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

Fifty-fourth Quarterly Report of Progress

Order No. W-13411

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INTRODUCTION

The experiments conducted to determine the heat resistance of Bacillus megaterium ATCC 6458 at 90 and 100°C were completed. Estimates from replicate experiments at eight percent relative humidities (< 0.001 to 100% RH) for each temperature were computed. A Bacillus cereus strain with high heat resistance was cultured and the resistance determined in phosphate buffer ($D_{121.1}$ = 2.16 min and z = 8.7°C).

This report summarizes the profile of the dry heat resistance of B. megaterium and also compares the most resistant condition to the three spores (Bacillus subtilis var. niger, ATCC 29669, and Bacillus stearothermophilus, strain 1518) previously reported (50th and 52nd Quarterly Reports).

The experimental conditions for B. megaterium are the same as reported in the 53rd Quarterly Report.

I. EXPERIMENTAL

A. Test organism

B. megaterium ATCC 6458 was obtained from the American Type Culture Collection (ATCC), Rockville, Md.

B. Spore preparation

An inoculum of actively growing B. megaterium cells was prepared from a harvested spore crop. Spore crops were then produced according to Angelotti et al. (1). Spore suspensions were washed five times, pooled, and the stock suspension was stored in double distilled water at 5°C until use.

C. Determination of dry heat resistance

The closed can system (19th Quarterly Report) was used for the dry heat inactivation studies. Duplicate cups were inoculated with a micropipette in

0.01 ml amounts to give about 1×10^6 spores per cup. The inoculated cups, cans, lids, and contents were dried in a vacuum oven for 100 min at 45 to 50°C (1.5-inch Hg pressure absolute).

An increased drying efficiency was achieved by purging the oven with dry nitrogen every 10 min for the first 90 min. This was followed by five consecutive purges of nitrogen, with a vacuum cycle between each purge. After the drying process, the cans, lids, and contents were removed from the oven and cooled to about 30°C in an equilibration hood overnight. An amount of water or dessicant (P_2O_5) was placed in the can to achieve the desired % RH at selected temperatures. The cans were sealed and removed from the hood, and the seams of each can soldered. Cans that were prepared to yield a selected % RH at a particular temperature were completely immersed in a silicone oil bath operating at the desired test temperature ($\pm$0.1°C). Cans were withdrawn at desired time intervals and plunged immediately into a refrigerated water bath (5°C) for 15 min. The cans were dried with sterile towels and opened with an electric can opener. Sample cups were placed in tubes of peptone water and sonified. Plate counts were made for each sample using fortified trypticase soy agar (0.1% soluble starch and 0.2% yeast extract) as the recovery medium. Plates were incubated for 2 days in a 35°C incubator prior to counting.

D. Determination of wet heat resistance

The spore suspension was diluted in 0.067 M phosphate buffer (pH 7.0) to give approximately 1×10^6 spores per ml. Two milliliters of this inoculum was dispensed into each of 13 x 100-mm borosilicate glass tubes and flamed sealed. The tubes were placed in metal racks and submerged completely in a constant-temperature oil bath. All thermal inactivation determinations were made in duplicate tubes. After each heating interval, the tubes were immediately

immersed in a 5°C-refrigerated water bath for 10 min. Samples were assayed by the pour plate method using fortified trypticase soy agar. Plate counts were obtained after 2 days' incubation at 35°C.

E. Statistical calculations

D values were estimated for the wet heat runs by computing the linear regression of $\log_{10}$ survivors versus time at each temperature. The D value is the absolute value of the inverse slope. A value that estimates the time to reduce the initial population 99.99% (F value) was calculated for each experiment in the closed can system. Since the initial concentrations were not constant, the end-point was computed using the fraction surviving versus time. The F value was determined by linear interpolation about 0.0001 and corrected for come-up time.

II. RESULTS AND DISCUSSION

The heat resistance of B. megaterium ATCC 6458 was observed at eight percent relative humidities (< 0.001, 0.06, 1.2, 6.7, 29.1, 49.5, 69.3, and 100% RH) for 90°C and (< 0.001, 0.04, 1.6, 7.1, 31.7, 51.5, 71.3, and 100% RH) for 100°C. Estimates of the time (h) to reduce the initial population 99.99% (F value) are shown in Table 1. The F values determined for 110°C were reported in the 53rd Quarterly Report. Percent coefficients of variation computed from replicate observations ranged from 0.8 to 19.2 (90 and 100°C data). The maximum resistance occurred at 30% RH.

