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

Fifth Quarterly Report of Progress

on

Research Project R-36-015-001

April 1 - June 30, 1966

GPO PRICE $ _____

CFSTI PRICE(S) $ _____

Hard copy (HC) 1.00

Microfiche (MF) .50

ff 653 July 65

Conducted by

Milk and Food Research, SEC
Division of Environmental Engineering
and Food Protection

for the

National Aeronautics and Space Administration
Washington, D. C.

U. S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
Public Health Service
Division of Environmental Engineering and Food Protection
Robert A. Taft Sanitary Engineering Center
Cincinnati, Ohio

July 1966

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

Fifth Quarterly Report of Progress

on

Research Project R-36-015-001

April 1 - June 30, 1966

CONTRIBUTORS

Food Microbiology	Milk Sanitation	Food Chemistry
Robert Angelotti	R. B. Read, Jr.	J. E. Campbell
Herbert E. Hall		J. E. Gilchrist
James Maryanski		
Susan G. Adams		

Statistics

James T. Peeler

Report Submitted by:

Robert Angelotti
Microbiologist

Report Reviewed and Forwarded by:

Keith H. Lewis
Principal Investigator

CONTENTS

32145

SUMMARY

D values for dry heat exposure temperatures of 115°C and 135°C were determined for Bacillus globigii spores encapsulated in plastic and dried on paper strips. These values are: plastic 115°C, D = 15.4 hours; 135 °C, D = 1.4 hours; paper 135°C, D = 16.1 minutes. The slope of the Decimal Reduction Time curve for spores encapsulated in Lucite and obtained by plotting the logarithms of D for 115, 125, and 135°C against temperature was calculated to be 18.5°C (33.3°F).

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

INTRODUCTION

In keeping with the goals set for this quarter, as outlined in the Fourth Quarterly Report of Progress, primary efforts were expended this quarter in determining the dry-heat resistance of Bacillus globigii spores encapsulated in Lucite and dried on paper strips.

EXPERIMENTAL

Two major modifications in the technique reported upon in the Fourth Quarterly Report of Progress for obtaining D values were employed this quarter. Results from replicate dry-heat inactivation experiments at temperatures of 125 and 135°C revealed that though the D values obtained at a given temperature often appeared not to differ grossly, they did differ significantly when subjected to statistical analysis. Difficulty was experienced in obtaining coincidence of the 95% confidence intervals for D values collected in duplicated experiments. In one such trial, for example, D_{135} values of 1.4 and 1.7 were obtained with 95% confidence intervals of 1.3 to 1.5, and 1.6 to 1.9, respectively. Information was presented during the April meeting of the Spacecraft Sterilization Advisory Committee of the AIBS held at Cape Kennedy to show that the water activity (A_w) of spores and the amount of moisture in a dry-heat system influenced the heat resistance of spores. Up to that time no precautions had been taken in this laboratory to assure that the moisture content of the spore-plastic system was uniform or controlled from experiment to experiment. To standardize the moisture content of the system and, theoretically, to

control the A_w of the spores, the following change in procedure was adopted: the granular methyl methacrylate is placed in a drying pan, inoculated, dried in a 50°C forced air oven, and ground in a motar as described earlier (2). At this point, however, the inoculated powder is weighed into a sterile, shallow stainless steel pan which in turn is placed over dry silica-gel in a dessicator and is held at room temperature for 1-1/2 hours followed by storage at 20°C for an additional 19 hours. Following the dry-storage period, during which it is assumed that a reproducible spore A_w, as well as a moisture equilibrium between plastic and air is achieved, the liquid monomer is added quickly to the system and the plastic is fabricated into rods as previously described (3). Since the adoption of this system, no difficulty has been experienced in obtaining statistically reproducible values in replicate heating experiments.

The second procedural modification was adopted in order to enumerate small numbers of spores surviving the heat treatments. In previous experiments, heat-treated plastic rods were sawed with hack saw blades to obtain shavings for dissolution in acetone and the resultant acetone-plastic solution was diluted in phosphate buffer solution for plate counting (2) or was passed through a Seitz filter pad (3) which in turn was ground in phosphate buffer solution, diluted and plated. Both of these procedures were fairly insensitive and no reliability could be placed upon colony counts falling below the range of 10^2 to 10^3. It was difficult, therefore, to establish the exact end-point (no survivor point) in a heating experiment. Additionally, because of the dilution factor involved, each colony on a plate derived from a rod sampled at the terminal end of an experiment represented between 100 and 1000 survivors and the introduction of a few spore contaminants from the

environment during the sawing and handling operations yielded results that appeared to establish exceptionally prolonged survival-times for the heated spores. In reviewing the technique, the major steps at which contamination appeared most likely to occur were during removal of the plastic rod from the TDT tube and during the sawing operation. To prevent contamination of the rods during the removal and sawing steps and to count small numbers of surviving spores without a dilution step, the following procedure was employed: following the desired exposure time in the oil bath, the TDT tubes were placed in a 4°C circulating water bath and held for 15 minutes. After cooling, the tubes were washed in detergent, rinsed in distilled water, and placed in a saturated alcoholic solution of iodine for 10 minutes. The tubes next were scored at the mid-point by means of a motorized steel cutting wheel and the bottom half of the glass tube was slipped-off the lower half of the plastic rod. The exposed portion of the rod was grasped with a sterile forceps and pulled from the upper section of the tube. The rod was then placed in a sterile screw-capped tube and weighed. After weighing, the rod was aseptically introduced through an entry port to a modified Waring Blendor cup containing 200 ml of sterile acetone. The blendor modification consisted of a stainless steel disc that had been cut from a vegetable grater and mounted in place of the rotary knives. The closure for the jar consisted of a threaded lid fitted with a piece of 1/2 inch diameter stainless steel tubing (the entry port) that projected through the lid. The exposed end of the steel tubing was fitted with a Morton closure and the open end, inside the blendor cup, was positioned approximately 3/16 of an inch above the grinding disc. The plastic rod is dropped down the steel tubing and a sterile steel rod is inserted that acts as a piston to drive the rod against the grinding disc.

