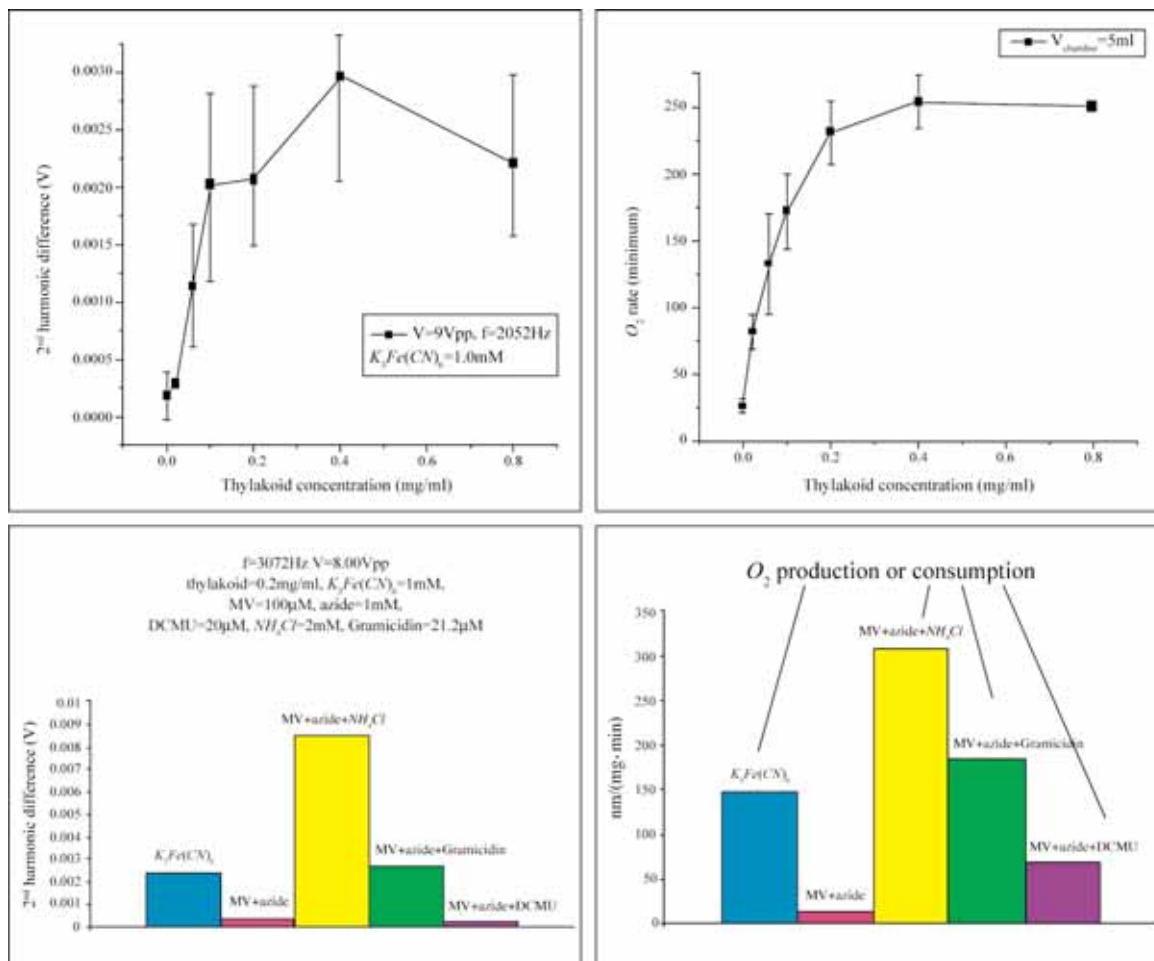


Figure 2. Summary of results on experiments correlating light-activated changes in second harmonic response with O_2 production or consumption of thylakoid membrane suspensions from spinach chloroplasts. The above data summarizes changes in both second harmonic response and oxygen production or consumption during two-minute time intervals, during which the organelles were exposed to white light. The bar graphs at the bottom show changes in second harmonic response (left) after adding various combinations of substrates, which correlate with the amount of oxygen produced or consumed (right) under the same conditions. These results suggest that changes in second harmonic response correlate with activity of the photosynthetic electron transport chain.

tosynthetic enzyme complexes are light-activated. Many of these experiments have been carried out in collaboration with the group headed by William R. Widger, Ph.D., in the Department of Biology and Biochemistry at the University of Houston. More recently, we have begun to collaborate with physicians and other investigators at The Methodist Hospital in Houston.

