Electrochemical Approaches for Chemical and Biological Analysis on Mars

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Obtaining in situ chemical data from planetary bodies such as Mars or Europa can present significant challenges. The one analytical technique that has many of the requisite characteristics to meet such a challenge is electroanalysis. Described here are three electroanalytical devices designed for in situ geochemical and biological analysis on Mars. The Mars Environmental Compatibility Assessment (MECA) was built and flight qualified for the now cancelled NASA Mars 2001 Lander. Part of MECA consisted of four "cells" containing arrays of electrochemical based sensors for measuring the ionic species in soil samples. A next-generation MECA, the Robotic Chemical Analysis Laboratory (RCAL), uses a carousel-type system to allow for greater customization of analytical procedures. A second instrument, proposed as part of the 2007 CryoScout *mission, consists of a flow-through inorganic chemical analyzer (MICA).* CryoScout *is a torpedo-like device designed for subsurface investigation of the stratigraphic climate record embedded in Mars' north polar cap. As the* CryoScout *melts its way through the ice cap, MICA will collect and analyze the meltwater for a variety of inorganics and chemical parameters. By analyzing the chemistry locked in the layers of dust, salt, and ice, geologists will be able to determine the recent history of climate, water, and atmosphere on Mars and link it to the past. Finally, electroanalysis shows its abilities in the detection of possible microorganism on Mars or elsewhere in the solar system. To identify an unknown microorganism, one that may not even use Earth-type biochemistry, requires a detection scheme which makes minimal assumptions and looks for the most general features. Recent work has demonstrated that the use of an array of electrochemical sensors which monitors the changes in a solution via electrical conductivity, pH, and ion selective electrodes, can be used to detect minute chemical perturbations caused by the growth of bacteria and with the correct methodology provide unambiguous detection of such life forms.*

KEYWORDS:

bacteria · electroanalysis · electrochemistry · geochemistry · sensors

During the past 40 years, a variety of scientific instruments have been used for missions to investigated planetary bodies within our solar system. Most of these missions have relied on remote sensing, typically based on optical or radiation detection techniques. Mars has been the focus of a large number of such missions.[1] The first successful flyby of Mars in 1964 returned 21 photos with subsequent flyby missions adding more. It was not until 1976, with the success of the two *Viking* Landers, and in 1997, with the *Pathfinder* Lander and Rover, that we obtained a detailed close up view of the surface and its chemical and physical properties.[2, 3]

The instruments on the *Viking I* and *II* Landers included three biology experiments, a gas chromatograph/mass spectrometer (GCMS), and an X-ray fluorescence spectrometer (XRFS). The results of the biology experiments have been interpreted by a majority of the science community as ruling out microbial life on the surface of Mars.[4] However, there are still some who are convinced the results leave no other conclusion but the presence of life.[5] The big surprise though was that the GCMS detected no organics in the soil samples down to the parts-per-billion (ppb) levels. Many hypotheses have been advanced to account for the absence of organics and the possible chemicals and reactions that could account for the ambiguous biology experiments. These have included reactions involving oxidants such as hydrogen or superperoxides, smectite clays, and superoxide radical ions. A recent study has shown that the *Viking* GCMS would not have been able to detected the degradation products from several million bacterial cells per gram of Martian soil at the ppb level.[6] The life detection and GCMS results form the basis for the prevalent opinion within the scientific community that it is probably unlikely that any microbial life forms were detected on the surface of Mars, and that, in addition, the chemical and physical conditions are such that it is probably unlikely that any organic-based life form could exist on the unprotected surface.

