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

Twelfth Quarterly Report of Progress

on

Research Project R-36-015-001

January 1 - March 31, 1968

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SUMMARY

During the twelfth quarter, D and z values for spores on paper, dried by lyophilization, was determined. In addition, the effect of epoxy coating on the z_D value of spores mated between steel washers was investigated. Also, the time required for dry spores to rehydrate to their maximum heat resistance was obtained. And, finally, a system employing epoxy resins was developed to determine the effect of water activity on the thermal destruction curve (z).

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INTRODUCTION

Previous dry heat resistance studies of *Bacillus subtilis* var. *niger* spores impregnated in filter paper indicated that a "wet-heat" kill mechanism was involved as evidenced by a z_D of 12.9°C. In spite of the drying process, sufficient water remained in the system to effect a wet-heat system. During the twelfth quarter, lyophilization was investigated as an improved drying method.

An unusually high z_D value (32.0°C) was reported last quarter for spores located between steel washers mated at 150 inch-pounds of torque (1). An explanation for this high value is presented in this report and is based on the slow loss of spore moisture to the hot gaseous environment in the TDT tube during heating. Sealing the mated surface units with epoxy resin prevented loss of moisture and resulted in a z_D value of 21.9°C which is comparable to that observed for the same spores heated in lucite, epoxy, and on stainless steel strips.

The rate at which dried spores absorb water from a humid environment and the effects of such absorption on thermal resistance was investigated. Spores dried on stainless steel strips were exposed to 100% relative humidity (RH) for various time intervals and were then exposed to a dry-heat temperature of 135°C for seven minutes. The results indicated that the spores did absorb water and that the thermal resistance was increased in proportion to the increased spore moisture content.

A dry-heat exposure system employing spores embedded in epoxy resin was developed. Results, to date, are comparable to those reported for spores embedded in lucite. This system will be used to determine the effect of a_w on z values.

EXPERIMENTAL

The dry heat resistance of spores lyophilized on paper strips

An explanation for the small D value (12.9) for spores dried on paper strips, as reported last quarter, was sought. The low value was indicative of a wet heat system which suggested that in spite of the drying procedures used, sufficient water was associated with the system to cause inactivation by wet heat. To test this hypothesis, these studies were repeated as follows: Whatman No. 2 filter paper strips were inoculated as described last quarter, dried at 50°C for one hour, and placed in thermal death time (TDT) tubes. The strips were then lyophilized five hours at 5 to 15 microns of mercury and sealed in vacuo with an oxy-gas flame. The sealed tubes were then exposed to dry heat as described last quarter. The D values for exposure temperatures of 115°, 125°, and 135°C are given in Table 1. From the thermal destruction curve (Fig. 1) a z_D value of 18.0°C was obtained. These results substantiate the hypothesis that residual water was associated with the paper strips used in last quarter's experiments. Lyophilization removes sufficient water from the system so that dry-heat conditions prevail as indicated by the z_D of 18°C.

The dry heat destruction of spores on mated surface units embedded in epoxy plastic

In the Eleventh Quarterly Report of Progress, a z_D value of 32°C was reported for spores located between steel washers mated at 150 inch-pounds of torque and exposed to dry heat (1). An explanation of this unusually high value was sought experimentally based upon the following premise.

The mated surface unit is not gas-tight, therefore, residual moisture associated with the dried spores would slowly escape to the hot gaseous environment within the TDT tube during heating. This process would continue until an equilibrium vapor pressure was achieved within the tube. At equilibrium, the spore moisture content would be extremely low because of the greatly reduced relative humidity. In practice, a mated surface experiment at 135°C requires a total experimental exposure time of three hours, whereas, one at 115°C requires 24 hours. The rate at which moisture would escape from spores is a function of the temperature - the higher the temperature, the faster the rate of moisture loss. Previous studies in this laboratory have demonstrated the protective effect of some moisture in association with spores during exposure to dry heat. If spores are continually drying as they are being heated, it follows that their heat resistance is changing (decreasing) as a function of the rate of water loss. The greater the length of time, out of the total experimental exposure interval, that some water remains in association with the spores, the greater will be the D value. At 135°C, moisture would be associated with the spores for a greater portion or percentage of the total experimental exposure interval than at 115°C. For example, if it took 0.5 hour, out of a 3 hour experiment, for the spores to completely dry at 135°C, then some moisture was associated with the spores for 16% of the total experimental exposure interval. Similarly, if it took one hour, out of a 24 hour experiment, for the spores to completely dry at 115°C, then some moisture was associated with the spores for only 4% of the total experimental exposure interval. This difference in time with which moisture was associated with the spores would tend to yield high D values at 135°C but almost normal D values at 115°C with a consequent flattening of the slope of the z curve. Such a phenomenon could explain the value of $z_D = 32$, reported last quarter for this system.

