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LIFE IN EXTRATERRESTRIAL ENVIRONMENTS

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IITRI Project L6023

I. INTRODUCTION

Simulated Martian environment experiments are being conducted with Staphylococcus aureus. The effects of the following barometric pressures and carbon dioxide concentrations were studied over a 56 day period:

(1) Earth atmosphere at pressures of 10, 25, and 40 mb with an 8- and a 20-hr daily freeze cycle

(2) Carbon dioxide concentrations and pressures of 37% at 40 mb, 76% at 25 mb, and 100% at 10 mb with 8- and a 20-hr daily freeze cycle.

(3) Carbon dioxide concentrations of 37, 67, and 100% at 98 mb with an 8- and a 20-hr daily freeze cycle.

S. aureus grew in all atmospheres at all barometric pressures with both an 8- and 20-hr daily freeze. Viable cell counts increased 3.0 to 3.5 logs in Earth atmospheres at different pressures, 2.0 to 2.9 logs in carbon dioxide atmospheres at different pressures, and 1.0 to 2.0 logs in carbon dioxide atmospheres at 98 mb pressure. In the latter two groups the greatest increase occurred in the tubes that received a 20-hr daily freeze. In general, maximum populations were reached within 7 days with an 8-hr daily freeze and within 28 days with a 20-hr daily freeze.

Additional studies with S. aureus were concerned with minimum moisture requirements in a variety of atmospheres. At present, three moisture concentrations of 0.49, 0.79, and 0.98 a_w with an Earth atmosphere at 25 mb pressure and an 8-hr daily freeze were used. Preliminary results indicated that 0.79 a_w was limiting at constant 35°C but was not limiting with an 8-hr daily freeze.

Soil ecology experiments are in progress on the growth response of Bacillus cereus, Lactobacillus plantarum, Pseudomonas aeruginosa, Putrefactive anaerobe (PA 3679), S. aureus, and Streptomyces albus in brunizemic, desertic, and podzolic soils with maximum and minimum relative humidities and a constant temperature of 35°C or a diurnal temperature cycle with an 8-hr freeze.

B. cereus, L. plantarum, P. aeruginosa, S. aureus, and S. albus grew in the brunizemic soil and L. plantarum, S. aureus, and S. albus grew in the podzolic soil while only PA 3679 grew in the desertic soil.

Population maxima resulting from growth in brunizemic and podzolic soils were 3 to 6 logs higher than initial numbers of viable cells which were as low as 100 per gram of soil.

Progress has been made on the phase of the program entitled "Design and Construction of Environmental Chambers." Final design and working drawings have been mutually accepted by IITRI Life Sciences and Engineering personnel. Construction and ordering of component parts are in progress.

II. EXPERIMENTAL PROCEDURES

Stock culture preparation of *B. cereus*, *P. aeruginosa*, PA 3679, and *S. aureus* were described in Report No. IITRI-L6023-5; *L. plantarum* in Report No. IITRI-L6023-6; and *S. albus* in Report No. IITRI-L6023-7. All stock cell suspensions were stored at 4°C until used. *B. cereus* and PA 3679 spore suspensions were heat-shocked at 80°C for 10 min just before use.

Bacterial counts are reported as averaged counts of two plates from each of two or three tubes. Incubation was at 35, 30, or 25°C for 1 to 5 days, depending on the bacterial species.

III. RESULTS AND DISCUSSION

A. Simulated Martian Environment Studies

All tubes in the experiments concerned with the effects of gaseous composition and pressure on *S. aureus* contained 1 g of felsite/limonite soil, 1% organic medium, and 9 to 10% moisture equivalent to 0.94 to 0.95 a_w. The tubes were flushed seven times with a particular gaseous atmosphere before pressure was established and the tubes were sealed. The balance of the 37% carbon dioxide atmosphere was 30% argon and 27% nitrogen. The balance of the 67% carbon dioxide atmosphere was 21% argon and 13% nitrogen. Diurnal freeze-thaw cycles of 8- and 20-hr freezes were used with each atmosphere. Control tubes were prepared in a similar manner, except they were flushed with Earth atmosphere and sealed at 98 mb. One-half of the control tubes were incubated at 35°C, and one-half received a diurnal temperature cycle with

an 8-hr daily freeze. Tubes from both control groups were sampled at 3 and at 7 days. Experimental tubes were sampled immediately and at 1, 3, 7, 28, and 56 days.

