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A Rational Model for Spacecraft Sterilization Requirements

J. P. Brannen, 2571

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ABSTRACT

COSPAR requirements that the probability of spacecraft contamination not exceed 10^{-4} demand an extrapolation of empirical data through four population decades beyond the range of possible measurement. The inherent dangers in such extrapolation prompts the introduction of maximum rationality in the models used. In this report, rationality is introduced through chemical reaction kinetics. The model is tested against empirical data; techniques for computation are investigated.

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1. Introduction. Observed nonlogarithmic survival of microbial populations exposed to a lethal thermal environment is often attributed to either sampling errors or population inhomogeniety [1]. If these explanations are valid, then sterilization cycles for various applications can be set by extrapolation of empirical data to the desired probability of contamination [2]. If nonlogarithmic survival is an intrinsic function of the organism involved, then serious doubts arise concerning the efficacy of this procedure. Our particular interest is in spacecraft sterilization cycles and the tentative COSPAR [3] objective of keeping the probability of a single viable organism aboard a vehicle intended for planetary landing or atmospheric penetration below 10^{-4}[4].

There are two distrubing aspects in the application of the logarithmic model in the planetary quarantine program. One, it is consistent with only one of the four types of survivor curves described by Schmidt [5] for the thermal inactivation of microorganisms; and, two, it needs a rational basis. The second of these is actually the most disturbing since empirical data must be extrapolated four decades beyond the range of measurability to obtain a contamination probability less than 10^{-4}. Lucas [6] points out that "empirical models usually lead to bad predictions for points ···not close to the region of the data used to fit the model. On the other hand, rational models often predict very well for points ···that are far from the experimental region."

This is a report on the derivation of a rational model for determining probability of microbial survival based on reaction kinetics. Using kinetics as observed in various organic reactions, the four types of survival curves described by Schmidt [5] may be obtained. This model provides a rational basis for the logarithmic model, but if the sterilization kinetics are of

a form found in the denaturation of protein, then probability of contamination will be greater than indicated from logarithmic extrapolation of empirical data.

Since many researchers have selected Bacillus subtilis var. niger as a standard organism for thermal inactivation studies, we give a formulation for survivors as a function of time and temperature which compares quite favorably with the results of Silverman [13] for dry heat sterilization of this organism.

2. Mathematical Model. Our initial assumptions are:

(i) The population is homogeneous.

(ii) Microbial deaths are independent.

(iii) There is no reproduction.

Under these conditions, the expected population, $E[\cdot]$, at time t is given by

$$E[X(t)] = X(0)p(t) \tag{1}$$

where p(t) is an organisms's probability of surviving to time t and X(0) is the initial population.

We next assume that microbial deaths in a thermal environment are the result of chemical reactions and that these reactions result in the inactivation of biologically vital molecular types. Each molecular type may contain more than one molecule and the cell is considered functional as regards the activity performed by a type as long as any one molecule of that type is still active. The cell is regarded as dead (sterile) if it is no longer functional relative to any one of these types.

Suppose there are N types and let n_i, $i = 1, \ldots, N$ denote the number of molecules belonging to the ith type. Let $q_i(t)$ be the probability that a given molecule of type i is active at time t. Then the probability that the cell contains at least one active type i molecule is given by

$$1 - [1-q_i(t)]^{n_i}$$

so that the probability that it is still functional in the N vital areas is given by

$$p(t) = \prod_{i=1}^{N} \{1-[1-q_i(t)]^{n_i}\}. \qquad (2)$$

Equation 2 also gives the reliability of a device of N components with a redundancy of n_i in the ith component with $q_i(t)$ the probability that a structure of the ith type is functional at time t.

We observe that equation 2 allows us to account for N different death mechanisms. If the cell is particularly vulnerable to thermal inactivation of the ith molecular type, the ith factor will dominate the determination of p(t). In this case, one may wish to use only the ith factor in equation 2 for a conservative estimate of p(t).

We would expect the molecules of a particular type to be extremely complex, for example, the protein of the cell wall or perhaps the nucleic acid, DNA. The number, n_i, of molecules of the ith type may vary greatly. A value of one to three seems appropriate for DNA, while ten thousand might be used for other types [7].

To find values for $q_i(t)$, we examine the kinetics of inactivation. Since we do not know the specific reactions,we cannot expect to proceed via the stoichiometry. However, we can compare survival curves resulting from inactivations of different orders. Protein denaturation, for example,is often of first order but the order may change with time and three-halves orders have been observed [8]. For the purpose of providing a kinetic basis for nonlogarithmic survival, consideration of first and second order reactions will suffice.

If the reaction inactivating the ith molecular type is first order, then from an initial concentration of $c_i(0)$ the rate of change of concentration is given, where $c_i(t)$ is concentration at time t, by

$$c_i'(t) = -kc_i(t). \tag{3}$$

If $c_i(0)$ is large, we would expect the probability that a molecule is active at time t to be $c_i(t)/c_i(0)$. It follows from equation 3 that $c_i(t) = c_i(0) \exp[-kt]$. Thus, $c_i(t)/c_i(0) = \exp[-kt]$. Intuitively, we take

$$q_i(t) = \exp[-kt] \tag{4}$$

in case the inactivating reaction is first order.

We note that for the 1st order model, N = 1 and $n_1 = 1$ equation 2 will give the familiar logarithmic model.

We may approach the determination of $q_i(t)$ in a less intuitive manner by again drawing an analogy to reliability theory [9]. Let $\beta(t)$ denote the conditional failure rate defined as follows:

> $\beta(t)dt$ is the probability that a given system fails (molecule is inactivated) in the time interval (t,t+dt), assuming it did not fail (was not inactivated) up to time t.