Approximately 1/2 inch of plastic is ground from the rod and the remainder of the rod is withdrawn from the tubing by impaling it upon an elongated sterile bodkin. The entry port is again sealed with the Morton closure and the blendor is placed upon a reciprocating shaker (144 strokes per min.) for 1/2 hour at room temperature during which time the plastic shavings are completely dissolved. The liquid contents of the blendor jar are passed through a Gelman alpha 6 metricel membrane filter and the membrane is placed in a sterile Petri dish to dry. Drying is usually completed within 30 seconds, after which the membrane is overlaid with approximately 20 ml of sterile tryptone, glucose beef extract agar. The membranes are incubated for 18 to 24 hours at 35°C and colonies counted. The weight of the plastic rod is again obtained after grinding and the number of surviving spores per gram of plastic is calculated.

Dry-heat resistance of B. globigii spores encapsulated in Lucite rods or dried on paper strips.

Employing the general procedures described last quarter but with the above described modifications included, thermal inactivation experiments were conducted at 115°C and 135°C. Spore resistance data were collected for both paper and Lucite at 135°C and for Lucite alone at 115°C.

The plots of the survival points for B. globigii spores encapsulated in Lucite (approximately 1×10^8 spores per gram) and heated at 115°C and 135°C are shown in Figures 1 and 4. The points in these plots represent the plate count values obtained from paired plastic rods sampled at each interval in duplicate experiments. It was observed once again that a non-logarithmic order of death occurred comparable to that reported last quarter for 125°C. An approximate 99% reduction in numbers of spores occurred

during the initial phases of the experiments followed by straight line, logarithmic decreases for the remainder of the heat treatment intervals. Linear regressions were calculated for all points after the initial 99% reduction for each experiment and these are presented in Figures 2, 3, 5, and 6. The percent sum of squares due to linear regression (R^2 = 86 and 92) for the 115°C experiments are comparable to those reported last quarter for 125°C, whereas, the R^2 value for the two 135°C experiments is better (97). The D values and corresponding 95% confidence intervals for the duplicate experiments at each temperature are: experiment 1, $D_{115°C}$ = 15.62 hours, 95% confidence interval of 13.40 to 17.84 hours; experiment 2, $D_{115°C}$ = 15.13 hours, 95% confidence interval of 13.65 to 16.62 hours; experiment 1, $D_{135°C}$ = 1.39 hours, 95% confidence interval of 1.26 to 1.52 hours; experiment 2, $D_{135°C}$ = 1.33 hours, 95% confidence interval of 1.24 to 1.42 hours. The 95% confidence intervals for the linear regression line are presented as the dashed lines in Figures 2, 3, 5, and 6.

The z_D value (the slope of the decimal reduction time line when the logarithm of D is plotted against temperature on a semi-logarithmic scale) is shown in Figure 7 and is: z_D = 18.5°C, 95% confidence interval of 14.3°C to 22.7°C. The data collected from all the replicate experiments at 115, 125, and 135°C were used in calculating the linear regression presented in Figure 7. On conversion of this z_D value to the Fahrenheit scale, a z_D of 33.3 is obtained which is approximately twice as large as the z_D (16 to 20 in the temperature range of 220 to 270°F) associated with spores subjected to wet-heat systems. Though the fit of the sums of squares of the regression line for z_D is high (R^2 = 97) the 95% confidence interval is wide (8.4°C).

This is due in part to the fact that this line is derived from only six pieces of data and interpolations for D at 120 and 130 °C will necessarily be estimates. Because of the wide confidence interval, it may be advisable to attempt to reduce the interval by determining experimentally D at 105 and 120 °C and recalculating z_D on the basis of 10 pieces of data.

Employing the same procedure reported last quarter (2) except for the step involving drying in a dessicator as described above in the plastic experiments, D was determined for B. subtilis spores dried on paper. These data are presented in Figure 8 from which it may be observed that a straight line could be fitted through the survivor points from 0 ~~hours~~ minutes through 120 minutes ~~hours~~ yielding a D_{135} of 16.1 minutes with a 95% confidence interval of 15.3 to 16.9 minutes. The logarithmic order of death is similar to that reported last quarter for these same spores on paper heated at 125°C. Death on paper occurs much more rapidly than in plastic and is characterized by a straight line rather than by multiphasic curves as in plastic. Explanations of the two types of death patterns (paper vs. plastic) are not presently available. Investigations of the nature of these two phenomena will be undertaken as time permits.

PROJECTED RESEARCH FOR SIXTH QUARTER

Activities during the sixth quarter will be divided between obtaining D values for B. globigii spores encapsulated in Lucite and heated at 105 and 120 °C and developing a model system for the recovery of spores from non-soluble plastics by the wet grinding system.