Unlike *Viking*, *Pathfinder* was the first mission to focus on Martian geochemistry and mineralogy. Its instruments and mobile rover were designed not to directly detect life but to primarily provide close up optical observation and determine the elemental chemical composition of the Martian rocks and surface material over hundreds of square meters using the alpha proton X-ray spectrometer (APXS). The *Viking* XRFS and *Pathfinder* APXS data have provided a reasonably clear picture of the elemental composition of the surface material. Even though the

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analyses were from different areas they present a picture of a rather homogeneous surface composition. The combined results of the *Viking* and pathfinder show the surface material to be roughly composed of 45% SiO_2, 18% FeO_2, 8% MgO, 6% SO_3, 7% Al_2O_3, 1.8% NaO, and 0.6% Cl.[7, 8]

The Advantages of Electroanalytical Devices

Chemical and biological analyses are routinely performed on Earth, however, to obtain valid in situ analytical data from remote harsh environments on planetary bodies, such as Mars or Europa, presents a truly unique and formidable challenge. Not only do constraints on such instrumentation include power, size, mass, cost, and robustness, but they it must also be able to survive high *g*-forces, an eight-month journey through a radiation permeated environment, and possible temperature variations ranging from $+60$ to $-100\,^{\circ}C$. Sensors based on electrochemical transduction schemes have many of the prerequisite properties and can withstand the environmental risks that will enable them to return significant scientific data under such severe constraints.

Because of the history and composition of Mars, electrochemical sensors may also be especially well suited in providing useful geochemical analyses and broader planetary scientific results. The surface of Mars, as described above, appears to contain a large fraction of sulfur and chlorine. These two elements are most likely in the form of sulfate and chloride salts. Based on what is known about the evolution of the solar system and the evidence returned by the Mars missions, planetary geologists have hypothesized that some time in its past Mars was covered by massive oceans and lakes that were eventually desiccated by some planetwide catastrophe or environmental change. Geochemical signatures of this wet period in Mars' history should be preserved in the form of layered salt-rich evaporite deposits that would have resulted from such large bodies of water and also from the geochemical weathering and transport of soluble minerals. Salts would also have been the byproducts of volcanic gases acting on the Martian soil or perhaps in areas where microbial activity existed.

The simplest technique for qualitative and quantitative analysis of ionic species is the use of potentiometric ion-selective electrodes (ISEs). These type of sensors possess several desirable characteristics, including a very wide dynamic detection range, availability for a large variety of chemical species (including gases) and properties, and their intrinsic simplicity. Most ISEs can be fabricated in simple, compact, and rugged configurations that will allow them to survive harsh chemical and physical environments, including the elevated levels of radiation encountered during flight. Combined with a subsurface sampling methodologies and sensors for conductivity and redox potential, ISEs can provide not only a picture of the ionic chemical composition but of the geological and climactic history of the planet.

Another type of electroanalysis which provides unique information is anodic stripping voltammetry (ASV). This preconcentration technique allows the analysis of several heavy metal ions, such as copper, lead, cadmium, mercury, and zinc, at sub-ppb levels. An array of microfabricated iridium ultramicroelectrodes was included in the recent MECA instrument for assessing the concentration of such potential toxic metals. The sensor measures only millimeters in size and can easily withstand harsh environments. There are no other devices available which "in total" can fit in a 50 mL volume, consume milliamps of electricity, and supply the concentration of metals such as mercury and cadmium at sub-ppb levels.

The Mars Environmental Compatibility Assessment (MECA)

The MECA instrument was originally designed, built, and flight qualified for the 2001 Mars Lander mission. The mission was subsequently cancelled due to the loss of the Mars Polar Lander in 1999. The MECA, and a newer version, the Robotic Chemical Analysis Laboratory (RCAL), have been proposed for the 2007 launch opportunity. MECA was designed to evaluate potential geochemical and environmental hazards to which future Mars explorers might be exposed and to return data that would help in understanding the geology, geochemistry, paleoclimate, and exobiology of Mars. The MECA instrument package contained a wet chemistry laboratory, an optical and atomic force microscope, an electrometer to characterize the electrostatics of the soil and its environment, and an array of material patches to study the abrasive and adhesive properties of soil grains. Because of payload limitations, the entire MECA package was limited to a mass of 10 kg, a peak power of 15 W, and a volume of $35 \times 25 \times 15\ cm^3$. The development of MECA for analyzing the surface material in a remote hostile environment posed a unique set of challenges, especially for remote chemical analysis and more specifically for electrochemical analysis.