Let $\underline{X}$ be a random variable equal to the time of failure of the system and let F be its distribution. Then,

$$F(t) = 1 - \exp\left[-\int_0^t \beta(s)ds\right].$$

We assume $\beta(t)dt$ is the concentration change in the interval $(t,t+dt)$ divided by the concentration at t, i.e.

$$\beta(t)dt = \frac{c_i(t)-c_i(t+dt)}{c_i(t)} .$$

Letting $dt \to 0$, we get

$$\beta(t) = - c_i'(t)/c_i(t).$$

Thus

$$\exp\left[- \int_0^t \beta(s)ds\right] = \exp \ \ln[c_i(t)/c_i(0)],$$

so that

$$F(t) = 1 - c_i(t)/c_i(0).$$

Hence,

$$q_i(t) = c_i(t)/c_i(0). \tag{5}$$

Strictly speaking, $c_i(t)$ is an expected concentration.

Since equation 5 was derived independently of reaction order, it will be used for all orders. Thus, for a second order reaction, i.e. $c_i'(t) = -k[c_i(t)]^2$, we set

$$q_i(t) = 1/[1+c_i(0)kt]. \tag{6}$$

In equation 6 we set the initial concentration equal to n_i, the number of molecules of the ith type, i.e., concentration = molec./cell.

A reaction of three-halves order has been observed by Lauffer [10] in the thermal destruction of Influenza A virus hemagglutinin. For this reaction

$$c'(t) = -k[c(t)]^{3/2}$$

so that

$$c(t) = c(0)/[2kt\sqrt{c(0)}+1]^2.$$

For this case we set

$$q_i(t) = 1/[2kt\sqrt{c(0)}+1]^2. \qquad (7)$$

In Figure 1 we show typical curves for p(t) obtained by use of equations 5 and 7 in equation 3 for various values of N and n_i. This is equivalent to plotting E[X(t)] for an initial population of 1. Curve A is for N = 1, a first order inactivation, and $n_1 = 3$. Curve B is for N = 2, $n_1 = 1$, $n_2 = 1$, the first molecular type inactivated by a first order reaction, and the second type inactivated by a second order reaction. Curve C is for N = 1, $n_1 = 1$, and the inactivation by a second order reaction. If we set N = 1, $n_1 = 1$, and inactivate by a first order reaction we obtain the logarithmic curve, Curve D.

Since empirical data exists, Figure 2, [11] that is similar to Curve C, we investigate the consequence of extrapolating such data if p(t) fits the model of equation 3 and the inactivating mechanism is a second order reaction. For demonstration purposes, we use only the simplest assumption.

Let N = 1, $n_1 = 1$, and let $q_i(t)$ be given by equation 6. Then

$$p(t) = 1-[1-q_i(t)] = q_i(t).$$

Let

$f(t) = \ln q_i(t) = \ln 1 - \ln[1+kt]$ so that

$f'(t) = - k/1+kt.$

Therefore, if $t_1 < t_2$, then $f'(t_1) < f'(t_2)$ so that a logarithmic extrapolation of $f(t)$, i.e., with a constant slope, will be below $f(t)$.

This could have some far reaching consequences for the planetary quarantine program if microbial death results only from a 2nd order reaction. However, it seems more likely that a collection of mechanisms is involved and that data such as in Figure 2 simply shows a dominant mechanism.

Figure 3 gives typical data for Bacillus subtilis var. niger from the laboratory of Angelotti, et.al. [12]. We observe that this data is not compatible with any of the curve types in Figure 1. Figure 4, shows a curve generated by the model of equation 2 under the assumption that N molecules are being destroyed by competing 1st and 2nd order reactions with probabilities of reaction orders also functions of time. For Figure 4, we simply set, see Appendix,

$$q_i(t) = [k_1/(k_1+k_2n_i)]u_i(t)+[k_2n_i/(k_1+k_2n_i)]v_i(t) \qquad (8)$$

where k_1 and k_2 are the reaction rate constants and $u_i(t)$ and $v_i(t)$ are the values of $q_i(t)$ from equations 4 and 6, respectively.

More investigation is needed in this area and we simply present equation 8 because of the similarity of these results to those of Angelotti, et.al.

Remarks (a) Values for $q_i(t)$ can be assigned via reliability theory. For example, the assumption that inactivation is the consequence of a first order reaction is equivalent to the assumption that the failure rate for the subsystems of the ith system is constant. We have chosen to concentrate on the kinetic approach because of the desirability of relating the model to temperature as a function of time. The Arrhenius equation,

$$k = AT^n \exp[-E_a/RT]$$

where k is the reaction rate constant; A, E_a, and R are constants; T is temperature in ^oK; and n may have any value dictated by experiment, provides the desired relationship.

(b) An adequate language for a report of this nature is not available. For example, an outside observer will be unable to distinguish, from an examination of the shape of survivor curves, between the case of $N = 1, n_1 = 1$ and the case $N = 100$, $n_i = 1$, $i = 1,\ldots,100$. Thus, perhaps the extensive use of the word molecule is unfortunate. However, a better term for covering both cases is not available.

Also, concentration is not used in the usual sense. We simply view concentration as molecules per cell, and all reactions occur in the individual cells.

(c) Our last remark is related to the application of reaction rates to reactions of low concentration. The assumption is that reactions in the "small" are much like reactions in the "large". Readers interested in investigating this area more closely could start with the Jachimowski thesis [14].

3. Applications. For this section we assume that Bacillus subtilis var. niger is a microbial standard of comparison and that the spacecraft sterilization environment is approximately that encountered by the microorganisms in the ovens used by Silverman [13]. The curves from Silverman's report which we shall use are given in Figures 5 and 6.