An experimental system was utilized in which epoxy resin was coated over the exterior surface of the mated units as a means of preventing the escape of moisture from the spores during heating. Heat trials were performed as previously described to note whether a z value comparable to that observed for steel strips and plastics ($z_D = 21$°C) would be obtained. The data from these experiments is presented in Table 2 and Fig. 2. A z_D of 21.9 was calculated from the thermal destruction curve. This value approximates that of the steel strip and plastic systems reported last quarter and indicates that the z_D of 32 formerly reported was related in some fashion to the rate of spore moisture loss during heating.

The effect of rehydration on the heat resistance of dried spores

It has been established that dried spores ($a_w < 0.2$) have less heat resistance than spores of intermediate moisture levels (a_w 0.2 - 0.4). In the experimental trials performed in which these findings were established, long exposures (2 weeks) in relative humidity chambers were used to permit spore equilibration. It became of interest to determine the rapidity with which spores absorb moisture from the environment and the extent to which such absorption affects thermal resistance. Such information would be useful in setting the environmental conditions for assembly areas, clean rooms, etc., so that spores contaminating such areas could be maintained in a minimally thermal resistant state.

Accordingly, spores were dried on stainless steel strips as described previously (1). These strips were then placed in tubes and dried in a flexible desiccator over silica gel for 48 hours at 25°C. The tubes were stoppered within the flexible desiccator before their removal and transfer to a second flexible chamber that contained a saturated water vapor atmosphere. By working from outside the bag, the strips were exposed to the 100% RH for various time intervals by removing the individual stoppers. After exposure to the saturated atmosphere, the tubes were stoppered again, removed from the plastic bag, sealed in an oxy-gas flame and heated for seven minutes at 135°C in a silicone bath.

The results of these experiments are given in Fig. 3. It is obvious from these data that spores rapidly absorb moisture from a water vapor saturated gaseous environment and that such water sorption affects dry heat resistance. Under the conditions of these experiments, maximum thermal resistance was noted for spores that had been exposed to 100% RH for 12 minutes or more. Similar studies will be performed at other relative humidity values and the results will be presented in subsequent reports.

<u>Influence of water activity on dry heat resistance of spores on glass encapsulated in epoxy</u>

Glass strips (1/4" x 7/8") were cut from microscope cover slips, inoculated with 0.01 ml of the aqueous stock spore suspension (approximately 1 x 10^8 spores), and dried one hour in the 50°C forced air oven. Following drying, the strip was placed in the equilibration chamber shown in Fig. 4 and allowed to equilibrate for two days at 25°C. Following equilibration, the tubing connecting the separate wells containing the salt solution and the glass strip was clamped off and the system was inverted. Upon removal of the stopper, the strip dropped into a TDT tube that had been previously coated with release agent. Sufficient epoxy syrup was poured immediately into the TDT to completely cover the glass strip and the epoxy was permitted to polymerize at 50°C for three hours. Following polymerization, the tubes were sealed in an oxy-gas flame and exposed to 135°C in a silicone bath for various time intervals. The number of survivors was determined as described in the Eleventh Quarterly Report of Progress. The results of these experiments are shown in Fig. 5.

It should be noted that the value for 0.0 a_w was obtained with spores dried on glass strips which were subsequently lyophilized (5 hours at 5 to 15 microns of mercury) and embedded in epoxy as described above.

