THE SEARCH FOR LIFE ON MARS: VIKING 1976 GAS CHANGES AS INDICATORS OF BIOLOGICAL ACTIVITY

V. I. OYAMA,* B. J. BERDAHL, G. C. CARLE, M. E. LEHWALT, and H. S. GINOZA

Ames Research Center, NASA, Moffett Field, Calif., U.S.A.

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Abstract. Gas compositional changes in the headspace of the Viking Biology Gas Exchange Experiment can originate from biological activity as well as redox chemical reactions, sorption and desorption phenomena, acid-base reactions, and trapped gas release. Biological phenomena are differentiated from the nonbiological gas changes by their dynamical qualities, notably by the ability of the M4 medium to sustain biological activity. Medium incompatibilities, with potential microbial types in soils, are demonstrated to be ameliorated by an incubation chamber design that provides thin films of medium around particulate soil masses and salt gradients when the soil is wet from below. Two phenomena in soils, the production and consumption of hydrogen and carbon monoxide, are coupled for a newly isolated *Clostridium sp.* A decrease in molecular nitrogen production by denitrifying organisms in the second and subsequent incubation cycles results from competitive nitrate utilization by anaerobic organisms. All soils tested from the cold, dry desert regions of Antarctica contain predominantly aerobic organisms while only six of the twelve soils respire using nitrate under anaerobic conditions. Although dry Antarctica soils are not the best simulations of Martian anoxic conditions, their responses show that long incubation times may be needed on Mars to demonstrate biological gas change phenomena.

1. Introduction

The objective of the gas exchange experiment (GEX) in the Viking lander biology instrument package is to determine whether biology exists in a 1-cc Martian soil sample delivered to it. We determine the presence or absence of that biology by interpreting the changes in the gas composition when that sample is perturbed first by water vapor then by liquid water containing organic nutrients and inorganic salts. A description of the development of the gas exchange experiment is described in *Icarus* (Oyama, 1972).

The GEX is capable of maximum flexibility while protecting the indigenous organisms from exposure to potentially physiologically incompatible medium (Figure 1). Martian soil is delivered to a soil distribution assembly in the biology instrument from a soil processing assembly that had earlier acquired soil from a surface sampler. The soil distribution assembly delivers soil to each of the three biology experiments. When lowered away from the distributor and rotated 180° and raised, the GEX incubation chamber is sealed by a fixed head end assembly. In this position, the incubation chamber is able to receive a volumetric gas mixture of carbon dioxide, krypton, and helium that raises the pressure of the incubation chamber to approximately 200 mbar. At this initial pressure, gas in the headspace

* Principal Investigator for GEX experiment.

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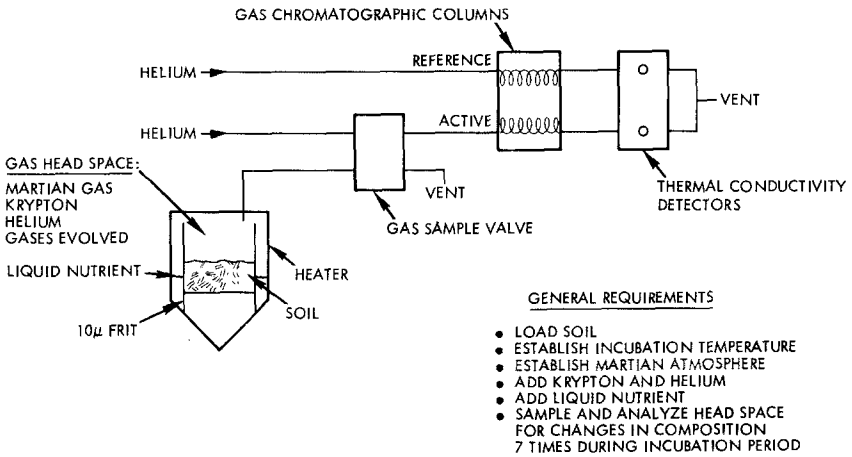

Fig. 1. Schematic of the gas exchange experiment.

can be simply transferred to the sample loop of a gas sampling assembly by utilizing the Martian ambient pressure of some 5 to 10 mbar and sequencing miniaturized solenoid valves. The trapped gas sample can be swept out of the gas sample loop directly into the chromatographic column by diverting helium carrier gas through this sample loop. A 7.6 m × 1.0 mm i.d. stainless steel tube filled with 100/120 mesh Poropak Q is utilized to separate gases of interest (Carle, 1970). The gases we have selected for measurement are shown in the chromatogram (Figure 2). All of the gases through nitrous oxide appear within the 16 min of chromatographic time allotted for a nominal analysis. However, the chromatographic capability extends through hydrogen sulfide, some 60 min following nitrous oxide. This chromatographic capability will be exercised on occasions as required. Krypton is used as internal

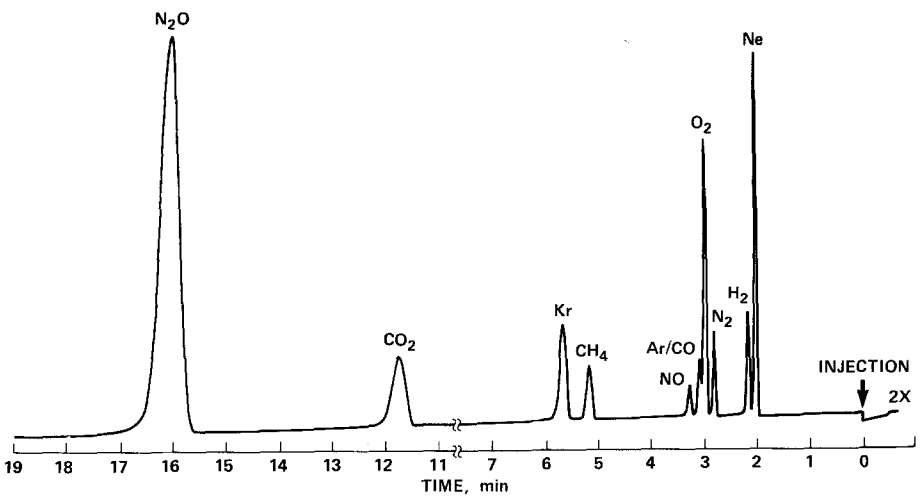

Fig. 2. Viking gas exchange chromatographic separations. Column: Poropak Q 100/120 mesh, 7.6 m × 1 mm i.d. Conditions: Helium carrier gas at 9.3-bar differential, temperature 297 K, flow rate ≈15.0 scc min^{-1}.

standard to calibrate the analyses and determine incubation chamber seal leak rates.

The incubation chamber (Figure 1) is equipped with a drain valve, a valve and restrictor for admitting low pressure helium (1.24 bar) for purging between incubation cycles, and a valve for delivering nutrient medium. A soil-colander (a perforated compartment) is suspended in the incubation chamber and holds the soil above the bottom. This allows a humid situation to prevail when a nominal 0.5 cc of fluid is delivered, and a wet situation when a nominal 2.5 cc of fluid has been delivered to the bottom of the incubation chamber. Since nutrient medium is carried to Mars in a leak-tight glass ampoule containing neon – another internal standard – measurement of actual delivery volume is ascertainable from neon solubility data.

2. Biological Premises

The biological premises that are implemented in the GEX are stated below:

First, perpetuation differentiates biological from nonbiological responses. Biological reaction could result in growth and multiplication, thus eliciting more responses when fresh medium is added. Chemical reactions on the other hand dissipate reactants thereby reducing responses.

Second, organisms are susceptible to drastic changes in the environment; when these changes are gradual or limiting, the organisms have a greater opportunity to adapt to them. Third, when the balance of nutrients and physical factors favor a particular species, it will grow and multiply until the environment becomes depleted of one or more essential components or becomes metabolically inhibiting (Odum, 1971).

In the discussions to follow, we try to illustrate these premises and describe some of the ways in which the GEX experiment is designed to react to them.