The experiments concerned with the effect of moisture on the growth of S. aureus utilized the same felsite/limonite soil with 1% organic medium, Earth atmosphere at 25 mb pressure, and an 8-hr daily freeze. Three moisture concentrations were used: 2.5, 4.5, and 9.6% equivalent to 0.49, 0.79, and 0.98 a_w, respectively. Control tubes for these experiments contained similar appropriate environments except that they were incubated at 35°C.

Figure 1 shows the effect of Earth atmosphere at different barometric pressures on S. aureus. With an 8-hr daily freeze the growth of S. aureus was rapid with maximum populations reached by 3 days. The growth of S. aureus at 10 mb reached maximum populations similar to the 25 and 40 mb by 3 days but there was an initial 0.4 log decrease in viable cells. An increase in viable cells counts of 3.0 to 3.5 logs occurred in 1 to 3 days. The 20-hr freeze groups reached similar population maxima, 3.2 to 3.4 log increase, but in 7 to 56 days.

The effect of 37, 67, and 100% carbon dioxide atmospheres at respective pressures of 40, 25, and 10 mb is shown in Figure 2. Numbers of S. aureus increased 2.0 to 2.2 logs within 7 to 56 days in the tubes that received an 8-hr daily freeze and a 2.8 to 2.9 log increase within 7 to 28 days in tubes that received a 20-hr daily freeze. The greater increase in viable cells in tubes

