System for Biological and Soil Chemical Tests on a Planetary Lander

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An approach for the remote detection and characterization of life in planetary soil samples is described. A mass spectrometer is used as the central sensor to monitor changes in the gas phase in eleven test cells filled with soil. Many biological assays, ranging from general "in situ" assays to monitoring specific metabolic processes (such as photosynthesis, respiration, denitrification, etc.) can be performed by appropriate additions to the test cell via attached preloaded injector capsules. The system is also compatible with a number of chemical assays such as the analysis of atmospheric composition (both chemical and isotopic), the status of soil water, and the determination of compounds of carbon, nitrogen, and sulfur in the soil.

I. Introduction

FOR the past several years we have been working on the development of an experimental package for a planetary landing craft that is capable of performing many biological and chemical experiments. Since it has been primarily directed toward a post Viking '75 Mars mission, we have attempted to design a system which will be responsive to the needs of a follow-on mission. Therefore an important aspect of our approach is its flexibility; it is possible to change the scientific emphasis both shortly because the flight and during the experiment on the planetary surface.

In the life detection system a mass spectrometer is used to monitor (as a function of time) the gas phase in test cells filled with soil. The system employs about a dozen test cells (≤ 10 ml), each of which has three reagent capsules. The contents of each capsule can be injected into its test cell upon remote command. In some cases, a test cell is also illuminated or subjected to an appropriate temperature regimen. Each test cell is connected independently to the mass spectrometer via an adjustable molecular leak. The use of nearly identical and self-sufficient units provides modular design, simplicity,[2] and operational reliability. Selection of the various assays (the content of the regent capsules) can be delayed until shortly before launch. The sequence and pattern of experiments can be controlled on line after landing on the Mars surface.

Since there is no real understanding of what constitutes life, we have attempted to converge on the planetary biology by performing a graded series of tests. These range from environmental assays (could the planet possibly sustain life?) and very general inferential experiments (that might discover strange life) to specific metabolic probes (oriented to Earth-like life). All of the bioassays are nondestructive, and rely on kinetic measurements rather than one-shot observations. We have also tested several chemical assays to analyze the soil and atmosphere for water and compounds of nitrogen, carbon, and sulfur. These assays could yield information concerning the capability of the Martian environment to sustain life and the possible existence of life in the past. In addition, measurements of a more geological and cosmological significance can be performed (e.g., $^{40}Ar/^{36}Ar$ in the atmosphere and $^{13}C/^{12}C$ isotope ratios of various carbon fractions).

In the following sections we will describe some laboratory results obtained during testing of biological and chemical assays. (Some earlier work along these same lines is described in Refs. 1 and 2). We will also briefly describe the flight hardware developed to perform these tests on a planetary lander.

II. Laboratory Technique

The bioassays described in this report were developed and tested using a quadrupole mass spectrometer (Extranuclear Laboratories, Pittsburgh, Pa.). The output was obtained with the aid of a PDP-8E mini-computer (Digital Equipment Corporation, Maynard, Mass.) that normalized the data (to argon 40) and provided some signal averaging. Soil samples were incubated in small glass tubes (6×50 mm Kimax) connected to a manifold. Nupro SS4SA valves were used to close the vessels as well as to provide a molecular leak to the mass spectrometer.

The chemical assays described in this report were carried out in Rittenberg tubes. The gas phase was analyzed using either the quadrupole mass spectrometer apparatus described above or a modified residual gas analyzer (CEC 21-613).

The soil samples used in these studies were acquired locally (3 Patapsco soils) or obtained from R. Johnson of NASA-Ames. All soils were characterized chemically by a commercial soil analysis laboratory. (The six soils obtained from NASA-Ames were originally acquired and chemically analyzed in 1967. The soils were subsequently stored air dried at room temperature until we obtained them in 1972).

III. Biological Assays

In Situ Experiments

In an "in situ" incubation, we attempt to observe existing phenomena with a minimum of perturbation. Several different types of "in situ" experiments can be envisioned.[2]

Due to spaceflight technical limitations, however, we have devoted our attention to an assay in which the soil sample is enclosed in a test cell and the gas phase composition analyzed as a function of time. Under these circumstances, the soil-

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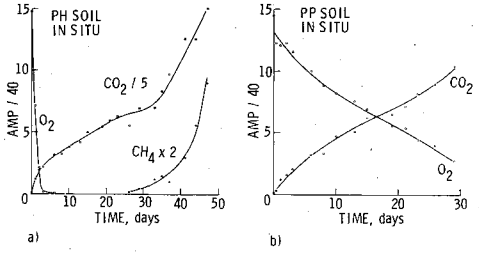

Fig. 1 "In situ" incubation. In this and in subsequent figures AMP/40 is the amplitude of the specie of interest normalized to argon 40. Soil weight: a) 0.4g; b) 0.8g.

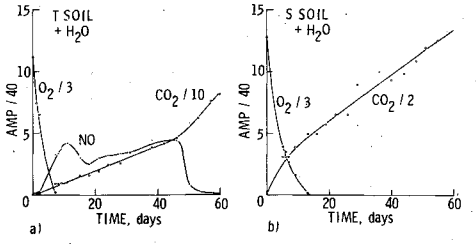

Fig. 3 Addition of H_2O. 0.1 ml H_2O added to a) 0.3g and b) 0.7g soil.

atmosphere system is completely isolated from the atmospheric buffer and the sensitivity of the measurement of biologically-induced changes greatly enhanced. Using this technique on Earth one can successively observe respiration (the uptake of O_2 and the evolution of CO_2), denitrification (the production of N_2 and N_2O from endogeneous soil nitrates) and fermentations (the production of H_2, CH_4, and volatile organic compounds under anaerobic conditions). With the addition of light, one can also observe photosynehetic oxygen evolution.