3. The GEX M4 Medium

The requirements for this medium are that it (i) survive spacecraft qualification temperature requirements in excess of the sterilization requirement, (ii) must not precipitate or have a high viscosity, (iii) support the growth and continued metabolic activity of soil microorganisms in soils, (iv) allow microorganisms in pure culture, capable of growing in it, to survive 21 days of incubation, and that no quantity of a constituent be present in such excesses as to be lethal to the anaerobic heterotrophic microorganisms in soils.

Out of these considerations, we excluded glucose and the amides, glutamine and asparagine, from the medium. Natural extracts and preparations known to elicit biological activity, such as yeast extract, beef extract, and tryptic digests of casein, were eliminated, because of the concern for reproducibility in preparing the medium and the necessity of quality assurance.

The M4 medium (Table I) is composed of 18 racemic amino acids, glycine, 17 vitamins, 5 generally easily biologically combustible carbon sources, 6 miscellaneous organic substances which include 4 nucleic acid bases, putrescine and a monoleate sorbitan as a fatty acid source, 13 elemental sources in ionic form, and EDTA to support the solubility of divalent cations. The pH of the final solution, compounded

TABLE I

Composition of the M4 medium

Amino Acids	(mM)	Salts	(M)	Vitamins and cofactors	(M)	CHO	(mM)
DL-Alanine	2.0	H_3BO_3	1×10^{-6}	Ascorbic acid	3×10^{-4}	Na acetate	30
DL-Arginine	1.0	$CaCl_2$	3×10^{-5}	Biotin	2×10^{-8}	Na citrate	0.7
DL-Aspartic acid	1.5	$CuSO_4$	8×10^{-8}	Choline CL	4×10^{-5}	Na formate	10
DL-Citrulline	0.5	$FeSO_4$	5×10^{-7}	Cobalamin	7×10^{-9}	Glycerol	39
DL-Cystine	0.3	$MgSO_4$	3×10^{-4}	i-Inositol	6×10^{-5}	DL-Na lactate	22
DL-Glutamic acid	2.0	$MnCl_2$	2×10^{-7}	Nicotinic acid	4×10^{-6}		
Glycine	1.3	$(NH_4)_6Mo_7O_{24}$	2×10^{-8}	Nicotinamide	4×10^{-6}		
DL-Histidine	1.3	KNO_3	1×10^{-3}	Ca pantothenate	1×10^{-6}	Miscellaneous	(M)
DL-Isoleucine	1.0	KH_2PO_4	8×10^{-3}	Pyridoxal	5×10^{-7}	Putrescine	5×10^{-5}
DL-Leucine	0.8	$ZnSO_4$	7×10^{-8}	Pyridoxine	2.5×10^{-6}	Tween 80	(0.1 mg/l)
DL-Lysine	2.0	NH_4VO_3	2×10^{-7}	Pyridoxamine	2×10^{-6}	Adenine	2×10^{-6}
DL-Methionine	0.7	$Co(NO_3)_2$	2×10^{-7}	Riboflavin	3×10^{-7}	Guanine	2×10^{-6}
DL-Phenylalanine	1.2	Na_2EDTA	3×10^{-5}	Thiamine	1.5×10^{-6}	Uracil	2×10^{-6}
DL-Proline	1.7			β-Alanine	6×10^{-6}	Xanthine	2×10^{-6}
DL-Serine	1.0			DPN	1×10^{-5}		
DL-Threonine	1.0			Folacin	3×10^{-8}		
DL-Tryptophan	0.3			PABA	8×10^{-9}		
DL-Tyrosine	1.0						
DL-Valine	1.7						

pH adjusted to 7.2

and de-aired, is 7.2. In equilibrium with 5 mbar of CO_2, the pH is 7.0. The final normality of NaCl is 0.11. The medium differs from the one described in *Icarus* (Oyama, 1972) mainly by a 20-fold decrease in nitrate, elimination of sodium sulfide, addition of EDTA, and reductions in the concentrations of putrescine, adenine, guanine, uracil, and xanthine ranging from 9.7- to 17.9-fold. Each of the medium constituents exclusive of the D forms of amino acids have been shown to be required as an essential growth factor for at least one isolated microorganism (fungus, yeast, bacteria, or protozoan) (Spector, 1961).

Considerations of the demonstrated toxicity of H_2S to a wide number of soil microorganisms (Wiame, 1958) have eliminated the use of Na_2S directly in the medium, and moderated the excessive use of sulfate as a respiratory electron accepting source. It is reasoned that, if such sulfate reducing organisms exist, sulfate in the environment would be present in sufficient quantities (supplemented by the 3×10^{-4} M in the medium) to demonstrate these sulfate reducers in the soil. Moreover, H_2S in the gas phase is not likely to appear until all excess iron ions are effectively precipitated as iron sulfides. (Alexander, 1964). With the same reasoning, a moderate level of nitrate (10^{-3} M) was determined to provide both a sustaining quantity for pure cultures of denitrifying bacteria and yet permit their respiratory product, either nitrogen and/or nitrous oxide, to be detected at reasonable levels.

The thermal lability of D-glucose is somewhat a blessing in disguise. Though noted for its universal applicability to stimulate biological activity, its failing is that its sustaining or maintaining aspects are accordingly diminished. Substitution of glucose by a mixture of acetate, citrate, formate, lactate, and glycerol does not elicit as acute a response, but provides the maintenance aspects that are desirable for differentiating biological from nonbiological chemical responses. No major changes in pH are discerned after days of incubating soil and its indigenous population with the M4 medium.

4. Incubation Chamber

Since it is presumptive to assume that a universal medium can ever be compounded that is capable of growing all heterotrophs directly, an incubation chamber was designed to provide a mechanism which allowed – according to the second of the biological premises – growth and metabolism of indigenous soil organisms with changes that these organisms can tolerate.

An example of a gradient accommodation for halophiles is demonstrated in the following experiment. The suspended soil colander (Figure 1) is wet when more than 1.1 cc of nutrient is injected into the sump. The soil wets by capillary action with only a small part of that soil column submerged in the nutrient. This procedure should yield a salt gradient and support the growth of halophiles even though the medium as such is hypotonic.

This concept was tested with a strain of *Halobacterium*, an obligate halophile (kindly provided by Dr Lawrence Hochstein), which was added to dry sterile samples of crushed basalt, dunite, peridotite, and a sterilized Death Valley soil. Previously

air oxidized M4 medium was added to test cells in a manner simulating GEX, and a gas mixture containing O_2, Kr, and He added. O_2 consumption and CO_2 production were followed.

The above observations (Figure 3) occurred during 32 days of incubation. CO_2 was not discerned in the dunite containing incubation chamber as expected because of the basicity of this material. A soil sample from the top of each of the colanders tested was placed in nutrient containing 15% NaCl. After 14 days at 303 K, all of the tubes showed the characteristic brick-red coloration of the halophile, thus, confirming the fact that the halophile survived at the apex of the salt gradient presumed formed in the soil column. This demonstration of gradient effects on biological populations is consistent with the literature as reviewed by Brock (1966).

That salt gradients could be formed by soils was demonstrated by eluting soil columns in the ascending mode (Figure 4). At feed rates of 0.25 and 1 cc/hr the histograms show that, with the slower of the two rates, the first elution increment has attained a sufficient NaCl concentration (>10%) to allow for the survival of obligate halophiles at the surface. The slower rate is nearly comparable to an ascending front wet by capillary action. A further demonstration of the second premise is shown in Figure 5.