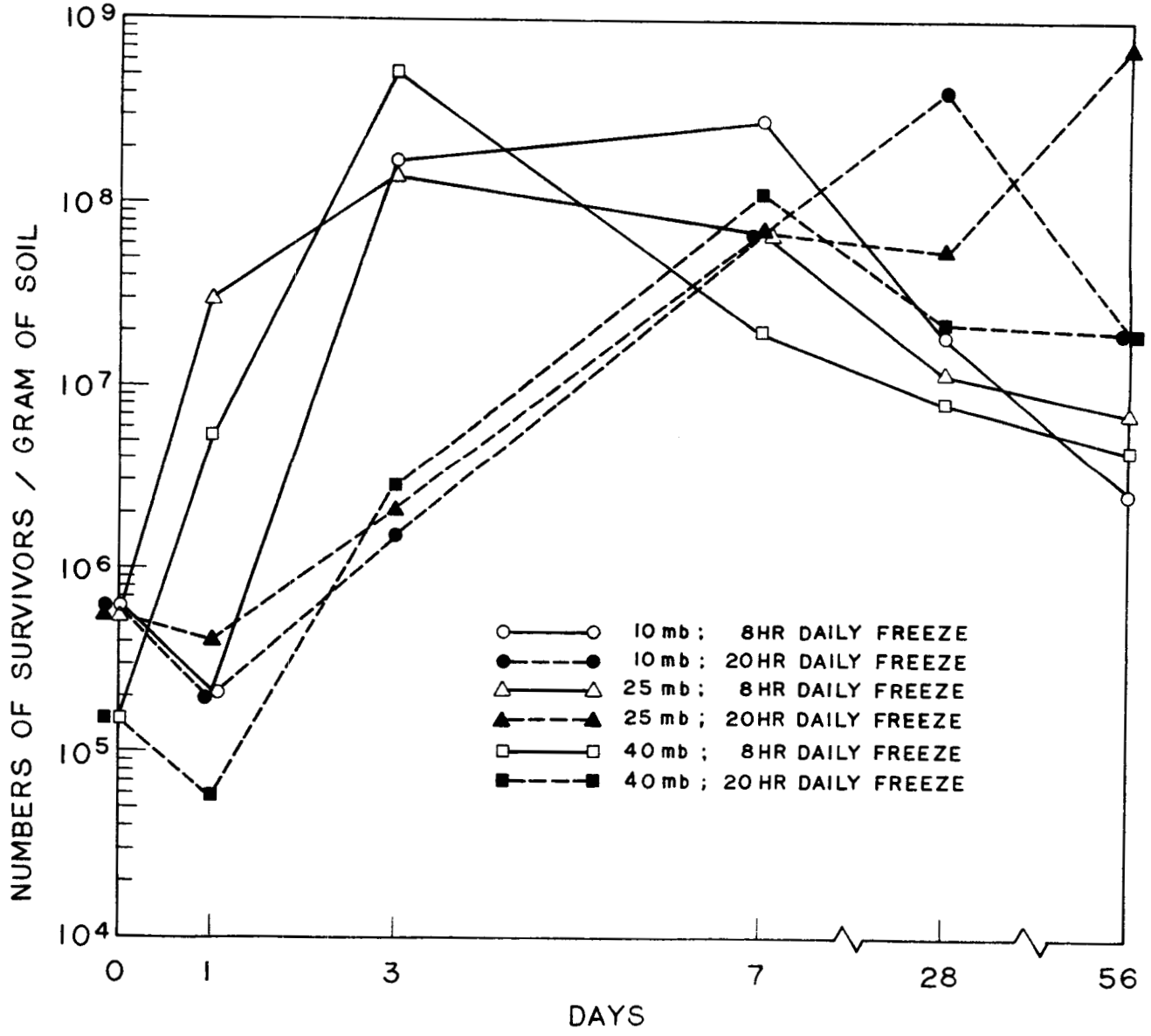

FIG. 1 EFFECT OF EARTH ATMOSPHERE AT DIFFERENT BAROMETRIC PRESSURES ON STAPHYLOCOCCUS AUREUS.