The Wet Chemistry Laboratory (WCL) Cell

Contained within MECA are four WCLs, each consisting of a thermally insulated, single-use, independent analysis cell, capped with a 30 mL pressurized water reservoir and actuator assembly. The actuator assembly consists of a water tank with a puncture valve, a sample loading drawer, a stirrer, and a solid reagent pellet dispenser. The sampling "drawer", which holds approximately $1.0\ cm^3$ of sample, receives the soil from the Lander's robotic arm and deposits it in the chamber. At the base is a spring-loaded flap which retracts and allows the soil to fall into the cell while a screen prevents particles >0.5 mm from falling into the receptacle.

Figure 1 shows the a single analysis cell with an inside view of the sensor array placement. Each cell was fabricated from an epoxy resin and measured $3 \times 3.5\ cm^2$ wide, 3.5 cm deep, and an internal volume of approximately 35 mL. The cells were designed to insure a leak rate of $<0.1\ cm^3\,min^{-1}$, corresponding to a partial pressure of water ten times lower than that of the Mars ambient contribution at 1 cm from the leak, so as to not cause any frosting on adjacent instruments. Each cell was surrounded by a printed circuit board containing preamplifiers for each sensor. Additional information regarding the design, fabrication, cali-

Figure 1. *A view of the MECA WCL components showing a) the actuator assembly containing the water reservoir, sample drawer, and WCL, b) the lower cell, and c) a close up of several sensors mounted on the interior of the WCL.*

bration, and evaluation of the cells has been previously published.[9]

To start a sample analysis, the Lander's robotic arm places soil in a sliding drawer, the drawer is closed, and the chamber and cell are resealed and pressurized to approximately 7 Torr. The water reservoir is heated to about 5 °C and the metal seal on the water tank is punctured to allow water to flow into the cell where it mixes with the soil sample. The temperature in the WCL is maintained at 20 ± 0.5 °C during the analysis and monitored throughout. Since some chemical reactions may occur instantaneously on addition of water to the soil, all the sensors are read immediately and then serially and repetitively. The stirring homogenizes the solution and drives it towards equilibrium in the shortest possible time. A small reagent pellet can be introduced at the end to provide an end-point calibration of the sensors.

The Ion Sensitive Electrode (ISE) Sensor Array

Figure 1 shows the placement of the sensor array within the WCL. The array consisted of 26 sensors with the majority being potentiometric, but also included voltammetric, amperometric, and conductivity based devices. The potentiometric devices included ISEs based on polymer membrane and solid pellet configurations. The ionic species which could be analyzed included H^+, Li^+, Na^+, K^+, Mg^{2+}, Ca^{2+}, NH_4^+, NO_3^-, ClO_4^-, HCO_3^-, Ag^+, Cd^{2+}, Cl^-, Br^-, and I^-. ISEs were also included for dissolved CO_2 and O_2. Figure 2 shows the configuration for a typical polymer membrane based ISE. The inner chamber, abutting against a Ag/AgCl reference electrode, is filled with a hydrogel containing an electrolyte and a fixed concentration of the analyte species. The hydrogel is covered by a plasticized PVC membrane into which an analyte specific ionophore is immobilized.

These PVC membrane based ISE sensors were stored at -20 °C for over 18 months, repeatedly frozen and thawed,

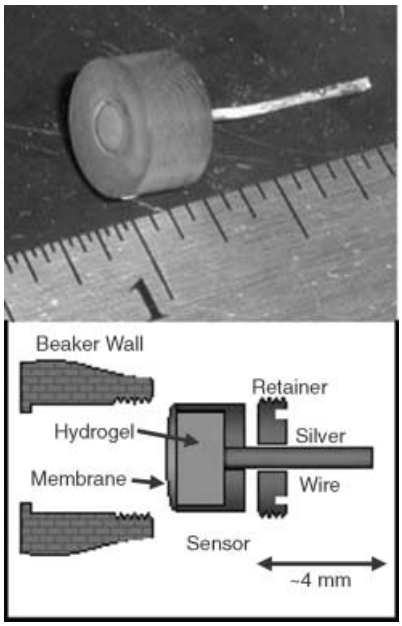

Figure 2. *A photographic and schematic view of a typical ISE sensor element used in the MECA.*