Figure 1 illustrates data obtained from two different soils incubated under "in situ" conditions. In Fig. 1A, we observe the metabolic processes in a rich soil; note that the oxygen is completely consumed within a few days. A few days later, we observe the onset of methane fermentation. In relatively rich soils such as this, one often observes that many other peaks appear, probably due to the production of organic volatiles. Several tested soils have also shown evidence of denitrification under "in situ" conditions. Figure 1B shows the oxygen uptake and concomitant CO_2 evolution in a poorer soil; although this soil shows a low respiration rate, it proved capable of a wide spectrum of metabolic processes.

Figure 2 illustrates a control procedure in which the change in gas phase composition above a soil sample is monitored *during* high temperature incubation (really heat sterilization). (For a discussion of some of the problems related to control procedures for life detection experiments in this context, see Ref. 2). The rate vs temperature profile of the processes in question is shown in Fig. 2A. Note that biological reactions show a pronounced temperature max; above about 40° the rate drops rapidly. Chemical reactions, in marked contrast, generally have an exponential relationship with respect to temperature. Consequently, if the rate of a reaction (such as O_2 uptake) is measured at two different elevated temperatures, one can (at least in principle) use an Arrhenius plot (Fig. 2B) to calculate the nonbiological rate at the physiological temperature; extrapolation of the high temperature rates will give the rate of the chemical reactions in the biological sample.

Several aspects of this control procedure (and control procedures in general) should be emphasized: 1) It is really only necessary to use a control procedure to check the validity of "downhill" reactions. Reactions that require an energy input (such as photosynthetic oxygen evolution) do not require a control. 2) The heated samples used in the "in situ" control procedure described above will also serve as sterile controls after cooling. 3) The composition of the atmosphere above a soil sample before and after heating can give information on the soil surface-atmosphere disequilibrium.

Addition of H_2O

Water is often a rate-limiting commodity for biological activity, and it is often a general stimulant for the metabolism of soil biota.§ Figure 3 illustrates two experiments in which H_2O was added to air dried soil samples. (These particular soils showed very low activity in the absence of added water). The added H_2O caused the induction of metabolic processes similar to those observed in the "in situ" experiment. One interesting facet of these data is the nitrogen oxide transient observed in Fig. 3A; this is due to the biological reduction of endogeneous nitrate in the absence of O_2.

§Under some circumstances (such as on Mars) it may be more judicious to add water as a vapor; at 15° liquid H_2O would double the atmospheric pressure over the soil at the Mars surface.

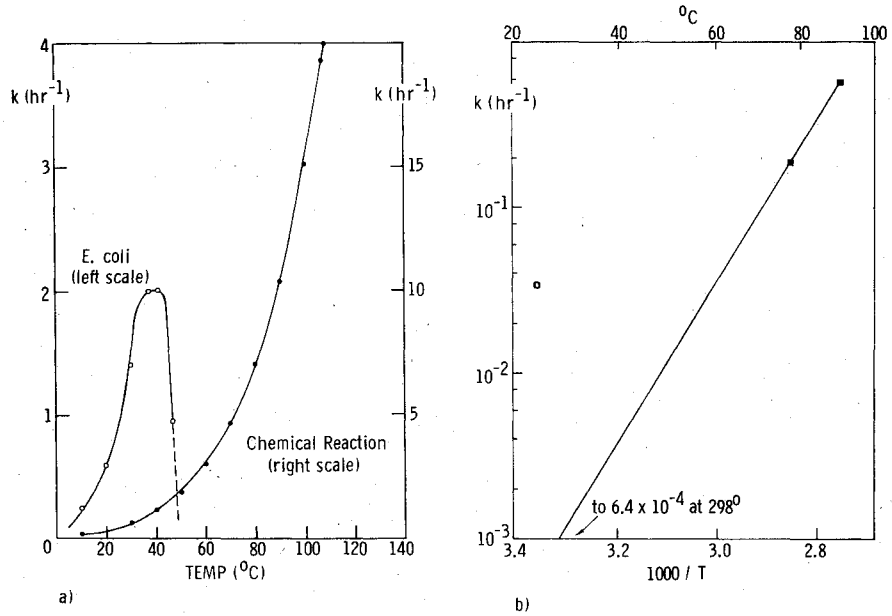

Fig. 2 An "in situ" control procedure. a) Typical rate vs temperature profile for a biological (open circle) and nonbiological (closed circle) system. The nonbiological curve was obtained from the expression: $k = A \exp(E_a/RT)$, where $A = 10^7$ hr^{-1} and $E_a = 10$ kcal. b) Arrhenius plot of data obtained for the rate of O_2 uptake by a soil sample at three different temperatures. The two O_2 uptake rates observed at high temperatures (closed squares) are used to predict the nonbiological rate at 25°C. The open square shows the observed rate of O_2 uptake.

Photosynthesis

Probably the most important biological process in the terrestrial biosphere is photosynthesis. This process is the only source of energy for the biosphere and is thus the ultimate driving process for all biological reactions.