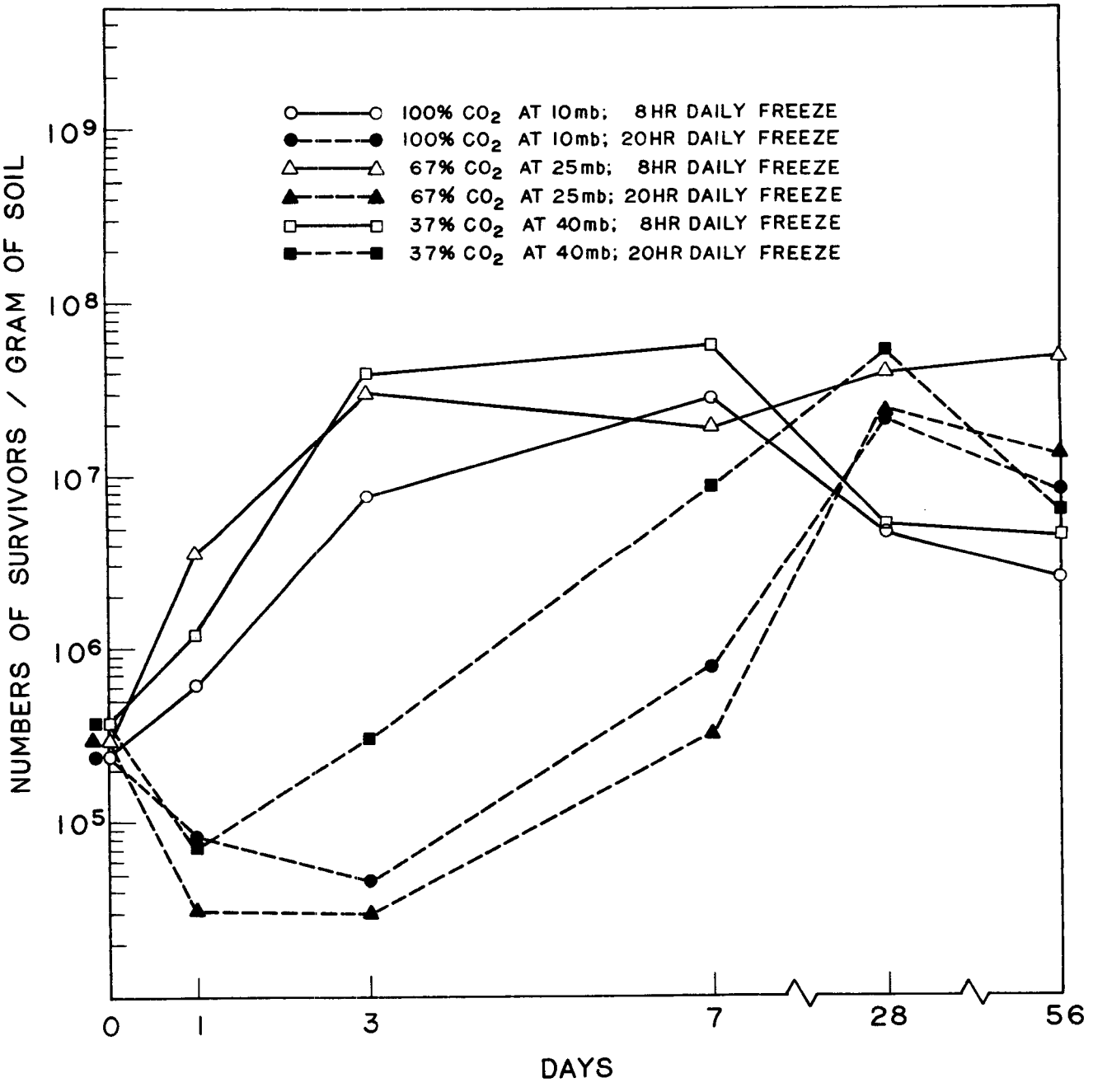

FIG. 2 EFFECT OF CARBON DIOXIDE AT DIFFERENT BAROMETRIC PRESSURES AND CONCENTRATIONS ON STAPHYLOCOCCUS AUREUS.

receiving a 20-hr daily freeze resulted from an initial decrease of viable cells which did not occur in tubes receiving an 8-hr daily freeze.

Population maxima were 0.5 to 1.0 logs lower in tubes with carbon dioxide atmospheres at 10, 25, and 40 mb pressures than in tubes with Earth atmosphere at these same pressures. These decreased population maxima resulted from a more severe environment that resulted in a greater depression of the growth response of S. aureus.

The effect of 37, 67, and 100% carbon dioxide atmospheres at 98 mb pressure is shown in Figure 3. With an 8-hr daily freeze population maxima 1.1 to 1.7 logs higher than initial numbers of viable cells were reached in 1 to 3 days and 1.0 to 2.0 logs higher with a 20-hr daily freeze within 7 to 28 days. The results indicated that an increase in relative abundance of carbon dioxide by an increase in barometric pressure further depressed the growth response of S. aureus. For, although the growth response rates were similar to the two previous groups, population maxima were decreased 2.0 to 2.5 logs when compared with Earth atmosphere maxima and 1.9 logs compared with maxima reached in carbon dioxide atmospheres with lower relative abundance of carbon dioxide.

Figure 4 shows the growth response to different moisture concentrations of S. aureus in an Earth atmosphere at 25 mb pressure with an 8-hr daily freeze. A moisture concentration of 0.98 a_w allowed rapid growth of the organism with both