calibrated and tested for six months. Even under such rigorous conditions all of the ISEs performed well, demonstrating that such sensors are suitable for geochemical measurements in harsh terrestrial and extraterrestrial environments. A fractional factorial calibration method successfully described the slope, intercept, and selectivity coefficients of the ISEs. The results were used to determine the activities of eight ions in complex samples of soil leachate simulants and soil samples. Although some small errors were encountered in the analyses, the overall conclusion was that the array successfully predicted the ionic concentrations. Simulants and samples consisting of several salts within a large concentration range were used to further evaluate the sensors. The selectivity issues, sensor noise, and incompatibilities that were experienced are not unlike what might occur if the sensor array had performed extraterrestrial chemical analyses. The results showed that the MECA/RCAL type of electrochemically based sensor array can accurately and reliably characterize the type of surface material expected on Mars.

The *CryoScout* Mars Inorganic Chemical Analyzer (MICA)

CryoScout has been defined as a deep subsurface mission to the north polar cap of Mars, which will explore the stratigraphic record of recent climate change in the underlying layered terrain. A "cryobot" thermally driven probe will penetrate the ice to examine the borehole wall's stratigraphy and the chemistry of the meltwater and entrained dust. Together with surface-station observations, these data will provide for a new understanding of the Martian polar meteorology; present climate and polar water exchange; recent polar volatile deposition and erosion; the scale, texture, structure, dust, and volatile content of subsurface layers; their accumulation rates; the origin of the entrained dust;

the evolution of the polar cap; and the role of orbital variations in this evolution. These high scientific priority goals, and this type of probe and landing site, represent important contribution to the Mars and planetary exploration program.

The "bubble" of meltwater that surrounds the cryobot while it descends will contain dissolved gases, dust, and soluble species extracted from the dust. The dust is derived from many sources, including ancient hydrothermal mineralization, chemical precipitation in lake beds, floodwaters episodically disgorged from the upper crust, or from moisture-driven mineral differentiation in the pedogenic surface.

The MICA system is an electrochemically based flow-through variant of the MECA WCL. Similar to the MECA, the MICA will contain a similar array of sensors that will analyze the soluble components of the Martian dust by measuring a variety of ionic species and properties, including conductivity (0.05 – 140 $\mu S\,cm^{-1}$), pH (0 – 14 to ± 0.5), cations (Na^+, K^+, Mg^{2+}, Ca^{2+}, and NH_4^+ to 10^{-8} M using ISEs), anions (Cl^-, NO_3^-, $HClO_4^-$, and HCO_3^- to 10^{-8} M), metals (Cu^{2+}, Cd^{2+}, Hg^{2+} and Pb^{2+} to ppb levels using ASV), reversible and irreversible oxidants in the meltwater (using cyclic voltammetry), dissolved CO_2 ($10^{-2} - 10^{-4}$ M), dissolved O_2 (0 – 14 ppm), oxidation reduction potential (ORP, from $+1$ to -1 V), and temperature (-40 to $+40\,^{\circ}C$).

As shown in Figure 3, MICA will normally sample the water from the meltwater surrounding the cryobot. After flowing through the sensor array unit the sample is injected into a waste

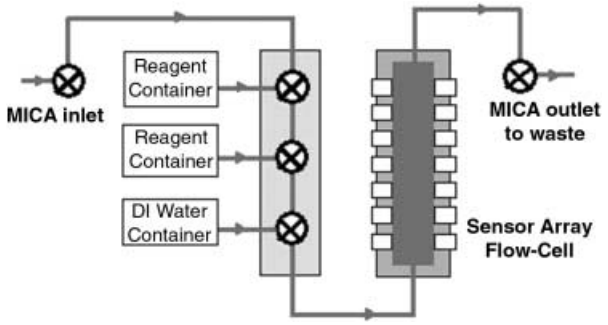

Figure 3. *Schematic view of the fluidics system consisting of valves, reagent containers, and flow-through sensor array.*

container to prevent contamination of the water "bubble" surrounding the cryobot. The control manifold includes valve-controlled injectors from reservoirs that, in addition to pure deionized water, may contain calibration solutions or specific reaction reagents. Unlike the MECA wet chemistry cells, these flow-through cells will require no stirring or heating.