A natural soil collected near a Leslie salt harvesting bed in the San Francisco Bay region was tested in a colander and compared with the same soil submerged, both in duplicate. Analyses of gases hydrogen, methane, and carbon dioxide show the

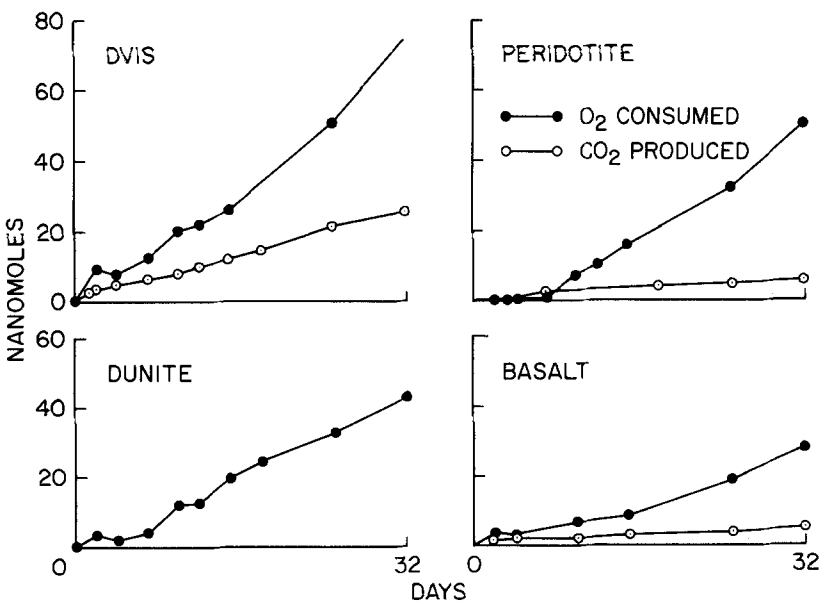

Fig. 3. Gas changes over sterile particles inoculated with an obligate halophile, Halobacterium, strain AR-1. Porous cell 1 × 1 × 4 cm was filled with preinoculated particles and incubated at room temperature in an atmosphere containing krypton, oxygen, and helium. Nanomoles of gas consumed or produced were measured in a 0.1-cc sample loop volume and normalized to krypton.

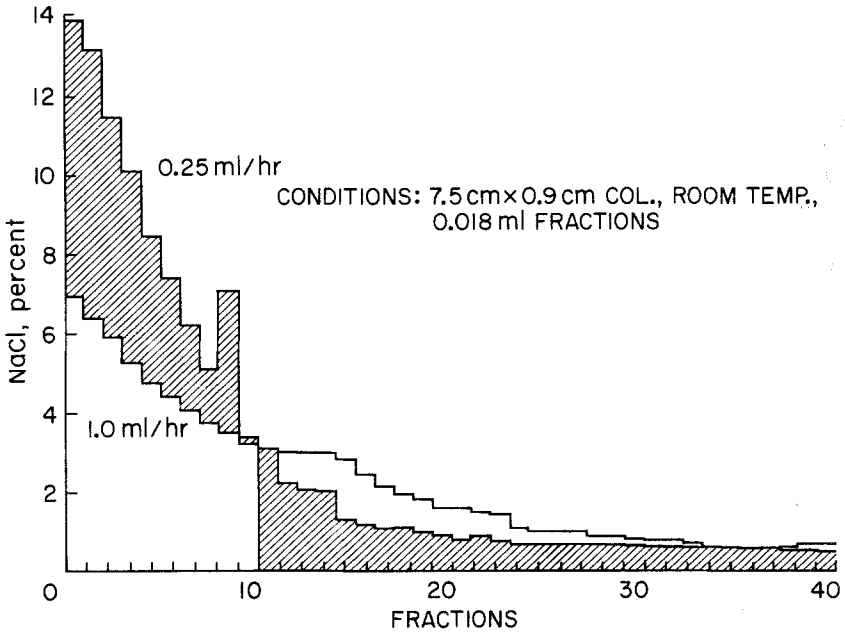

Fig. 4. Sodium ion gradient (expressed as NaCl) from H_2O elution of Death Valley 1S soil at two flow rates.

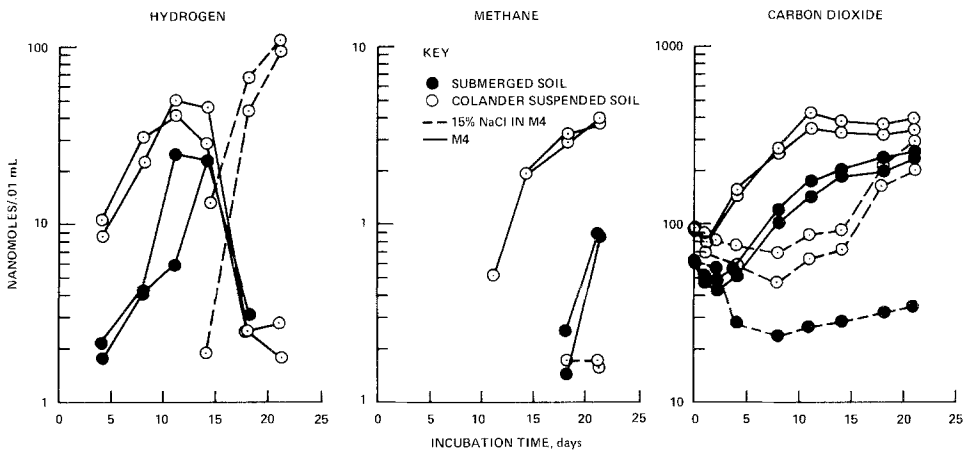

Fig. 5. Effects of soil colander on anaerobiosis. 0.9-cc soil in 1 × 1 × 4 cm colander incubated at 295 K with 2.5-cc M4 medium and 0.9 cc of soil submerged in 2.5-cc M4 medium.

mediating effects of the colander when M4 is used directly or when M4 is supplemented with 15% NaCl. These data are consistent with the hypothesis that surface films of normally physiologically intolerable nutrient provided by capillary feed become tolerable to microorganisms in soils.

5. Nonbiological Gas Changes

We now attack the question of what constitutes nonbiological gas changes so that a basis is established for recognizing the potential chemical reactions and physical phenomena that could occur with virgin planetary material.

It is clear that, if water or water vapor were available on a planetary surface, thermodynamical considerations would not allow water reactive materials exposed to this water vapor to exist for long. On the other hand, a planet devoid of water may be expected to have substances that could react with water. In our studies of lunar material, the latter case was demonstrated (Oyama *et al.*, 1971 and 1972).

Repeated administration of nutrient to the lunar material produced decreasing amounts of hydrogen gas concurrent with the absorption of carbon dioxide. Very small amounts of methane also appeared. These chemical reactions can be simulated under similar conditions of incubation by additions of nutrient to metallic elements and carbides as shown in Figures 6 and 7. The first of these show that the elements

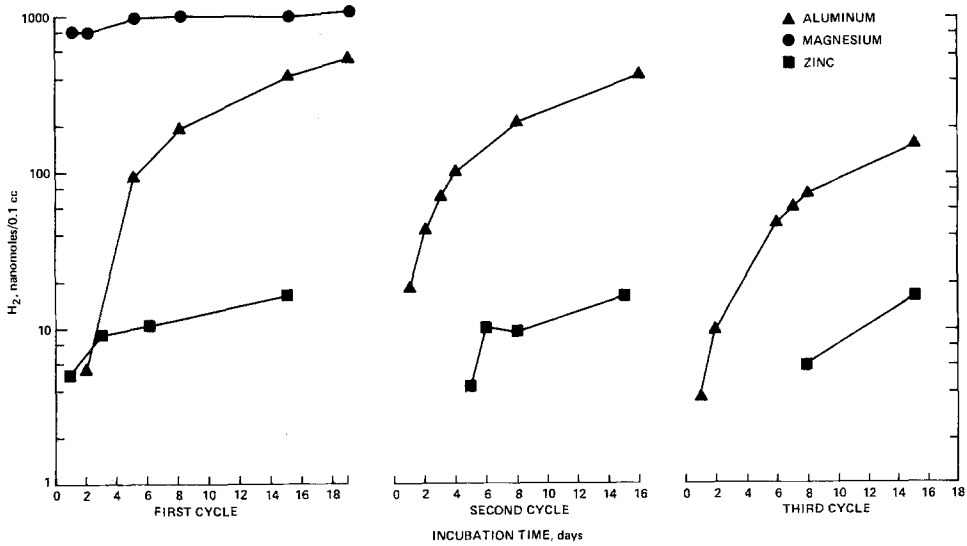

Fig. 6. Metal reactions with GEX M4 medium (hydrogen production). 2 cc of the M4 medium were added to 28 mg of the metal in a closed test chamber after the chamber was evacuated and back-filled to 1 atm with a gas mixture containing 1.38% Kr, 1.39% CO_2, balance He. At the end of a cycle, the test chamber was drained and recharged with M4 medium and the above gas mixture.

magnesium, zinc, and aluminum react with water to produce hydrogen gas during each of three wet cycles. The most reactive of elements shown is magnesium which is totally oxidized by water in the first cycle. Other reactive elements are sodium, potassium, calcium, cerium, manganese, and iron. Metallic iron, an abundant element in lunar fines, is the major but probably not the only reducing agent in the lunar fines. Elements such as Ag, As, Bi, Cd, Co, Cu, Hg, In, Mo, Ni, Sb, Sn, and W show no activity in the GEX.