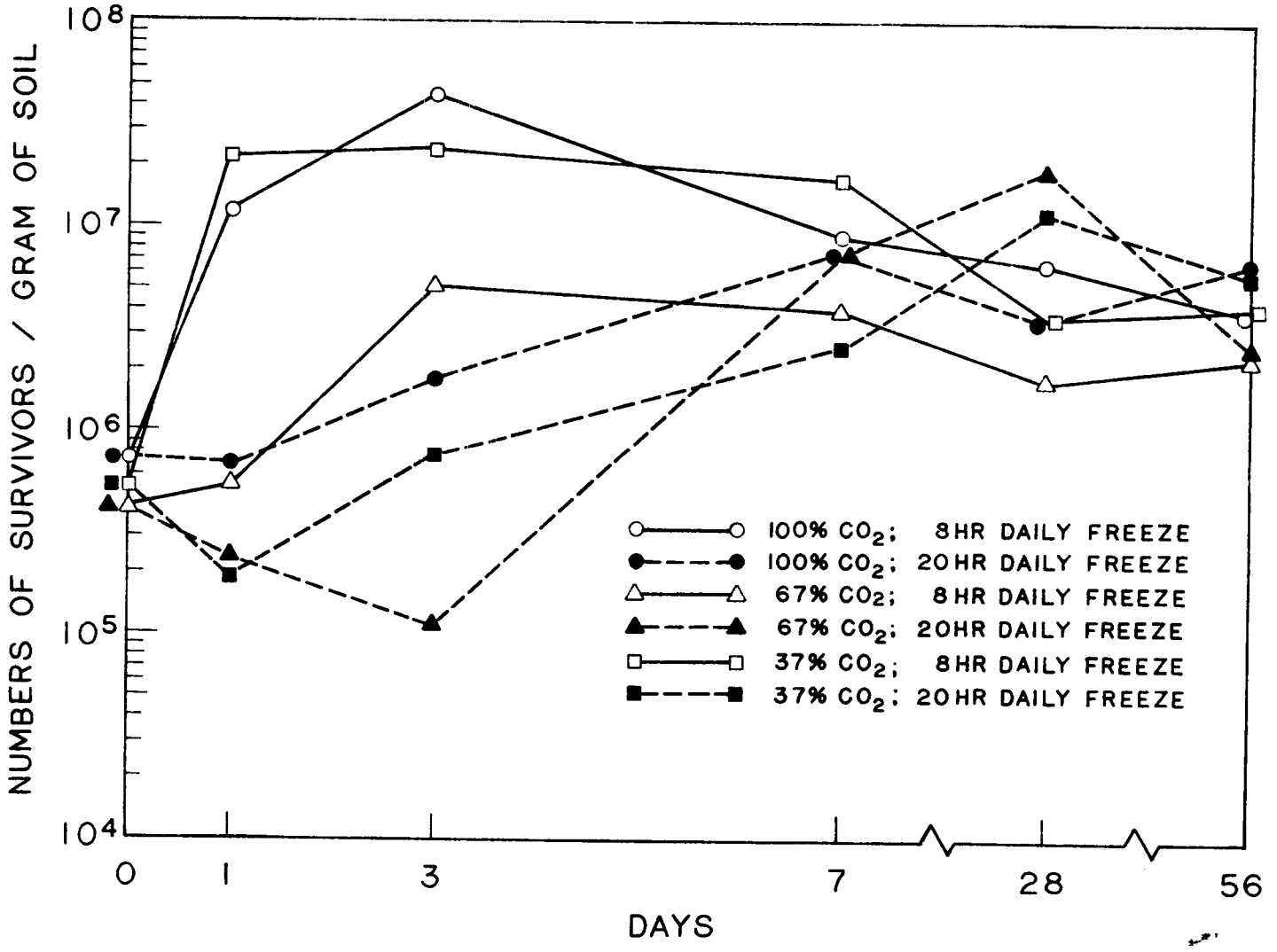

FIG. 3 EFFECT OF DIFFERENT CARBON DIOXIDE CONCENTRATIONS AT 98mb PRESSURE ON STAPHYLOCOCCUS AUREUS.