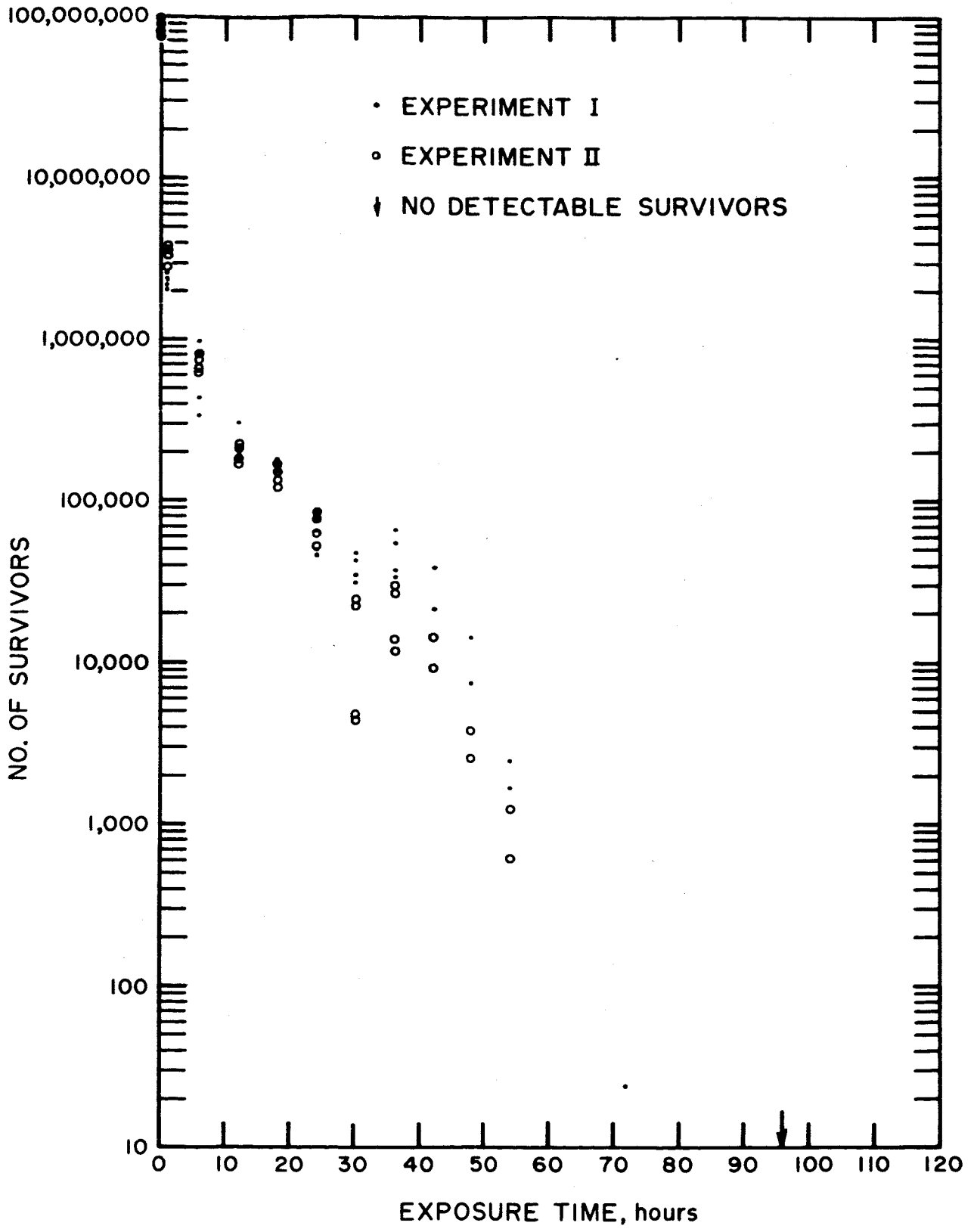
REFERENCES

1. First Quarterly Report of Progress, April 1 - June 30, 1965, NASA Research Project R-36-015-001.

2. Fourth Quarterly Report of Progress, January 1 - March 31, 1966, NASA Research Project R-36-015-001.

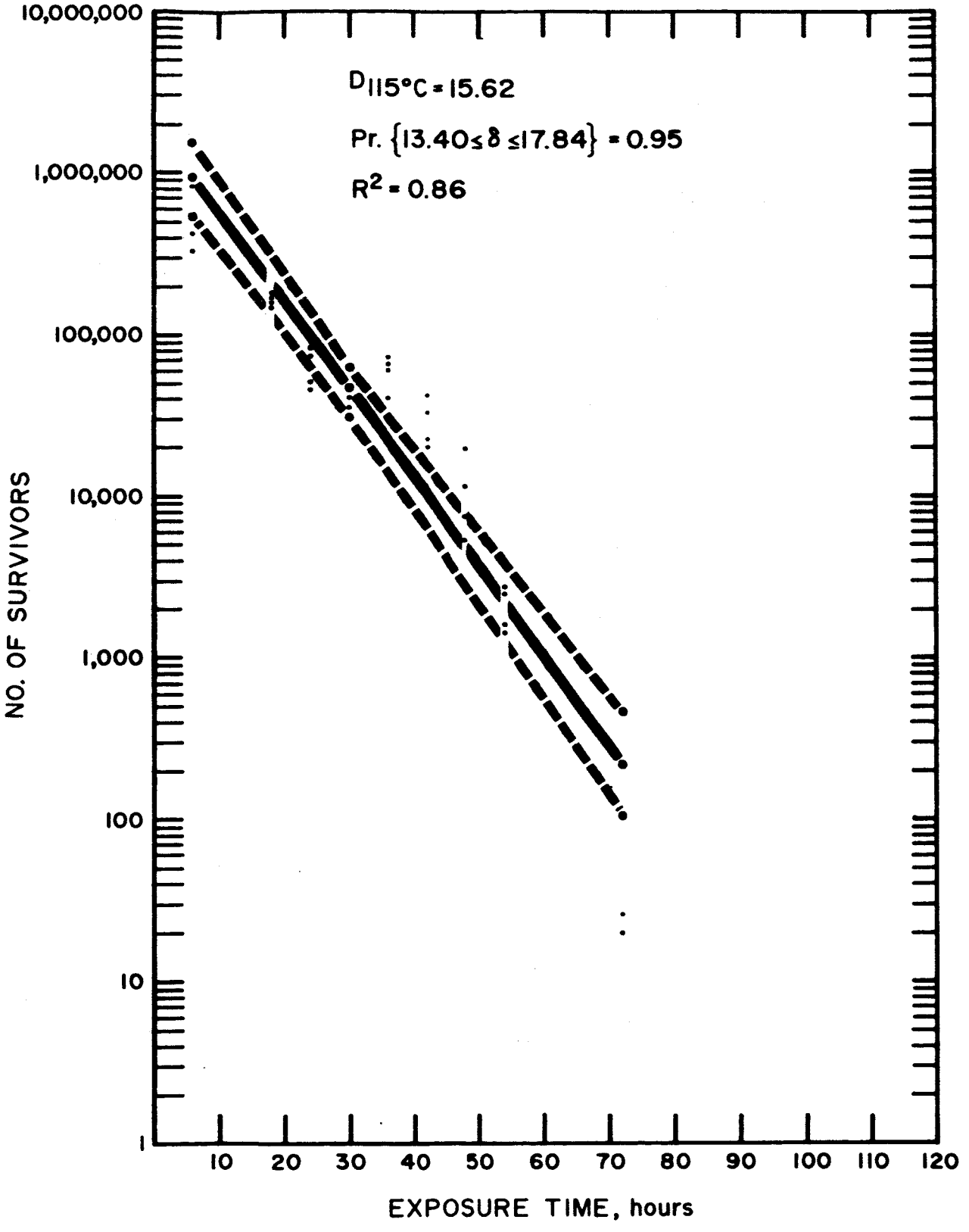