Aerobic photosynthesis (green plant photosynthesis) involves the oxidation of water and the reduction of CO_2, the products being molecular O_2 and reduced carbon (at the redox level of carbohydrate); with ^{18}O labelled water, $^{18}O_2$ gas is evolved.

$$2H_2{}^{18}O + CO_2 \rightarrow {}^{18}O_2 + [CHOH] + H_2O$$

Anaerobic photosynthesis is carried out by photosynthetic bacteria and occurs in many forms; it is a relatively insignificant process on a global scale. Although many different light driven reactions have been observed with these bacteria, the reaction most likely to be observed by our technique is the production of hydrogen and CO_2 from organic substrates;

$$\text{organic substrate} \xrightarrow{h\nu} H_2 + CO_2$$

Figure 4 shows the photosynthetic activity observed starting from two different initial conditions. The data in Fig. 4A show an experiment in which light was added at time zero to a soil enclosed in a glass test cell. Note that after a distinct lag, there is evidence of profuse photosynthetic activity, as shown both by the production of oxygen and the consumption of CO_2 (really a suppression of its evolution). After the light was removed (at about 13 days) CO_2 increased and O_2 decreased due to the respiratory processes.

Figure 4B shows an experiment in which the same soil was first allowed to become anaerobic before light was administered. Note that after about 10 days large quantities of methane appeared. However, after a lag period in the light, O_2 increased dramatically and the methane and CO_2 were completely consumed. (No O_2 appeared until the methane was consumed, probably due to the reaction $CH_4 + 2O_2 \rightarrow CO_2 + 2H_2O$ catalyzed by bacteria of the genus *Pseudomonas*.) The CO_2 produced plus the CO_2 originally present in the test cell were apparently consumed during the subsequent photosynthetic processes.

The Addition of "Yeast Extract"

One of the most effective ways to stimulate terrestrial metabolism is by the addition of an aqueous nutrient solution (referred to here as "yeast extract"). The phenomena observed often are similar to those of the "in situ" and H_2O addition experiments on a greatly compressed time scale. This experiment is analogous to the gas exchange experiment aboard Viking '75.[3]

Figure 5 illustrates results obtained under these conditions. A comparison of these data with some of the above data illustrate the often striking effect of nutrient addition. A few salient points might be noted: 1) In PP soil the oxygen consumption rate was increased more than one order of magnitude (compare Fig. 5A with Fig. 1B). 2) PP soil in this reaction showed pronounced anaerobic activity compared to the "in situ" incubation. 3) T soil contained substantial amounts of endogeneous nitrate as evidenced by the large NO transient seen in Fig. 5B. 4) The unstable, almost oscillatory, behavior of CO_2 shown in Fig. 5B apparently has a legitimate basis, and is not due to machine or technical instabilities. We routinely observed that CO_2 is evolved and taken up in fits and starts once methane begins to be produced.

Addition of ^{13}C-Labelled Organic Nutrients

The metabolism of labelled organic compounds by soil biota results in the evolution of labelled CO_2 and organic volatiles due to respiration and fermentation. The use of ^{13}C-labelled nutrients allows the sensitive detection of the evolved CO_2 even in the presence of a large CO_2 background (such as in the Mars atmosphere). In addition, labelling the nutrient allows one to pinpoint the source of the CO_2 evolved. This experiment is an analog of the ^{14}C-label release experiment aboard Viking '75[4]; in our system, it can be combined with the "yeast extract" experiment described above.

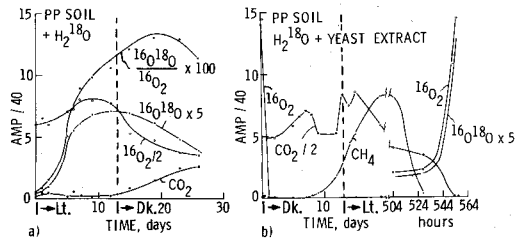

Fig. 4 Photosynthesis. a) 0.1 ml $H_2{}^{18}O$ (10 A%) added to 0.4g soil. b) 0.1 ml of 1% yeast extract in $H_2{}^{18}$ (10A%) added to 0.4g soil.

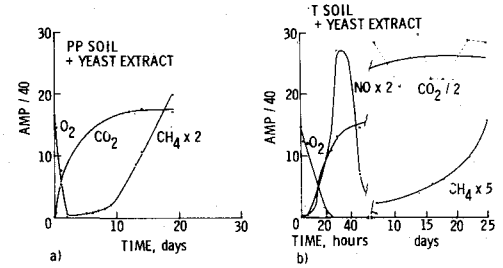

Fig. 5 Addition of yeast extract. 0.1 ml of 1% yeast extract added to a) 0.9g and b) 0.2g soil.

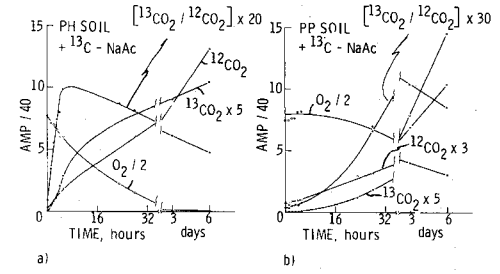

Fig. 6 Addition of ^{13}C-labelled organic nutrient. 0.1 ml of 10 mM ^{13}C-sodium acetate (61.2 A%) added to a) 0.4g and b) 0.8g soil.