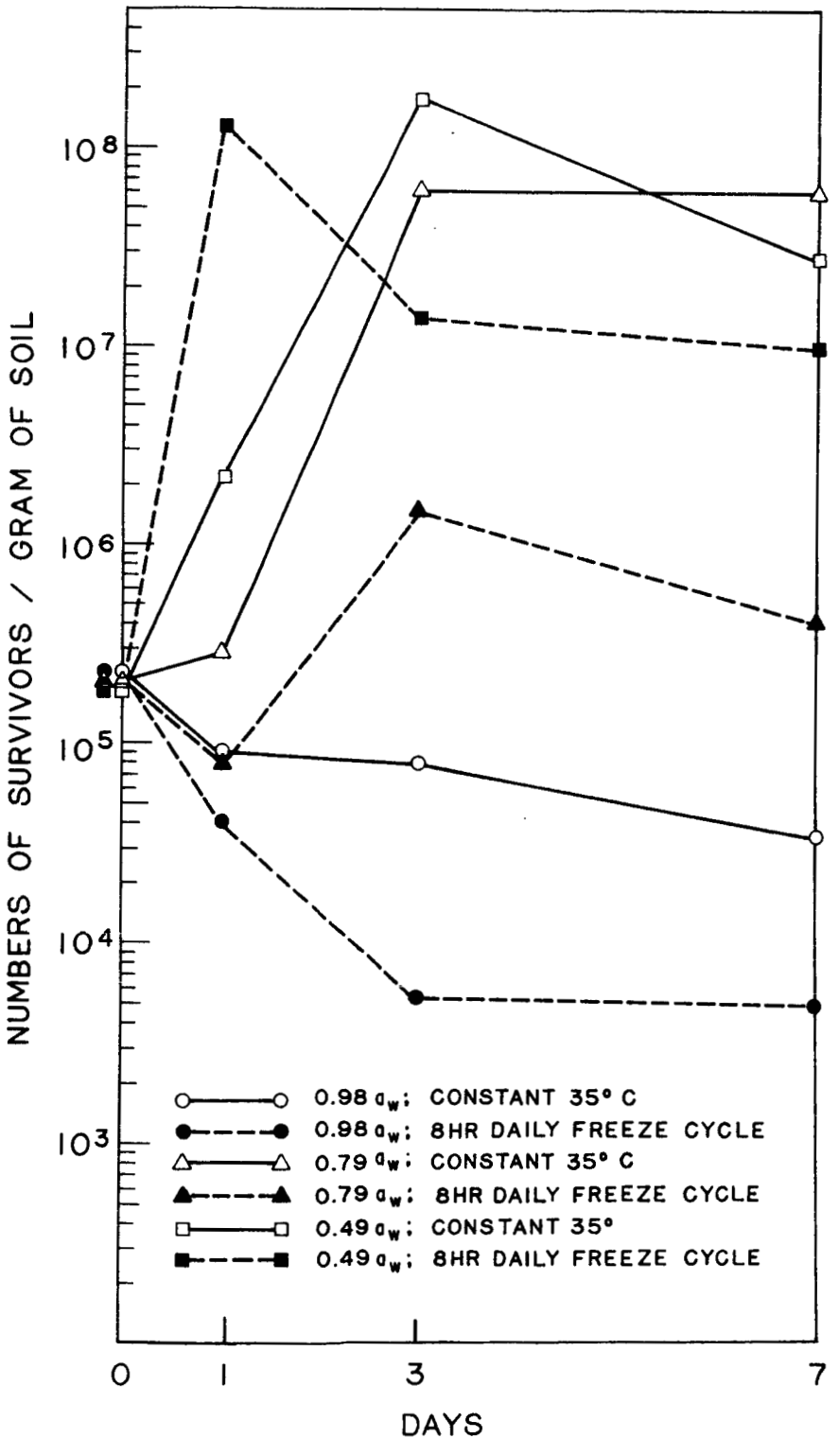

FIG. 4 EFFECT OF MOISTURE ON STAPHYLOCOCCUS AUREUS IN AN EARTH ATMOSPHERE AT 25 mb PRESSURE AND DAILY FREEZE THAW CYCLE.

8-hr daily freeze and constant 35°C temperature conditions. While with a moisture concentration of 0.49 a_w the viability of S. aureus decreased much more rapidly in a constant 35°C environment than with an 8-hr daily freeze. The significant finding was that at a moisture concentration of 0.79 a_w S. aureus growth rate was greater, and higher population maxima reached, with an 8-hr daily freeze than with constant 35°C.

Minimum a_w requirements of this organism by the methods of Scott (Austral. J. Biol. Sci. 6, 549-564, 1953) using mixtures of triple salts, NaCl:KCl:Na_2SO_4, in a molar ratio of 5:3:2 and concentrations of trypticase soy broth (BBL) to establish different a_w's indicated that 0.86 to 0.88 a_w was limiting for growth. With daily freeze-thaw cycles the organism was able to grow at a lower a_w. These results are a confirmation of previous work with B. cereus (Hagen, Hawrylewicz, and Ehrlich, Appl. Microbiol., in press) which demonstrated that the earlier theories of both Scott and Gunderson (Campbell Low Temperature Microbiology Symposium, p. 195, 1961, Ibid, p. 299-312) were true: i.e., a substrate with water, when frozen and thawed repeatedly, could result in areas, or microenvironments, of higher water concentrations.

B. Soil Ecology Studies

The main objective of the soil ecology studies were concerned with determining the minimum number of an organism required to establish itself in an environment with a particular soil type.

These studies dealt with the growth responses in three types of soil of selected organisms that could be present in spacecraft

assembly rooms. The organisms used were B. cereus, L. plantarum, PA 3679, P. aeruginosa, S. aureus, and S. albus. The soils were brunizemic with high organic content and a pH of 7.1, desertic with high clay and a pH of 8.0, and podzolic with low organic content and a pH of 5.7.

All tubes in the experiments contained 1 g of previously sterilized soil, sufficient water to establish an a_w of 0.99 or a limiting concentration depending upon the organism, and an earth atmosphere at standard pressure (approximately 1013 mb). The tubes were inoculated with particular organisms at predetermined cell populations and sealed. Half the tubes were incubated at 35°C, and half received a duirual freeze-thaw cycle with an 8-hr freeze. Tubes were sampled immediately and at 7, 28, and 56 days.

The experiments are not complete, but certain trends can be presented at this time.

Table 1 shows that the brunizemic soil was the best substrate for growth and survival of the selected bacterial species. A greater number of the bacterial species were able to grow in this soil at all moisture concentrations and temperature conditions. Fewer of the bacterial species died off completely in this soil than in either the desertic or podzolic soils.

The bacterial species that were able to grow in the podzolic soil L. plantarum , S. aureus, and S. albus could only do so at the maximum moisture concentration with either constant 35°C or 8-hr daily freeze cycle.