3. Third Quarterly Report of Progress, October 1 - December 31, 1965, NASA Research Project R-36-015-001.

Figure 1.



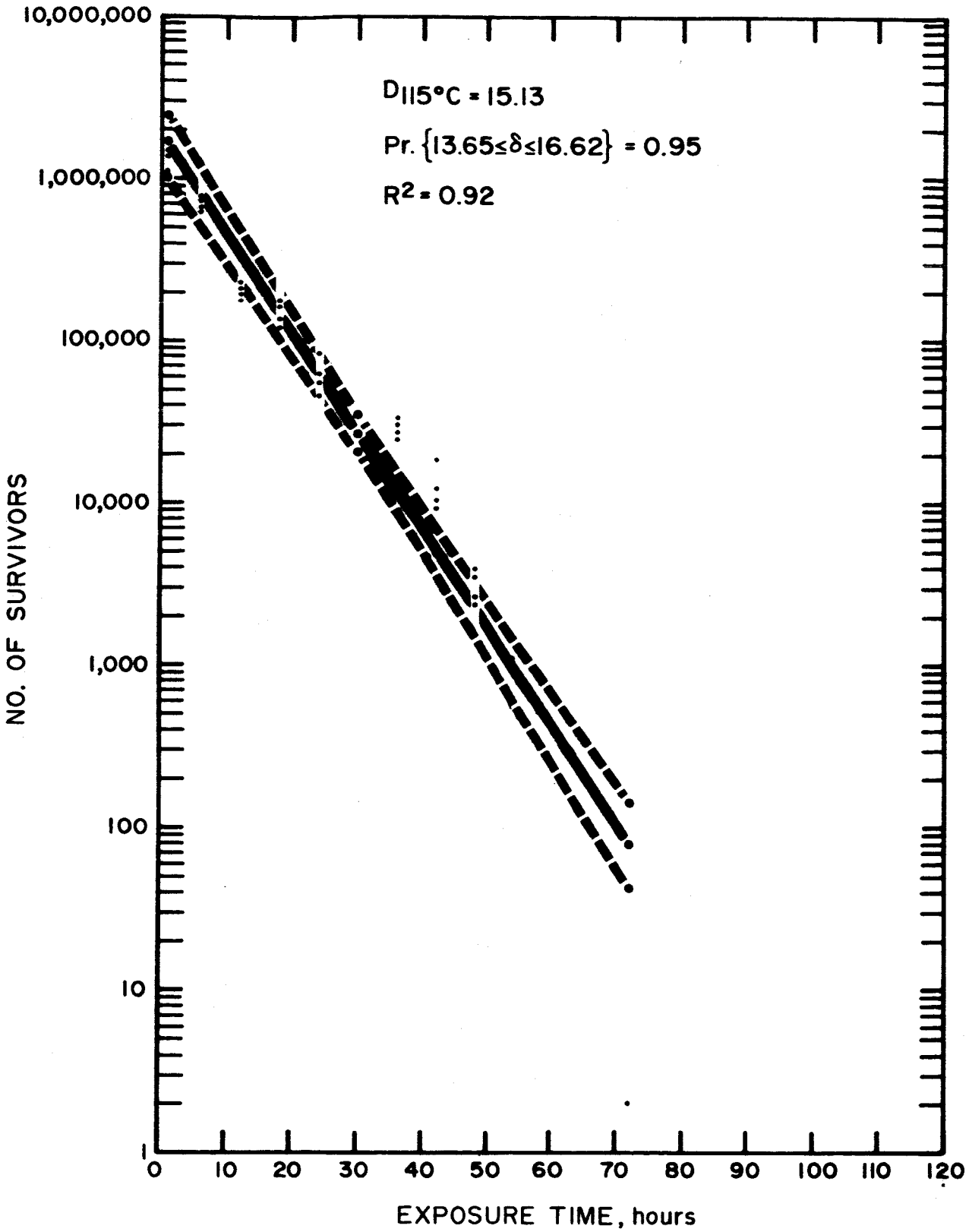
SURVIVOR CURVE OF Bacillus globigii SPORES ENCAPSULATED IN LUCITE AND EXPOSED TO A DRY HEAT TEMPERATURE OF 115°C.