First we observe that Silverman's data is convex so that if our model is to fit this data it will be with a first order reaction (Curve A, Figure 1). Experimentation at the computer console, G.E. 235, led to the conclusion that if this model is to fit Silverman's data it will be under the assumption that two vital molecules are being deactivated in each cell by a first order reaction. A reaction rate constant of .55 $hr.^{-1}$ gives the fit illustrated in Figure 5 to Silverman's 106^0 data and a rate constant of 2.65 $hr.^{-1}$ gives the fit to Silverman's 120^0 data illustrated in Figure 6. We next turn to the Arrhenius equation which relates reaction rate constants to temperature. If rate constants k_1 and k_2 are known for temperatures T_1 and T_2, in ^0K, then the rate constant k for temperature T, in ^0K, is given by

$$k = \exp[(1/T-1/T_1)(\ln k_2-\ln k_1)/(1/T_2-1/T_1)+\ln k_1]. \qquad (9)$$

Using the rate constants for 106^0C, .55 $hr.^{-1}$, and 120^0C, 2.65 $hr.^{-1}$, in equation 9, we get a constant of 12.49+ $hr.^{-1}$ for $T = 408^0K \approx 135^0C$. A comparison of Silverman's 135^0C data and the curve resulting from this constant is given in Figure 6. We thus conclude that an accurate prediction of survivors can be made for Silverman's laboratory conditions.

Given a temperature T in ^0K, k is determined from equation 9 with $T_1 = 106+273$, $T_2 = 120+273$, $k_1 = .55$, and $k_2 = 2.65$. Then expected survivors at time t from an initial population X(0) is given by

$$E[X(t)] = X(0)\{1-[1-\exp(-kt)]^2\}. \qquad (10)$$

Some workers in the planetary quarantine program are already using the logarithmic model; however, the program modifications required to convert to equation 10 are not prohibitive. The program, in G. E. Basic, used in this study is given in the Appendix.

At this time, our available data is for constant temperature conditions. Since a non-constant temperature profile is to be expected during spacecraft sterilization, we investigate numerical methods, based on the general model of equation 2, for predicting the probability of contamination when the lethal temperature varies with time. Recall that the logarithmic model is a special case of equation 2 so that the techniques discussed here are applicable even if equation 10 is not adopted.

Since the function varying with time will be the probability of single spore survival,we begin with equation 5, i.e.

$$q_i(t) = c_i(t)/c_i(0).$$

From this it follows that

$$q_i'(t) = c_i'(t)/c_i(0).$$

Assuming first order kinetics for Bacillus subtilis var. niger, we have

$$c_i'(t) = -k(t,T(t))c_i(t) \tag{11}$$

where $k(t,T(t))$ is the reaction rate constant which is a function of time and temperature, T, in degrees Kelvin.

Suppose we hypothesize a temperature profile T(t). It follows from equation 5 and 11 that

$$q_i'(t) = -k(t,T(t))q_i(t). \tag{12}$$

Thus, knowing T(t) we may solve for $k(t,T(t))$ by equation 10 and our problem becomes that of solving the differential equation 12.

We know that $q_i(0) = 1$. Therefore, an approximate solution can be obtained by simple difference methods. For example, let h be the step size for approximation and let $q_i(hj) = q_j$, i.e., the value of q_i at the jth step will be denoted by q_j. Then, forward differencing gives

$$\frac{q_{j+1}-q_j}{h} = -k(t,T(t))q_{j+1} \tag{13}$$

and backward differencing gives

$$\frac{q_{j+1}-q_j}{h} = -k(t,T(t))q_j. \tag{14}$$

Solving equations 13 and 14 for q_{j+1} and averaging yields

$$q_{j+1} = \frac{q_j}{2}\{2-h^2[k(t,T(t))]^2\}/\{1+h[k(t,T(t))]\} \qquad (15)$$

Equation 15 then, provides a simple step by step procedure for finding $q_j(t)$ for varying temperatures.

In Figure 7, we show p(t) for different temperature profiles and for both the logarithmic assumption and equation 10. Since the reaction rate constants obtained by Silverman, .505 $hr.^{-1}$ at 106°C, 2.88 $hr.^{-1}$ at 120°C, and 18.35 $hr.^{-1}$ at 135°C do not vary greatly from the ones we obtained from his data and equation 10, we have used our rate constants, .55 $hr.^{-1}$ at 106°C, 2.65 $hr.^{-1}$ at 120°C, and 12.5 $hr.^{-1}$ at 135°C, for the logarithmic case. Curve A shows p(t) for the logarithmic case and a linear heat up time of 8 hrs. from 100°C to 135° and constant thereafter, Curve A' has the same temperature profile as curve A but we used equation 10 instead of the logarithmic assumption. Curves B and B' are for the two mathematical models used for A and A' but the temperature profile is for 4 hrs. from 100° to 135°.

The program used to generate the curves of Figure 9 is shown in the Appendix. In all cases, $q_j(t)$ was approximated by equation 15. The ease of computation is obvious from the program.

In the computation of $q_j(t)$, one can easily use equation 12 with Runge-Kutta methods with no appreciable additions to the complexity. As an alternative one could use integration to obtain from equation 12 the equation

$$q_i(t) = \exp[-\int_0^t k(s,T(s))ds].$$

However, it may be simpler to use equation 15 or a Runge-Kutta method. A Runge-Kutta program is given in the Appendix.