Table 1

EFFECT OF SOIL TYPE ON GROWTH RESPONSE OF BACTERIA[a]

Organism	Number of Cells Inoculated/g of Soil	Ability to Establish an Ecological Niche[b]											
		Brunizemic Soil				Desertic Soil				Podzolic Soil			
		Constant[c]		Diurnal[d]		Constant[c]		Diurnal[d]		Constant[c]		Diurnal[d]	
		Max[e]	Min[f]	Max[e]	Min[f]	Max[e]	Min[f]	Max[e]	Min[f]	Max[e]	Min[f]	Max[e]	Min[f]
B. cereus	10^4	+	+	+	+	-	-	-	-	+	-	0	-
	10^3	+	+	+	+	-	-	-	-	+	-	0	-
	10^2	+	+	+	+	+	-	-	-	+	-	0	-
PA 3679	10^4	-	-*	-	-	+	+	+	+	-*	-	-*	-
	10^3	-	-*	-	-	0	+*	+	+	-*	-	-*	-
	10^2	-*	-*	-	-	0	+	+	+	-*	-*	-*	-*
L. plantarum	10^5	+	+*	+	+	-*	-*	-*	-*	+	-	+	-
	10^4	+	+*	+	+	-*	-*	-*	-*	+	-	+	-
	10^3	+	+*	+	+	-*	-*	-*	-*	+	-	+	+
P. aeruginosa	10^4	+	+	+	+	-	-	-*	-	+	-*	-*	-*
	10^3	+	+	+	+	-*	-	-*	-	-*	-*	-*	-*
	10^2	+	+	-*	+	-*	-	-*	-	-*	-*	-*	-*
L. aureus	10^5	+	-	+	+	-*	-*	-*	-*	+	-*	+	-*
	10^4	+	-	+	+	-*	-*	-*	-*	+	-*	+	-*
	10^3	+	-	+	-	-*	-*	-*	-*	+	-*	+	-*
S. albus	10^5	+	+	+	+					+	-	+	-
	10^4	+	+	+	-*					+	-*	+	-
	10^3	+	+	+	-*					+	-*	+	-

[a]These results are based upon experiments in progress for 7 or 28 days, or completed after 56 days.

[b]+ indicates increase, - indicates decrease, and 0 indicates no change in viable cells as compared to initial numbers.

[c]Incubations at constant 35°C.

[d]Incubation with diurnal 8-hr freeze (-65°C) and 16-hr thaw (30°C) cycle.

[e]Maximum water requirement for a particular organism added to the tubes.

[f]Minimum water requirement for a particular organism added to the tubes.

*Signifies that no viable cells were recovered at the most recent sampling time.

The desertic soil was the poorest substrate. Only PA 3679 showed a very slight increase in numbers of viable cells, a 0.5 log increase. Also, L. plantarum, P. aeruginosa, and S. aureus did not survive in this soil.

Population maxima of the organisms that did grow in the brunizemic and podzolic soils were from 3 to 6 logs higher than initial cell counts which were as low as 100 to 1,000 cells per gram of soil depending on the organism and the experiment.

In terms of ability to establish an ecological niche as a function of amount of inoculum, moisture concentration, soil type, and temperature condition L. plantarum was the best followed by S. albus, S. aureus, B. cereus, P. aeruginosa, and PA 3679.

C. Design and Construction of Environmental Chambers

Figure 5 represents the accepted design of the 150 psi environmental chamber. Work has begun in ordering the construction of the component parts.

IV. SUMMARY

Growth of S. aureus was not inhibited in Earth atmospheres at barometric pressures of 10, 25, or 40 mb. Growth was rapid and abundant.

Carbon dioxide concentrations of 37, 67, and 100% at pressures of 40, 25, and 10 mb respectively, did not adversely affect the growth of S. aureus although the population maxima were slightly lower than the Earth atmosphere maxima.