Figure 2.



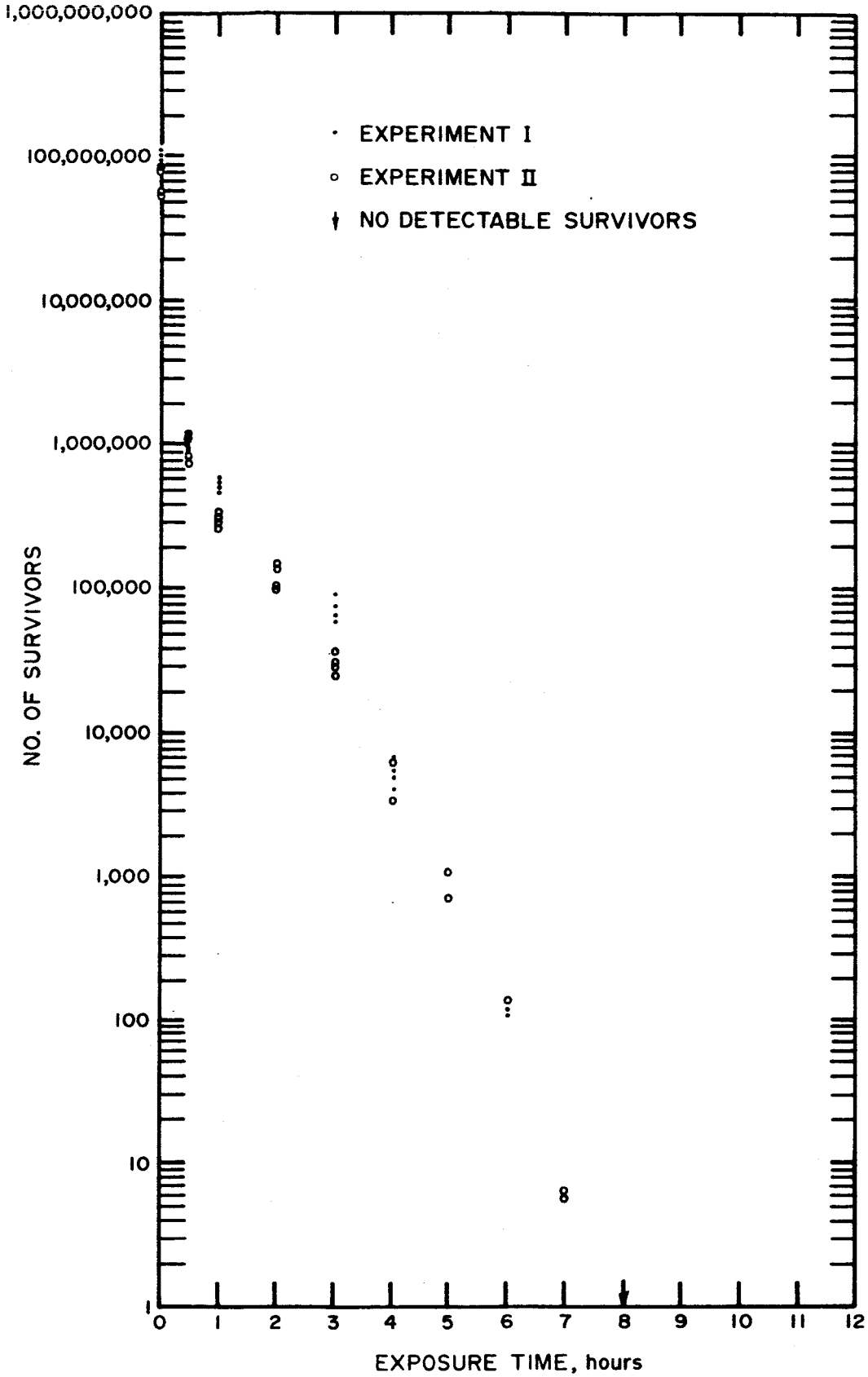
DRY HEAT INACTIVATION AT 115°C OF THE MOST RESISTANT POPULATION OF Bacillus globigii SPORES ENCAPSULATED IN LUCITE. (EXPERIMENT I).

Figure 3.



