Evaluation of a Stochastic Inactivation Model for Heat-Activated Spores of Bacillus spp.*

Maria G. Corradini,1 Mark D. Normand,2 Murray Eisenberg,3 and Micha Peleg2*

Instituto de Tecnología, Facultad de Ingeniería y Ciencias Exactas, Universidad Argentina de la Empresa, Ciudad de Buenos Aires, Argentina; Department of Food Science, University of Massachusetts, Amherst, Massachusetts 01003; and Department of Mathematics and Statistics, University of Massachusetts, Amherst, Massachusetts 01003

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Heat activates the dormant spores of certain Bacillus spp., which is reflected in the “activation shoulder” in their survival curves. At the same time, heat also inactivates the already active and just activated spores, as well as those still dormant. A stochastic model based on progressively changing probabilities of activation and inactivation can describe this phenomenon. The model is presented in a fully probabilistic discrete form for individual and small groups of spores and as a semicontinuous deterministic model for large spore populations. The same underlying algorithm applies to both isothermal and dynamic heat treatments. Its construction does not require the assumption of the activation and inactivation kinetics or knowledge of their biophysical and biochemical mechanisms. A simplified version of the semicontinuous model was used to simulate survival curves with the activation shoulder that are reminiscent of experimental curves reported in the literature. The model is not intended to replace current models to predict dynamic inactivation but only to offer a conceptual alternative to their interpretation. Nevertheless, by linking the survival curve’s shape to probabilities of events at the individual spore level, the model explains, and can be used to simulate, the irregular activation and survival patterns of individual and small groups of spores, which might be involved in food poisoning and spoilage.

Heat inactivation kinetics of bacterial spores is a well-researched field. Much of the work on its relation to foods has focused on the heat-resistant spores of Clostridia, particularly those of Clostridium botulinum, which to this date serves as the reference organism in sterility calculations of low-acid foods (8, 32). The thermal resistance of Bacilli spores, although also extensively studied, has received less attention in the literature on food preservation. This is primarily because they are unlikely to germinate and produce cells that will survive and divide under the anaerobic conditions in a sterilized food container. Yet the mere possibility of viable Bacillus spores being present in processed foods has become an issue of food safety and a security concern. For this reason, there is a renewed interest in these spores’ heat resistance (2, 3, 6, 7, 16, 30). One of the peculiarities of certain Bacillus spores, like those of Bacillus sporothermodurans or Bacillus stearotherophilus, is that many of them can remain dormant unless activated by heat. The result is a survival curve that exhibits an “activation shoulder,” as shown schematically in Fig. 1 and with published data in Fig. 2. Thus, modeling this survival pattern, where the number of spores initially grows rather than declines, must account for the heat’s dual role of being a lethal agent and activator at the same time.

Traditionally, the thermal inactivation of both Clostridia and Bacilli spores has been thought to follow first-order kinetics (9, 12, 31), an assumption that has been frequently challenged in recent years (18, 21, 33, 35). The most publicized model of the simultaneous heat activation and inactivation of Bacillus spores in food is that proposed by Sapru et al. (24, 25), which is an improved version of models proposed earlier by Shull et al. (29) and Rodriguez et al. (23). All of these authors and others (1, 17) assumed that the activation of dormant spores follows first-order kinetics and so does their inactivation before and after activation. The temperature dependence of the corresponding exponential rate constants was assumed to follow the Arrhenius equation.

Peleg (18, 20) and van Boekel (33, 35) have shown that none of the above assumptions was necessary and that the same survival data on Bacillus stearotherophilus reported by Sapru et al. (25) and other investigators (5) can be described by different kinds of alternative four-parameter empirical models, which have a slightly better fit. This was evident not only visually (Fig. 2) but also as judged by statistical criteria (34). Fig. 2 shows the fit of the “double Weibullian” model proposed by van Boekel (33). It has the following form:

\[
\log S(t) = b_1 t^{n_1} - b_2 t^{n_2}
\]

where \( S(t) = N(t)/N_0 \) is the survival/activation ratio, \( N_0 \) and \( N(t) \) are the initial and momentary number of countable spores, respectively, and \( b_1, b_2, n_1, \) and \( n_2 \) are adjustable temperature-dependent constants. Figure 2 also shows the fit of an ad hoc empirical model, a hybrid between the double Weibullian model and one previously proposed (20) that can be written in the following form:
The equation for the log survival ratio is given by:

$$\log S(t) = \frac{a_1 t}{b_1 + t} - b_2 e^{t c_2}$$  \hspace{1cm} (2)

or

$$\log S(t) = \frac{a_1 t}{b_1 + t} - \left(\frac{t}{t_{c_2}}\right)^{m_2}$$  \hspace{1cm} (3)

where $a_1, b_1, t_{c_2},$ and $m_2$ are adjustable temperature-dependent parameters. According to this model, $a_1$ is the asymptote of the first term on the right, $b_1$ is a time characteristic of the activation, $t_{c_2}$ is a characteristic time of the inactivation, and $m_2$ is a parameter that represents the curve’s postpeak concavity. The structure of equation 2 or 3 dictates that the number of dormant spores must be finite and cannot exceed $N_0 \times 10^{a_1},$ if the logarithm is base 10, or $N_0 \times \exp(a_1),$ if it is base $e.$ (A demonstration that generates realistic-looking activation/inactivation curves using equation 3 as a model is available from Wolfram Research [http://demonstrations.wolfram.com/SurvivalCurvesOfBacilliSporesWithAnActivationShoulder/].)

