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

Twenty-first Quarterly Report of Progress

Research Project R-36-015-001

April 1, 1970 - June 30, 1970

Conducted by

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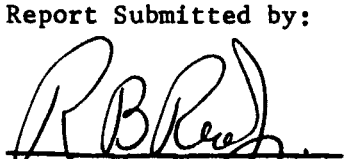

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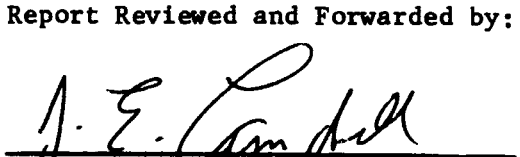

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CONTENTS

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

Introduction

This quarter our efforts were concentrated upon identifying thermal inactivation curves for Bacillus subtilis var. niger spores with 2.6, 10, and 100 μg of water per ml of headspace. In our last report we gave inactivation curves for the same organism with 0.25 μg of water present per ml of headspace.

I. THERMAL INACTIVATION STUDIES

Several experiments were conducted involving thousands of spore populations to identify the nature of the thermal inactivation curve of B. subtilis var. niger spores under 2.6, 10, and 100 μg of water per ml of headspace air. B. subtilis var. niger spores suspended in 95% ethyl alcohol were diluted in sterile double distilled water and were dispensed with a microburette in 0.01 ml amounts into stainless steel cups to give about 10^6 spores per cup. The cups were arranged in circular shelves and placed in 206 x 300 tin cans. Thirty cups were on each shelf and four shelves were used in each can for a total of 120 cups per can. The cans, lids, and contents were dried in a vacuum oven for 90 minutes at 46-50 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 70 minutes and this was followed by five consecutive purges of nitrogen. A vacuum cycle was used 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. A weighed amount of hydrated sodium tungstate was placed in the bottom of each can and the cans were sealed by a conventional can sealing machine. The sealed cans were removed from the equilibration hood and at designated time intervals were dropped in a 75 C oil bath and preconditioned for 5 hours. After preconditioning, the cans were heated immediately in a 125 C oil bath at various time intervals and cooled in a refrigerated bath.

Spore survivors from cups containing > 10 spores per cup were assayed by sonifying the cups in peptone water and plating. The most probable number technique for assaying cups having < 10 spores per cup was used. This was determined by adding 0.5 ml of tryptone glucose beef extract broth to each cup and incubating these cups at 35 C for 7 days. Cups were scored for growth or no growth and the most probable numbers of survivors per cup calculated from these data.

Typical thermal inactivation curves are shown in Figures 1, 2, and 3.

Nonlinear curves of $\log_{10}$ number of survivors versus time were observed in the range studied, i.e., 1×10^{6} to 1 to 10^{-2} spores per cup. Thus, the D value concept is inadequate to summarize these data. The data were examined by a second-degree polynomial model. Tests were performed using this emperical model to determine whether data from paired runs could be pooled together. All paired runs were poolable.

II. MOISTURE MEASUREMENTS

Since using sodium tungstate dehydrate as a source of moisture in sealed tin cans, we have monitored the moisture content of several experiments by using the Solids Moisture Analyzer. The procedure for doing this was reported in the 19th Quarterly Report.

At one time the moisture recoveries from sodium tungstate at levels of 1 and 10 μg per ml were low. Attempts to find a source of these losses were u successful and we were unable to repeat the findings of low recovery, but in the process of looking for low recoveries, we found some high recoveries. These we were able to trace to solder holding the shelves together. This was corrected by forming the shelves by crimping the edge rather than soldering the bottoms to the edges.

The abnormalities in recoveries disappeared, thus we were able to speculate that the two were interrelated.

D values were determined on B. subtilis var. niger spores at the levels of 0, 1, 10, and 100 μg of water per ml of headspace. Duplicate moisture recoveries from added sodium tungstate dehydrate were determined at each of these levels. The results are shown in Table 1.