Aluminum carbide and calcium carbide show the production of methane (Figure 7). Metal carbides tested in GEX showing no discernible activity are: B, Cr, Hf, Mo,

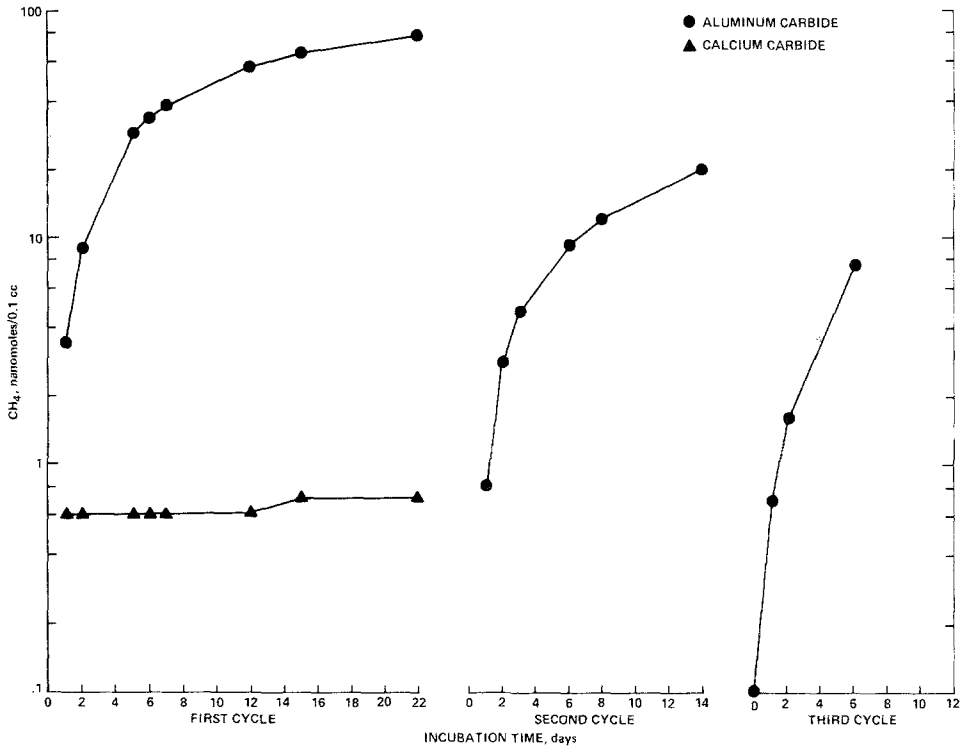

Fig. 7. Metal carbide reactions with GEX M4 medium (methane production). 2 cc of the M4 medium were added to 28 mg of the metal carbide in a closed test chamber after the chamber was evacuated and backfilled to 1 atmosphere with a gas mixture containing 1.38% Kr, 1.39% CO_2, balance He. At the end of a cycle, the test chamber was drained and recharged with M4 medium and the above gas mixture.

Nb, Si, Ta, Ti, V, W, and Zr. These graphs show that, given time and fresh charges of medium, the element or elemental carbide will be dissipated. The rate of dissipation is a function of the intrinsic nature of the element, and the physical setting. If the reduction in rate during subsequent nutrient recharges is slow, it will take a long time to show this dissipation. However, it can be stated categorically that the decrease in the production rate of hydrogen and methane by inorganic reactions is a monotonic function. All of the above phenomena are examples of the nonregenerative aspects that nonbiological reactions provide and contrast with the first of the premises.

Besides chemical reactions, physical sorption and desorption processes occur. These are highly susceptible to the presence of water vapor and are virtually swamped when liquid water wets the surfaces. Thus, the first several days of the GEX experiment calls for humidification, which is initiated when a 0.5 cc of nutrient, a quantity insufficient to wet the soil, is delivered to the bottom of the incubation chamber. Figure 8 shows CO_2 concentration in nanomoles/0.1 cc during incubation of four materials: a sterile Aiken soil, powdered basalt, limonite, and finely crushed quartz under Martian CO_2 partial pressures. The soil, basalt, and limonite show CO_2 uptake during the early part of the dry incubation, but quartz does not. When, however, humid conditions are obtained, CO_2 is desorbed from the three material surfaces, whereas quartz surfaces now adsorb CO_2 tenaciously. Even though the data suggest

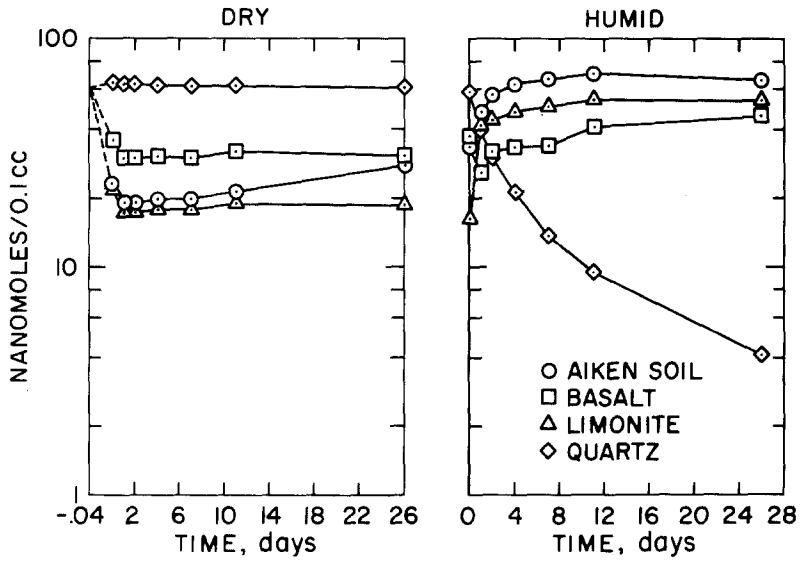

Fig. 8. H_2O_v effects on the sorption/desorption of CO_2 by particulates of various sources. 2.6 cc of the particulate material was placed in a test chamber and the chamber evacuated and backfilled to 1 atmosphere with a gas mixture containing 1.38 % Kr, 1.39 % CO_2, balance He. Humidity was attained by transferring 1.3 cc H_2O to the test chamber, below the soil colander.

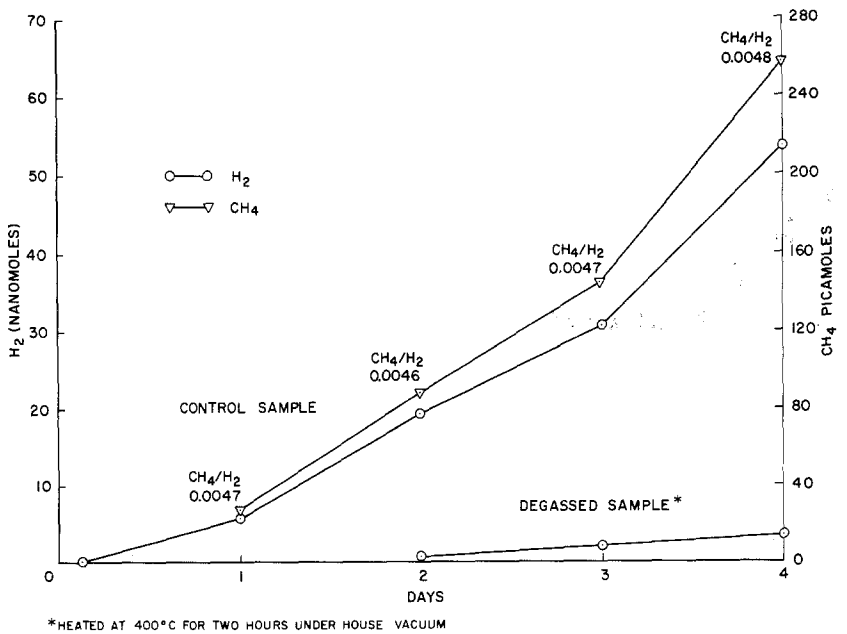

Fig. 9. H_2 and CH_4 desorption from Bowers Clay after addition of medium but before biological production of gases. 2 cc of M4 medium were added to 1 cc of the control and degassed samples after evacuating and backfilling the test chamber to 1 atmosphere with a gas mixture containing 1.38 % Kr, 1.39 % CO_2, balance He.

that interesting information may be obtained about surface material on Mars from CO_2 data, it has to be tempered with the fact that humidification limits the water available to dissipate the aforementioned chemical elements and carbides in a reasonable time, if they are present, and would make it extremely difficult to differentiate biological activity. What, then, could humidification provide?