Figure 6 shows results obtained when a rich and poor soil were incubated in the presence of ^{13}C-labelled sodium acetate. In these assays, the most significant parameter is the $^{13}C/^{12}C$ ratio. A few salient aspects of these data are: 1) In both cases the $^{13}C/^{12}C$ ratio appears to go through a max.; however, this max. occurs much earlier in the PH (rich) than in the PP (poor) soil. 2) The occurrence of this max. suggests that the addition of ^{13}C-labelled acetate turns on biological processes to the point where other endogenous compounds are metabolized at a significant rate. 3) The relative biological activity induced in these two soils can be deduced in two ways: a) the time at which the $^{13}C/^{12}C$ ratio reaches its max. (that is, about 8 hrs. vs about 2 days); and b) the fact that in PH soil the system was completely anaerobic in about a day, while in PP soil the system was not anaerobic after 6 days.

Alternate Electron Acceptors

Many terrestrial organisms are able to use terminal electron acceptors other than O_2; for example, in the process of denitrification, nitrate is reduced to N_2 and N_2O; i.e., $^{15}NO_3 \rightarrow {}^{15}N_2, {}^{15}N_2O, {}^{15}NO$ (by the use of ^{15}N-labelled nitrate, these reduction products can be measured at relatively empty positions in the mass spectrum). Other terminal elec-

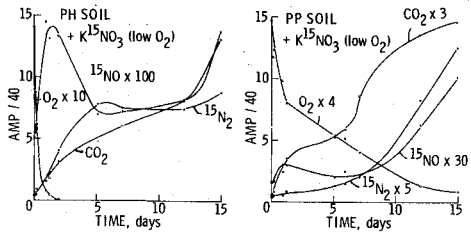

Fig. 7 Biological reduction of $^{15}NO_3^-$. 0.1 ml of 10 mM $K^{15}NO_3$ (95A%) added to a) 0.6g and b) 0.9g soil. Initial gas phase was approximately 95% N_2, 3% O_2, 2% Ar.

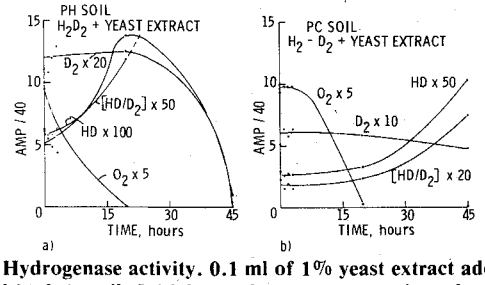

Fig. 8 Hydrogenase activity. 0.1 ml of 1% yeast extract added to a) 0.4g and b) 0.4g soil. Initial gas phase was approximately 18% H_2, 7% D_2, 38% N_2, 24% O_2, 13- Ar.

tron acceptor reactions are also known; for example, sulfate: $SO_4^= \rightarrow H_2S$, and phosphate: $PO_4^= \rightarrow PH_3$. Since the O_2 availability on some of the planets is known to be limited (e.g. on Mars), these alternate pathways could be important. Figure 7 shows results obtained when soil samples were incubated with ^{15}N-labelled potassium nitrate under conditions of limited O_2. In both cases, a transient burst of nitrogen oxides (NO and N_2O) is produced followed by a steady production of labelled molecular nitrogen. Note that no "yeast extract" or other organic nutrient was added (i.e., additions were entirely inorganic); consequently this experiment is not Earth oriented.

(If the planetary atmosphere has a low O_2 partial pressure, the addition of O_2 (or $^{18}O_2$) to the gas phase might stimulate respiration-like processes. Then one might observe gas exchange patterns like those in Figs. 1, 3, and 5).

Hydrogenase Activity

Many terrestrial organisms are known that are able to metabolize molecular hydrogen. This metabolism can take the form of isotope scrambling ($H_2 + D_2 \rightarrow 2DH$) or a net uptake or evolution of H_2 (or D_2). Figure 8 shows results obtained when two soils were incubated in an H_2-D_2 atmosphere. The two soils seem to react similarly, although on a much different time scale.

Probably more than any other experiment, this assay requires a sterile control. The nonbiological activation of H_2 is quite possible under anticipated experimental conditions.

Nitrogen Fixation Assayed by Acetylene Reduction

A widely used assay for the nitrogen fixation system (the reduction of molecular N_2 to NH_3) involves the reduction of acetylene (an analog of N_2) to ethylene. To date, we have only observed this process in soil samples which have been illuminated, suggesting that we are observing light-driven nitrogen fixation due to blue-green algae. Our failure to detect this process in the dark is not unexpected; most reports suggest that there is very little endogeneous N_2 fixation in unamended dark-incubated soils.

In our assays we use ^{13}C-labelled acetylene so that the reduction product, ethylene, appears at mass 30, a relatively clean position in the mass spectrum, instead of mass 28 (where it can be obscured by N_2 and a fragment of CO_2). Figure 9 shows the results using two illuminated soils in the presence of $^{13}C_2H_2$. Note that in Fig. 9A, oxygen is consumed at a rate similar to that observed in the "in situ" experiment, and not evolved in the process of photosynthesis as one might expect (compare with Figs. 1B and 4A). We have no ready explanation for this observation. In the experiments shown in Fig. 9B we can observe the light-induced proliferation of photosynthetic organisms; note the $^{12}CO_2$ transient and the inverse O_2 transient. Apparently, it is these organisms (probably blue-green algae) that are responsible for the production of $^{13}C_2H_4$ from $^{13}C_2H_2$. In addition the $^{13}C_2H_2$ is apparently being oxidized (either directly or indirectly) to $^{13}CO_2$.