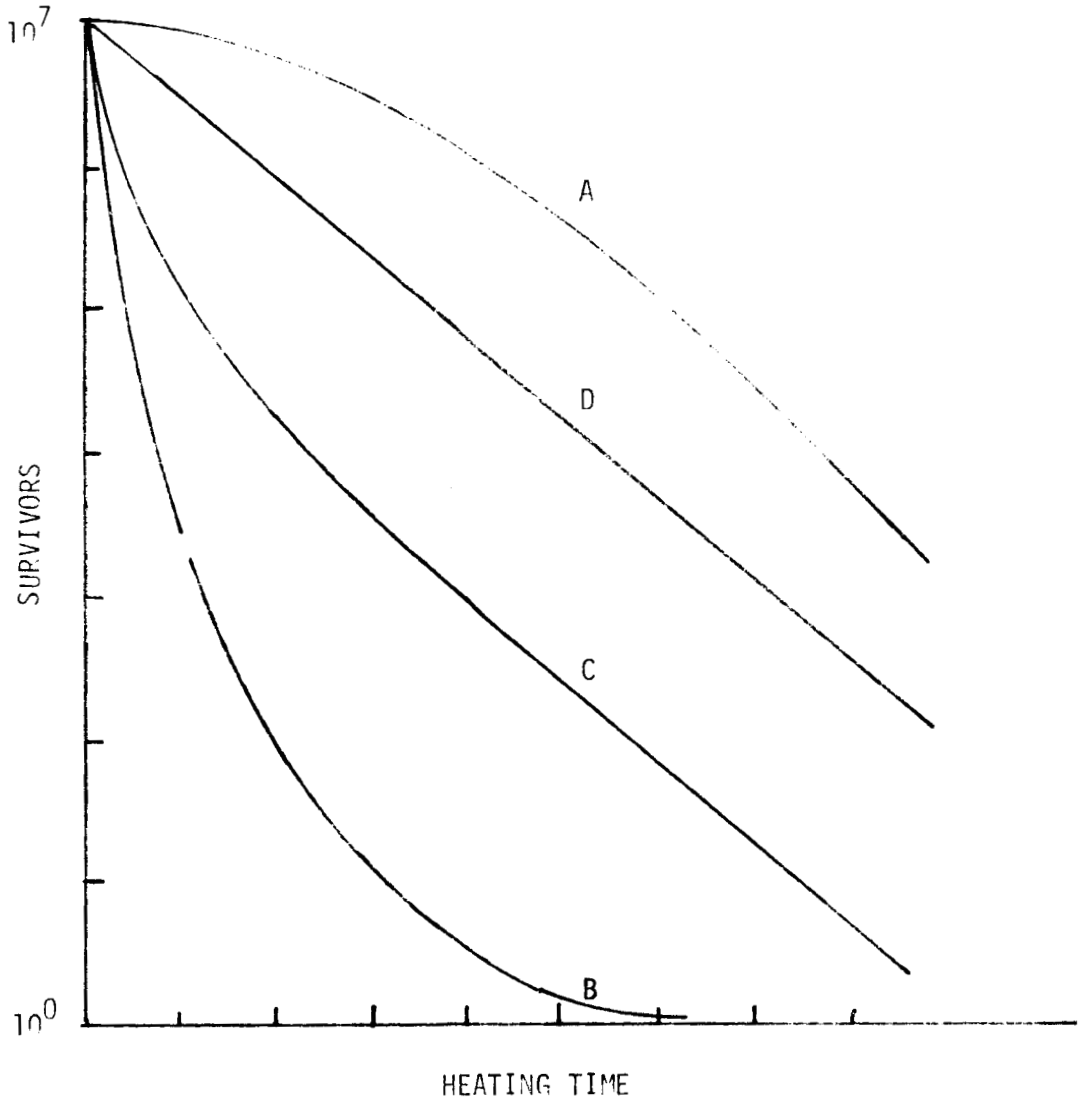


FIGURE 1. Expected survivors for various assumptions regarding number of vital molecular types and inactivation orders. Curve A: 1st order inactivation of 3 molecules of one type. Curve B: 2nd order inactivation of 3 molecules. Curve C: 1st order inactivation of 1 molecule, 2nd order inactivation of 1 molecule. Curve D: 1st order inactivation of 1 molecule.

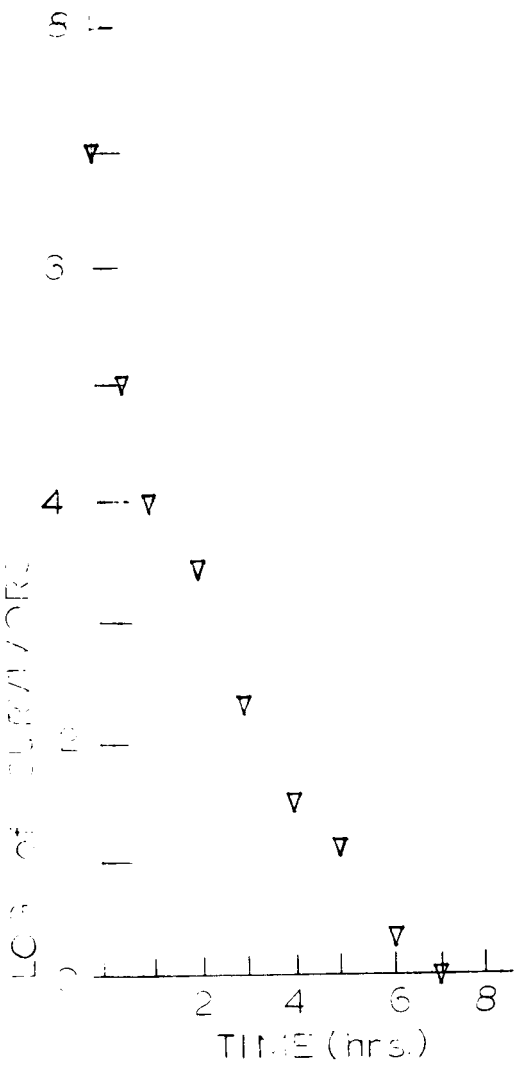


FIGURE 2

Effect of dry heat treatment at 125°C on dry spores of Bacillus coagulans on paper strips.

