

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

Sixth Quarterly Report of Progress

on

Research Project R-36-015-001

July 1 - September 30, 1966

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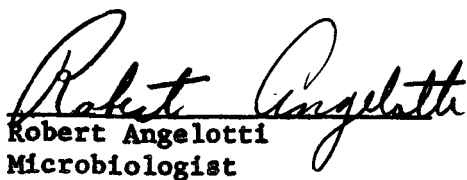
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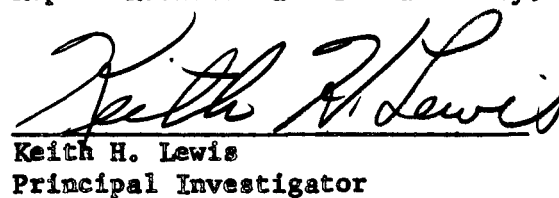
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SUMMARY

The dry heat resistance of Bacillus subtilis var. niger spores encapsulated in Lucite rods at an inoculum of approximately 1×10^8 spores per gram and exposed to temperatures of 105, 120, and 160° C was determined. The D values and their corresponding 95% confidence intervals were found to range respectively: $D_{105} = 1.2$ to 1.3 days, 95% C.I. of 1.1 to 1.5 days; $D_{120} = 5.9$ to 6.2 hours, 95% C.I. of 5.0 to 6.8 hours; $D_{160} = 4.6$ to 4.8 minutes, 95% C.I. of 3.8 to 4.9 minutes. A Decimal Reduction Time curve was constructed employing the D values from all temperatures studied to date. The slope of this curve over the temperature range of 105° C to 160° C yielded a value of $z_D = 20.8$ Centigrade degrees.

Author

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INTRODUCTION

In keeping with the suggestions of the Interplanetary Quarantine Office, additional experiments were performed this quarter on the dry heat resistance of Bacillus subtilis var. niger encapsulated in Lucite to obtain a Decimal Reduction Time curve (z_D) over the temperature range of 105° C to 160° C. Because the 95% confidence interval for the z_D curve reported in the Fifth Quarterly Report of Progress was wide (8.4 Centigrade degrees) due to the limited number of experimental values reported, additional data points were collected to note whether the 95% confidence interval would be narrowed and whether the linear order of death would continue throughout the wider temperature range.

EXPERIMENTAL

The procedures employed for inoculating methyl methacrylate powder, fabricating plastic rods, performing the heating trials, and enumerating the viable spores in the heated rods were identical to those described in the Fifth Quarterly Report of Progress.

Dry-heat resistance of B. subtilis var. niger (B. globigii) spores encapsulated in Lucite rods.

The plots of the survival points for B. globigii spores encapsulated in Lucite (approximately 1×10^8 spores per gram) and heated at

105, 120, and 160° C are shown in Figures 1, 2, and 3. The points in these plots represent the plate count values obtained from paired plastic rods sampled at each interval in duplicate experiments.

In performing linear regressions of these survival points, all data were included since the rate of die-off during the initial stages of the heat treatments was not as rapid as that reported upon earlier for 115, 125, and 135° C. Consequently, linear regressions were calculated from all the survival points obtained at 105, 120, and 160° C and are presented in Figures 4 through 9. As has been customary in past reports, the per cent sum of squares due to linear regression (R^2) are presented and these values are comparable to those reported upon for 115, 125, and 135° C.

The D values and corresponding 95% confidence intervals for the duplicate experiments performed to date for each temperature are shown in Table I.

TABLE I

D Values for Bacillus subtilis var. niger encapsulated
in Lucite rods

Dry-heat Exposure Temperature ° C	D Value	95% Confidence Interval
105	1.2 days 1.3 days	1.1 to 1.4 days 1.1 to 1.5 days
115	15.6 hours 15.1 hours	13.4 to 17.8 hours 13.7 to 16.2 hours
120	6.2 hours 5.9 hours	5.6 to 6.7 hours 5.0 to 6.8 hours
125	3.1 hours 3.4 hours	2.5 to 3.6 hours 2.9 to 3.9 hours
135	1.4 hours 1.3 hours	1.3 to 1.5 hours 1.2 to 1.4 hours
160	4.8 minutes 4.6 minutes	4.6 to 4.9 minutes 3.8 to 4.6 minutes

The z_D value was calculated from a linear regression of the semi-logarithmic plot of D values and their corresponding temperatures and is presented in Figure 10. The z_D value is 20.8 Centigrade degrees and is a measure of the temperature coefficient for the change in rate of destruction with temperature. The 95% confidence interval (8.0 Centigrade degrees) for the z_D value obtained when all the D values were plotted did not change appreciably from that reported last quarter (8.4 Centigrade degrees) for a similar plot of D values for the temperatures 115, 125, and 135° C. On the other hand, the value of z_D did increase from 18.5 to 20.8.

At all temperatures tested to date, with the exception of 160° C, the thermal lag for the heat-up and cool-down of Lucite rods has ranged between 0.09% and 3.0% of the total experimental exposure time. For this reason, no corrections for thermal lag have been applied to the D values obtained at 105, 115, 120, 125, and 135° C. The thermal lag of Lucite rods heated at 160° C represents, however, approximately 55% of the total experimental time period and a correction for the D value obtained at this temperature is necessary. Though the correction was not applied to the values for 160° C shown in Table I and Figure 10, it is expected that the corrected z_D value will decrease from that reported here. The corrected values for D 160° C and z_D will be reported as soon as the calculations are completed. In the interim the slope of the Decimal Reduction Time curve shown in Figure 10 may be used as a tentative guide to establishing dry heat cycles with some degree of safety.