Additional Remarks

In many of the bioassays described above a complex array of gas compositional changes is observed, and it is not always possible to uniquely identify the metabolic transformation and/or type of organism involved. However, in these cases it is readily apparent that the observed gas-phase changes are unexplainable by physico-chemical mechanisms. For example, the occurrence of complex, energetically "uphill" reactions, such as the light-driven evolution of O_2 with concomitant CO_2 uptake (e.g., Figs. 4 and 9B), or the reduction of nitrate to molecular N_2 in an (initially) oxidizing atmosphere (e.g., Fig. 7) would probably be impossible to rationalize in terms of a nonbiological soil reaction. Similarly, the often observed occurrence of transient changes in gas phase composition and the production of methane by a soil sample that was initially under 1/5 atm of O_2 (Fig. 1A) are strong indicators of biological activity, even in the absence of a control sample.

IV. Chemical Assays

In principle, any experiment based on the analysis of the atmospheric composition within the test cell can be performed. Thus, the test cells can be used as reaction chambers for any analysis that is based on a change in gas phase composition. The main restriction pertaining to this system is that there is little opportunity to perform standard chemical manipulations. A number of chemical assays of the soil and atmosphere have been tested in our laboratory that could yield information concerning the capability of the planetary environment to sustain life and/or the possible existence of life in the past. In general these procedures have a sensitivity comparable to that obtainable by standard wet chemical methods.

Atmospheric Analysis

A detailed analysis of a planetary atmosphere is one of the most important facets of any life detection investigation. Since our proposed life detection system is really based on the atmospheric analysis of enclosed gas-soil samples, it is relatively simple to adapt this apparatus to provide a detailed analysis of a remote planetary atmosphere.

In some instances a single mass spectrometer scan of a planetary atmosphere would not be sufficient to uniquely determine its constituents. For example, a detailed mass spectrometric analysis of the atmosphere of Mars is complicated by the high percentage of CO_2; a spectrometer-generated fragment of CO_2 appears at $m/e=28$, thus interfering with the determination of CO and N_2. In this case a detailed analysis of the atmosphere before and after removing CO_2 and CO would allow the detection and measurement of species masked by these compounds (e.g., N_2). (This procedure is being used above Viking '75).[5]

Tests in our laboratory have shown that CO_2 and CO can be sequentially removed from the test cell to very low residual levels. Using crystalline LiOH in a "getter module" we found that CO_2 was depleted from an initial pressure of 15 Torr to 10^{-3} Torr within an hour. In tests using AgO (to oxidize the

CO), LiOH (to remove the CO_2 formed) and anhydrous magnesium perchlorate (to remove the H_2O generated) we observed that ≥99.5% of the CO was removed in 6 hrs.

The implementation of this procedure aboard a planetary lander would require the use of two "getter modules" that would be fitted in place of reagent capsules on the test cell cap. The complete procedure would then involve three sequential mass-spectrometric atmospheric determinations interleaved with gettering, i.e.: 1) analysis of complete atmosphere; 2) removal of CO_2 with LiOH getter; 3) analysis of CO_2-depleted atmosphere; 4) removal of CO with AgO-LiOH-Mg $(ClO_4)_2$ getter; 5) analysis of CO_2-CO depleted atmosphere. The variable molecular leak to the mass spectrometer used in this system should result in an increased dynamic range of several orders of magnitude for the analysis of trace constituents (compared to Viking '75).

Determination of Atmospheric, Organic, and Inorganic Carbon Fractions (also soil $SO_3^=$ and $S^=$)

The opportunity to determine the amount and isotopic composition of various carbon fractions on a planetary surface has elicited a great deal of interest among the scientific community. We have tested a procedure that complies with the technical constraints of a prototype instrument and allows the determination of the amount (and possibly isotopic composition) of a) atmospheric CO_2, b) inorganic carbon, and c) soil organic carbon. In addition, this procedure allows the determination of soil sulfite and sulfide.

This procedure utilizes two reagent capsules and a chemical CO_2 "getter" contained in an auxiliary module (fitted in place of a reagent capsule) on the cap of the test cell. The general procedure is as follows: after introducing soil (and atmosphere) into the test cell, 1) the atmospheric CO_2 ("A") is analyzed mass spectrometrically; 2) this CO_2 is removed by the "getter"; 3) mineral acid is added to release CO_2 ("B") from the soil carbonate and bicarbonate; 4) CO_2 fraction "B" is analyzed; 5) this CO_2 fraction is removed; 6) $K_2Cr_2O_7$ is added and the sample heated to oxidize the soil organic carbon to CO_2 ("C"); 7) CO_2 fraction "C" is analyzed.

Figure 10 shows the time course of the CO_2 partial pressure during an experimental sequence. Three distinct phases are apparent: 1) the uptake of atmospheric CO_2 by the trap; 2) the evolution and subsequent uptake of CO_2 derived from inorganic carbon; and 3) the evolution of CO_2 derived from soil organic carbon. In our laboratory tests, no attempt was made to determine the $^{13}C/^{12}C$ ratio of the various CO_2 fractions, since the results obtained would not be applicable to the spaceflight hardware.

In some soil tests it appeared that this assay was reagent limited. This limitation correlates with the amount of available protons, and is apparently unrelated to the incomplete reaction observed when the soil is not completely wetted. In our experiments using soils we never observed acid-limitation of the inorganic carbon determination; however, the organic carbon determination became acid limited at about 6 mg (6000 ppm) organic carbon. The problem of acid limitation may or may not be significant; for the case of Mars useful preflight information might be obtained from the GC-MS experiment aboard Viking '75. If the problem is deemed significant, it could be alleviated by 1) an increase in the amount of acid added, 2) an increase in the acid concentration (≤2 fold), and/or 3) the use of less soil.

