ECOLOGY AND THERMAL INACTIVATION OF MICROBES IN AND ON INTERPLANETARY SPACE VEHICLE COMPONENTS

Thirty-first Quarterly Report of Progress

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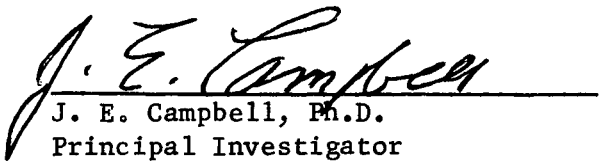
Conbributors:

A. L. Reyes
A. J. Wehby
R. G. Crawford
J. C. Wimsatt
J. E. Campbell
J. T. Peeler

I

Report Submitted and Forwarded by:



J. E. Campbell, Ph.D.
Principal Investigator

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Introduction

The maximum allowable water vapor in the sterilizing gas to be used in the thermal sterilization of the Viking lander shall be less than 25% relative humidity (RH) at standard conditions of 0°C and 760 mm Hg pressure.

Data in the 29th Quarterly Report of Progress showed that this constraint has not significantly changed the D and z values for Bacillus subtilis var. niger spores currently used for calculating the time and temperature of the thermal sterilization cycles. We also demonstrated that by lowering the humidity of the system to < 0.001% RH (over P_2O_5) at all temperatures, the D value was reduced by a factor of 3.

For this quarter, we directed our efforts to extend data on the thermal inactivation of B. subtilis var. niger spores at 113°C with 0.134% RH (1.3 μg H_2O/cm^3) and < 0.001% RH (over P_2O_5) over the range of 10^6 to 10^{-3} test organisms per sample.

Thermal Inactivation Studies

The experiments were carried out in the conventional manner. The spores were suspended in 95% ethyl alcohol, diluted in sterile double-distilled water, and dispensed with a repeating dispenser in 0.01 ml amounts in stainless steel cups to give about 10^6 spores per cup. The cups were arranged on circular shelves and placed in 206 mm x 300 mm tin cans. Each can

contained four shelves (30 cups per shelf) for a total of 120 cups per can. The cans, lids, and contents were dried in a vacuum oven for 110 minutes at 45° to 50°C (at 1.5-inch Hg pressure absolute). To increase the drying rate, the oven was purged with dry nitrogen every 10 minutes for the first 100 minutes, followed by five consecutive purges of nitrogen, with a vacuum cycle between each purge. After drying, the cans, lids, and contents were removed from the oven and cooled to about 30°C in the equilibration hood to give 1.3 $\mu g\ H_2O/cm^3$ per can. In another set of cans an appropriate amount of desiccant was placed in each can to give a near zero per cent relative humidity.

The cans were sealed and removed from the equilibration hood. The seams on each can were soldered and wiped to prevent leakage of water vapor during the heating cycle. The cans were heated at 113°C for varying times and cooled in an ice bath. Spore assays were made on survivor counts of 71 per cup by sonification of the cup containing the spores in peptone water followed by conventional plating. When the survivor count was < 1 per cup, the sample cups were assayed by the most probable number method. This was done by adding 0.5 ml of TGE broth to each of the cups, scoring for growth or no growth after 7 days' incubation at 35°C, and calculating the most probable number of survivors.

Results

The thermal inactivation curve for *B. subtilis* var. *niger* spores at 113°C and 25% RH (STP) is presented in Figure 1. The

survivor curve appears to have two slopes, with the second slope having a larger D value. Data are also shown in Figure 2. In this experiment, the spores were treated under a "drier" condition by the addition of P_2O_5 to each can prior to heat treatment. The slopes of the survivor curves in Figure 1 and Figure 2 show a similar pattern except that the slope of the curve in Figure 2 under a drier condition is much steeper than the slope shown in Figure 1.

In both cases with temperature held constant, the D value was reduced approximately by a factor of 3 by lowering the relative humidity of the system to near zero.

It is also seen in each case, based on MPN estimates, that there were no spore survivors after 30 hours of the heating cycle.