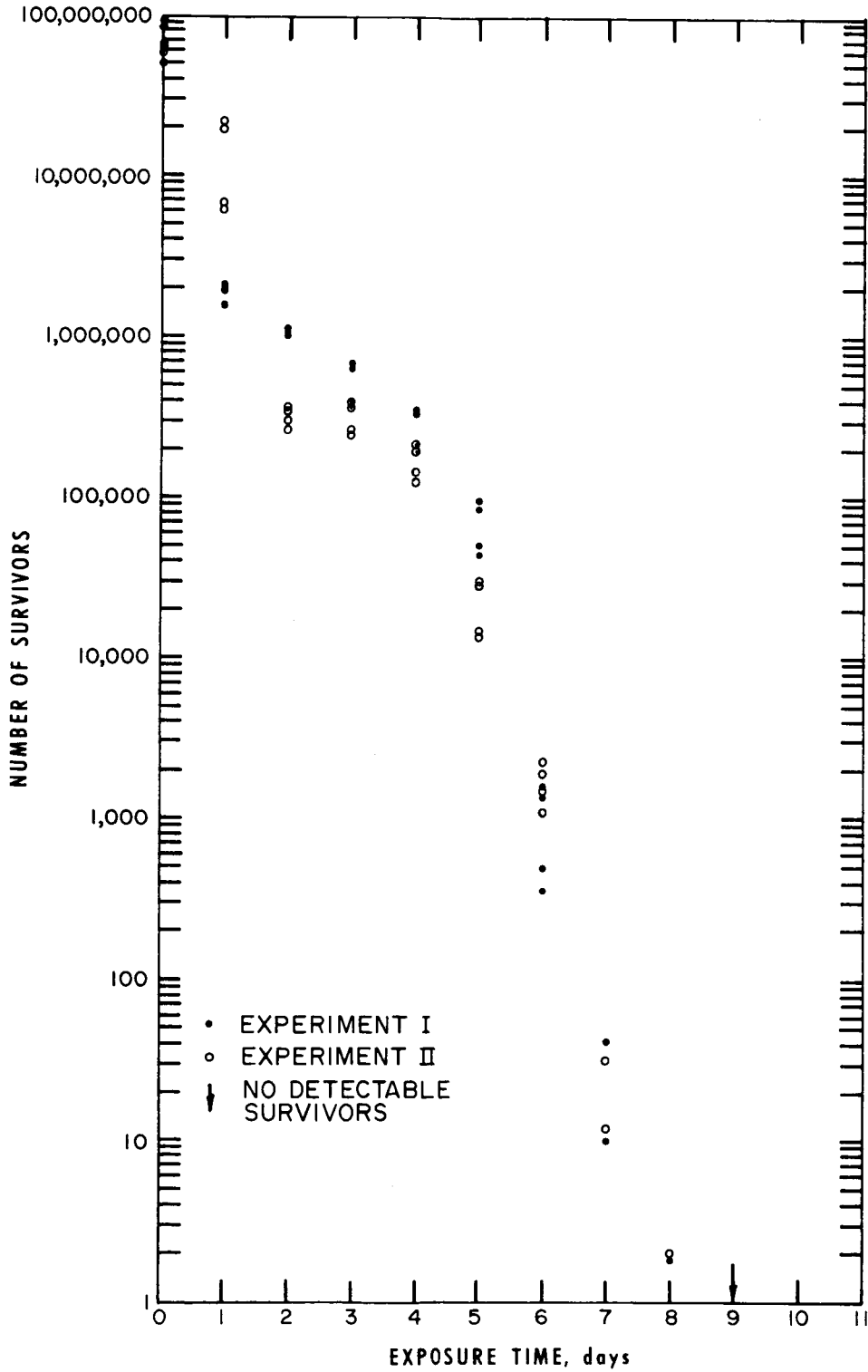
PROJECTED RESEARCH FOR SEVENTH QUARTER

During the next quarter continuing efforts will be maintained to develop and evaluate a system for recovering spores from epoxy resins and to obtain D values for B. subtilis var. niger spores encapsulated in such resins.

REFERENCES

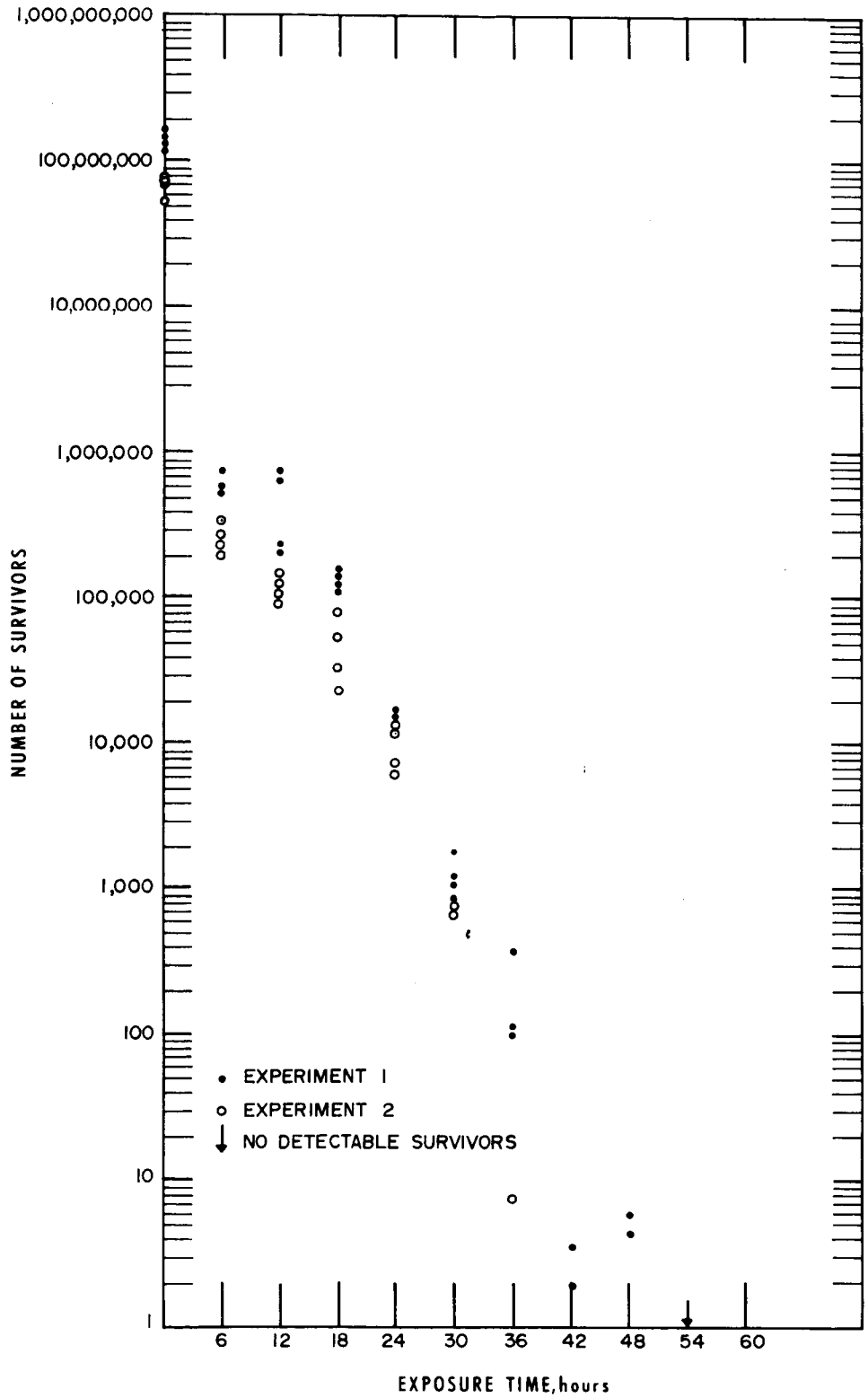
1. Fifth Quarterly Report of Progress, April - June 30, 1966, NASA Research Project R-36-015-001.