The results for the a_w values of 0.1 through 0.8 are in agreement with those for lucite. It is interesting to note, however, that no difference was observed for a_w values of 0.0 and 0.1. The significance of this finding is not understood presently, however, additional studies are planned for intermediate levels of a_w between 0.0 and 0.1. Similar studies will be conducted at 115°C and 125°C as a means of determining the effect of a_w on z value.

PROJECTED RESEARCH FOR THE THIRTEENTH QUARTER

During the next quarter, research will be directed toward determining the time required for dry spores to rehydrate at various relative humidities. In addition, D values for spores on glass that are adjusted to various a_w and are embedded in epoxy will be determined at 115°C and 125°C.

REFERENCES

1. Eleventh Quarterly Report of Progress, October - December 31, 1967. NASA Research Project R-36-015-001.

TABLE 1

D Values for Bacillus subtilis var. niger Spores*
Inoculated on Paper and Dried by Lyophilization

Dry-Heat Exposure Temperature °C	D Value (minutes)	95% Confidence Interval (minutes)
115	48.3	46.1 - 50.6
125	10.1	9.6 - 10.7
135	3.8	3.5 - 4.2

* Inoculum = 1×10^8 per gram.

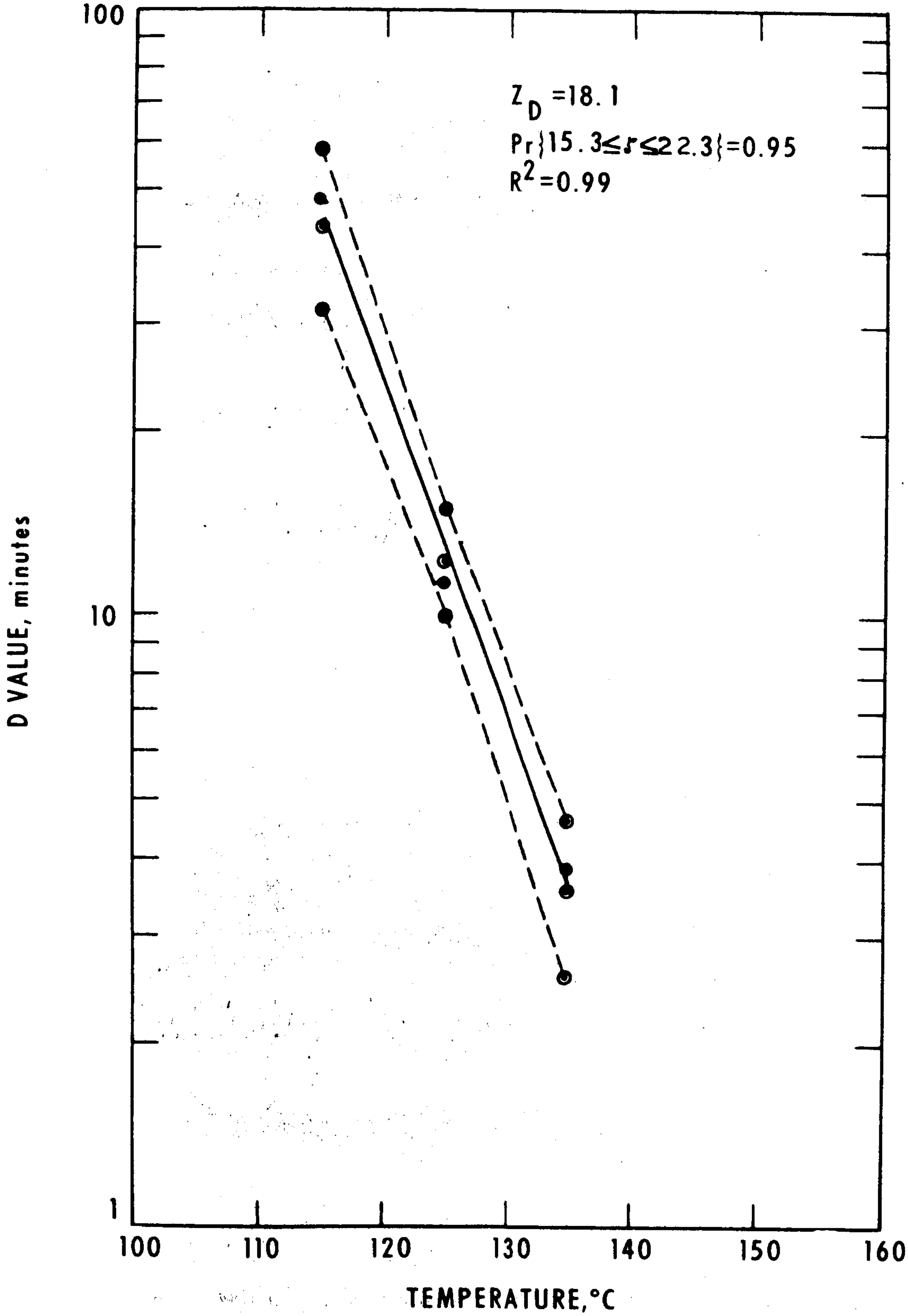
TABLE 2

D Values for *Bacillus subtilis* var. *niger* Spores*
Located Between Steel Washers Mated at
150 Inch-Pounds Torque and Sealed in Epoxy

Dry-Heat Exposure Temperature °C	D Value (minutes)	95% Confidence Interval (minutes)
115	310.8	292.2 - 331.8
125	165.0	150.0 - 183.0
135	52.47	48.52 - 57.14

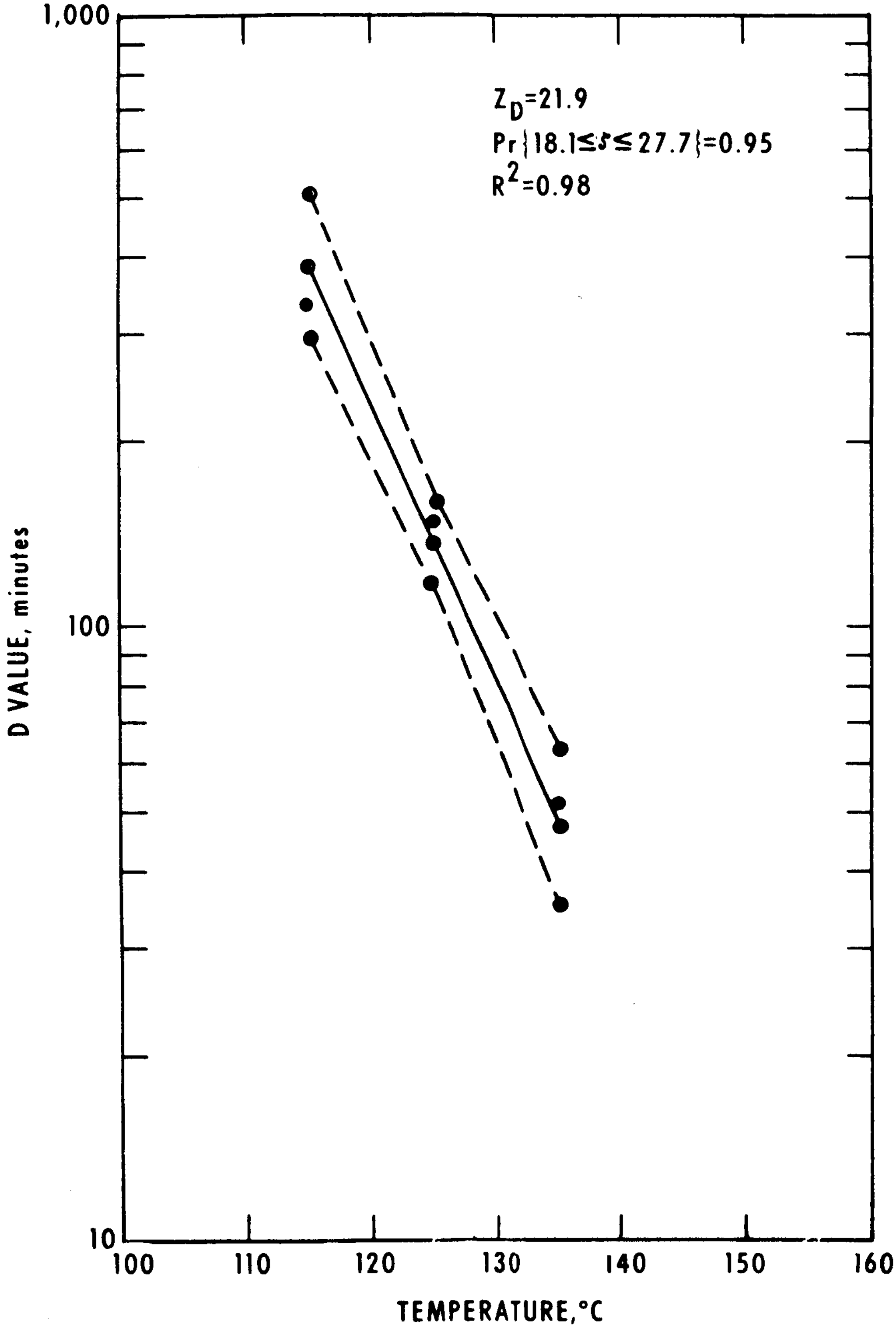
* Inoculum = 1×10^8 per gram.