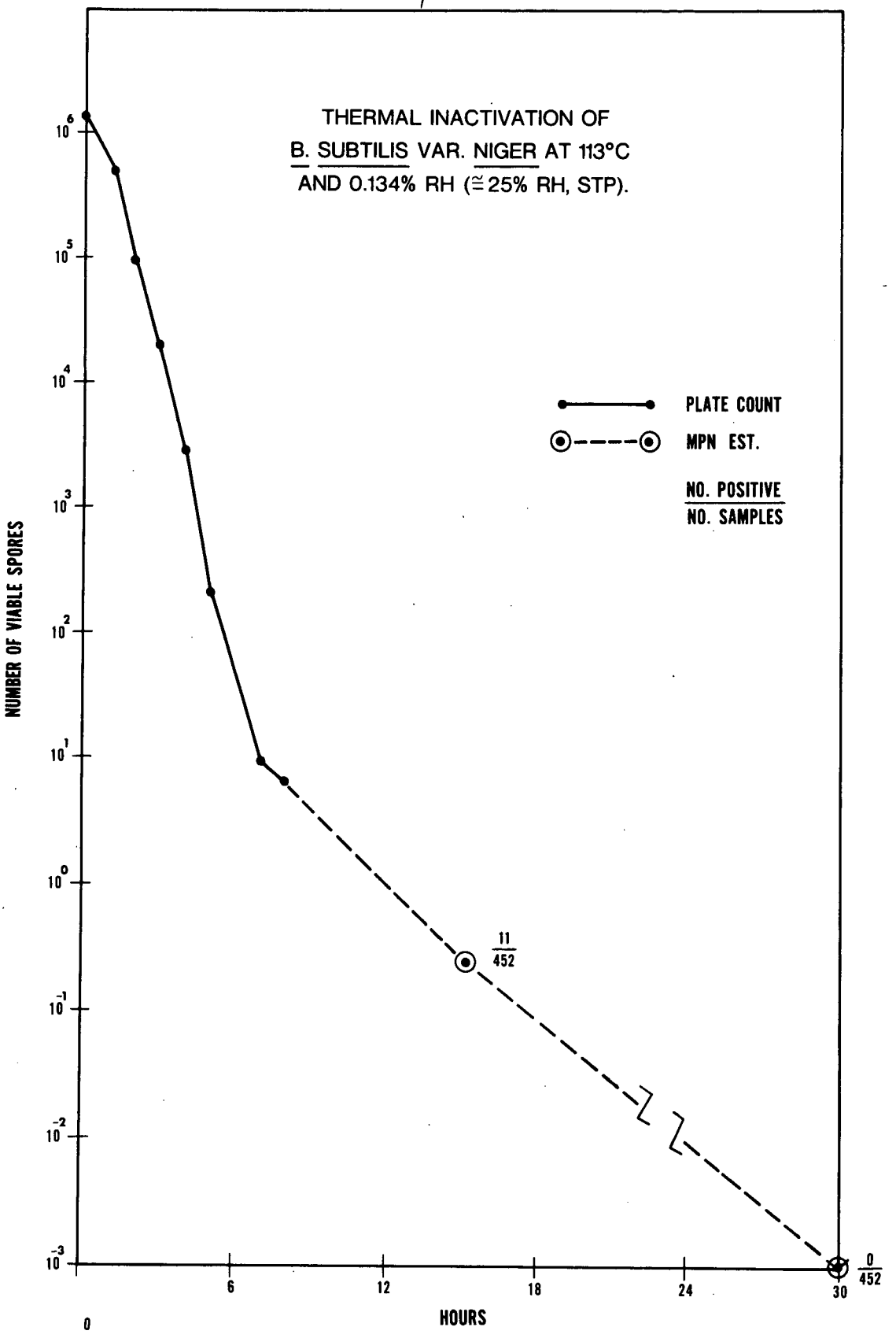
THERMAL INACTIVATION OF
B. SUBTILIS VAR. NIGER AT 113°C
AND 0.134% RH (≅25% RH, STP).
PLATE COUNT
MPN EST.
NO. POSITIVE
NO. SAMPLES
NUMBER OF VIABLE SPORES
10^{6}
10^{5}
10^{4}
10^{3}
10^{2}
10^{1}
10^{0}
10^{-1}
10^{-2}
10^{-3}
11/452
0/452
0
6
12
18
24
30
HOURS