It is clear that electrochemically based sensors have the inherent simplicity and robust characteristics that will allow them to survive and perform chemical analyses in the type of environments found in space and on Mars. Even though these sensors are relatively simple, the data they can return will provide information that will be vital to both future astronauts and to a variety of planetary scientists.

An Electrochemical Microbial Growth Detection System (LIDA)

The discovery of any life form elsewhere in the solar system, which proved to have an independent history from life on Earth, would help elucidate some of the most perplexing questions at the boundaries of biology and chemistry, and could reveal much about the origin and evolution of life. It could reveal what characteristics are particular to life on Earth and which are common everywhere. Is Earth's biology and biochemistry unique or is it possibly ubiquitous in the Cosmos?

The characteristics that microbial life may take on a planet such as Mars, other than the need for water, carbon, and energy, is a totally open question. In recent years our understanding of the limits of habitability have undergone a drastic revision. Researchers have discovered life distributed throughout the Earth's crust in a variety of previously unthinkable environments. Microbial life has been found in such places as hot springs 200 m below the surface, generating metabolic energy by combining hydrogen from the rocks with carbon dioxide;[10] buried deep in Antarctica's hyperarid, ultracold ice-free valleys;[11] and near deep ocean volcanic vents at temperatures of 110 °C and pressures of 150 atmospheres.[12]

Life of course is intimately linked with both the geochemical and, more importantly, with the electrochemical redox environment. Organisms obtain energy by coupling energetically favorable redox pairs with a negative net redox potential. The electroanalytical instruments described above, RCAL and MICA, both have the capability to determine if an environment on Mars has the required "chemical potential" to sustain life. However, we have recently discovered the RCAL and MICA sensor arrays also have the ability to detect small perturbations caused by the growth of microbes near or on their surfaces. What is an intractable problem of biofouling on Earth may be the key to detecting extraterrestrial microorganisms.

The Mars *Viking* Results

As mentioned above, the Mars *Viking I* and *II* Landers included three biology experiments. In the Labeled Release (LR) experiment, the soil was moistened with nutrients (H_2O and ^{14}C-labeled organics) and then incubated. Microorganisms would consume the nutrients and produce detectable ^{14}C-containing gases. The Gas Exchange (GEX) experiment partially submerged the sample under a simulated Martian atmosphere in a mixture of organic and inorganic compounds. Gases such as CO_2, O_2, CH_4, H_2, and N_2 produced by organisms were to be detected by GC. The Pyrolytic Release (PR) experiment incubated the soil sample with a $^{14}CO_2/^{14}CO$ mixture and UV light provided by a xenon lamp. No nutrients or water were added. After five days the gases were flushed, the sample heated to 625 °C, and emitted gases passed through a ^{14}C detector. The GCMS, to everyone's surprise, detected no organics in the soil samples down to the ppb levels. Later analysis however has shown that the GCMS would probably not have detected microbial cells or products at the sub-ppb levels.

The *Viking* experiments though, made several assumptions which contributed to the ambiguity of the results. In the LR experiment, it was assumed that microorganisms on Mars would possess biochemical and metabolic systems similar to Earth organisms and would consume the organic compounds supplied and produce ^{14}C-containing gases. The LR results, even though positive on first analysis, became inconclusive and ambiguous when interpreted along with the other experiments and the GCMS data. It appeared that the Martian soil had chemically reacted with the added water and the ^{14}C-labeled organics to release ^{14}C-labeled gases. The reaction was rapid and did not appear biological in origin. The GEX experiment made the same assumptions as the LR one. Again, the possibility of abiotic emission of O_2 and other gases made the results non-definitive. Finally, even though no water or nutrients were added in the PR experiment, the same assumptions as the LR experiment were also made. The possibility of gas exchange with the soil and the high-temperature heating produced non-definitive results.