Corradini and Peleg (5) proposed a way to estimate the initial number of dormant spores from survival curves having an activation shoulder using a similar model, which was originally described in Peleg (20). They suggested that the intersection of a tangent to the survival curve drawn at its postpeak region with the time axis (Fig. 1) is not a recommended method to estimate the number of dormant spores and that it can render unrealistically high values if used. Also, where there is no evidence that the survival curve in the postpeak region ever becomes a straight line; the same survival curve will yield different estimates of the dormant spores’ initial number depending on the experiment’s duration. Moreover, if in the postpeak region the survival ratio drop rate progressively increases, as it most probably does (Fig. 2) (20, 33), then the number of dormant spores estimated by the tangent extrapolation method will grow indefinitely, despite the fact that it must be finite (1). Also, since the exponential inactivation rate can be a function of time as well as of temperature, the applicability of the Arrhenius equation as a secondary model might come into question. The same can also be said about the log-linearity of the $D$ value’s temperature dependence if used instead of the Arrhenius equation.

The question that arises in light of all the above is whether one can construct a conceptual population dynamic model of the activation/inactivation of spores without assuming any fixed kinetic order. The biochemical and biophysical mechanisms that govern bacterial spore germination, activation, and inactivation have been thoroughly investigated (11, 13, 14, 15, 22, 26–28). Still, it is not clear how processes within an individual spore can be translated into activation and survival patterns at the population level and how their manifestation can be expressed in a mathematical model. Whenever a system has inherent variability and knowledge of its working is incomplete or merely insufficient to develop a model from basic principles, one can, and sometimes must, resort to a probabilistic modeling approach. The general objective of this work has been to explore the merits and limitations of this option by developing a stochastic model of Bacilli spores’ heat activation and inactivation and examining its properties. The goal has not been to
develop a new method to predict the spores’ survival under dynamic conditions—rate versions of the existing empirical models such as equation 1, 2, or 3 seem to be quite suitable for that—but to offer an alternative interpretation of the patterns reported and discussed in the literature.

**METHODS**

Theoretical background. Consider a *Bacillus* spore population consisting of active and dormant spores only, i.e., with no vegetative cells. For simplicity, we shall assume that within their respective groups, the active and all the dormant spores can be treated as initially indistinguishable. We shall assume also that once exposed to heat at levels used in thermal preservation of food, a spore can be in only three distinct states: active, countable and dormant, and therefore uncountable, or inactivated ("dead"), in which case it will also be uncountable. In other words, we shall deal only with scenarios where adaptation, injury, and recovery from injury play no role. We shall discuss first the potential fate of individual active and dormant spores and then the fate of their assemblies.

(i) The inactivation of an initially active spore. A schematic view of the potential states of an individual active spore is given in Fig. 3 (4, 10). It shows that after a short time interval, $\Delta t$, the spore can be inactivated (become dead) with a probability of $P_n(i)\Delta t$ (the subscript $n$ stands for mortality) or survive with a probability of $1 - P_n(i)\Delta t$, where $P_n(i)$ is the probability of inactivation within that time interval. Because $P_n(i)$ is the inactivation probability per unit time, it has time-reciprocal units. The probability rate $P_n(i)$ can, but need not, be constant; it would most likely vary with time as the heat process proceeds, especially if the temperature rises or falls. Also, while the inactivation probability rate $P_n(i)$ can assume values greater than 1, the inactivation probability $P_n(i)\Delta t$ cannot; i.e., $0 \leq P_n(i)\Delta t \leq 1$ (10). If a spore survived the first exposure (Fig. 4), then after a second time interval $\Delta t$, it can be inactivated (die) with a new (or same) probability, $P_n(i + \Delta t)\Delta t$, or remain viable with a new (or same) probability, $1 - P_n(i + \Delta t)\Delta t$. After a third time increment $\Delta t$, the probability "game" will be repeated, with a new (or same) $P_n(i + 2\Delta t)\Delta t$, and so on. The iterations will continue until the spore is inactivated, at which point the process will come to an end. The effect of different $P_n(i)$ functions on the survival patterns that emerge is discussed elsewhere (4, 10). Suffice it to say here that the survival probability of an active spore at $t = 0$ is $1$ by definition, and after $i = 1, 2, 3 \ldots n$ time increments of equal duration $\Delta t$ (Fig. 4), the survival probability becomes

$$P(n) = \prod_{i=1}^{n} (1 - P_n(i)\Delta t)$$ (4)

For a noninteger time, we can use an interpolation function as an approximation.