Fig. 1



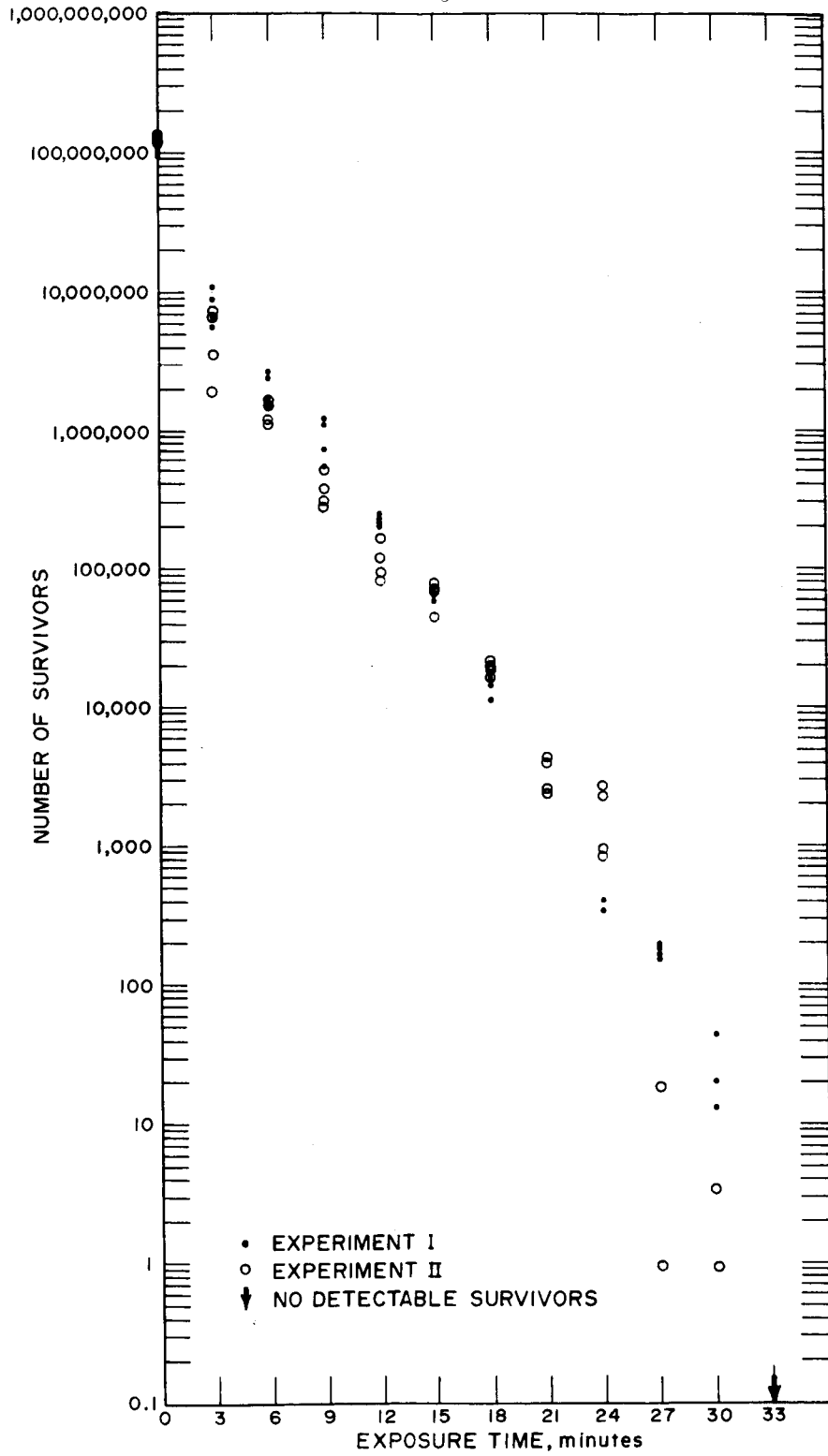
SURVIVOR CURVE OF *Bacillus subtilis* var. *niger* SPORES ENCAPSULATED IN LUCITE AND EXPOSED TO A DRY HEAT TEMPERATURE OF 105° C.