A plot of the mean F values versus %RH is shown in Fig. 1 for all three temperatures. Each mean was connected by a line. The individual estimates were fitted to a linear regression of $\log_{10}$ F versus temperature (°C), and the absolute value of the inverse slope was calculated. This is the z value given in Table 2. The z values ranged from 15 to 30.5.

Plots of the average F values (h) and the estimated regression lines are shown in Fig. 2 for four typical %RH. The resistance of B. megaterium ATCC 6458 spores is about the same for <0.001% RH (P_2O_5) and 100% RH. However, at 100°C the 30% RH condition produced resistances more than 20 times those observed at <0.001 and 100% RH.

The pattern of resistance is similar for all the spores examined to date (B. subtilis var. niger, ATCC 29669, B. stearothermophilus, and B. megaterium ATCC 6458). The area of highest resistance has been observed extending from 1% RH to about 50% RH. The peak resistance for the first two species was about 10% RH, with 30% RH being the most resistant condition for the latter two.

A plot of the most resistant conditions for spores of the four species studied to date is shown in Fig. 3. The dotted lines represent an extrapolation outside of the range of experimental conditions. When compared at 100°C, the F values range from 25 h for B. megaterium ATCC 6458 (30% RH), the least resistant of the four species, to 720 h for ATCC 29669 (7% RH), the most resistant species. At this temperature in the closed can system, the greatest resistance of ATCC 29669 was almost 30-fold higher than that of B. megaterium.

The relative order of heat resistance was different when spores of these species were studied in wet heat conditions in phosphate buffer. B. stearothermophilus was then the most resistant strain.

III. FUTURE STUDIES

The estimates of resistance for B. cereus in phosphate buffer will be reported along with results from 120°C for eight percent relative humidities.

REFERENCE

1. Angelotti, R., J. H. Maryanski, T. F. Butler, J. T. Peeler, and J. E. Campbell. 1968. Influence of spore moisture content on the dry-heat resistance of Bacillus subtilis var. niger. Appl. Microbiol. 16:735-745.

Table 1. Thermal resistance of B. megaterium ATCC 6458 at eight percent relative humidites (% RH)

Temp. (°C)	% RH	Time to reduce N_o 99.99% (h)	Mean (h)	% coefficient of variation
90	< 0.001	0.94, 1.07	1.01	9.1
	0.06	2.98, 2.53	2.76	11.5
	1.2	10.05, 11.29	10.67	8.2
	6.7	46.00, 60.45	53.23	19.2
	29.1	69.76, 78.37	74.07	8.2
	49.5	31.83, 26.62, 29.44	29.30	8.9
	69.3	12.06, 11.77	11.92	1.7
	100.0	1.71, 1.73	1.72	0.8
100	< 0.001	0.41, 0.34	0.38	13.2
	0.04	0.87, 1.28, 1.17	1.11	19.2
	1.6	4.14, 3.69	3.92	5.7
	7.1	19.50, 18.67	19.09	3.1
	31.7	19.77, 17.77	18.77	7.5
	51.5	9.17, 6.84, 8.37	8.13	14.6
	71.3	2.62, 2.27	2.44	10.1
	100.0	0.83, 0.85	0.84	1.7

Table 2. Summary of z_F values over three temperatures (90, 100, 110°C) for eight percent relative humidities (% RH)

B. megaterium ATCC 6458

% RH	z value (°C)	Correlation coefficient (r)
< 0.001	19.1	-0.990
0.04	22.3	-0.984
1.4	21.4	-0.997
6.9	20.8	-0.993
29.9	21.5	-0.985
50.9	19.8	-0.993
69.8	15.0	-0.995
100.0	30.5	-0.999
Average	21.3	
% CV	20.4	

Fig. 1. Relationship of % RH to the time (h) to reduce an initial population of B. megaterium ATCC 6458 99.99% at three temperatures (90, 100, and 110°C).

