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Report No. IITRI-L6023-4
(Quarterly Status Report)

LIFE IN EXTRATERRESTRIAL ENVIRONMENTS

Contract No. NASr-22

National Aeronautics and
Space Administration

IIT RESEARCH INSTITUTE

Report No. IITRI-L6023-4
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LIFE IN EXTRATERRESTRIAL ENVIRONMENTS

November 15, 1965 to February 15, 1966

National Aeronautics and Space Administration

Contract No. NASr-22
IITRI Project L6023

I. INTRODUCTION

It has been established that spores of a strain of Bacillus cereus, isolated from California desert soil, germinate and grow vegetatively in the absence of oxygen in a simulated Martian environment with diurnal temperature cycles (-65 to 25°C) and 8% soil moisture. It has also been established that B. subtilis spores not germinating anaerobically germinate and grow vegetatively with subsequent sporulation when the partial pressure of oxygen is 10 mm of Hg in the same environment. B. cereus requires an oxygen partial pressure of 15 mm of Hg for sporulation.

Determination of the effect of 8-, 16-, and 20-hr diurnal freeze cycles on the growth response of B. cereus and B. subtilis spores during 56 days in a simulated Martian environment modified by 8 to 10% soil moisture and an oxygen partial pressure of 15 mm of Hg was completed during the past

year. Detailed data are reported in Reports No. IITRI-L6023-1, -2, and -3. In summary, we found that:

(1) Extension of the freeze cycle from 8 to 16 hr delayed *B. cereus* spore germination and subsequent vegetative cell growth 2 days and sporulation at least 4 days. *B. subtilis* spore germination, vegetative cell growth, and sporulation were delayed at least 2 days.

(2) Extension of the freeze cycle from 8 to 20 hr delayed *B. cereus* spore germination and vegetative cell growth 6 days. *B. subtilis* spore germination and vegetative cell growth were delayed at least 3 days. Sporulation of neither organism was apparent during 56 days.

(3) The growth response (vegetative cell growth and sporulation) of *B. subtilis* was more rapid, with maximum populations reached before those of *B. cereus*.

(4) After 56 days in the simulated Martian environment, greater than 50% of the viable *B. cereus* cells were spores except those subjected to 20-hr freeze cycle. Less than 50% of the viable *B. subtilis* cells were spores.

(5) B. cereus and B. subtilis spores produced in the simulated Martian environment retained their viability and were able to reestablish an ecological niche when transferred into a similar environment.

(6) Thermal-death time studies with B. subtilis indicated that no change in thermal resistance occurred in spores produced in the simulated Martian environment. Similar studies with B. cereus were not conclusive because of initially low spore populations.

(7) Dry-heat studies with B. cereus spores in simulated Martian soil indicated that exposure to either 100 or 130°C for as long as 30 min was not sufficient to sensitize the spores to a simulated Martian environment with diurnal freezing and thawing cycles. Although spore germination, vegetative cell growth, and sporulation were delayed, population densities after 28 days of exposure to the environment were the same as those of unheated spores.

Other studies were concerned with possible anaerobic sporulation of B. cereus and vegetative cell growth and sporulation of B. subtilis in the simulated Martian environment, with 2, 0.2, and 0.02% potassium nitrate as a terminal electron acceptor in place of oxygen. Possible vegetative cell growth of B. cereus occurred at 0.02% nitrate concentration, while B. subtilis was able to grow vegetatively at all three concentrations. Sporulation of neither organism occurred.

Studies during the past quarter were concerned with the growth response of B. cereus and B. subtilis spores in a simulated Martian environment with low barometric pressures between 5 and 12 mm of Hg. Preliminary studies indicated that the growth response of these organisms was unusually delayed or inhibited by the low barometric pressure. Further studies showed that soil particle size and/or pH are contributing factors; therefore further work is required before conclusions concerning the effects of barometric pressure are drawn.