Fig. 2



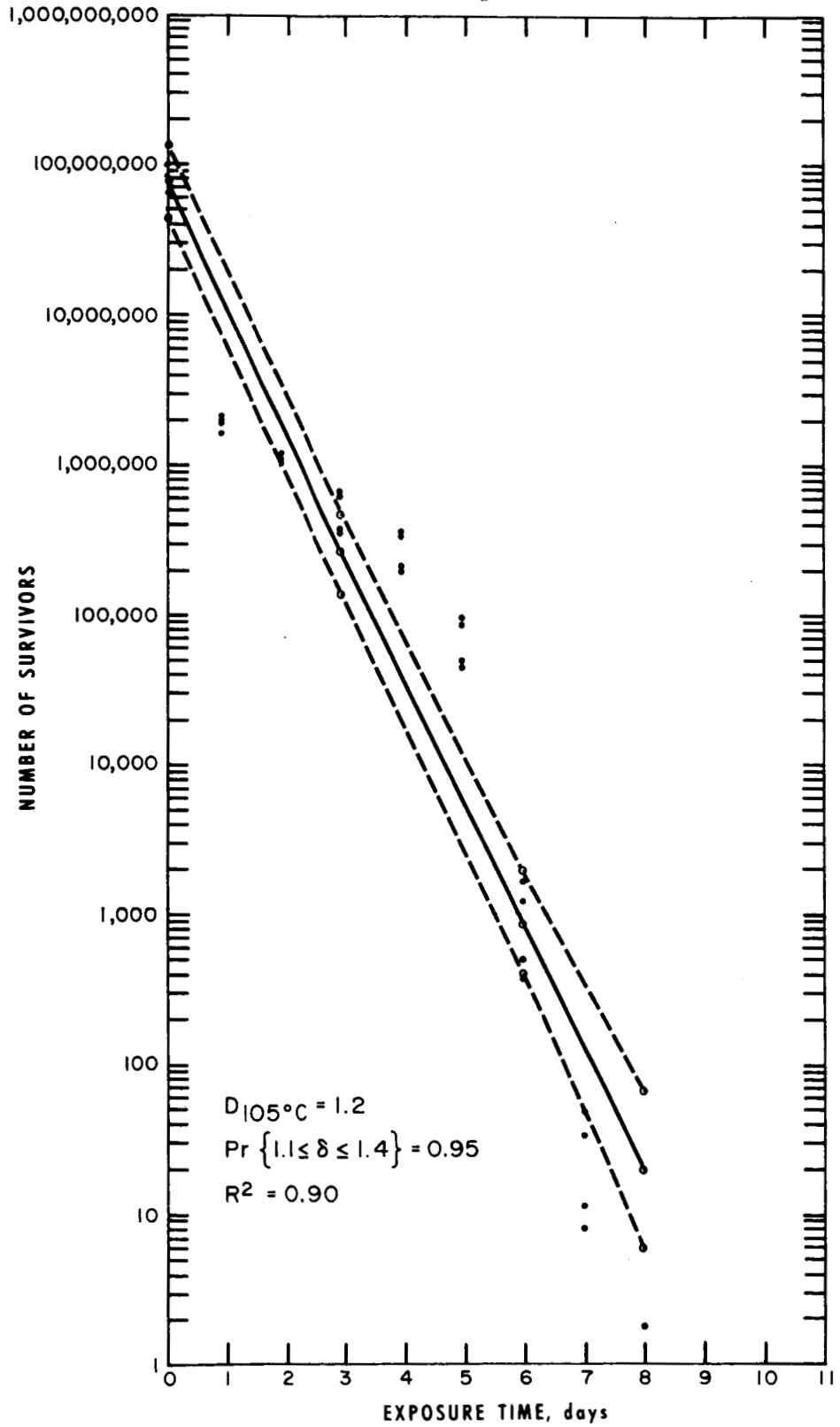
SURVIVOR CURVE OF *Bacillus subtilis* var. *niger* SPORES ENCAPSULATED IN LUCITE AND EXPOSED TO A DRY HEAT TEMPERATURE OF 120° C.