One somewhat more subtle problem is that, as currently envisioned, the isotope ratio of the atmospheric CO_2 can be directly determined, but not its absolute abundance, since the argon standard is not added until the addition of mineral acid (the first addition). This shortcoming may or may not be important. It should be possible to get an indirect determination of the CO_2 abundance in the atmosphere by comparing the CO_2 signal to another gas present (e.g., O_2). This secondary standard could then be calibrated by determining the Ar-O_2 ratio after the addition of acid and Ar.

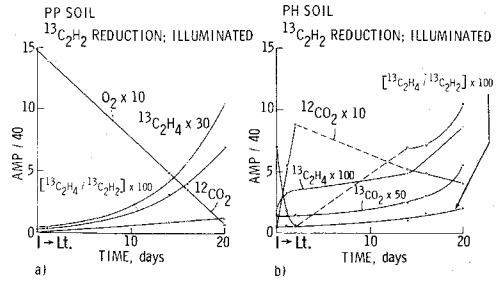

Fig. 9 Nitrogenase activity assayed by acetylene reduction. 0.1 ml of 30 mM sodium acetate added to a) 0.6g and b) 0.3g soil. Initial gas phase was approximately 67% $^{13}C_2H_2$ (90 A%), 19%O_2, 14% Ar.

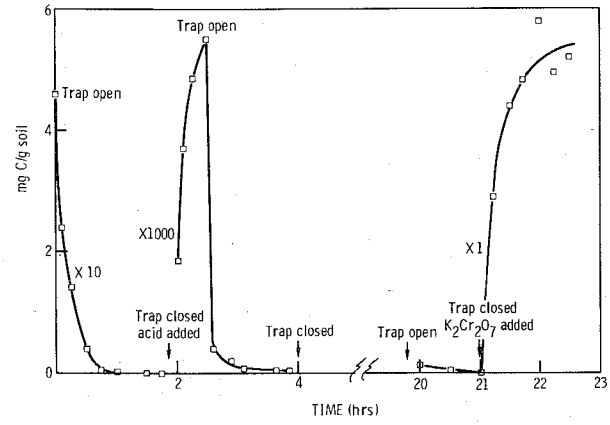

Fig. 10 Time course of CO_2 partial pressure during a complete experimental sequence (using S soil) expressed in mgC/g soil. A known amount of CO_2 was present in the gas phase at time zero.

Determination of H_2O—Gaseous, Free, and Bound

The status and availability of water on a planet is of prime importance with respect to the possible presence of life. Consequently, the determination of various forms of water at the planetary surface is of prime biological (as well as geological) interest.

Water is quite difficult to measure directly using a mass spectrometer because it is easily condensible, and consequently the apparatus has a memory for H_2O. However, these problems can be alleviated to a large extent by proper design of the mass spectrometer and sample inlet system.[6]

Figure 11 shows the results of some experiments performed on the prototype flight instrument mass spectrometer to examine its H_2O measuring characteristics. (We should emphasize that this experimental apparatus was not optimized for H_2O determinations, and had a lot of superfluous plumbing; thus the experiments are really a worst-case illustration). Figure 11A illustrates the progressive increase of the H_2O signal (normalized to argon) in which the mass spectrometer was sampling the gas phase of a test cell equilibrated at 25° with liquid H_2. In this particular experimental configuration the equilibration time was 30-40 min. The pumpdown characteristics of this apparatus are shown in Fig. 11B; the sample of H_2O-saturated air was terminated at $t=0$. The distinct multiphasic nature of the kinetic curve indicates the existence of several different H_2O sources with disparate H_2O affinities. The background level attained after 16 hrs was only about 5% of the initial level, sufficiently low for the semiquantitative applications intended.

An alternative approach for the mass spectrometric determination of H_2O is to transform the water stoichiometrically to a compound that is more easily measured. For example, using calcium carbide one would observe the production of acetylene

$$CaC_2 + H_2O \rightarrow CaO + C_2H_2$$

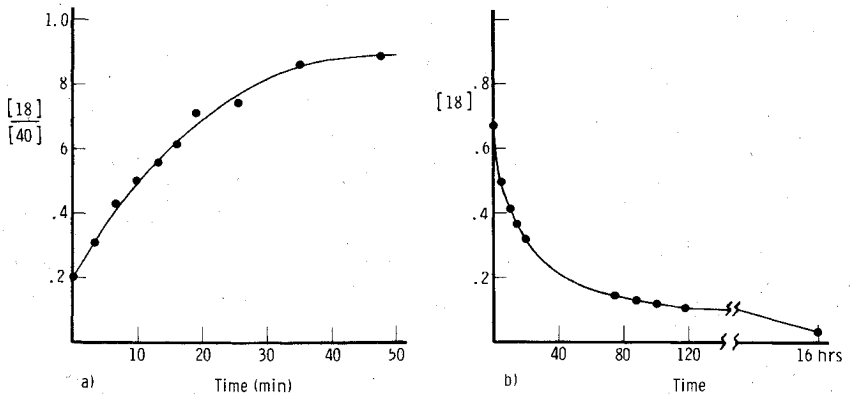

Fig. 11 a) Time course of the normalized H_2O signal (18/40) obtained when H_2O saturated air (at 25°C was continuously sampled by the prototype flight instrument. b) H_2O pumpdown characteristics of prototype instrument after prolonged exposure to H_2O saturated air. Inlet valve closed at $t = 0$.