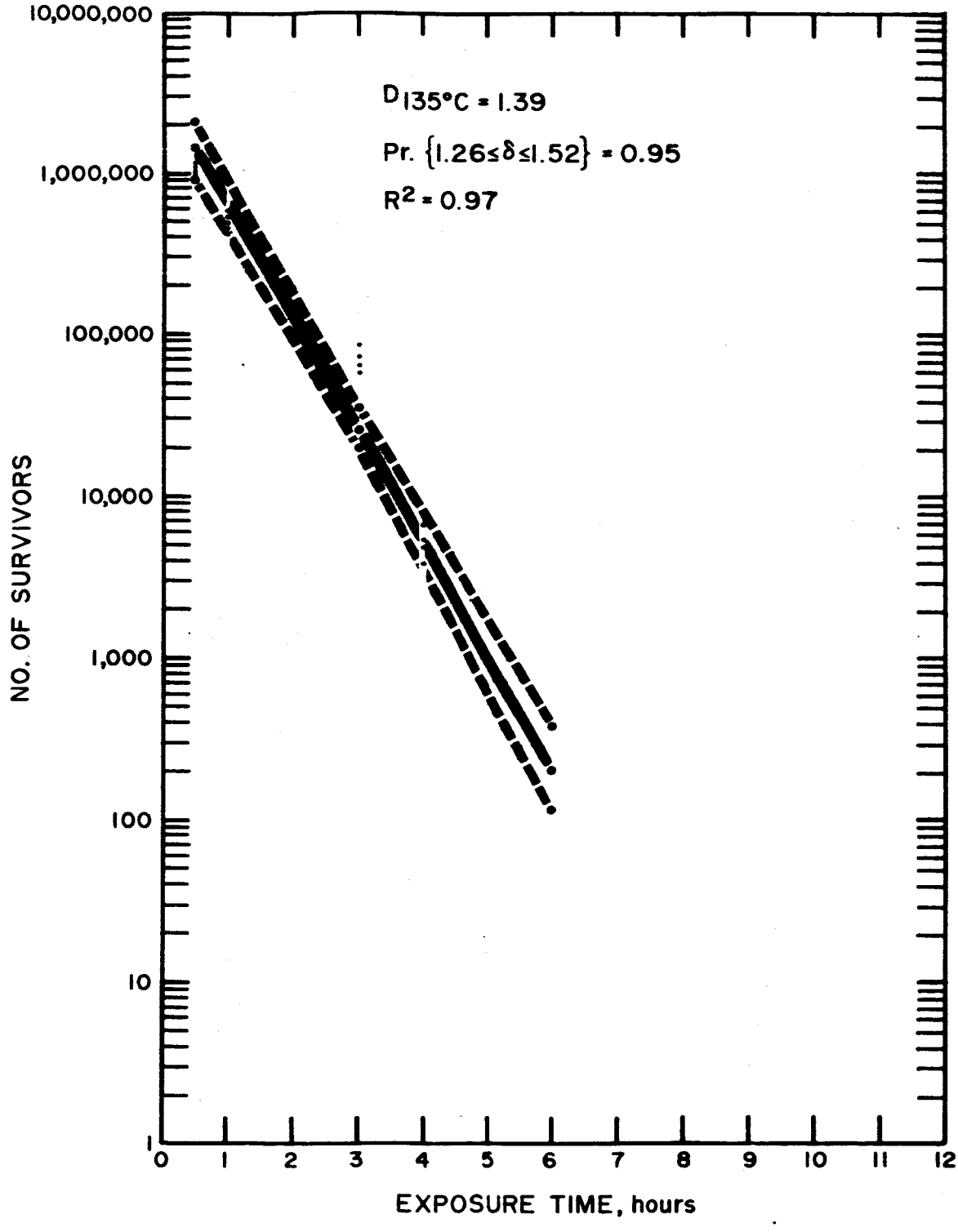
DRY HEAT INACTIVATION AT 115°C OF THE MOST RESISTANT POPULATION OF Bacillus globigii SPORES ENCAPSULATED IN LUCITE (EXPERIMENT II).

Figure 4.