RESULTS AND DISCUSSION

Our most recent experiments involve measurements of harmonics generated by suspensions of whole cells, mitochondria, and thylakoid membranes in response to applied sinusoidal electric fields at kilohertz frequencies. The frequency- and amplitude-dependences of the induced (e.g., second and third) harmonics exhibit features that appear to correlate with the activity of complexes in the mitochondrial (or photosynthetic) electron transport chain. This suggests that sensors based on harmonic generation spectroscopy could be developed to detect mitochondrial activity and possible dysfunction,

which has been implicated in obesity, type-2 diabetes, heart disease, cancer, and numerous specific mitochondrial disorders. Thylakoid membrane suspensions (from spinach chloroplasts) have also proved useful model organelles for preliminary studies because the generated harmonics depend strongly on the presence or absence of light in such photosynthesizing organelles.

Several examples of observed harmonic generation spectra in the kilohertz range are illustrated by Figure 1, which shows generated harmonics vs. applied frequency for *S. pombe* (fission yeast), which has been activated by adding glucose. The changes in harmonic response are observed to correlate with oxygen consumption, which was measured at the same time. (Note that the oxygen concentration began to drop precipitously when glucose was added at $t = 18$ min.) The changes in harmonic response may thus be caused by respiratory activity in the mitochondrial electron transport chain. Note the change in second-harmonic spectral responses as the cell suspension continues to consume both glucose and oxygen. Recent modeling studies suggest that this behavior may reflect changes in

the mitochondrial membrane potential.

We have also studied the harmonic responses of suspensions of thylakoid membranes from spinach chloroplasts, which engage in photosynthesis and whose physiological processes are light-activated (Figure 2). In addition to exposing the organelles to light, electron acceptors that triggered either oxygen production (potassium ferricyanide ($K_3[Fe(CN)_6]$) or oxygen consumption (methyl viologen (MV) + sodium azide) were added to the aqueous media in which the thylakoid membranes were suspended.

The results, summarized in Figure 2, strongly suggest that the observed temporal changes in second harmonic response reflect activity of the photosynthetic electron transport chain in the thylakoid membrane. Identical colors of the bars on the left and right sides at the bottom of Figure 2 correspond to identical conditions. When the electron acceptor potassium ferricyanide was added (blue bars, left) the change in second harmonic and oxygen production levels were both about mid-range as compared to the other situations shown.

The combination of methyl viologen and sodium azide acts as an electron acceptor, as for the case of ferricyanide. A key difference, however, is that the MV-azide combination leads to consumption of more oxygen than that produced during photosynthesis. In addition, a build-up of the thylakoid membrane potential can inhibit the rate of activity in the photosynthetic electron transport chain (ETC). This slow ETC activity is reflected by the extremely small bars at the bottom of Figure 2, which show the small change in second harmonic and the small net amount of oxygen consumed. Adding a membrane depolarizer, either ammonium chloride (NH_4Cl) or gramicidin, greatly reduces the ETC inhibiting effects of the membrane potential, which greatly increases ETC activity. This behavior is reflected by the dramatically enhanced sizes of the yellow and light-blue bars.

CONCLUSIONS

Our studies to date suggest that harmonic generation spectroscopy reflects enzyme activity in live cells and organelles. Future objectives will include further development of electromagnetic sensors to detect signatures of life, such as enzyme activity. Potential astrobiology applications, including detection of possible extant life in the Mars subsurface, will continue to provide incentive to study the unusual approaches discussed here for monitoring biological systems.

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BIOSENSORS—Jie Fang, graduate student in physics, is conducting experiments that involve nonlinear responses of live organisms to sinusoidal electric fields in Professor Miller's laboratory.

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