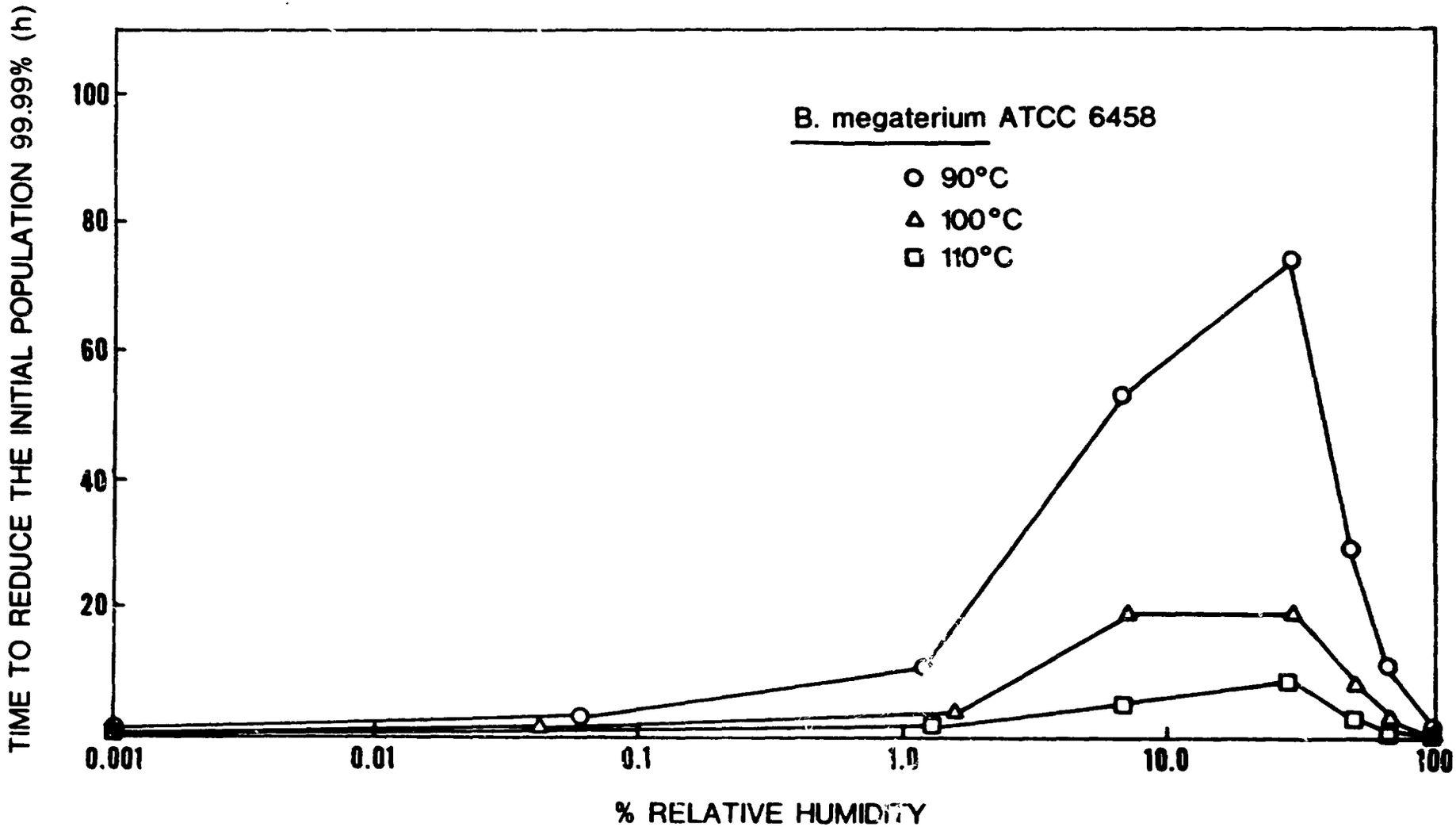

B. megaterium ATCC 6458
90°C
100°C
110°C
TIME TO REDUCE THE INITIAL POPULATION 99.99% (h)
100
80
60
40
20
0.001
0.01
0.1
1.0
10.0
100
% RELATIVE HUMIDITY

Fig. 2. Relation of the time (h) to reduce an initial population of B. megaterium ATCC 6458 99.99% versus temperature (°C).