It could provide a transition from extremely arid conditions and acclimatize microorganisms to the impending wetting to follow. It can be argued, that Mars' microorganisms may be susceptible to sudden wetting.

Now, another kind of gaseous liberation is possible at least from soils that have clays. We have found for example that Bowers clay, a dry sedimentary soil rich in montmorillonite, after being heat sterilized and vacuum pumped, will liberate hydrogen and methane when wet (Figure 9). That these are trapped gases and are not formed during wetting is evidenced by the constant relative ratio of the two gases during the release period.

Unfolding and swelling of the lattice structures is demonstrated in these soils by timed titration (Figure 10). During titration with mild acid, Bowers clay expands with moisture, exposing more basic surfaces so that the pH, at times, paradoxically, increases with additions of dilute acid. It is clear that the origin of the methane and hydrogen was the result of earlier biological activity. This sediment material has copious amounts of organisms that produce these gases. Trapping of these gases must occur during the gradual drying of the soil, at some critical point, during collapse of the lattice structure. If periods of wetness were accompanied by biological activity in Martian soils, it is possible that during episodes of drying, gases generated by the metabolic processes could be trapped. Thus, subsequent wetting in the GEX

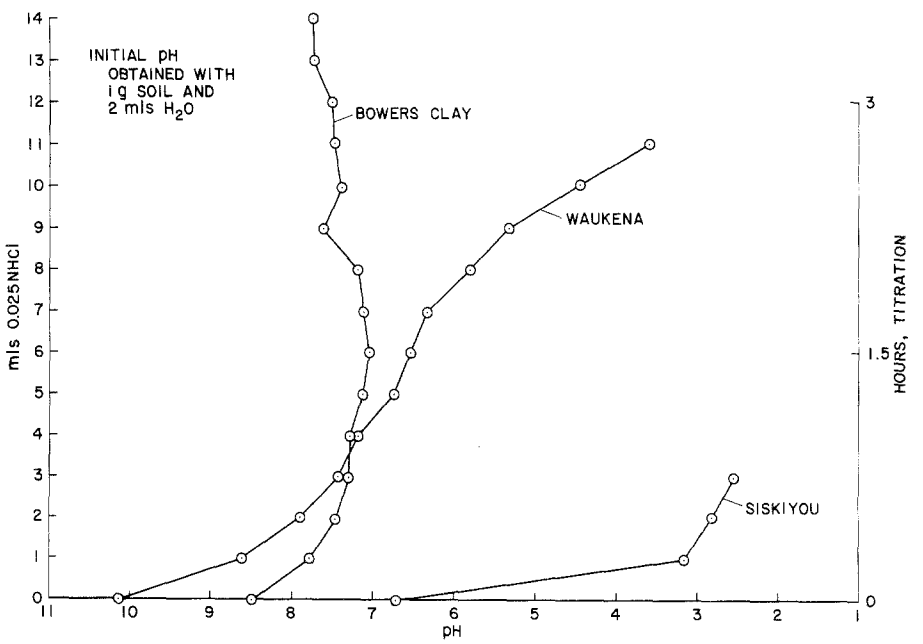

Fig. 10. Titration curves for three California soils.

could release these gases providing portents of biological activity to follow. The desorption and wet release processes are basically one-time phenomena that should occur within a given cycle of activity. On the other hand, wet chemical reactions may require more than one cycle to dissipate.

6. Biological Gas Changes

The biological gas changes that we are now going to discuss are dynamical ones that could be altered by adjustments to the scheduling of the recharging cycles and theoretically could be sustained forever, as long as the nutrient is complete, and there is an infinite supply. Obviously, the selection of medium constituents was based upon the need to have as many of the essential components as was practical, while medium quantity was limited in practice by spacecraft weight and volume constraints.

In our studies of terrestrial soils using the M4 medium, gaseous changes measured in the headspace of the incubation test chambers are caused by biological activities associated with soil organisms. Exceptions to this are the very early gas changes due to nonequilibrium phenomena when nutrient is added to soil. Carbon dioxide is absorbed by the medium and the soil buffers, and traces of gases not removed at the start of the experiment are slowly desorbed from soil. The time required for equilibrium to be obtained is about one to two days. Outside of these minor contributions, gas changes are a result of biological activity.

A typical example of diverse activity in rich soils, is seen in Staten Island soil (Figure 11). Three cycles of 15 days of nutrient wetting are shown followed by a sterile cycle. The significant features of this graph are the early, 3-day appearance of methane on the first cycle, followed by increasing production rates during subsequent cycles, the constancy of carbon dioxide production curves for the three

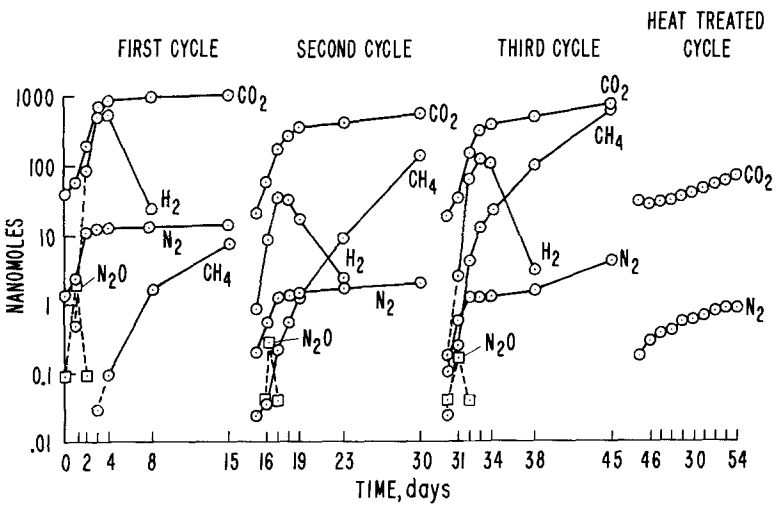

Fig. 11. Gas changes over Staten Island soil incubated with M4 medium. 2 cc of the M4 medium were added to 1 cc of Staten Island soil in a closed test chamber. The chamber was evacuated and backfilled to 1 atm with a gas mixture containing 1.38% Kr, 1.39% CO_2, balance He. At the end of a cycle, the test chamber was drained and recharged with M4 medium and the above gas mixture.