II. EXPERIMENTAL PROCEDURES

The Bacillus species used were the IITRI strain of B. subtilis and a B. cereus strain isolated from California

desert soil. The spore suspensions, prepared in the manner described in Reports No. IITRI-C194-12 and -13, were heat-shocked for 10 min at 80°C immediately before use.

Experiments investigating the effect of barometric pressure on the growth response of B. cereus and B. subtilis were conducted under the following environmental conditions:

(1) Daily freeze cycle of 8 hr

(2) Felsite/limonite soil containing 1% organic medium

(3) Moisture concentrations of 7, 9, and 10%

(4) Barometric pressures of 5, 9, 12, and 75 mm of Hg.

Water is in the vapor state at 25°C under barometric pressures of 5, 9, and 12 mm of Hg. The moisture in the tubes was frozen in a dry ice-acetone bath prior to establishment of pressure. Freezing was conducted to maintain the moisture at a constant level although we realized that when the frozen moisture in the tubes thaws, the water vapor contributes to the pressure of the system in accordance with Dalton's law of partial pressures.

The pH of the simulated Martian soil was adjusted by altering the ratio of limonite to felsite; limonite is acidic and felsite is alkaline.

The particle size of the simulated Martian soil was adjusted by altering the relative amounts of particles in the following size ranges: 590 to 297 μ, 297 to 210 μ, 210 to 149 μ, 149 to 105 μ, 105 to 74 μ, 74 to 44 μ, and < 44 μ.

Total and spore counts were performed in all studies at 0, 1, 2, 7, and 28 days unless otherwise stated. The recovery medium was trypticase soy agar (BBL). B. cereus was incubated for 24 hr at 35°C, and B. subtilis was incubated for 48 hr at 35°C.

III. RESULTS AND DISCUSSION

Figures 1 and 2 appeared in Report No. IITRI-L6023-3 and are shown here to introduce the problem encountered during this quarter. At the time the preliminary results were obtained it did not seem unusual that the growth responses of B. cereus and B. subtilis had been severely repressed since, we reasoned, a lowering of the barometric pressure effects the water available to a microorganism. Water is in the vapor state above 2 and 14°C at barometric pressures of 5 and 12 mm of Hg, respectively. If liquid water were not available to the organism, the growth cycle would be confined to short time periods during freezing and thawing in that region below the temperature maxima of 2 or 14°C.

IIT Research Institute
10 West 35 Street, Chicago, Illinois 60616
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L6023
February 18, 1966

Miss Winnie M. Morgan
Technical Reports Officer
Office of Grants and Research Contracts
Office of Space Science and Applications
National Aeronautics and Space Administration
Washington, D.C. 20546

Dear Miss Morgan:

This is to call attention to several errors in the last Quarterly Status Report No. IITRI-L6023-4, Contract No. NASr 22, entitled, "Life in Extraterrestrial Environments."

(1) Page 7, Figure 1: axis of ordinate should be entitled, "Number of Survivors/gram of Soil."

(2) Page 8, Figure 2: axis of abscissa should be entitled, "Days;" axis of ordinate should be entitled, "Number of Survivors/gram of Soil;" logarithmic numbers reading from the top down should be 10^7, 10^6, 10^5, 10^4, and 10^3; and page number 8 is missing.

Sincerely yours,

Charles A. Hagen
Research Bacteriologist
Life Sciences Research

CAH/bla

Figure 1

EFFECT OF BAROMETRIC PRESSURE ON THE GROWTH RESPONSE OF BACILLUS CEREUS IN A SIMULATED MARTIAN ENVIRONMENT

Experimental Conditions: No flushing of tubes prior to sealing, 7% moisture, diurnal temperature cycle (-65 to 25°C) with 8 hr freeze cycle.