Fig. 3



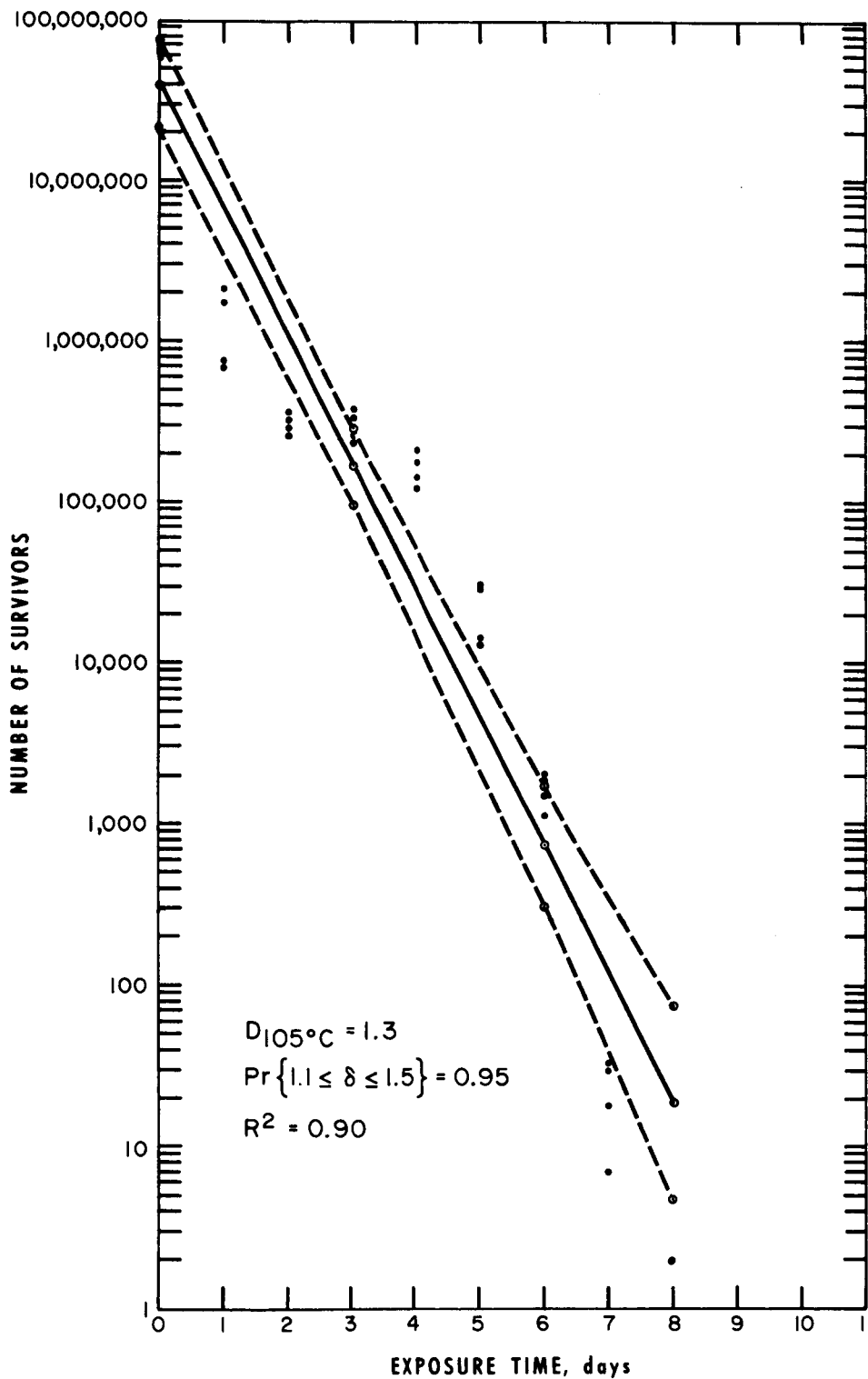
SURVIVOR CURVE OF *Bacillus subtilis* var. *niger* SPORES ENCAPSULATED IN LUCITE AND EXPOSED TO A DRY HEAT TEMPERATURE OF 160° C.

Fig. 4



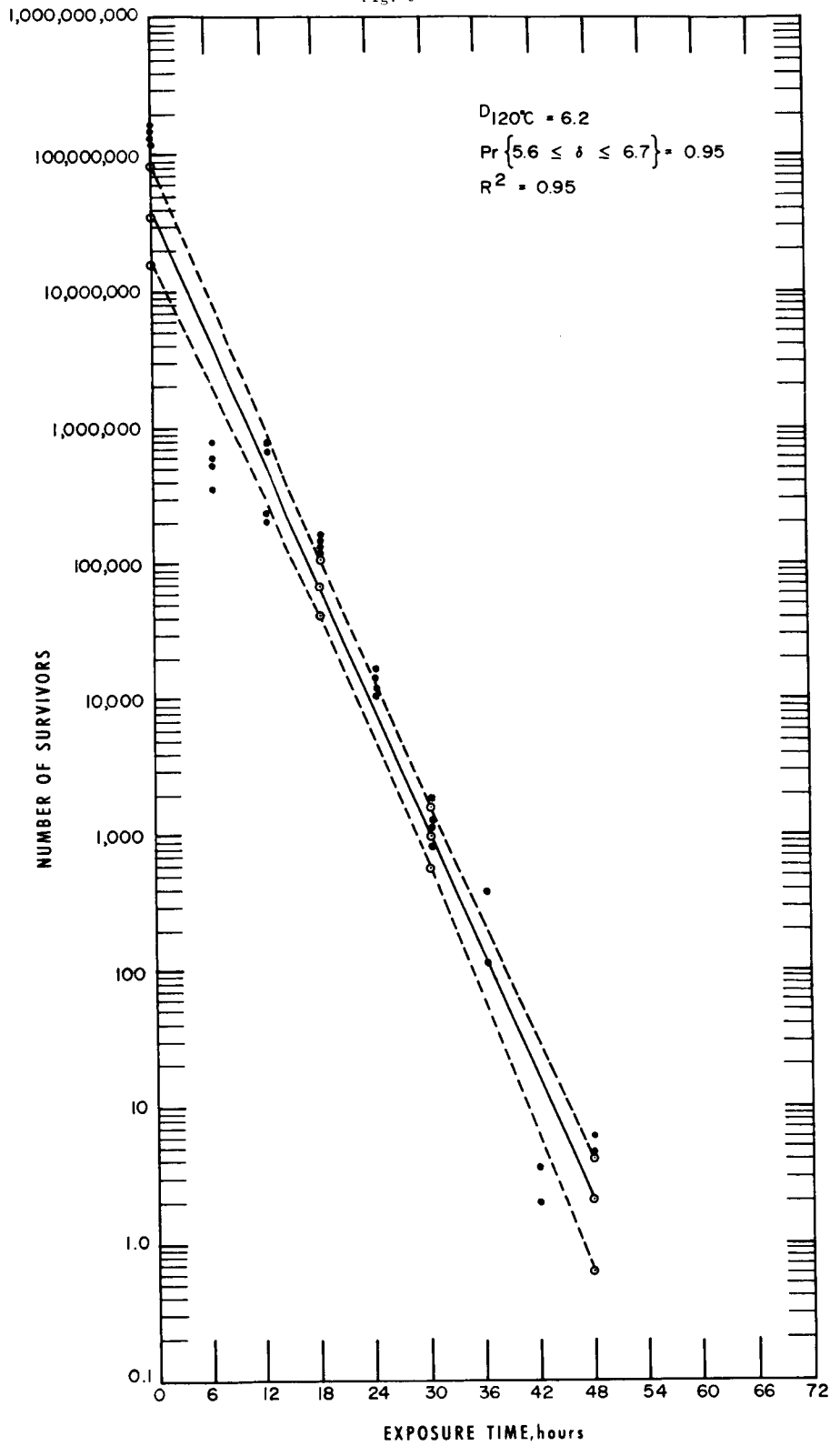
DRY HEAT INACTIVATION AT 105° C OF THE MOST RESISTANT POPULATION OF *Bacillus subtilis* var. *niger* SPORES ENCAPSULATED IN LUCITE (EXPERIMENT I).