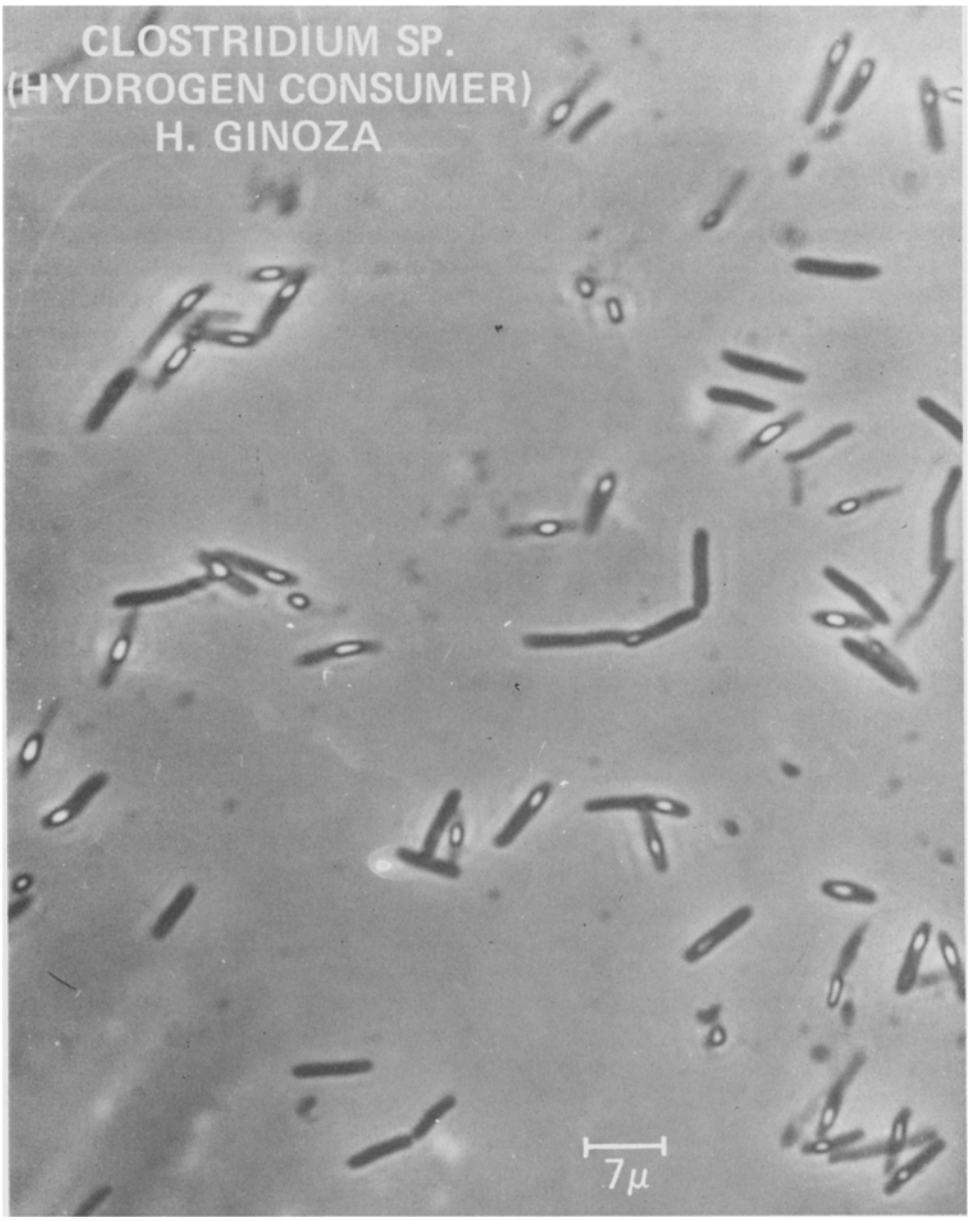

Fig. 12. *Clostridium sp.*

cycles, the production and consumption of hydrogen in all three cycles, the production of nitrogen but at decreasing rates following the first cycle, and the production and consumption of nitrous oxide. The fourth cycle is a thermal control cycle using the same soil. The soil is drained, dried under helium, and heated at 433 K for 3 h under helium, then cooled, gassed, and wetted with nutrient.

These are typical gas changes of the 12 great soil groups in California. Samples from various parts of the world are generally typified by these same gas changes with some variation in amplitude or participating gas or lag time. Occasionally, some soils that show hydrogen production show no hydrogen consumption. The gas changes involving increases in carbon dioxide and methane are associated with microbial activity. However, the production and consumption of hydrogen in a given system has not been noted in the soil literature, nor is there an explanation for the decreasing production of molecular nitrogen in most of the soils.

One of objectives, therefore, has been to examine why hydrogen consumption occurred. Because of all the reactions listed, this dynamical change in hydrogen, i.e., the production and consumption, cannot be easily explained by unstable inorganic mixtures, and its occurrence on Mars would be unequivocal evidence of life. A search for an organism that consumes hydrogen was sought in a soil. A *Clostridium sp.* that oxidizes hydrogen has been isolated and characterized by one of us, H. Ginoza (Figure 12).

This organism is not capable of growth and hydrogen consumption in M4 alone. However, in M4 with soil extract, nearly half the hydrogen at the start is consumed when the mixture is inoculated with the organism (Figure 13). With sterile soil plus M4 plus organisms, hydrogen uptake corresponds to M4 plus native soil. It appears that the soil provides an essential component or components that support growth and the metabolism of hydrogen.

When electron accepting substrates are added, carbonate appears to be the most active in the oxidation of hydrogen, while sulfate shows no differences from the control and nitrate appears to depress hydrogen uptake.

Titration of carbonate shows that maximum hydrogen uptake occurs at 0.025 M with stimulation effects seen even at 1/5 of that level.

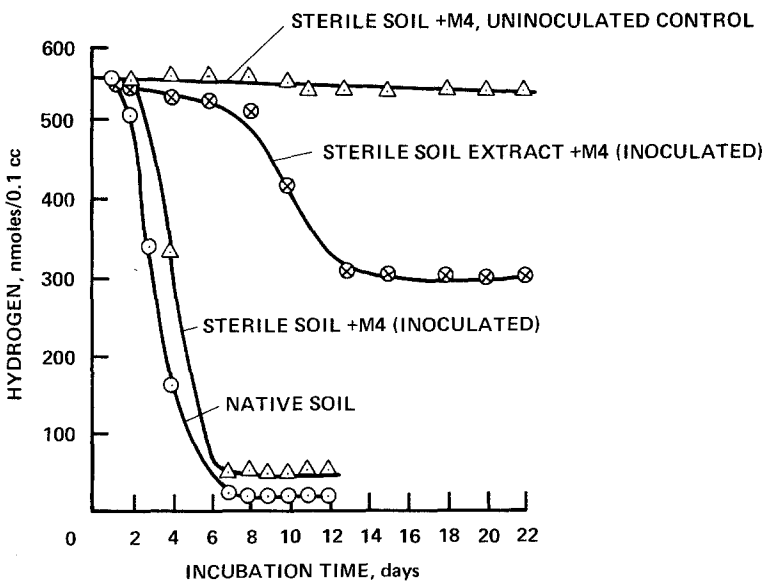

Fig. 13. Utilization of molecular hydrogen by *Clostridium sp.*

With formate titration, a feed-back mechanism is illustrated (Figure 14). With initial hydrogen partial pressure of approximately 223 mbar, 0.04 M formate is estimated to maintain the level of hydrogen. Concentrations in excess of that molarity produce more hydrogen, and lesser levels provide for its consumption. It would appear, therefore, that we have a hydrogen lyase system involving formate, carbon dioxide, and hydrogen (Stephenson and Strickland, 1931 and 1933; and Stephenson, 1937).