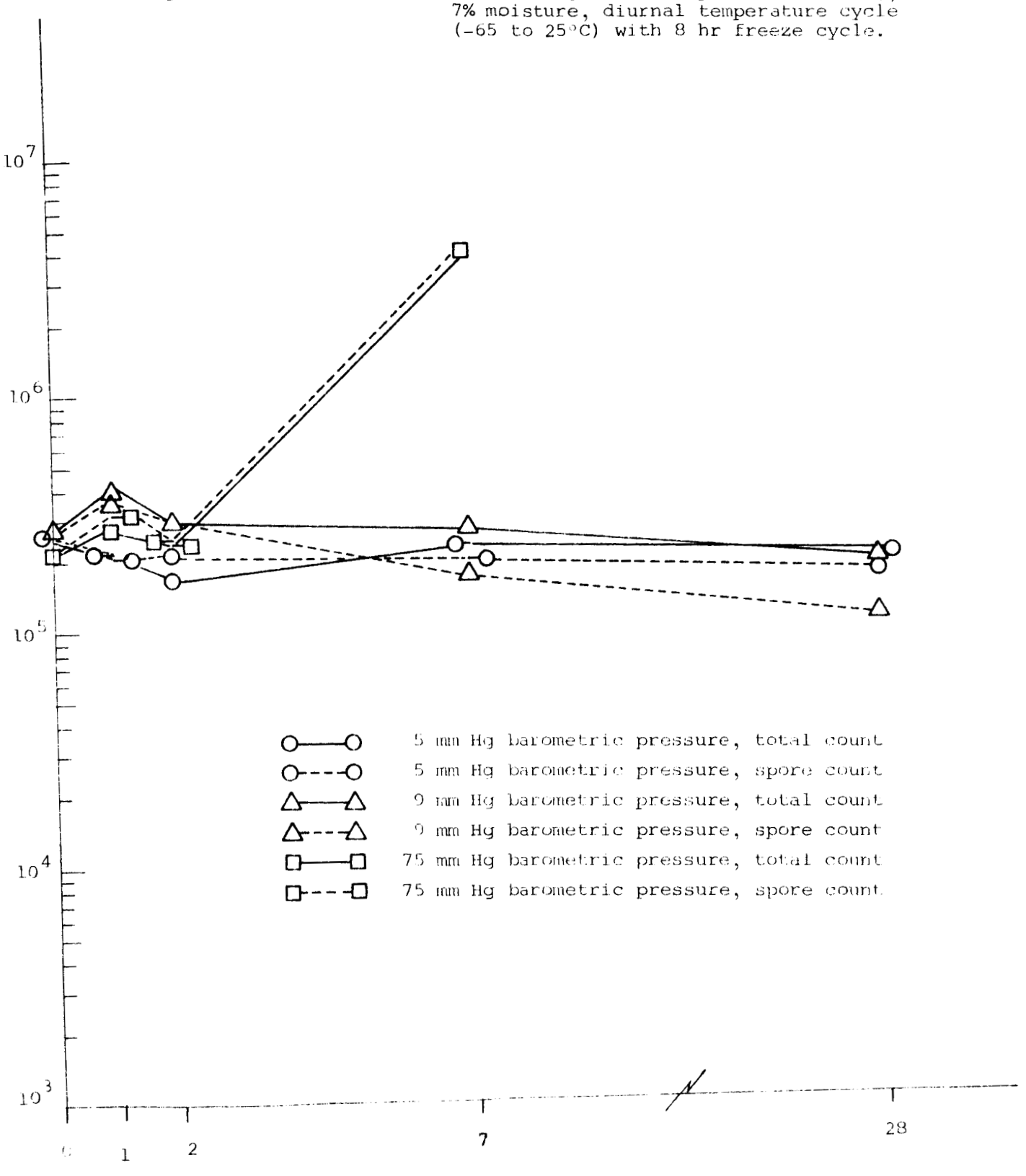