Table 1. Moisture analysis of cans containing crimped stainless steel shelves, cups, spores, and sodium tungstate

Can #	Water concentration (μg/ml)	Total water (mg)	Recovery (%)
B_1, B_2	104.9	19.620 ± 0.029	103 ± 1
C_1, C_2	9.7	1.812 ± 0.054	89 ± 3
D_1, D_2	11.5	2.145 ± 0.065	104 ± 4
E_1, E_2	2.6	0.482 ± 0.063	157 ± 31

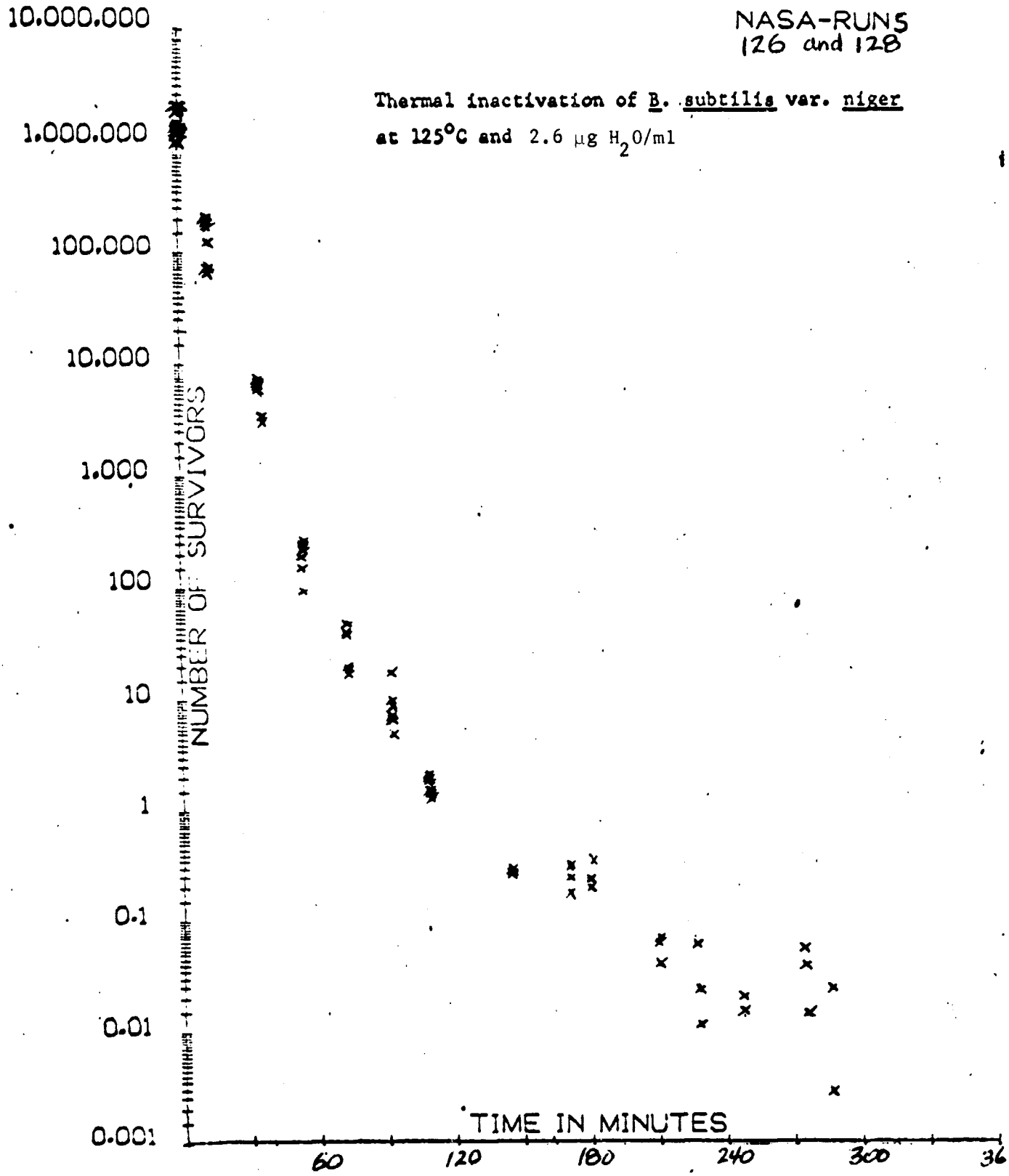
NASA-RUNS
126 and 128
Thermal inactivation of B. subtilis var. niger
at 125°C and 2.6 μg H_2O/ml
10.000.000
1.000.000
100.000
10.000
1.000
100
10
1
0.1
0.01
0.001
NUMBER OF SURVIVORS
TIME IN MINUTES
60
120
180
240
300
360

FIGURE 1.