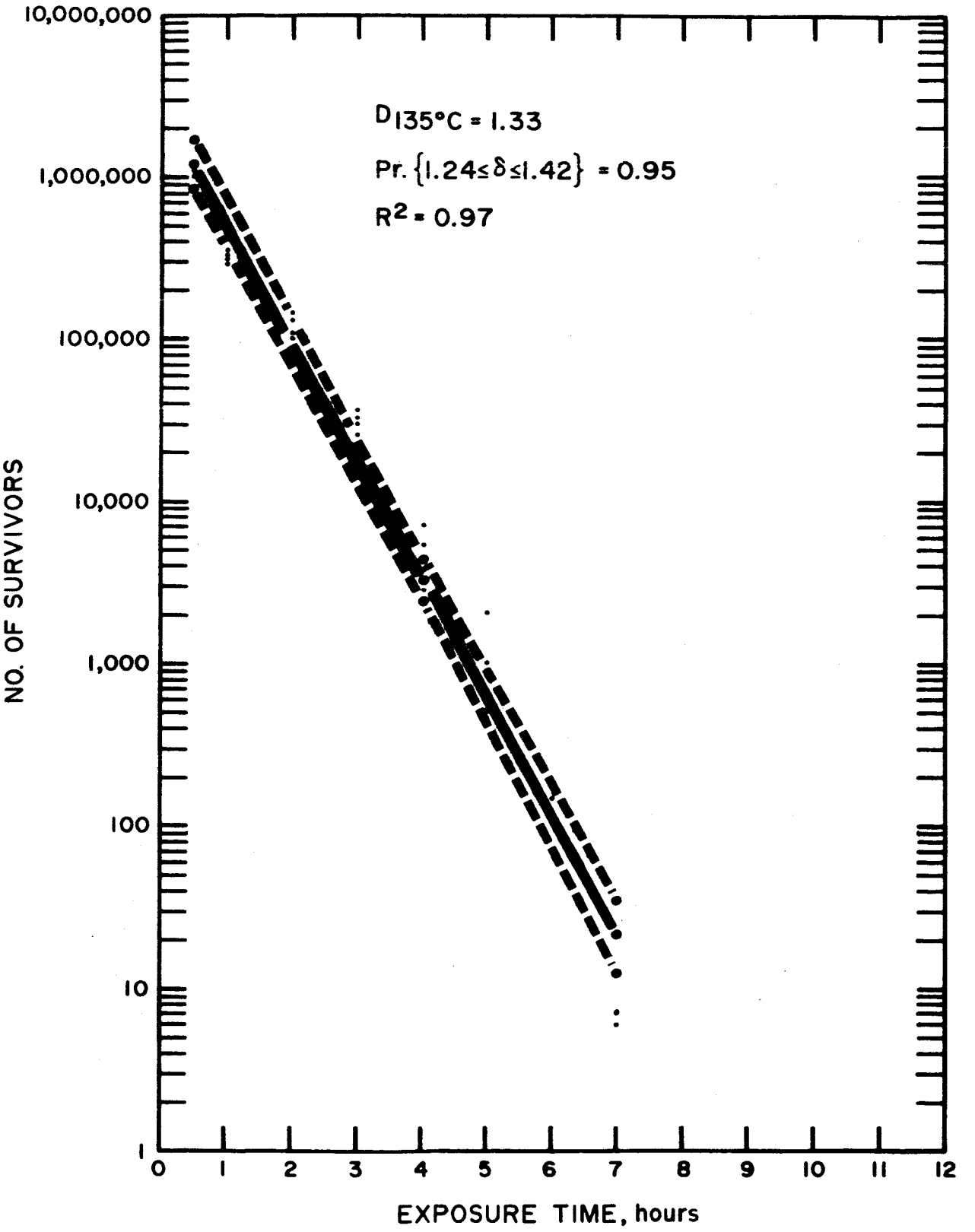
SURVIVOR CURVE OF Bacillus globigii SPORES ENCAPSULATED IN LUCITE AND EXPOSED TO A DRY HEAT TEMPERATURE OF 135°C.

Figure 5.



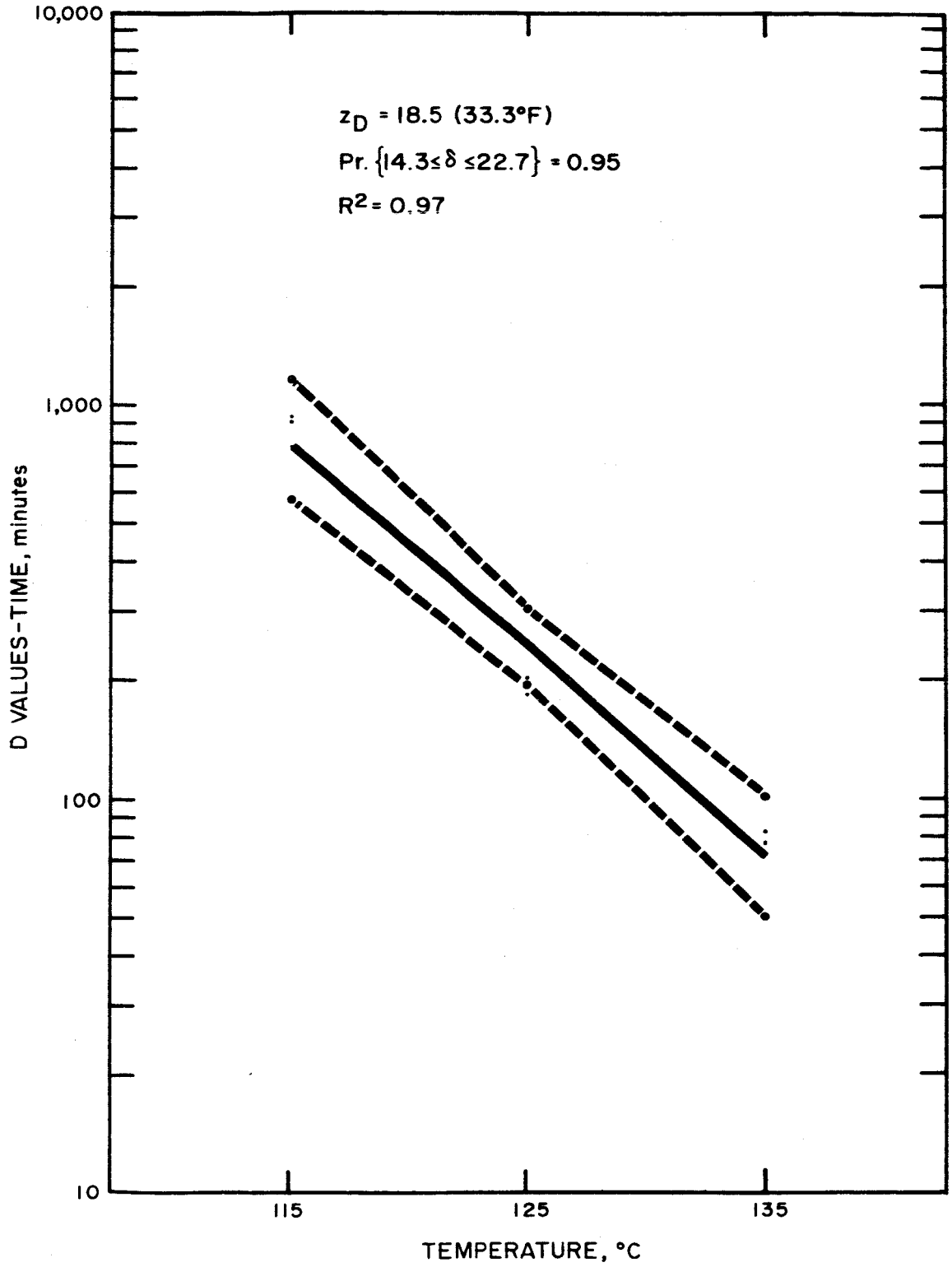
DRY HEAT INACTIVATION AT 135°C OF THE MOST RESISTANT POPULATION OF Bacillus globigii SPORES ENCAPSULATED IN LUCITE. (EXPERIMENT I).