It is against this backdrop that we must ask ourselves: What assumptions should be made? Where should we look? And what type of instrumentation should be used that will enable us to unambiguously characterize the surface chemistry and detect any microorganisms on Mars? There is no evidence that extraterrestrial life must necessarily be built on terrestrial biochemistry. Thus, we should not assume any commonality except water, carbon chemistry, an energy source, and reproduction. The question then arises: How do we detect reproduction? Direct microscopic observation and culturing would of course provide definitive evidence, but this is not likely to happen soon. Detection of chemical species such as amino acids, PAHs, lipids, proteins, or DNA/RNA might work, but the major drawback is that many organics can be formed abiotically and there is no basis to assume that DNA/RNA/proteins exactly as we know them are required. Thus, we propose that the best method for detecting microorganisms is to monitor the amplification of the "chemical disequilibrium" that is caused by the reproduction and growth of an organism within a transposed portion of its habitat.

Electrochemical Growth Detection

Bacterial growth in culture media has typically been monitored optically, by measuring turbidity, or electrochemically, by conductivity, pH, or capacitance.[13, 14] Although never flown, several of these methods have been proposed for detection of extraterrestrial microbial life.[15–17] Optical turbidity does not appear to be a viable technique because of the problems associated with analyzing a particulate soil sample. Modifications have been proposed to resolve this dilemma,[15] but very little is gained and the final results may still allow ambiguous interpretation. Even though more reliable, each of the electrochemical techniques by themselves may also be prone to interferences or ambiguous interpretation. However, we propose that integrating the conductivity, pH, and several ion-selective electrodes as a sensor array and incorporating them into a multisample micro-laboratory will provide a reliable, robust, low-mass, and low-power device for monitoring microbial growth—the Life Detection Array (LIDA).

LIDA is based on several crucial components which ensure a definitive conclusion that changes in the growth chambers were biologically induced. These components include two chambers containing a differentially monitored pair of sensor arrays with one chamber for control and the other for the inoculation, a special sterilization and inoculation procedure, use of the local soil as a growth medium, and multiple replications. A schematic of the growth chambers is shown in Figure 4. The chambers are identical and each contains sensors for conductivity, oxidation–reduction potential (ORP), pH, Na^+, K^+, Ca^{2+}, Mg^{2+}, Cl^-, and NO_3^-. More sensors can be added but, as will be seen from our preliminary experiments, they may not be needed. Any global changes due to temperature, pressure, or soil chemistry should affect both chambers identically and thus the differential measurements will remain "zeroed".

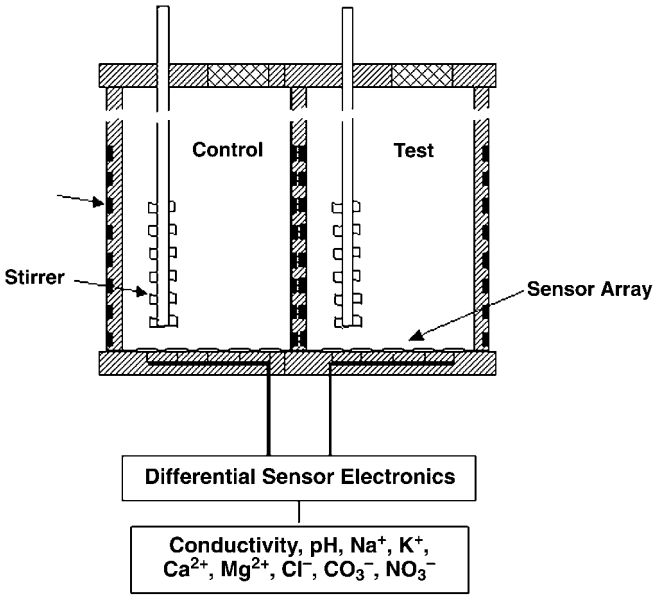

Figure 4. *Schematic diagram of the differential life detection chambers. Both the control and test chambers are identical in every respect until the unsterilized nanogram sample is added to the test chamber.*

The experiment starts by delivering into the chambers of an equal amount of homogenized soil sample. The chambers are then filled with equal amounts of pure sterilized water. Once the water is added, the solutions are then continuously monitored. The stirrer and sterilizing heaters are then turned on and the temperature is increased to at least 110 °C and maintained for a predetermined period of time. The temperature is then decreased to just above freezing and the chambers allowed to equilibrate and the atmosphere above the solution is maintained at about 8 Torr CO_2.