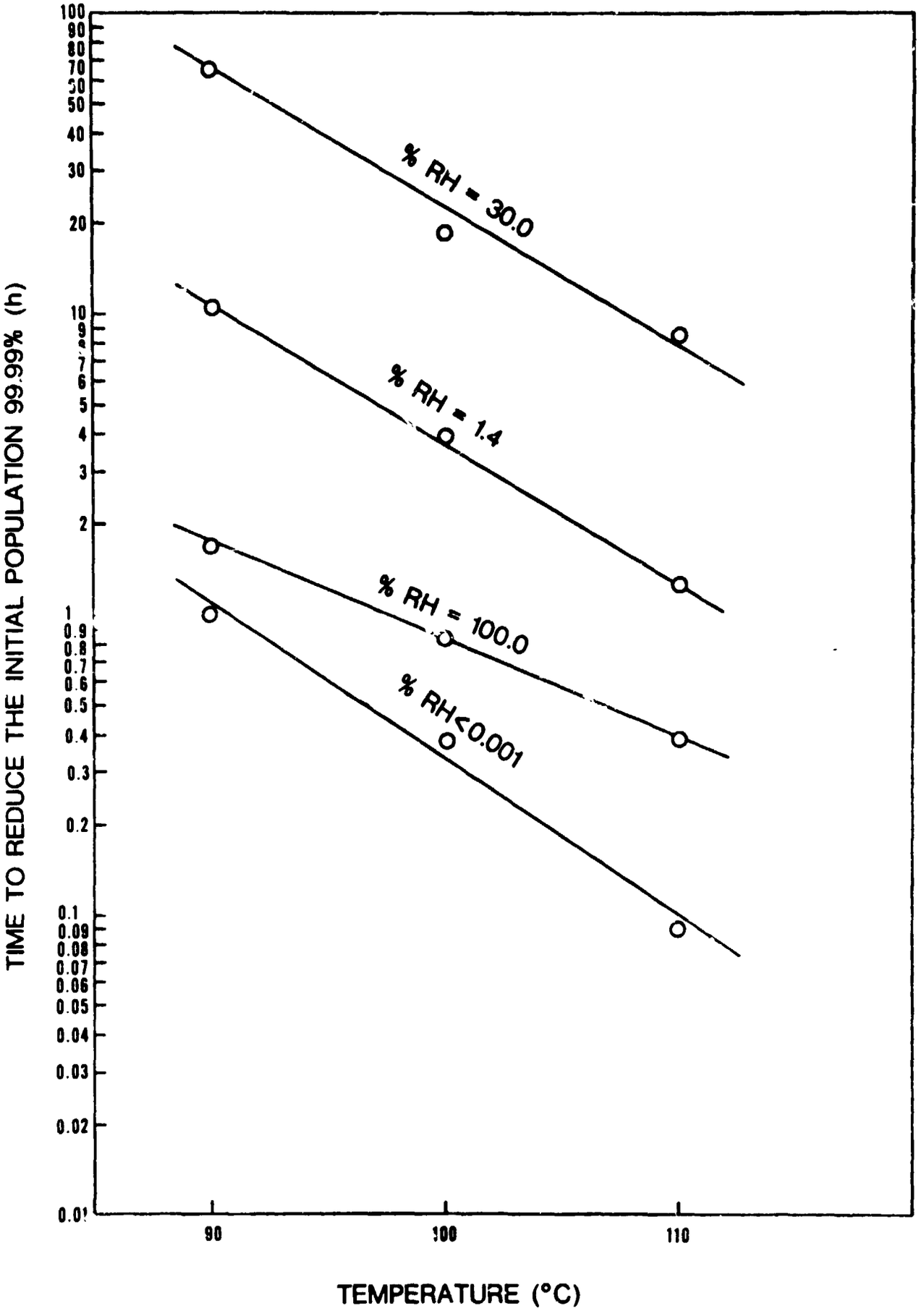
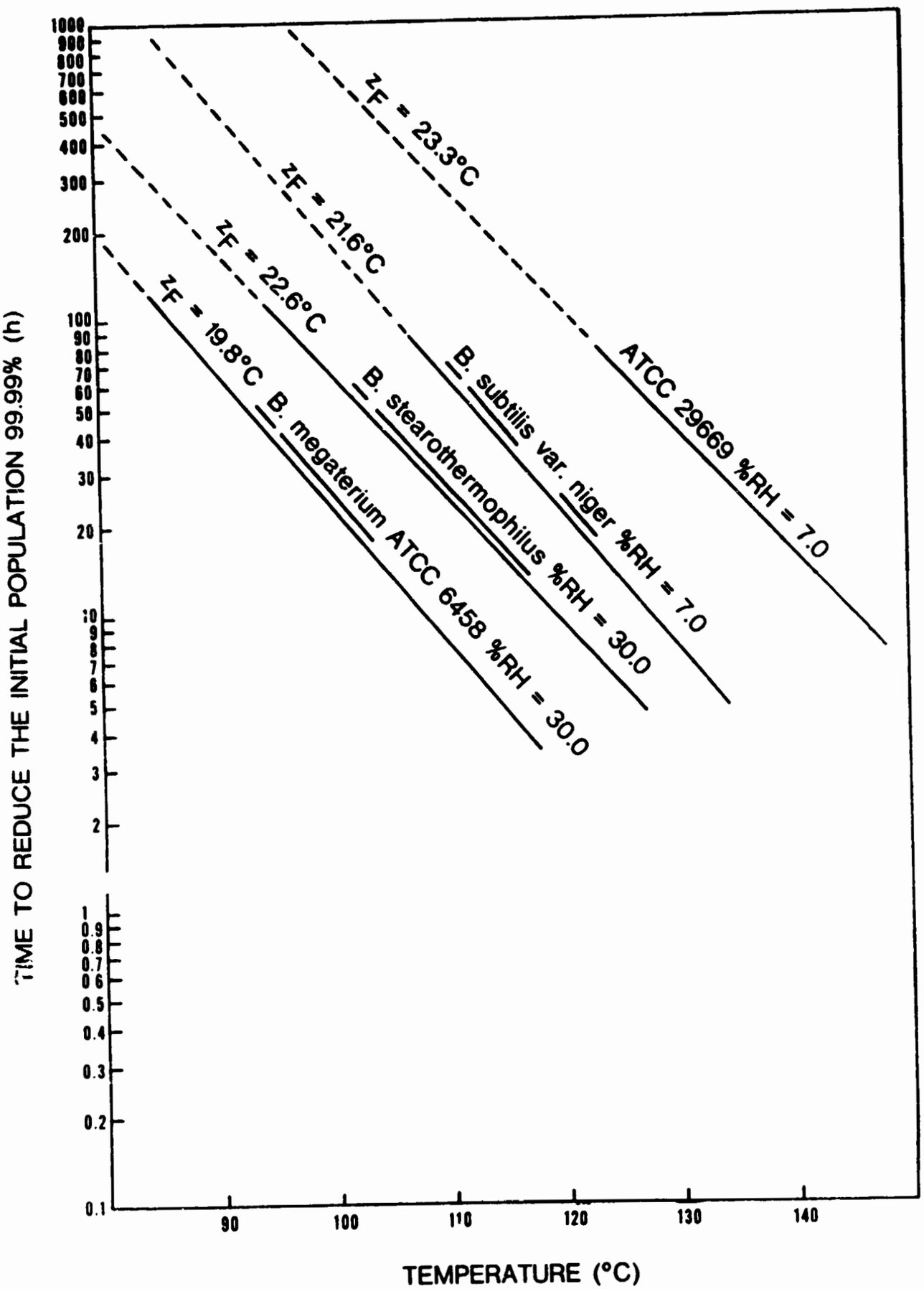

TIME TO REDUCE THE INITIAL POPULATION 99.99% (h)
100
90
80
70
60
50
40
30
20
10
9
8
7
6
5
4
3
2
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.09
0.08
0.07
0.06
0.05
0.04
0.03
0.02
0.01
% RH = 30.0
% RH = 1.4
% RH = 100.0
% RH<0.001
90
100
110
TEMPERATURE (°C)

Fig. 3. Relation of the maximum time (h) to reduce an initial population of four spores 99.99% versus temperature (°C).


TIME TO REDUCE THE INITIAL POPULATION 99.99% (h)
1000
900
800
700
600
500
400
300
200
100
90
80
70
60
50
40
30
20
10
9
8
7
6
5
4
3
2
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
90
100
110
120
130
140
TEMPERATURE (°C)
zF = 23.3°C
zF = 21.6°C
zF = 22.6°C
zF = 19.8°C
ATCC 29669 %RH = 7.0
B. subtilis var. niger %RH = 7.0
B. stearothermophilus %RH = 30.0
B. megaterium ATCC 6458 %RH = 30.0