Figure 1



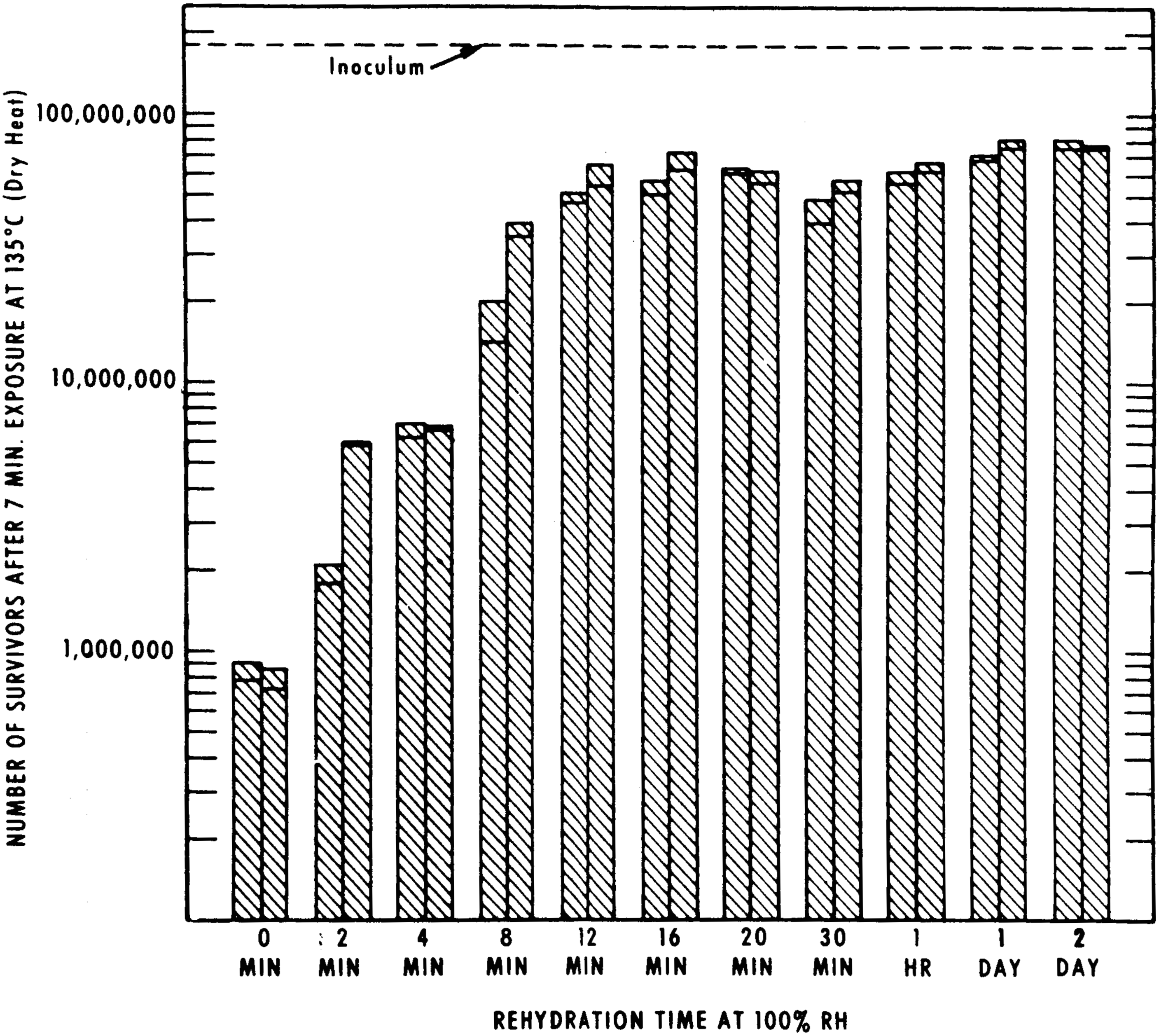
THERMAL DESTRUCTION CURVE FOR B. subtilis var. niger SPORES LYOPHILIZED ON PAPER STRIPS