Tests of this reaction in the laboratorty have shown that this is a feasible procedure under the anticipated conditions. In the context of the prototype spaceflight apparatus, the procedure would involve the addition of pure CaC_2 to a sealed test cell containing planetary atmosphere (and soil). The amount of C_2H_2 generated would then be compared with a known amount of argon (added along with the CaC_2) using the mass spectrometer.

In laboratory experiments we have obtained C_2H_2/H_2O stoichiometries varying between 0.5 and 1.0, the higher values being observed at low H_2O partial pressures. We suspect that the lower values reflect the reaction

$$H_2O + CaO \rightarrow Ca(OH)_2$$

and that the overall reaction at high H_2O partial pressure is best described by the equation

$$CaC_2 + 2H_2O \rightarrow Ca(OH)_2 + C_2H_2$$

Both of the above methods should have sufficient sensitivity to measure H_2O in planetary atmospheres. For example, although the partial pressure of H_2O in the Mars atmosphere is very low, the total pressure is also two orders of magnitude lower than that of Earth, so that on a mole-for-mole basis the Mars atmosphere is about as wet as that of Earth.

In addition to the determination of liquid and atmospheric water, a good deal of additional information can be obtained by raising the temperature of the vessel to release various species of bound water. (The instrument presented being designed and constructed provides for a programmed heating regimen to sequentially release the various forms of bound water.) For example, if the temperature were raised to ~100°, adsorbed water would be released. At higher temperatures, the water sequestered in clay, silicates, and micas could also be determined. The use of the calcium carbide-acetylene method for these high temperature determinations would be complicated by the polymerization of acetylene above 150°. We should note that it should be possible to determine the H/D ratio of the water by measuring the 26/27 ratio of the acetylene obtained; the measurement of the H/D ratio in the direct H_2O determination is complicated by the spurious appearance of H_3O^+ in the spectrometer.

Determination of Soil Nitrogen Compounds

A biologically important aspect of planetary surface chemistry is the status of nitrogen. We have tested a series of assays to determine nitrogen compounds that are (biologically) convertible; i.e., nitrate, nitrite, ammonia, and primary aliphatic amines (including amino acids and protein amino nitrogen). Combined with a detailed atmospheric analysis, these assays should yield a good deal of information concerning the nitrogen balance at the planetary surface.

Soil Nitrate and Nitrite

We have tested an assay for the determination of soil nitrate in which NO_3 is reduced to N_2O by sulfamic acid in acid solution according to the reaction[7]

$$NH_2SO_3H + HNO_3 \rightarrow N_2O + H_2SO_4 + H_2O$$

Under weakly acid conditions, nitrite is also reduced (to N_2) according to the reaction

$$NH_2SO_3H + HNO_2 \rightarrow N_2 + H_2SO_4 + H_2O$$

Isotopic studies using ^{15}N-labelled nitrate, nitrite, and sulfamic acid in our laboratory have shown that the reduction product (N_2O or N_2) contains one nitrogen atom derived from nitrate and one from sulfamic acid. Thus, the reaction of ^{15}N sulfamic acid with ^{14}N substrates (and vice versa) result in reaction products that appear at relatively empty positions in the mass spectrum (i.e. $m/e = 45$ and 29 instead of 44 and 28, the positions of CO_2 and CO).

Experimental results obtained using ^{15}N-labelled potassium nitrite are shown in Fig. 12 and Table 1. The less-than-perfect recoveries obtained are probably due to the high reactivity of nitrite.

Figure 13 shows the results obtained when $K^{15}NO_3$ is reduced in solution or with a typical soil sample. A comparison of the two cases indicates that the quantitation of the assay is degraded somewhat by the presence of soil (other experiments also bear this out); we suspect that this is due to the soil catalyzed reduction of NO_3^- to products other than N_2O.

Primary Aliphatic Amines (Amino Acids and Protein)

We have also tested a chemical assay in which primary aliphatic amines are determined by a variant of the Van Slyke method. In this procedure the amines are oxidized to N_2 by nitrous acid. With prior hydrolysis, protein amino acids also can be determined. Without the use of ^{15}N-labelled substrates, this assay is complicated by extraneous sources of N_2 and a MS-generated fragment of CO_2. By the use of ^{15}N-labelled sodium nitrite, the molecular nitrogen obtained from the reaction appears at $m/e = 29$, a relatively clean position in the mass spectrum. Reciprocal experiments in which either the

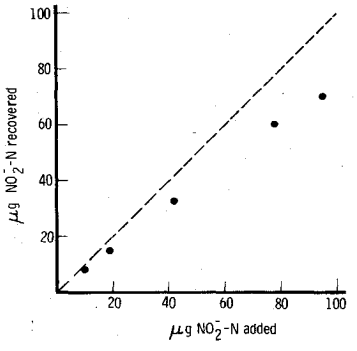

Fig. 12 $K^{15}NO_2$ added vs nitrite N recovered (as N_2) after reduction by sulfamic acid in 1 ml H_2O solution. Dashed line indicates theoretical 100% recovery.

Table 1 Recovery of nitrite N (as N_2) after reduction by sulfamic acid in the presence of 1 cm^3 of various soils

Soil	μg ^{15}N added	Percent recovery
A	54	42
BC	52	101
S	52	28
T	52	95
W	54	78

amino acid or the nitrite was labelled with ^{15}N corroborate the generally accepted scheme; i.e., one nitrogen is derived from the amino group, the other from nitrite according to the overall reaction

$$HNO_2 + RHN_2 \rightarrow ROH + N_2 + H_2O$$

Simple variants of this procedure also allow the determination of other nitrogen compounds; longer incubation times release N_2 from ammonia and urea, while prior hydrolysis using 0.5M NaOH allows the determination of protein amino nitrogen.