Figure 6.



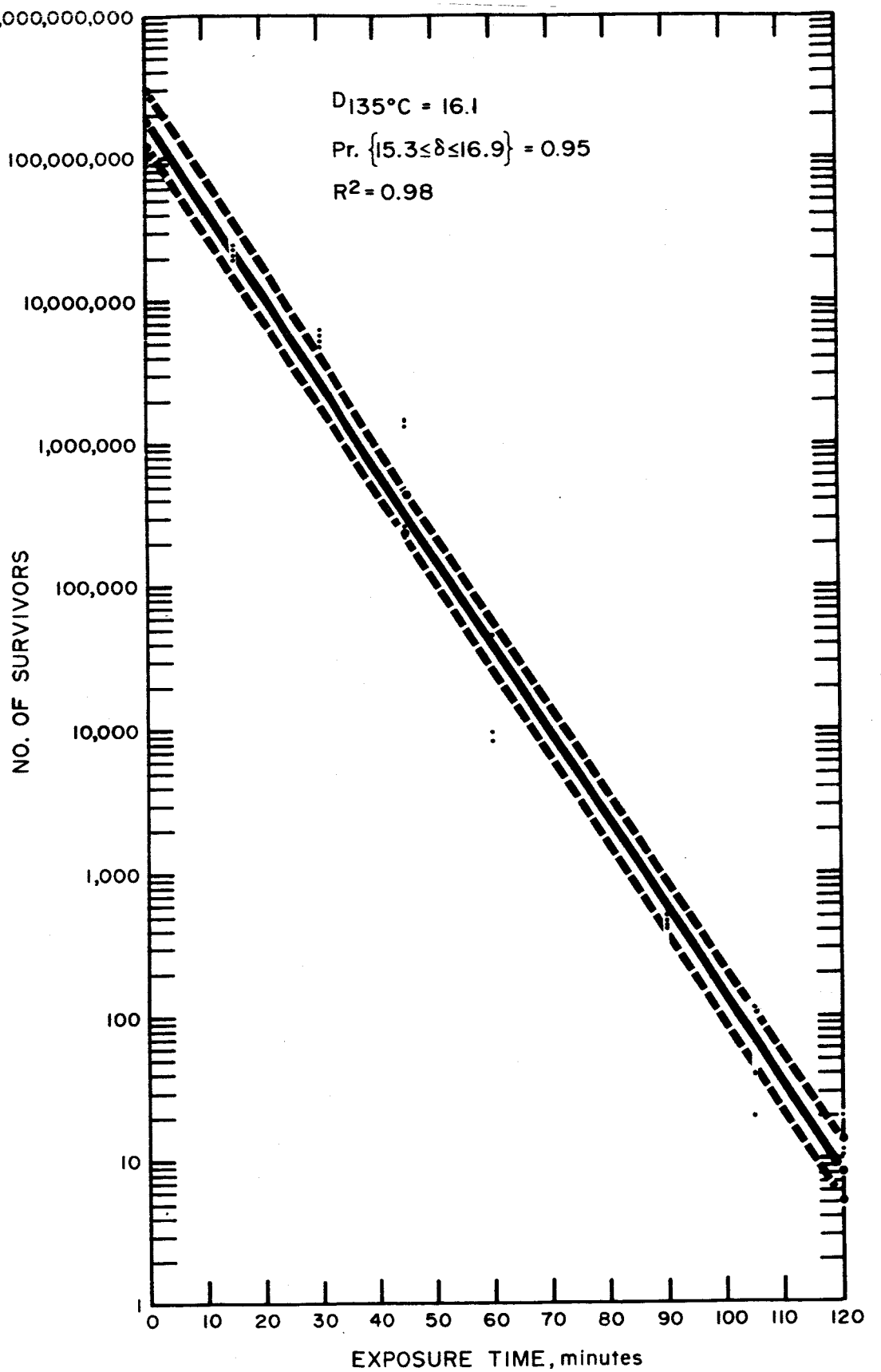
DRY HEAT INACTIVATION AT 135°C OF THE MOST RESISTANT POPULATION OF Bacillus globigii SPORES ENCAPSULATED IN LUCITE. (EXPERIMENT II).

Figure 7.



DECIMAL REDUCTION TIME CURVES FOR Bacillus globigii SPORES ENCAPSULATED IN LUCITE.

Figure 8.



DRY HEAT INACTIVATION AT 135°C OF Bacillus globigii SPORES DRIED ON PAPER STRIPS.