Figure 2



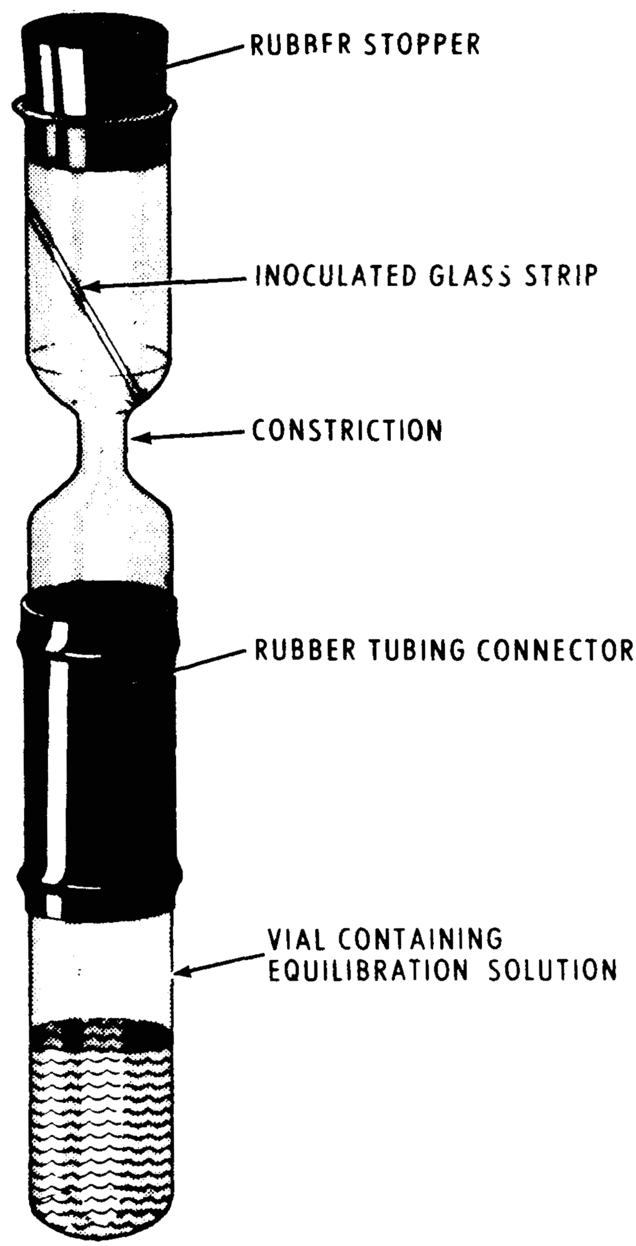
THERMAL DESTRUCTION CURVE FOR B. subtilis var. niger SPORES LOCATED BETWEEN STEEL WASHERS MATED AT 150 IN.-LBS. OF TORQUE AND SEALED IN EPOXY