Fig. 5



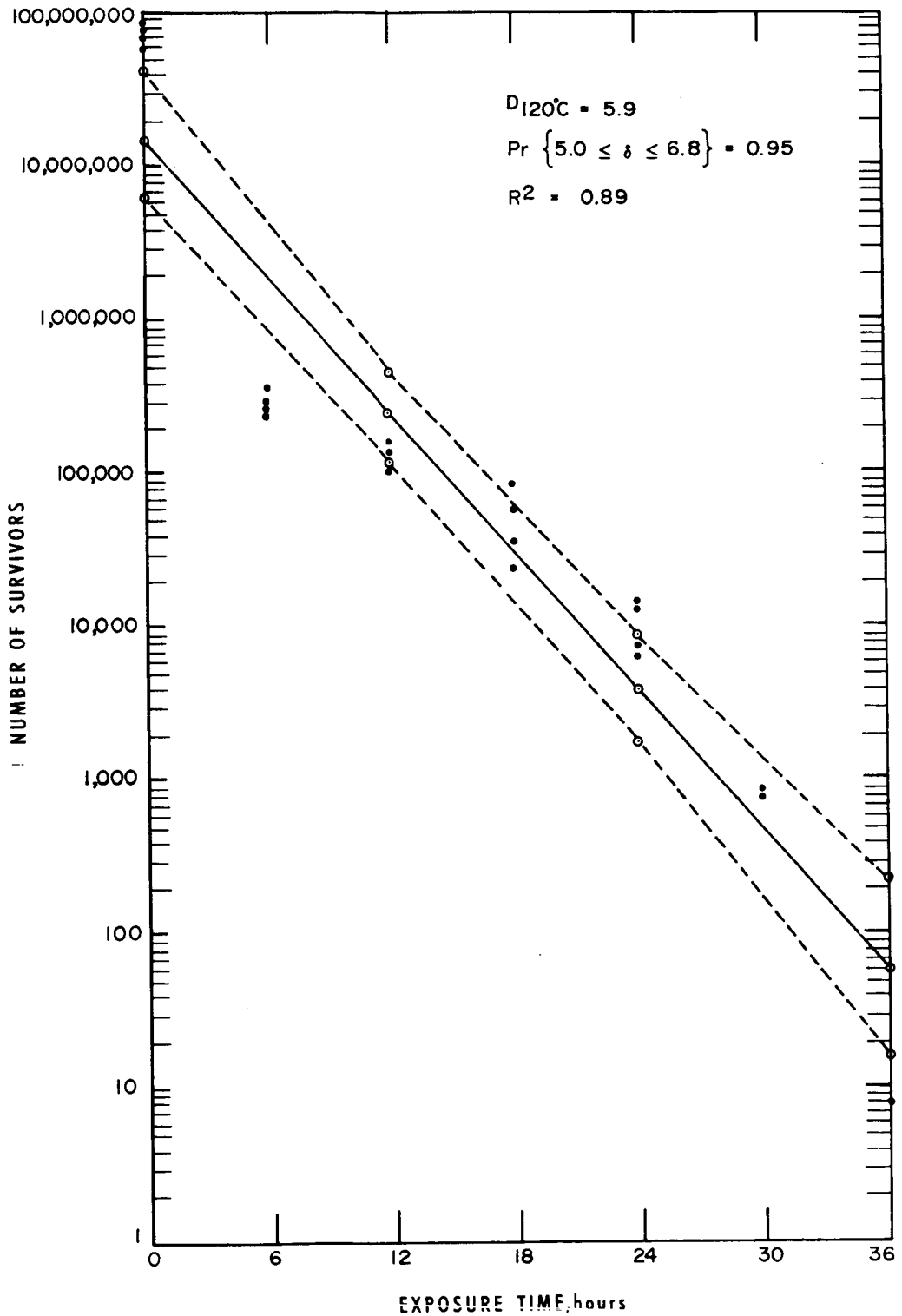
DRY HEAT INACTIVATION AT 105° C OF THE MOST RESISTANT POPULATION OF *Bacillus subtilis* var. *niger* SPORES ENCAPSULATED IN LUCITE (EXPERIMENT II).