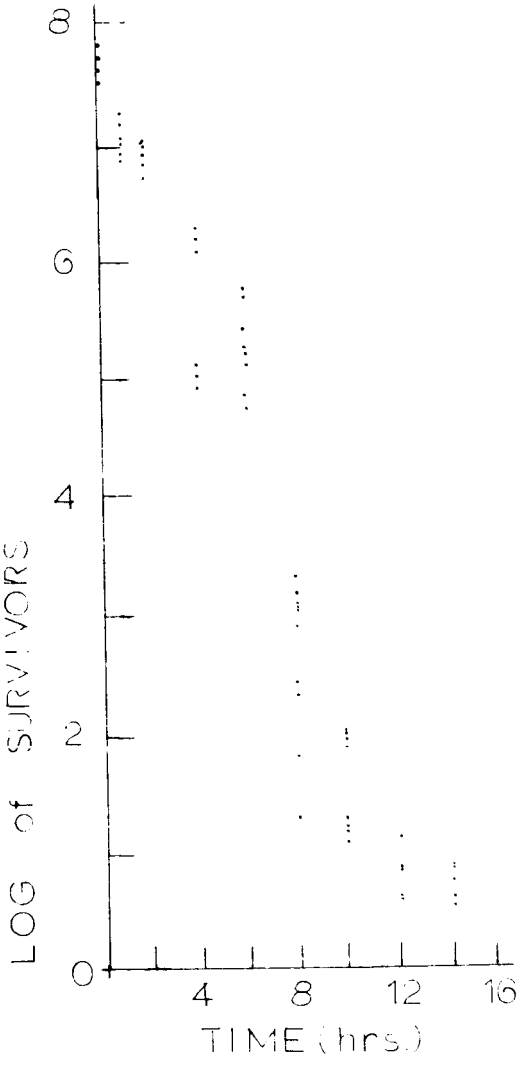


FIGURE 3

Effect of dry heat treatment at 125°C on dry spores of Bacillus subtilis var. niger on paper strips.

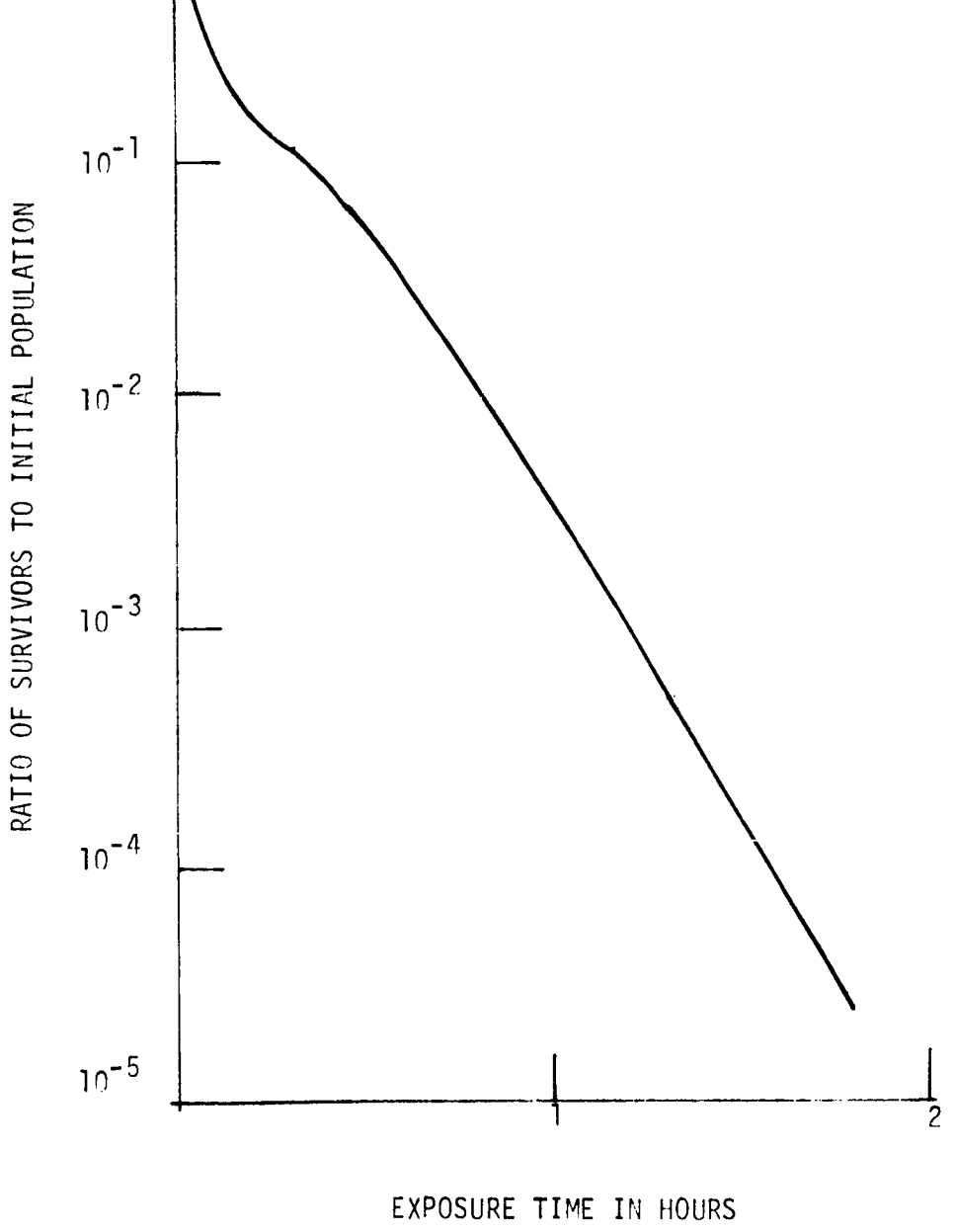


FIGURE 4. Expected survivors if death results from deactivation of 1 molecule by either 1st or 2nd order reactions with rate constants 6 hr^{-1} for the 1st order reaction and 50 $molec^{-1}hr^{-1}$ for the 2nd order reaction.