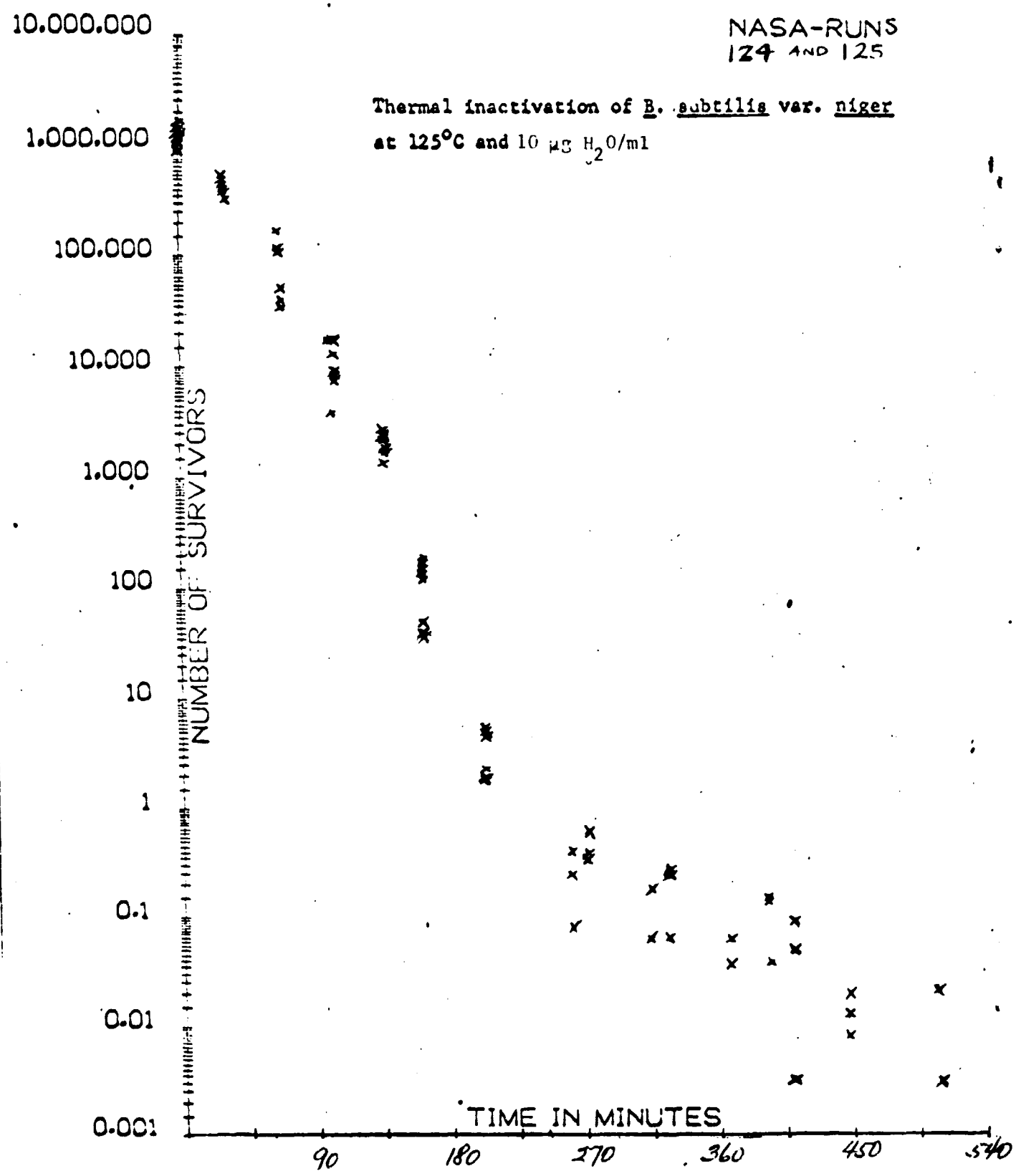
NASA-RUNS
124 AND 125
Thermal inactivation of B. subtilis var. niger
at 125°C and 10 µg H_2O/ml
10.000.000
1.000.000
100.000
10.000
1.000
100
10
1
0.1
0.01
0.001
NUMBER OF SURVIVORS
TIME IN MINUTES
90
180
270
360
450
540

FIGURE 2.