After equilibrium has been ensured, a nanogram quantity or surface "swipe" is introduced into the test chamber. Introducing such a minute quantity decreases the probability that a chemical reaction with the water or newly solvated components would be responsible for a significant disequilibrium of the bulk solution.

The chambers are the monitored for the maximum time allowable. Microbial metabolism and excretion in the test chamber will change the conductivity of the solution and may alter many chemical parameters which can be detected by the various sensors in the array. More importantly, the biofouling of the sensors caused by the metabolic products or by the organisms sensor membranes will result in a signal. Even partial monolayer coverage can effect the transport and charge properties of ISE membranes.

If this experiment was performed in a totally sterile environment, there should be no difference between the two monitored chambers. Any differential between the two chambers, that changes as a function of time, must necessarily be the result of the "substance" introduced into the test chamber and whose effects are being "amplified" by some process. If we insure that chemical processes such as catalysis are not responsible, the conclusion can only be a reproducing and growing life form. This substance and/or entity must thus be possess the ability to cause (reproducibly and exponentially) extensive slow changes in the chemistry of the sample even though it was introduced in nanogram quantities.

The results in Figure 5 show some of the preliminary data obtained with *Lactobaccilus casei*. Both chambers were sterilized and conductivity was monitored for a day to insure stability and sterility. One chamber was then inoculated with a small amount of *L. casei*. Within about 12 h the conductivity for the inoculated chamber began to show a slow increase. During the next 48 h it was clear that growth was proceeding in the inoculated

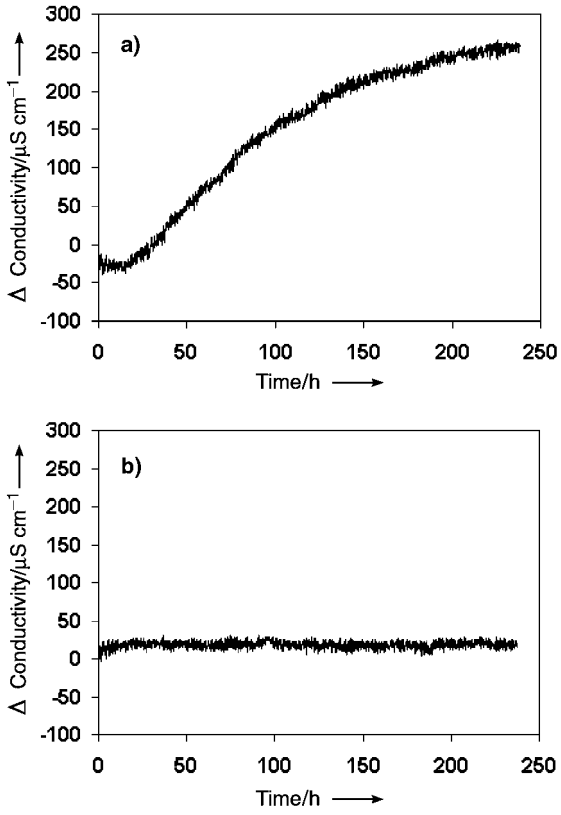

Figure 5. *Results for monitoring conductivity in the test chamber inoculated with* L. casei *and in the sterile control chamber over a period of 240 h.*