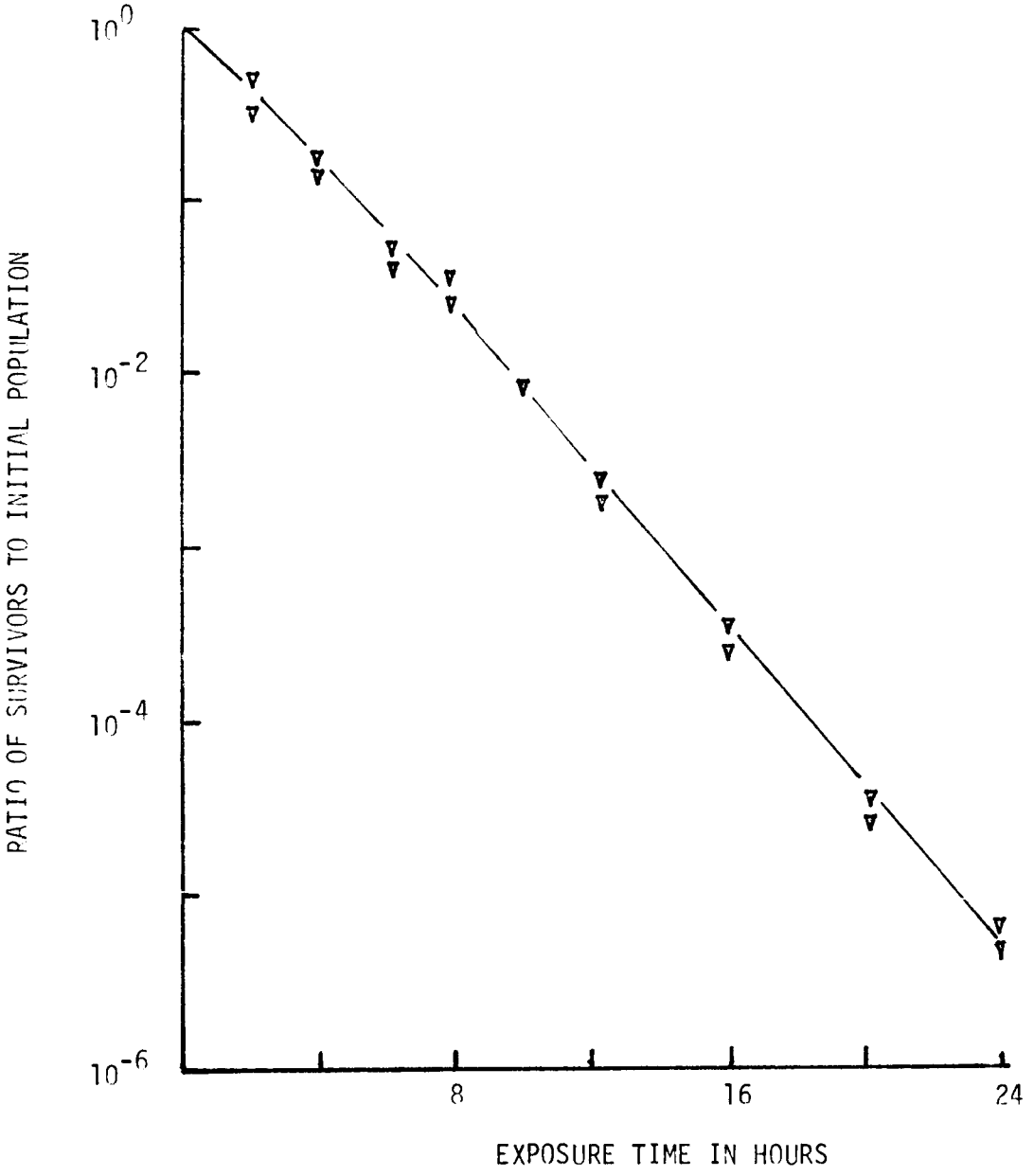


FIGURE 5. Comparison of empirical data for inactivation of Bacillus subtilis var niger at 106°C, ▽, to expected survivors if death results from inactivation of 2 molecules by 1st order reaction with rate constant .55 hr^{-1}. The solid curve represents computed expected survivors.