Figure 14 illustrates the quantitative recovery of DL-alanine using this technique in solution and in the presence of soil Comparable results were obtained with ten other amino acids. Experiments to date suggest that the mass spectrometric determination of soil amino nitrogen is quite reliable and sensitive. Even in relatively poor (i.e., biologically inactive) soils, amino compounds can be detected with a sensitivity that is several orders of magnitude higher than the background of the system.

V. Implementation

Hardware Development

A hardware development program has been underway to produce a system capable of performing the tests described above on a future planetary lander.[8] This system comprises an array of eleven test cells, with selectable internal volumes ranging from 1 to 10 cm^3. Soil from a single funnel on top of the landing craft can be distributed upon remote command to each of eleven test cells (up to 8 cm^3 in 1 cm^3 increments). After loading, the test cell with its charge of soil and atmosphere is permanently sealed via a gold gasket; this seal has a leak rate that is negligible for the purposes of our experiments. Each of the eleven test cells is contained in a module that plugs into the assembly as a separate unit, and can be independently tested.

The cap to each test cell contains three sealed quartz capsules, each containing up to 1 cm^3 of reagent to be added to the soil in that cell. These capsules can be ruptured individually on command, so that the contents are added directly to the soil. Special test cells can also be provided for other experiments; the contents of the test cell can be illuminated (for photosynthesis experiments), and CO_2 can be removed from the atmosphere (for the analysis of soil carbon).

Each cell cap is connected to the inlet manifold of a mass spectrometer by way of a variable leak valve. The manifold connects the eleven leak valves to the ion source of the mass spectrometer via gold tubes which contain emergency pinch off mechanisms, so that a tube can be sealed in the event that one of the leak valves fails in the open position.

The variable leak valve most suitable for this system is the piezo-electrically actuated valve developed by the University of Colorado. It has no organic seats or seals, and is capable of a wide dynamic range of flow control (five orders of magnitude); when closed, it has a leak rate well below the limit required for our experiment.

The mass spectrometer used in this system is a slightly modified version of one developed by Nier et al., and flown as an atmospheric analysis instrument on NASA's Atmospheric

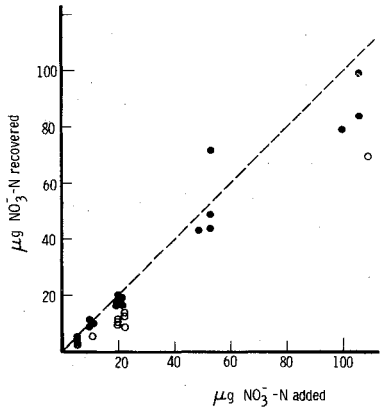

Fig. 13 $K^{15}NO_3$ added vs nitrate N recovered (as N_2O) after reduction by sulfamic acid in 1 ml solution (closed circles) and 1 cm^3 A soil (open circles). Dashed line indicates theoretical 100% recovery.

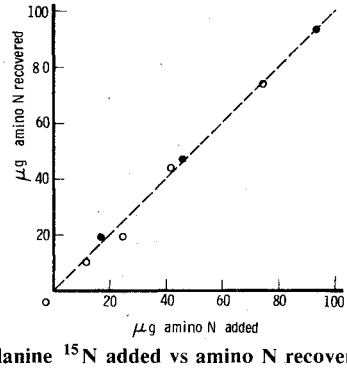

Fig. 14 DL-alanine ^{15}N added vs amino N recovered (as N_2) after reaction with nitrous acid in 1 ml solution (open circles) and in the presence of 1 cm^3 A soil (closed circles). Dashed lines indicates theoretical 100% recovery.

Explorer (AE) satellite.[9] A number of tests, performed in collaboration with Dr. Nier, have demonstrated the suitability of the mass spectrometer for this life detection system.

In this system each cell can be maintained at an incubation temperature of 10°C ± 10 or heated to temperatures as high as 160°C. Thermal control is maintained by circulating nitrogen gas through a closed loop comprising a heat exchanger, surrounding the incubating test cells, and a radiator, on the outside of the lander. Each test cell is mounted in a water jacket that is in close thermal contact with the heat exchanger. The nitrogen is circulated through the loop at night to freeze the water in the water jackets, and thus provide cooling for the next day. The Martin Marietta Thermal Analysis System (MITAS) program was used to generate a computer thermal model of this system; the results indicated that this thermal control method could cope with even the worst-case situations.

Integration of Assays: Experimental Strategy

The assays described in the preceding sections can be integrated into experimental strategies that take advantage of the flexibility and real-time capabilities of this life detection system. Within a strategy, test cells can be assigned several experiments on a contingency basis; after each primary experiment a second experiment compatible with the perturbation imposed by the first would be performed. In some cases, all three reagent capsules would be used in a single experiment. In other cases, three different experiments could employ the same test cell, with the primary experiment selected on the basis of its scientific importance and minimum perturbation of the sample. In this instance the success of a primary experiment might preclude a subsequent determination.

An alternate strategy might be employed in the event that a mobile lander is available. In this case, several test cells might be dedicated to the same life detection experiments (e.g., "in situ," $+H_2O$, and/or "yeast extract"). Sampling sites could be selected with the aid of the camera and the soil samples obtained tested for biological activity by the chosen detection bioassays. If the results were negative, the soil would be discarded and a new soil obtained. In the event of a positive or interesting result, a soil sample could be retained for further experimentation.

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