Fig. 6



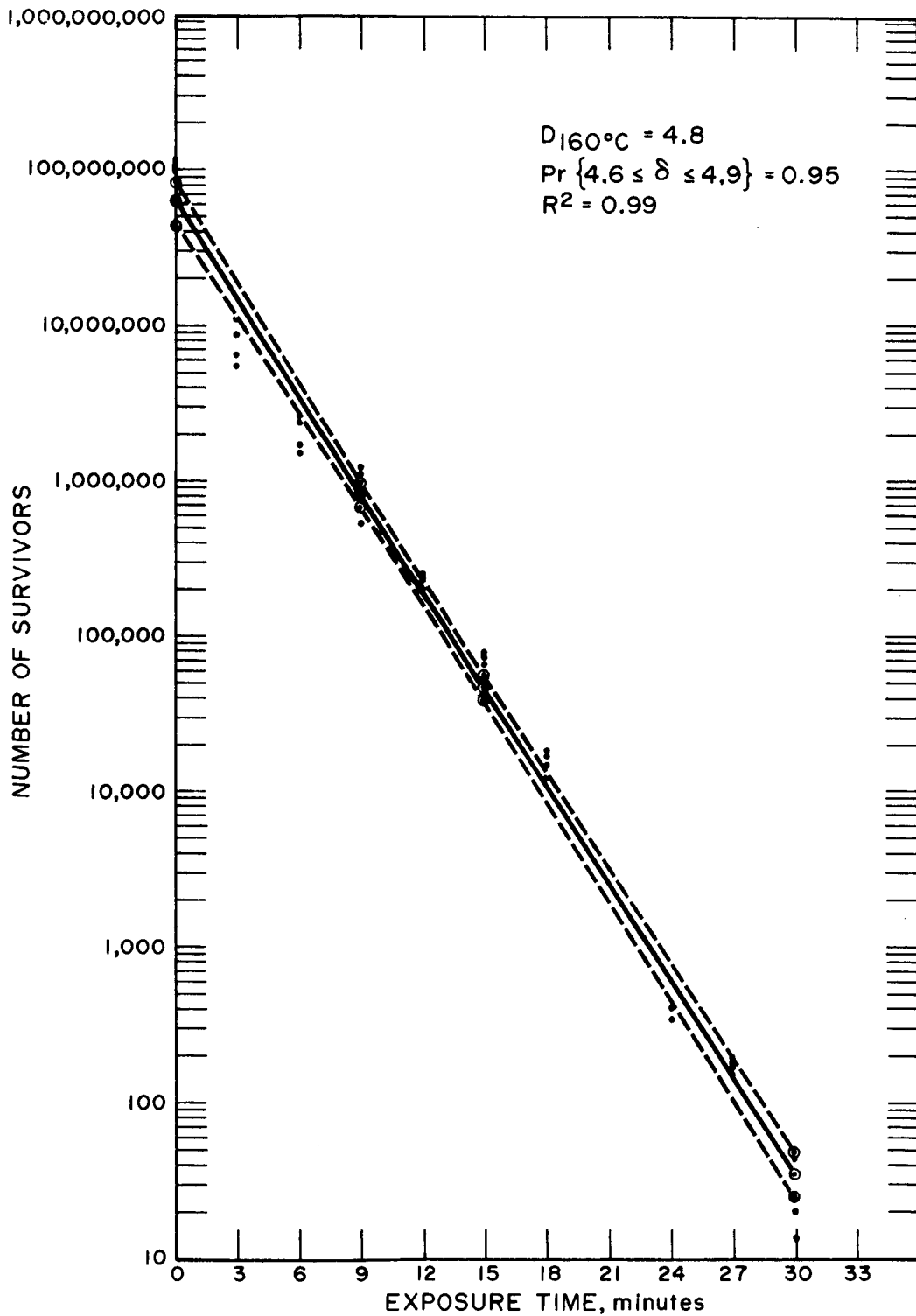
DRY HEAT INACTIVATION AT 120° C OF THE MOST RESISTANT POPULATION OF *Bacillus subtilis* var. *niger* SPORES ENCAPSULATED IN LUCITE (EXPERIMENT 1).

Fig. 7



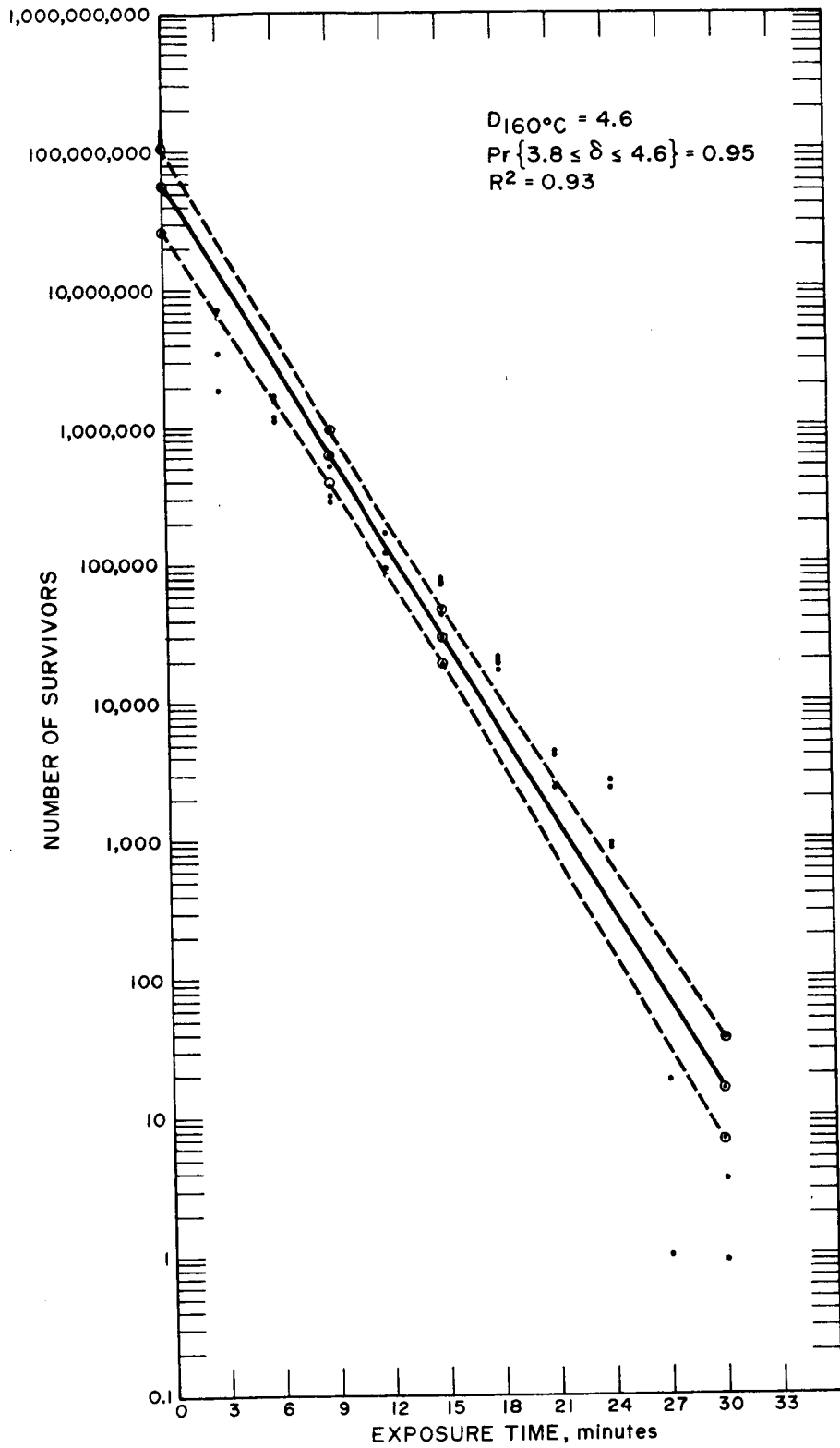
DRY HEAT INACTIVATION AT 120° C OF THE MOST RESISTANT POPULATION OF *Bacillus subtilis* var. *niger* SPORES ENCAPSULATED IN LUCITE (EXPERIMENT 2).