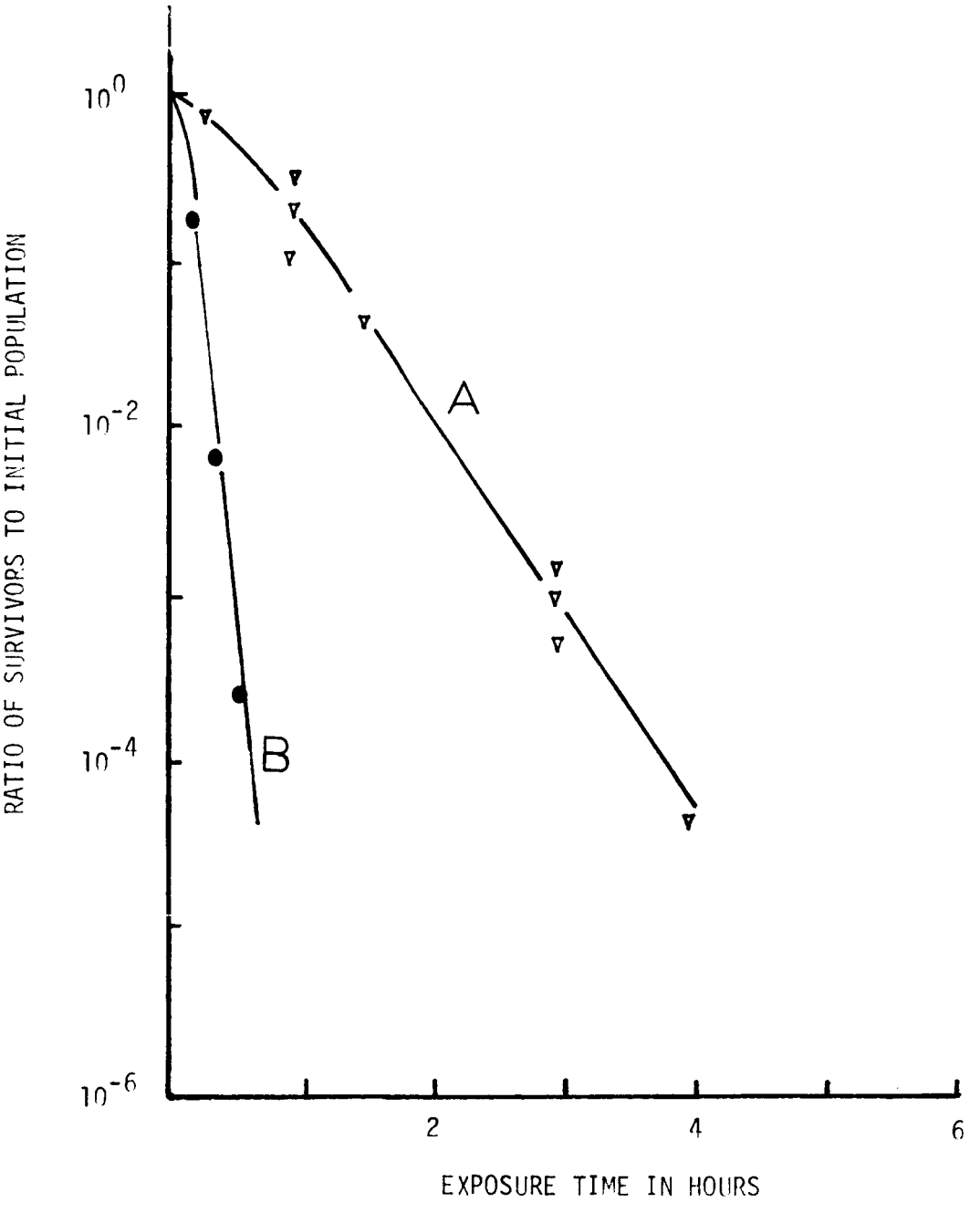


FIGURE 6. Comparison of empirical data for Bacillus subtilis var. niger at 120°C, ▽, and 135°C, ●, to expected survivors if death results from inactivation of 2 molecules by 1st order reaction with rate constants 2.65, Curve A, and 12.5, Curve B. The solid curves represent computed expected survivors with Curve B predicted by the Arrhenius equation.

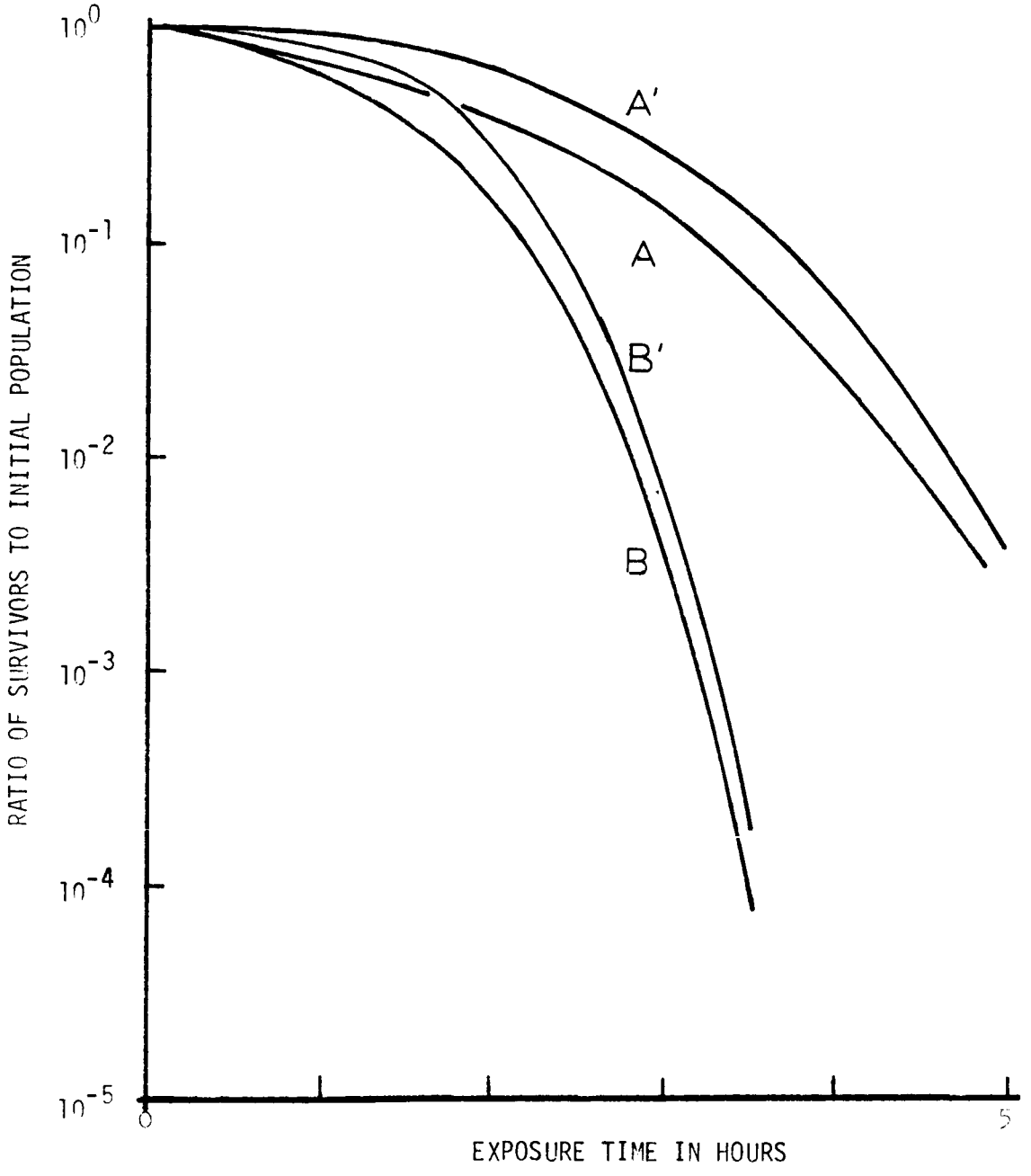


FIGURE 7. Survivor curves for various temperature profiles. A' and A - 8 hour heat up from 100°C to 135°C. B' and B - 4 hour heat up from 100°C to 135°C. A' and B' - 2 molecules inactivated by 1st order reaction. A and B - 1 molecule inactivated by 1st order reaction.