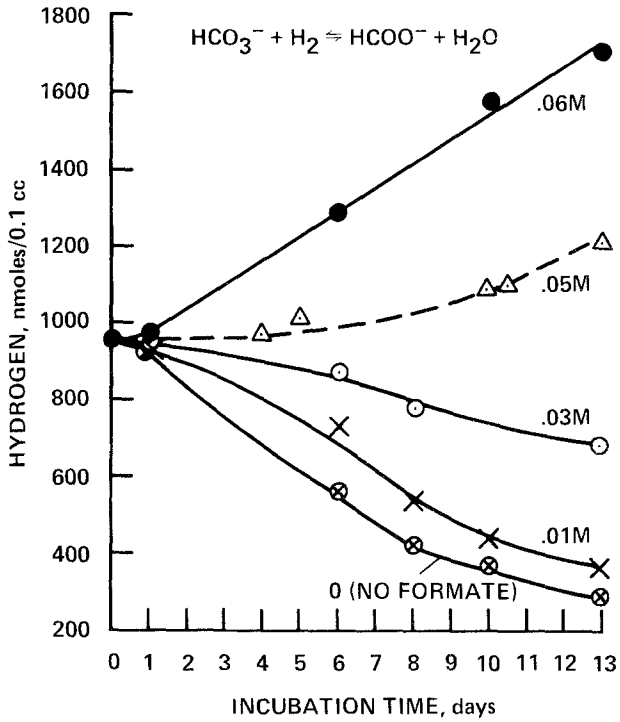

Fig. 14. Formate/hydrogen dependent relationship with sterile Staten Island soil inoculated with *Clostridium sp.*

Moreover, our interest in carbon monoxide, because it is one of the Martian atmospheric gases, has led us into converging paths. We have earlier demonstrated that CO uptake in soils occurred under anaerobic conditions. H. Ginoza's *Clostridium sp.*, on the other hand, produced the most carbon monoxide at the highest nitrate concentrations tested, as shown in Table II. At 0.05 M initial concentration of nitrate, 23 nanomoles of CO per 0.1 cc appeared in the headspace in 2 weeks of incubation. It was reasoned that, if an oxidant such as nitrate inhibited the consumption of hydrogen and produced carbon monoxide, while anaerobic conditions allowed for carbon monoxide uptake, it was likely that carbon monoxide or an intermediary substance was also directly linked in hydrogen oxidation. Preliminary experiments have revealed that hydrogen oxidation is linked to carbon monoxide consumption and leads one to speculate that nitrate blocks the reduction of carbon monoxide to formate or acetate and, in the normal course of events,

TABLE II

Effect of nitrate as a terminal electron acceptor for oxidation of molecular hydrogen in sterile Staten Island soil inoculated with *Clostridium sp.*

Nitrate conc. molar	Evolution of gases nmole/0.1 ml			*Hydrogen uptake nmole/0.1 ml
	N_2O	N_2	CO	
0.05	6.16	10.27	23.00	110.00
0.025	53.70	6.38	—	20.00
0.010	44.90	1.38	6.54	125.00
0.005	10.45	2.40	2.40	404.00
0.0025	3.03	3.77	0.025	596.00
0.0012	0.80	2.77	0.08	500.00

*750 nmole/0.1 ml initial concentration of hydrogen

carbon dioxide is reduced to an intermediary substance closely resembling, if not, carbon monoxide. These observations may have implications on determining sinks and sources of carbon monoxide in the terrestrial realm.

The only other phenomenon which remained to be understood amongst the gas changes was the reduced production of nitrogen during the second and subsequent cycles with the M4 medium that mimic gaseous changes due to chemistry (see first premise). Since reduction in the initial concentration of nitrate from approximately 2×10^{-2} M to the present 10^{-3} M were made to expressly improve the medium for the obligate anaerobes, a reexamination of nitrate utilization was made on the second cycle of Staten Island inoculated media ranging from 3×10^{-1} to 1×10^{-3} M nitrate (Table III). The data show that only with the two highest initial concentra-

TABLE III

Percent conversion of added NO_3 to evolved nitrogeneous gases on cycle 2

Source of denitrifier	Initial concentration nitrate (molar)			
	3×10^{-1}	1×10^{-1}	1×10^{-2}	1×10^{-3}
Staten Island soil	100%	100%	60%	30%
Pseudomonas denitrificans				100%

tions of nitrate was all of the nitrate stoichiometrically converted to the nitrogenous gases, while only 30% of the nitrate was converted at the M4 concentration of 1×10^{-3} M. On the other hand, *Pseudomonas denitrificans* tested in the M4 medium converted all of the nitrate to the nitrogenous gases.

This inefficient utilization of nitrate for respiration on the part of the denitrifiers in soils was shown not to be because of the loss of denitrifiers (Figure 15). In this experiment, for the first cycle, duplicate samples of soil were incubated with M4 medium. When incubation was recycled with M4, it served as the control. The graphs show the normal increased production of methane, the characteristic hydrogen production and consumption, while levels of nitrogen reached are lowered in the

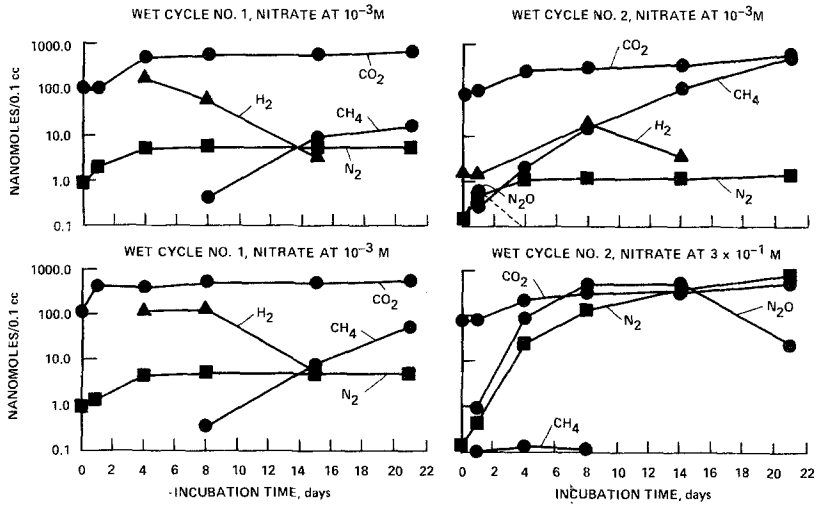

Fig. 15. Nitrate effects on gas changes in Staten Island soil.

second cycle. When, however, the medium incorporates nitrate at the 3×10^{-1} M concentration (lower right graph), copious amounts of nitrous oxide and nitrogen are released, while methane and hydrogen production are virtually eliminated.

In experiments with the same soil, it has also been shown that dialysis bags containing *Pseudomonas denitrificans* immersed in M4 medium survive and maintain their viability.

From these observations, it is concluded that nitrate unavailability is the cause of the reduced level of nitrogeneous gases evolved. When nitrate is sufficiently high (greater than 1×10^{-3} M level), a large number of anaerobic bacteria are inhibited and full use can then be made of the nitrate for respiration. When the level of nitrate is at 1×10^{-3} M initially, the anaerobic bacteria are competitively utilizing nitrate so that less is available for respiration.

7. Antarctica Soils

Now, we would like to dwell briefly on some Antarctica soil experiments which have helped to formulate plans for coping with low frequency soil populations that are coupled to low temperature environments. Although at present we are working on submerged soils that were gathered below the thermocline by Dr Richard Morita's group, we shall restrict our discussion to the dry desert Antarctica soils which were gathered by Dr Roy Cameron and which are now under the management of Dr Edward L. Merek at the ARC (Cameron 1969, 1970, 1971).

Samples of 12 soils with but one exception have exclusively aerobic populations. Fifty percent of these soils can respire by utilizing nitrate as an electron-accepting source in the absence of molecular oxygen. Without exception, all of the soils show aerobic respiratory activity, and, interestingly, oxygen uptake appears to be an extremely sensitive measure.

The grain-like sandy characteristics of these soils and the extreme dryness may

indicate a lack of anaerobic niches. But these soils demonstrate the length of incubation at 283 K required to elicit recognizable anaerobic and aerobic responses. Table IV shows the elapsed time required for a definitive anaerobic response (aerobes utilizing nitrate under anaerobic conditions). It ranges from 21 to 120 days. The mean time was 44 days. For aerobic responses, the range was 11 to 106 days and a mean time of 26 days. Therefore, unless missions are extended, the incubation time may not be adequate to detect life if it is as severely limited as in the Antarctica.