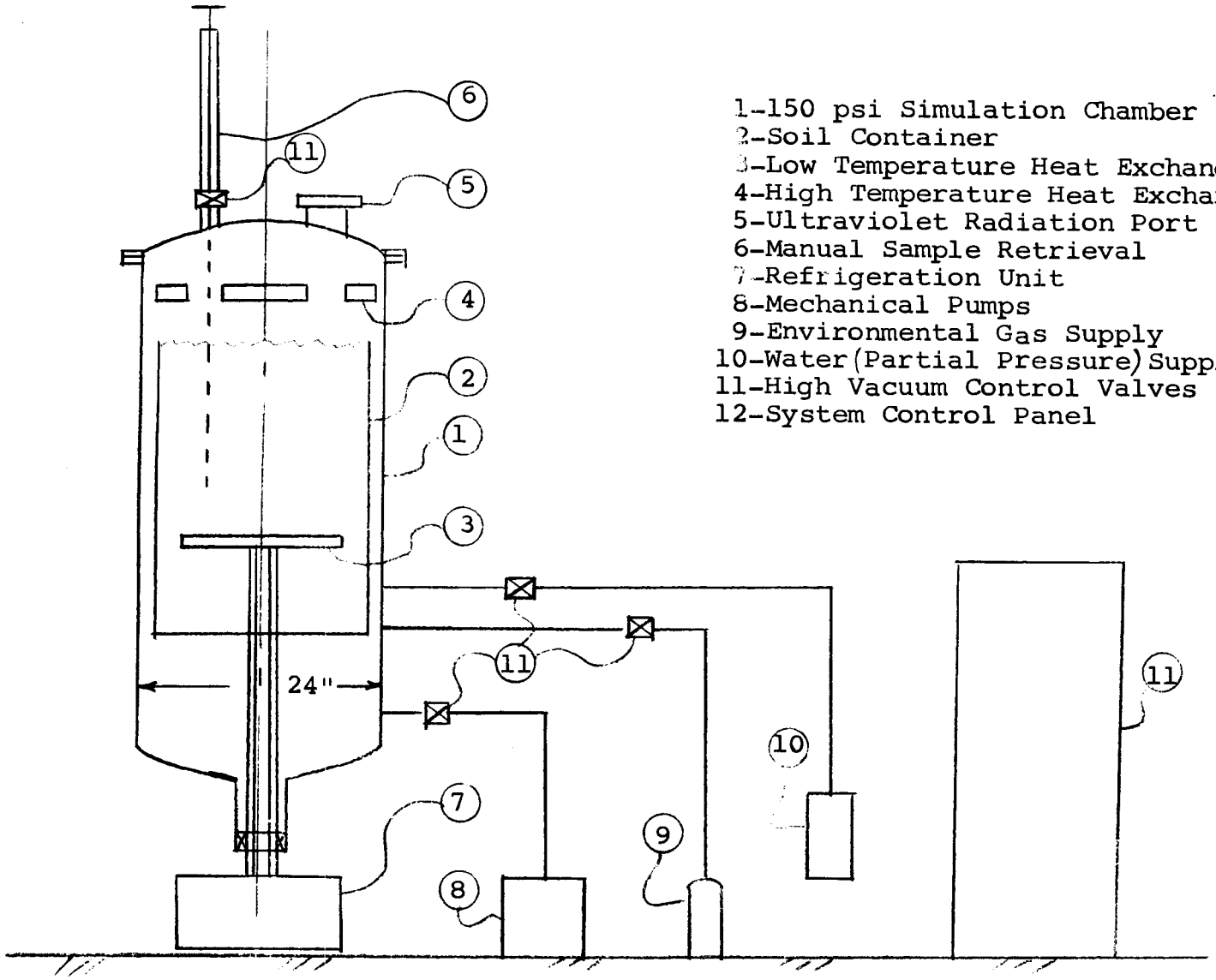

Figure 5 - 150 psi Chamber Schematic

Carbon dioxide concentrations of 37, 67, and 100% at 98mb pressure did not inhibit growth of S. aureus but population maxima were lower than both the Earth atmosphere group and the carbon dioxide atmosphere at different pressure groups.

An 8-hr daily freeze lowered the minimum water requirement for growth of S. aureus below that required for growth at constant 35°C.

The ability to establish an ecological niche as a function of amount of inoculum, moisture concentration, soil type, and temperature, L. plantarum was best, followed by S. albus, S. aureus, B. cereus, P. aeruginosa, and PA 3679.

Initial numbers of 100 viable cells per gram of soil of B. cereus, L. plantarum, P. aeruginosa, and S. albus increased 3 to 6 logs; and the lowest number of S. aureus tested, 700 per gram of soil, increased 4 logs.

V. PERSONNEL AND RECORDS

The experiments were planned with the counsel of Dr. E. J. Hawrylewicz, and the technical assistance of Mr. Bruce Anderson, Miss Marjorie Ewing, and Miss Vivian Tolkacz.

Experimental data are recorded in IITRI Logbooks C16684, C C16876, C16889, C16938, C17092, C17094, C17096, C17260, C17271, C17272, C17497, C17091, C17097, C17266, and C17587.

Respectfully submitted,

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