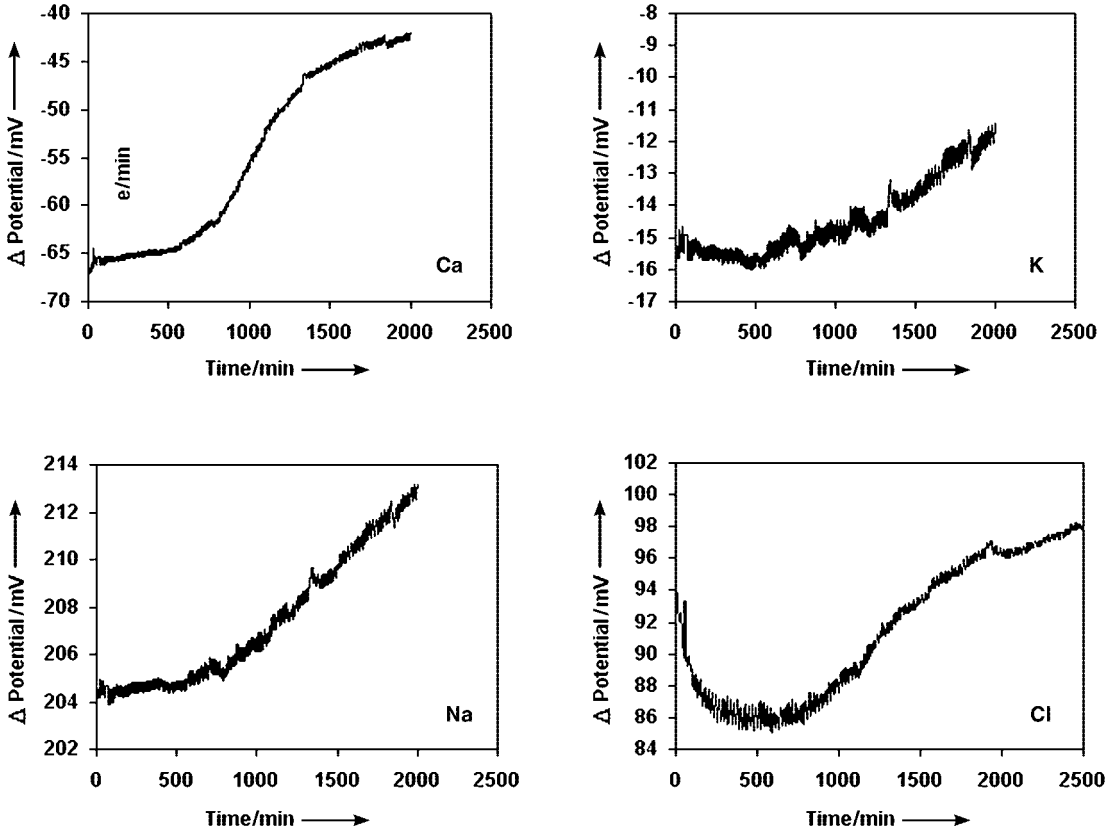

Figure 6. *Typical changes in the potential of Ca^{2+}, K^{+}, Na^{+}, and Cl^{-}, ISEs after monitoring a chamber inoculated with* B. subtilis *for approximately 36 h.*

chamber. This experiment has been repeated numerous times and is a good indication that even conductivity by itself is sufficient to monitor growth for *L. casei*. However, for other bacteria, such as *Bacillus subtilis*, conductivity was not sufficient to always indicate growth.

Figure 6 shows the results for *B. subtilis* when monitored with ISEs for Ca^{2+}, K^+, Na^+, and Cl^-. To our surprise, these electrodes indicated drastic changes in ionic concentration. However, seeing that the bacteria could not produce or consume such quantities of ionic species that would be needed to cause these changes, it is clear that the changes in potential are being most probably induced by bacterial products adsorbing to the ISE membrane surfaces. Whatever the cause of these changes (which were not seen in the control), they give an indication that something is most likely growing in the chamber. It is interesting to note that after about 500 min the Ca^{2+} ISE is producing a curve typically seen for bacterial growth while at the same time the curves for the Cl^-, K^+, and Na^+ ISEs are also starting to show changes. These type of results are reproducible and the sterile chambers showed no such changes.

These preliminary experiments demonstrated that an array of electrochemical sensors was capable of detecting bacterial growth at reasonably short times. Continuing experiments with these and other microorganisms will use a larger number of ISE sensors and will investigate ways to increase sensitivity, decrease the limits of detection and response time, and increase reliability. Even though some may not be satisfied that microbial life has been discovered until a group of microbiologists lands on Mars, these type of devices can give a reasonably good indication of what may or may not exist.

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