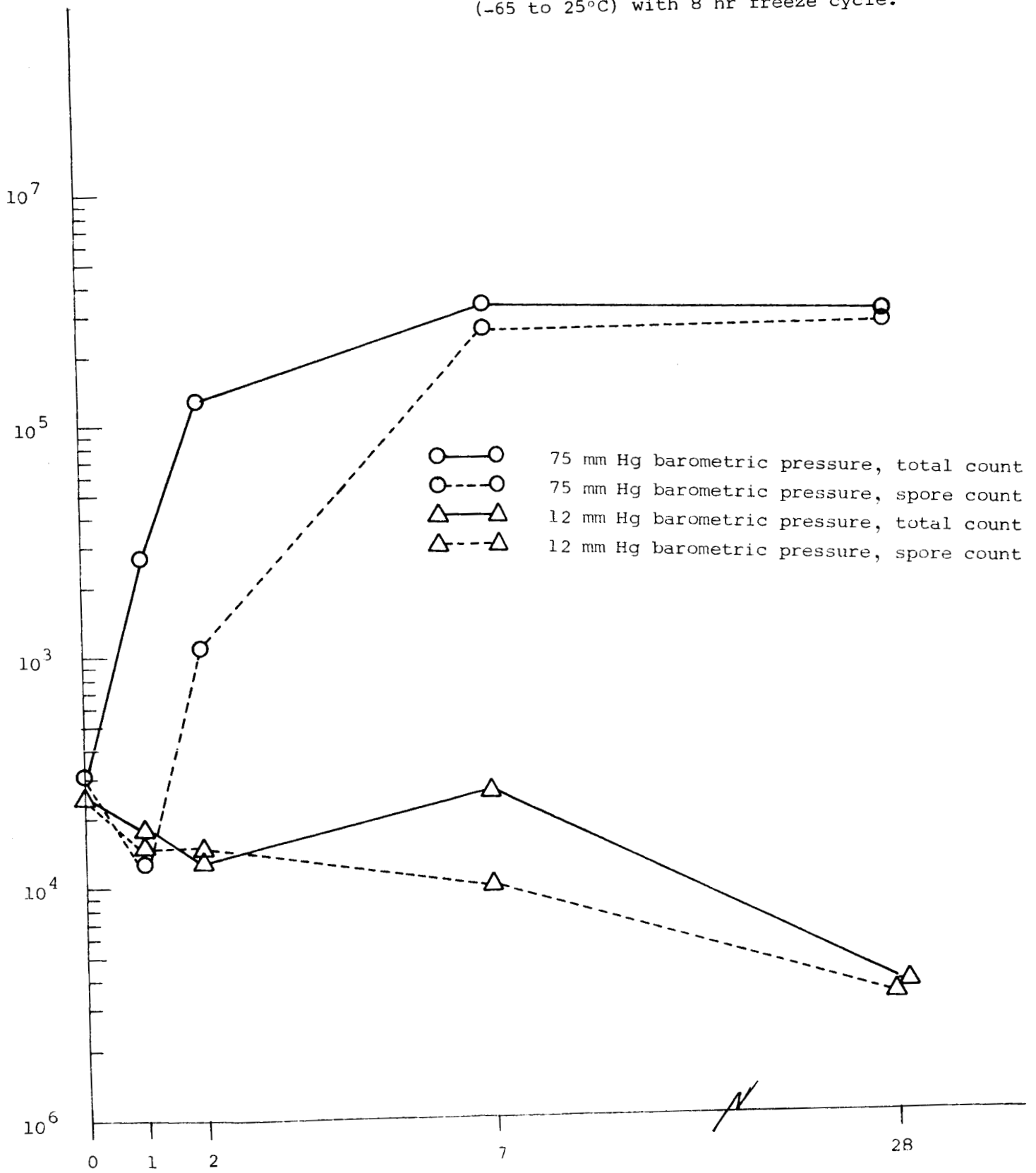