Figure 3



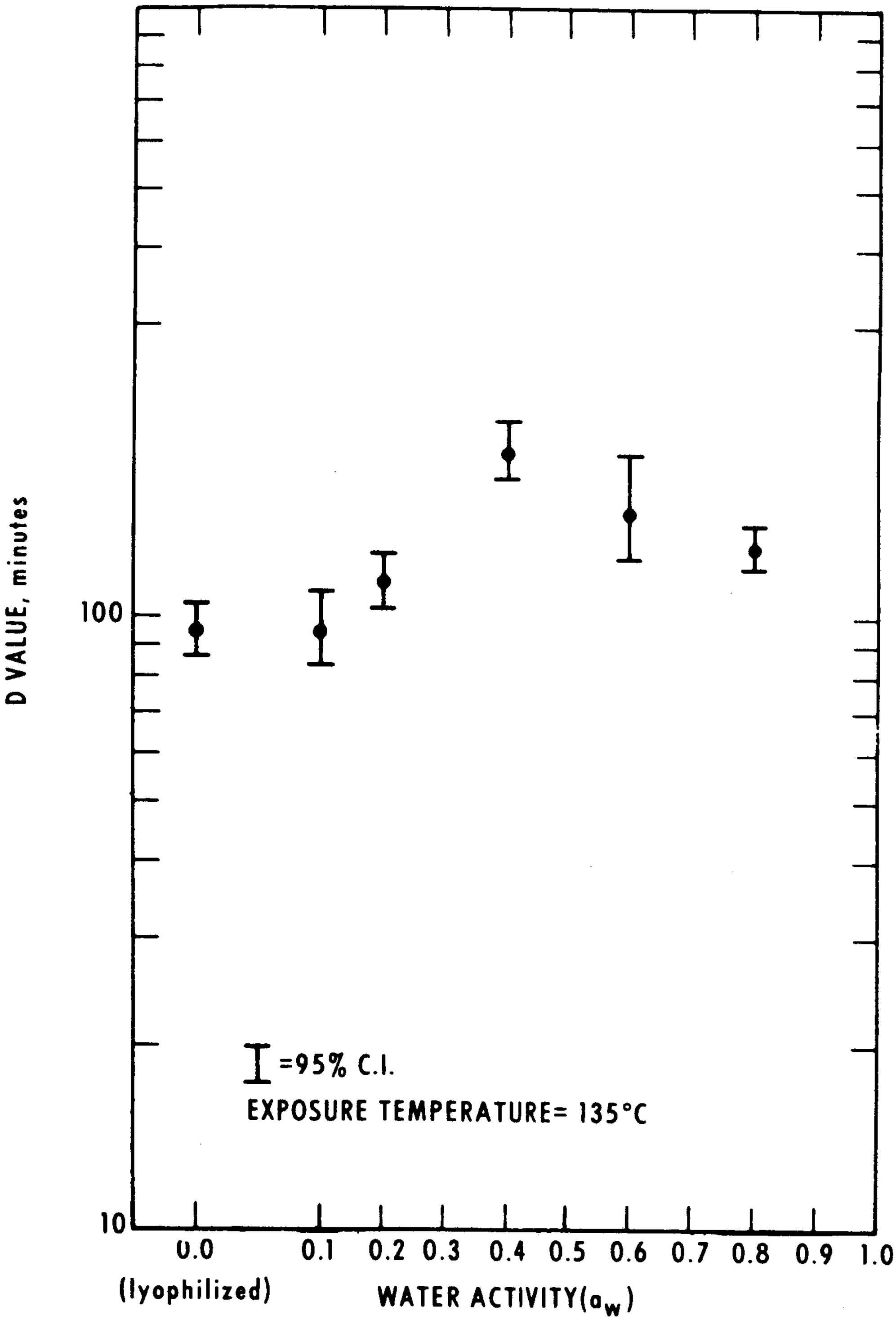
INFLUENCE OF WATER ABSORPTION FROM A SATURATED ATMOSPHERE ON THE DRY HEAT RESISTANCE OF *B. subtilis* var. *niger* SPORES

Figure 4



Equilibrium Relative Humidity Chamber for Controlling Spore Water Activity

Figure 5



INFLUENCE OF WATER ACTIVITY ON DRY HEAT RESISTANCE OF *Bacillus subtilis* var. *niger* SPORES ON GLASS ENCAPSULATED IN EPOXY