TABLE IV

Elapsed time before definitive gas changes seen with Antarctic soils

	Anaerobic incubation		Aerobic incubation	
Soil	Elapsed time, days	Cycle	Elapsed time, days	Cycle
500	–			
	–		42 (O_2 uptake)	2
	–		21 (O_2 uptake)	1
537	41 (N_2)	2	11 (O_2 uptake, N_2)	1
	36 (N_2)	1	11 (O_2 uptake, N_2)	1
538	–		29 (O_2 uptake)	2
	84 (N_2O)	1	27 (O_2 uptake)	1
571	54 (N_2, N_2O)	3	106 (O_2 uptake)	3
	21 (N_2O)	1	25 (O_2 uptake, N_2, N_2O)	1
	28 (N_2O)	1	42 (N_2)	1
600	–		21 (O_2 uptake, N_2O)	1
	37 (N_2)	1	21 (O_2 uptake, N_2, N_2O)	1
	28 (N_2, N_2O)	1	28 (O_2 uptake)	1
603	–		32 (O_2 uptake)	2
	–		28 (O_2 uptake)	1
	–		28 (O_2 uptake)	1
610	–		18 (O_2 uptake)	1
	–		14 (O_2 uptake)	1
	–		14 (O_2 uptake)	1
618	–		14 (O_2 uptake)	1
	–		21 (O_2 uptake)	1
	–		18 (O_2 uptake)	1
625	–		18 (O_2 uptake)	1
	–		14 (O_2 uptake)	1
	–		14 (O_2 uptake)	1
632	–		25 (O_2 uptake)	2
	–		49 (O_2 uptake)	1
	–		–	
638	25 (N_2, N_2O)	2	21 (O_2 uptake)	1
	22 (N_2)	1	21 (O_2 uptake)	1
	–		14 (O_2 uptake)	1
742	120 (N_2O)	3	20 (O_2 uptake)	1
	–		20 (O_2 uptake)	1
	–			

Figure 16 shows an example of gas changes over an Antarctica soil from Bull Pass which was incubated for 160 days. It Illustrates the third premise; that specific organisms will grow and multiply until the environment becomes depleted of one or more essential components or becomes metabolically inhibiting. Such an example is shown by nitrogen production starting on the second cycle which begins on the

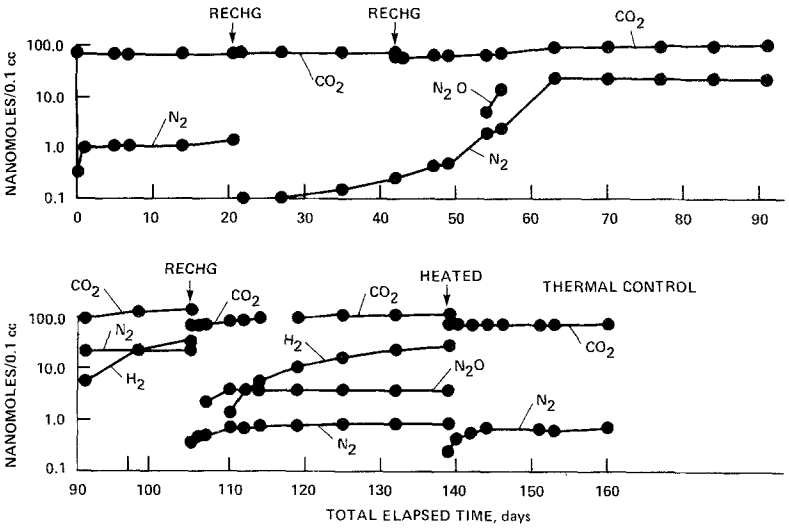

Fig. 16. Gas changes in Bull Pass soil (Antarctic #571) incubated with M4 medium anaerobically at 283 K.

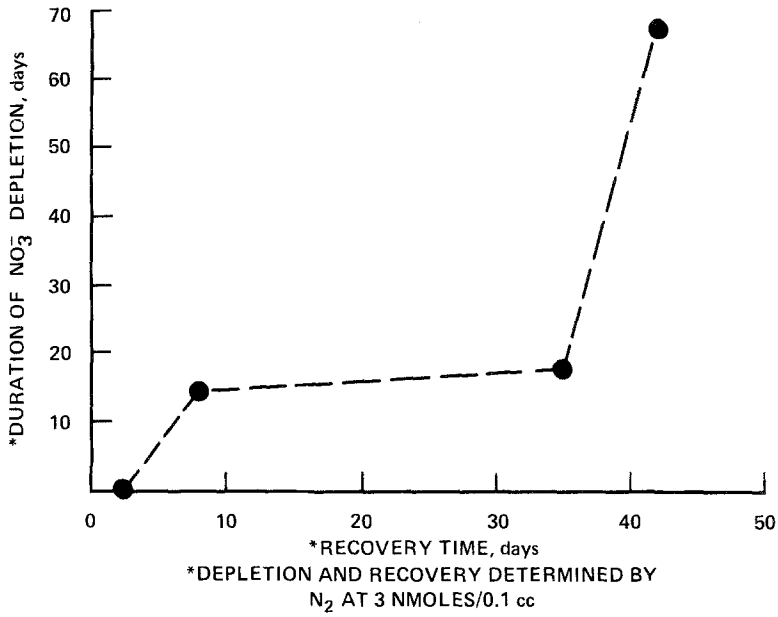

Fig. 17. Recovery of nitrate respiring organisms from nitrate depletion in Antarctic soil #537.

21st day. Nitrogen reaches its maximum on the 63rd day after 43 days of incubation. From day 21 to day 106, a total of 85 days, no medium changes were made. No significant amount of nitrogen is produced in the cycle following, whereas hydrogen which was generated in the third cycle and had probably not plateaued, was effectively regenerated in the fourth cycle.

That various organisms have differing survival and viability characteristics is shown in Figures 17 and 18. Figure 17 plots 'duration of nitrate depletion' on the ordinate

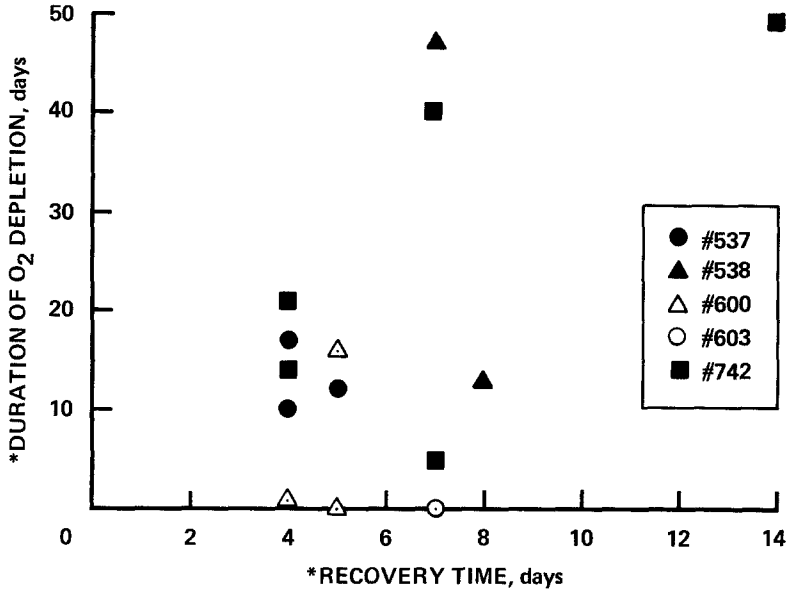

Fig. 18. Recovery of oxygen utilizing organisms from oxygen depletion in Antarctic soil.

as the duration of time taken to obtain a significant nitrogen production. Here, nitrate depletion time is that duration of time after the onset of nitrogen production that nitrogen gas remains constant. It would appear that nitrate depletion must be recognized early, i.e., within a 14-day period, if it is necessary to reobserve the phenomenon for confirmation within 10 days following recharge. In like manner, Figure 18 shows a scattergram of 'duration of oxygen depletion' vs recovery time in days. Here, we see that oxygen depletion up to 40 days could occur, and the recovery time is within a 10-day period.

These data show that sequence changes during landed mission operations is an essential requirement for the GEX experiment. It would allow us to recharge or extend the cycle durations, obtain more analyses, etc., as is warranted by progress of the experiment.

8. Summary

In summary, we have discussed biological gas changes, their sources, and how they can be differentiated from nonbiological ones. Incompatibilities between medium and the indigenous microbial populations are ameliorated by special incubation chamber design. From cold incubation of low-frequency soils, it is concluded that decisive negative tests of GEX may require extended incubations beyond the nominal mission plan of 60 days barring any outright information that negates the presence of life on Mars.

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