APPENDIX

PROGRAM FOR COMPUTING PROBABILITY OF SINGLE SPORE SURVIVAL TO TIME X UNDER LETHAL TEMPERATURE $T^{o}K$. THE PROGRAM IS IN G.E.BASIC.

```
5LETR=2
6REM R=NUMBER OF MOLEC.TO BE DEACTIVATED
10LETT=398
15LETW=(LOG(.55)-LOG(2.65))*379*392/14
20LETW=W*(1/T-1/379)+LOG(.55)
25LETD=EXP(W)
26REM D=REACTION RATE CONSTANT FOR TEMPERATURE T
30LETS=.1
35LETV=3
40PRINT"R=";R;"D=";D
45FORX=S TO V STEP S
50LETZ=(1-EXP(-D**))↑R
55LETY=1-Z
60PRINTX;Y
65NEXTX
70 END
```

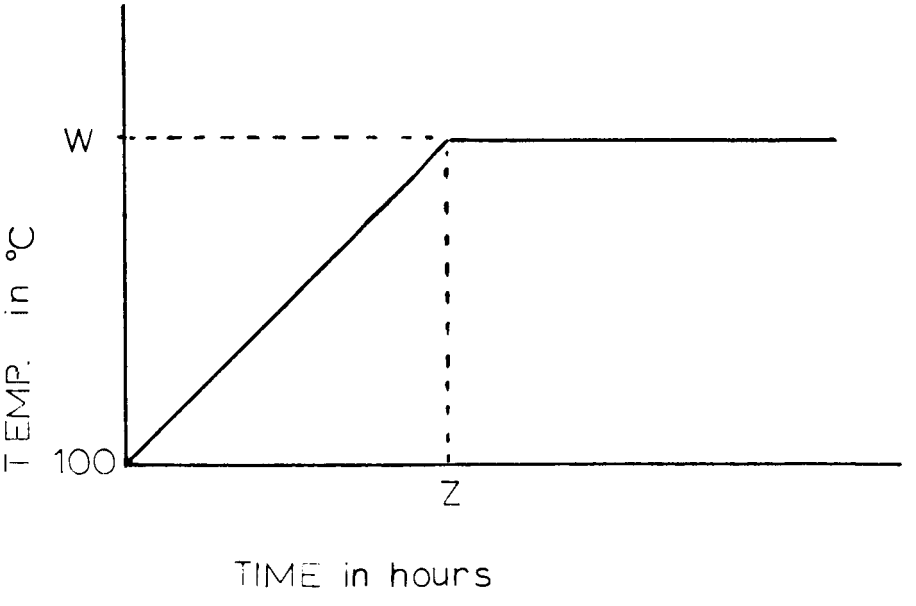
THIS IS THE PROGRAM USED TO COMPUTE THE $135^{o}C$ CURVE IN FIGURE 6 FROM THE REACTION RATE CONSTANTS FOR 106^{o} AND $120^{o}C$.

THIS IS THE PROGRAM USED TO COMPUTE THE CURVES IN FIGURE 7 FOR TEMPERATURE A VARIABLE.

```
5LETR=2
6REM R=NUMBER OF MOLEC.BEING DEACTIVATED
10LETTI=4
15LETS=.5
20LETA=(LOG(2.65)-LOG(.55))/(1/393-1/379)
25LETQ=1
26REM Q IS THE VALUE OF Q SUB I.
30FORX=S TO T1 STEP S
35LETZ=7/6
40LETW=135
41REM Z IS THE TIME TO GO FROM 100°C TO W°C
45LETW=273+W
50IFX>=Z THEN 70
55LETV=(W-373)/Z
60LETT=X*V+373
66REM T IS THE TEMP. AT TIME X
65 GO TO 75
70LETT=W
75LETB=(1/T-1/379)*A+LOG(.55)
80LETB=EXP(B)
81REM B=REACTION RATE CONSTANT FOR TEMP. T
85LETQ=.5*Q*(2-S*S*B*B)/(1+S*B)
90LETY=1-(1-Q)↑R
```

```
95PRINTX,Y
100NEXTX
105 END
```

THIS IS THE PROGRAM USED TO GENERATE THE CURVES IN FIGURE 7. THE TEMPERATURE PROFILE IS AS FOLLOWS:



Fourth order Runge-Kutta approximation for $q_i(t)$.

From equation 12,

$$q_i'(t) = -k(t,T(t))q_i(t).$$

$$t_n = nh$$

$$q_n = q_i(nh)$$

where h is the step size.

$$q_{n+1} = q_n + k_1/6+k_2/3+k_3/3+k_4/6+0(h^5)$$

where

$$k_1 = -hk(t_n,T(t_n))q_n,$$

$$k_2 = -hk[t_n+h/2,T(t_n+h/2)][q_n+k_1/2],$$

$$k_3 = -hk[t_n+h/2,T(t_n+h/2)][q_n+k_2/2];$$

$$k_4 = -hk[t_n+h,T(t_n+h)][q_n+k_3].$$

$k(t_n, T(t_n)$ is determined from Equation 9.

Intuitive basis for Equation 8.

Assume that a molecule may be inactivated by either a first or second order reaction but not by both. Assume that the probability of reaction of ith order, i = 1, 2, occurring between time t and t + dt is related to the rates at which the separate reactions occur. Thus we take, $\rho_1(t)$, the probability that first order reaction will occur to be

$$\rho_1(t) = \frac{-k_1 c_i(t)}{-k_1 c_i(t) - k_2[c_i(t)]^2} = \frac{k_1}{k_1 + k_2 c_i(t)} \qquad (a)$$

Similarly, we set

$$\rho_2(t) = \frac{k_2[c_i(t)]^2}{k_1 c_i(t) + k_2[c_i(t)]^2} = \frac{k_2 c_i(t)}{k_1 + k_2 c_i(t)} \qquad (b)$$

For Equation 8, we simplify equations a and b by setting

$$\rho_1 = k_1/(k_1 + k_2 n_i)$$

and

$$\rho_2 = k_2 n_i/(k_1 + k_2 n_i).$$

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