Fig. 8



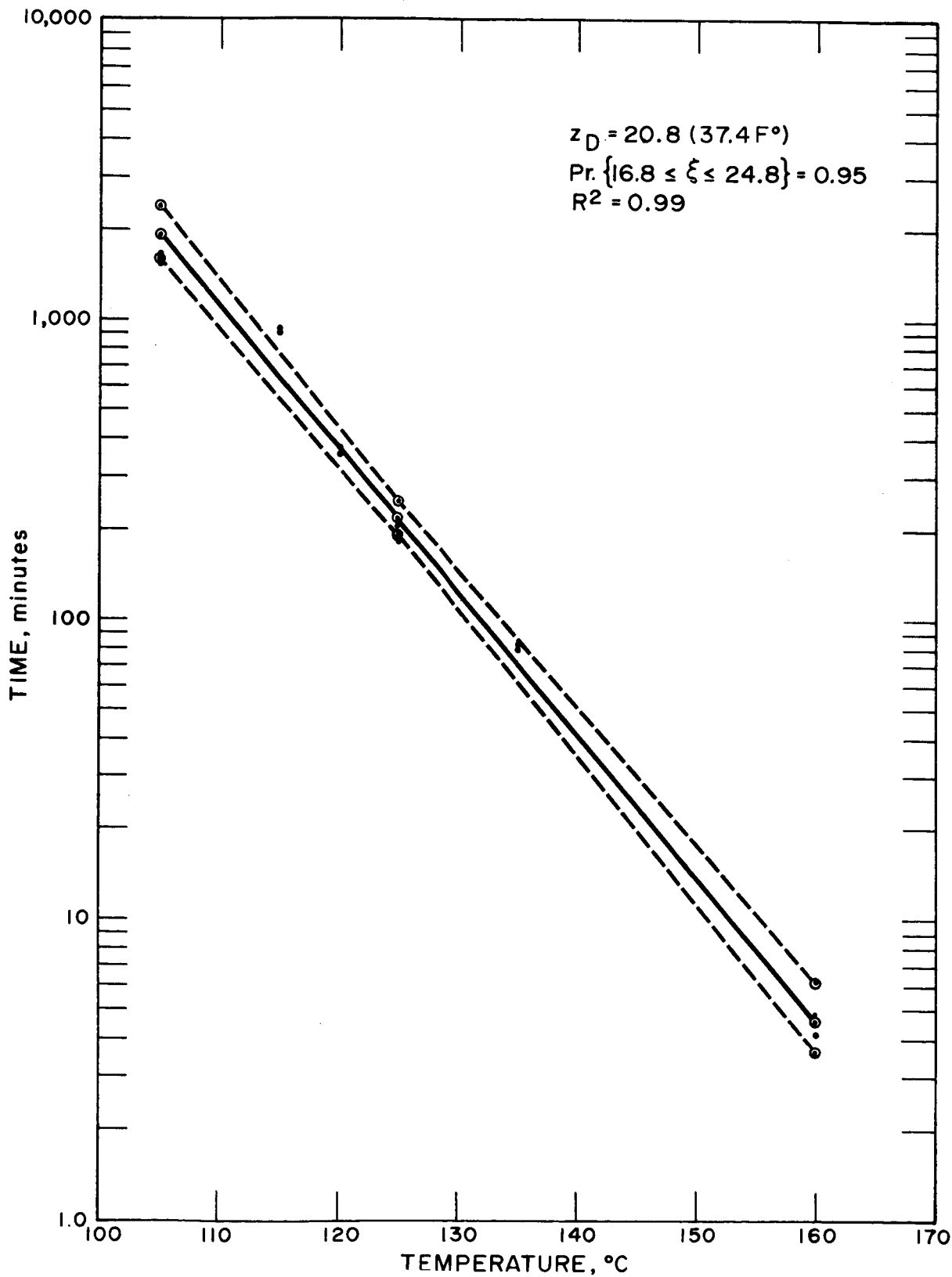
DRY HEAT INACTIVATION AT 160° C OF THE MOST RESISTANT POPULATION OF *Bacillus subtilis* var. *niger* SPORES ENCAPSULATED IN LUCITE (EXPERIMENT I).

Fig. 9



DRY HEAT INACTIVATION AT 160°C OF THE MOST RESISTANT POPULATION OF *Bacillus subtilis* var. *niger* SPORES ENCAPSULATED IN LUCITE (EXPERIMENT II).

Fig. 10



DECIMAL REDUCTION TIME CURVE FOR *Bacillus subtilis* var. *niger* SPORES ENCAPSULATED IN LUCITE.