FIGURE 1

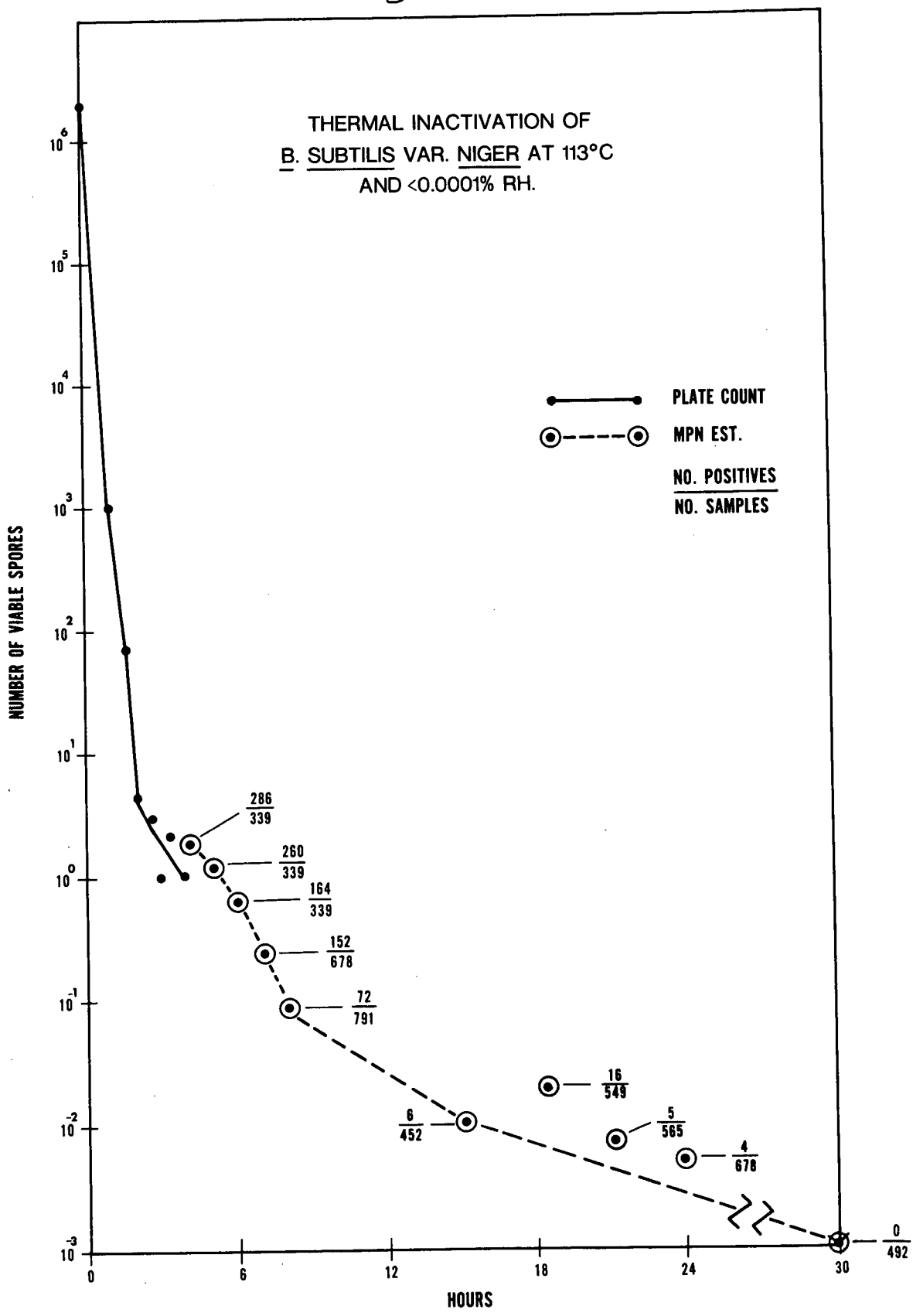
THERMAL INACTIVATION OF
B. SUBTILIS VAR. NIGER AT 113°C
AND <0.0001% RH.
PLATE COUNT
MPN EST.
NO. POSITIVES
NO. SAMPLES
NUMBER OF VIABLE SPORES
HOURS
286/339
260/339
164/339
152/678
72/791
6/452
16/549
5/565
4/678
0/492

FIGURE 2