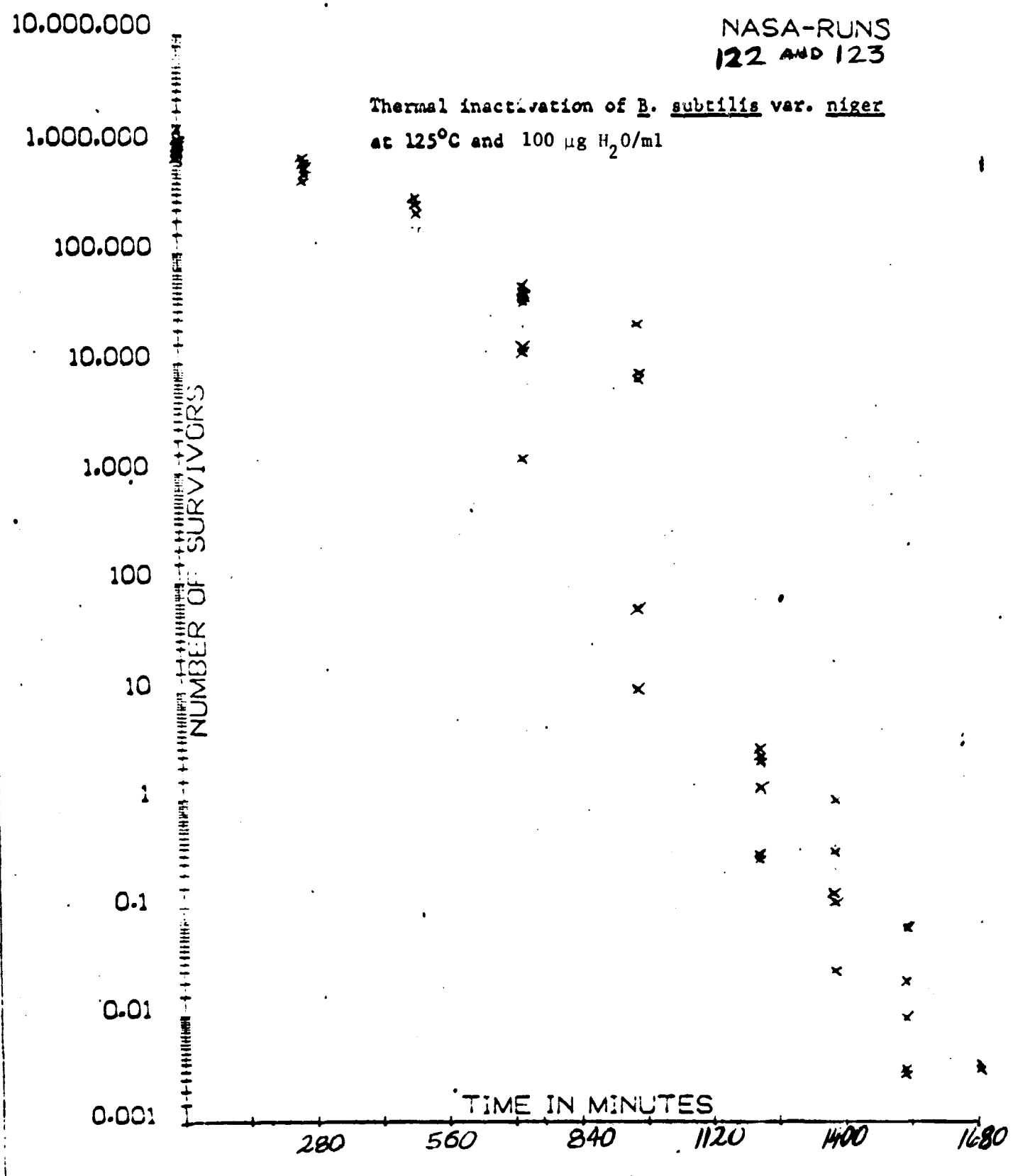
NASA-RUNS
122 AND 123
Thermal inactivation of B. subtilis var. niger
at 125°C and 100 µg H_2O/ml
10,000,000
1,000,000
100,000
10,000
1,000
100
10
1
0.1
0.01
0.001
NUMBER OF SURVIVORS
TIME IN MINUTES
280
560
840
1120
1400
1680

FIGURE 3.