Figure 2

EFFECT OF BAROMETRIC PRESSURE ON THE GROWTH RESPONSE OF BACILLUS SUBTILIS IN A SIMULATED MARTIAN ENVIRONMENT

Experimental Conditions: No flushing of tubes prior to sealing, 9% moisture, diurnal temperature cycle (-65 to 25°C) with 8 hr freeze cycle.



However, experiments with environmental conditions that should have permitted growth of B. cereus were negative. The following factors were considered:

(1) Change in the growth response of the bacterial cultures

(2) Improper soil substrate

(3) Too little available water

(4) Absence of organic medium.

While checking the above factors we learned that a new felsite/limonite soil with a pH of 8.7 to 8.8 had been recently prepared. Maximum initiation of spore germination usually occurs between pH 6 and 8, but the optimum pH may vary with the initiating substance. Lawrence (ref. 1) found an optimum near pH 8 for B. cereus. Vas and Proszt (ref. 2) found that below pH 6 the initiation of B. cereus spores fell slightly but decreased suddenly at pH 5. Wolfe and Mahmoud (ref. 3) obtained more initiation of B. subtilis spores at pH 8.5 than at pH 7 or 6. However, Wolfe and Thorley (ref. 4) found that more germination was initiated with B. subtilis spores by glucose at pH 5.5 than at pH 7.5 or 8.5, but with L-alanine more initiation occurred at 8.5.

Therefore the optimal pH for initiation of spore germination appears to be dependent upon the bacterial strain and the substrate. The pH of 8.7 to 8.8 found in our

experiments is a limiting value for optimal spore germination of B. cereus and B. subtilis.

Figure 3 shows the growth response of different stock spore suspensions of B. cereus in the alkaline soil and of one such suspension in soil with the pH adjusted to 7.2 by the addition of more limonite. The two spore suspensions inoculated into the more alkaline soil did not germinate during the 7 and 28 days of the experiment. The spore suspension inoculated into the soil with a pH of 7.2 did germinate, with subsequent vegetative cell growth occurring between 2 and 7 days. No sporulation occurred with this culture, and we believe that this finding is related to one or more of the following factors:

(1) Size of soil particles

(2) pH of soil

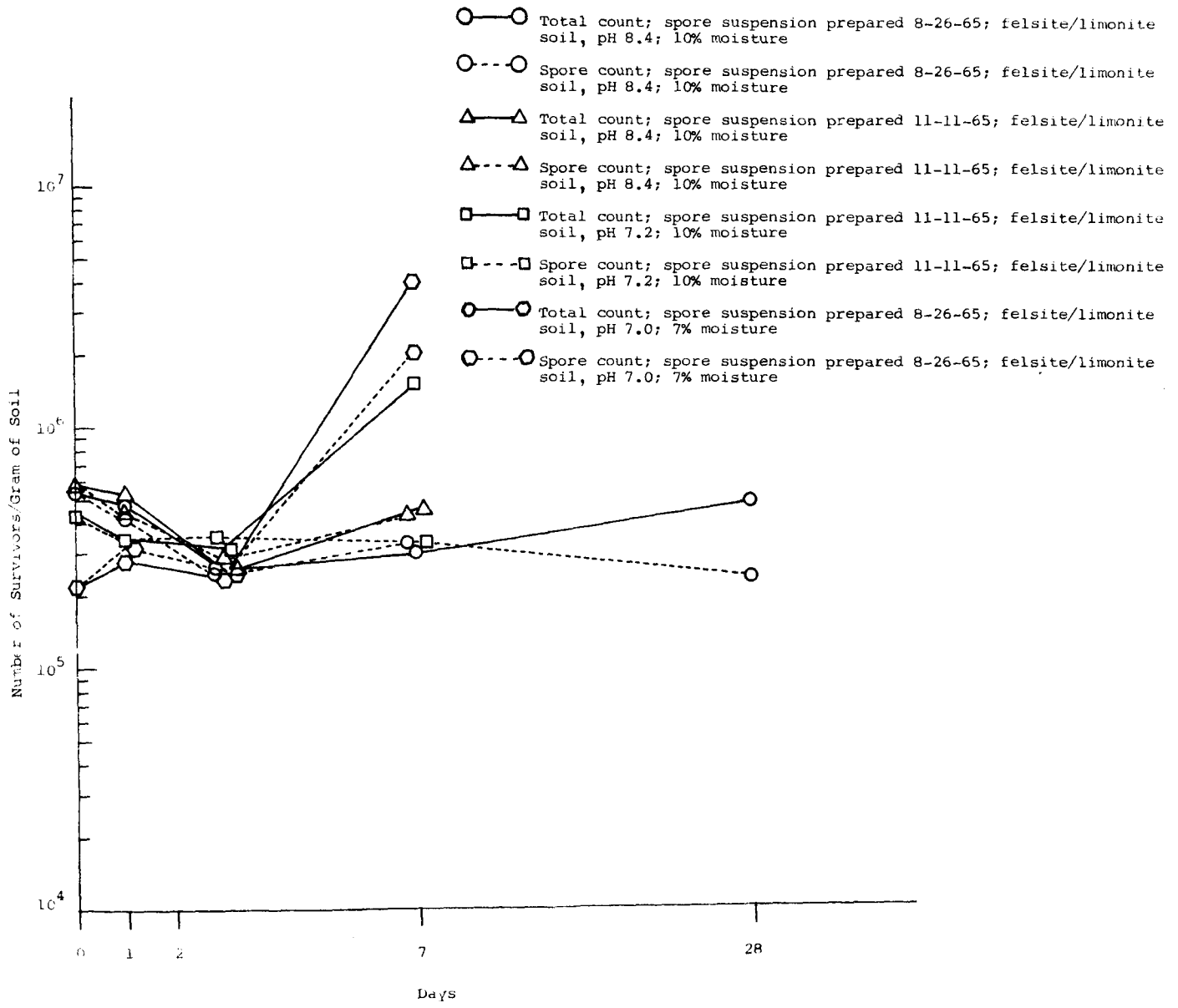
(3) Available soil moisture.

Preliminary conclusions indicate that increasing the limonite fraction affects the amount of bound water. Limonite is readily hydrated to goethite and competes with the microorganisms for available water. This and the other factors above are being investigated.

Figure 3

THE EFFECT OF pH AND SOIL TYPE ON BACILLUS CEREUS SPORE GERMINATION IN A SIMULATED MARTIAN ENVIRONMENT

Experimental Conditions: Diurnal temperature cycle (-65 to 25°C) with 8-hr freeze cycle; 15-mm Hg oxygen pressure.



IV. SUMMARY

A summary of the results from the past year were presented in Section I. This data showed that *B. cereus* spores germinate with subsequent vegetative cell growth in a simulated Martian environment devoid of oxygen, but they required oxygen for sporulation.

Recent data indicate that *B. cereus* and *B. subtilis* can sustain an ecological niche in a simulated Martian environment modified by 8 to 10% moisture and an oxygen partial pressure of 15 mm of Hg.

The Martian environment was not lethal to heat-treated spores of *B. cereus*.

The inhibition of *B. cereus* and *B. subtilis* spore germination by reduced barometric pressures simulating recent estimates of the environment of Mars is being reexamined in terms of soil pH and particle size.

V. PERSONNEL AND RECORDS

Experiments were planned with the counsel of Dr. E. J. Hawrylewicz and with the technical assistance of Miss Marjorie Ewing and Miss Vivian Tolkacz.

Experimental data are recorded in IITRI Logbooks C16394, C16413, and C16590.

Respectfully submitted,

IIT RESEARCH INSTITUTE

Charles A. Hagen
Associate Bacteriologist
Life Sciences Research

Approved by:

E. J. Hawrylewicz
Assistant Director
Life Sciences Research

CAH/cg

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