In Mathematica (Wolfram Research, Champaign, IL), the software used in this work, we can write the model (equation 3) in the following form: $pn = \text{Table}[(i, \text{algorithm}(i, \Delta t), (i, 0, n))$, where $pn$ is the probability of finding a viable spore after $n$ unit time intervals. Once done, the program calculates and returns the values of the probability $pn$ for all integer values of time between zero and the chosen integer time ($t = n$). We now define the semicontinuous version of $P(t)$ as Interpolation[$pn$]. Its execution by Mathematica renders $P(t)$ as an InterpolatingFunction object, which can be plotted and which for all practical purposes is treated by Mathematica as an ordinary function (like Log[x] or Exp[x], for example). Readers who are interested in the program can contact the authors for a more detailed explanation. Since $P(t)$ is the probability of finding a viable spore after time $t$, the plot of $P(t)$ versus $t$ in this case is the spores’ survival curve. Examples of survival curves produced by the procedure with equation 4 as a model are shown in Fig. 5. For large populations, one can construct the alternative presentation of the survival curve, i.e., as $N(t)$ versus $t$, by treating $P(t)$ as the survival ratio $\Delta t = N(t)/N(0)$, where, as before, $N(t)$ and $N(0)$ are the initial and momentary number of spores. Similarly, log $P(t)$ can represent log $S(t)$ for constructing the semilogarithmic survival curve, as shown in Fig. 5.

For an individual spore or small group of spores, one has to use the stochastic, discrete, form of the model in the following manner (4, 10). Suppose for the first unit time ($\Delta t = 1, P_n(0) = 0.3$). The first step is to draw a random number, $0 \leq R_{10} \leq 1$, with equal probability distribution. If $R_{10} > 0.3$, the spore is inactivated and leaves the game. If $R_{10} > 0.3$, the spore survives and passes to the next round. We now draw a new random number, $R_{02}$. Suppose that for the second $\Delta t$, $P_n(1 + \Delta t) = 0.4$. Then, if $R_{02} \leq 0.4$, the spore dies off, and the process ends. Otherwise, if $R_{02} > 0.4$, the spore survives and passes to the next round. The iterations continue in this manner, with a newly drawn $R_n$ and a corresponding $P_n(i)$ at each one, until the spore is inactivated and brings the process to its end. Generated survival curves of small numbers of individual spores produced by this algorithm are shown in Fig. 6. The figure demonstrates that the irregular and irreproducible pattern exhibited by a small group of spores becomes more regular and deterministic as the group size increases. (The plots having different shades of gray and Roman numerals represent different experiments.)

(ii) The activation and inactivation of dormant spores. After a time interval $\Delta t$, a dormant spore exposed to heat can be dead (inactivated), still dormant and uncountable, or activated and hence countable. According to the stochastic ap-
whether it is inactivated. Since we allow for only the above three states to exist, the activation modes of dormant activated and initially active spores are hard to find. Therefore, for simplicity, we will consider a scenario where \( P_m(t) \) is the same for dormant and activated spores and where the activation probability function, \( P_a(t) \), like \( P_m(t) \) is a function of time only; i.e., it is the same in all the branches of the probabilities tree. As before, we shall set all the \( \Delta \) values to be of a unit time duration, i.e., \( \Delta t = 1 \), and define the probability rate functions, \( P_m(t) \) and \( P_a(t) \), accordingly.

For this simplified scenario, the probability of finding a viable spore after an integer number of time intervals \( n \), i.e., after time \( t = n \times 1 = n \) is calculated as follows:

\[
P(0) = 0
\]
\[
P(1) = P_a(1)
\]
\[
P(2) = [1 - P_a(1) - P_m(1)P_a(2) + P_m(1)][1 - P_m(2)]
\]
\[
P(3) =
\]
\[
[1 - P_a(2)]P_m(3) + [1 - P_a(1)][1 - P_m(2)][1 - P_m(3)] + P_m(1)[1 - P_m(2)][1 - P_m(3)]
\]
\[
P(4) =
\]
\[
[1 - P_a(1) - P_m(1)][1 - P_a(2) - P_m(2)][1 - P_m(3) - P_m(4)]P_m(4) + [1 - P_a(1) - P_m(1)][1 - P_a(2) - P_m(2)][1 - P_m(3)][1 - P_m(4)] + [1 - P_a(1) - P_m(1)][1 - P_m(2)][1 - P_m(3)][1 - P_m(4)] + P_m(1)[1 - P_m(2)][1 - P_m(3)][1 - P_m(4)]
\]
\[
P(5) =
\]
\[
[1 - P_a(1) - P_m(1)][1 - P_a(2) - P_m(2)][1 - P_m(3) - P_m(4)][1 - P_m(5)] + [1 - P_a(1) - P_m(1)][1 - P_a(2) - P_m(2)][1 - P_m(3)][1 - P_m(4)][1 - P_m(5)] + [1 - P_a(1) - P_m(1)][1 - P_m(2)][1 - P_m(3)][1 - P_m(4)][1 - P_m(5)] + P_m(1)[1 - P_m(2)][1 - P_m(3)][1 - P_m(4)][1 - P_m(5)]
\]
\[
P(n) =
\]
\[
[1 - P_a(1) - P_m(1)][1 - P_a(2) - P_m(2)][1 - P_m(3) - P_m(4)][1 - P_m(5)][1 - P_m(n)] - P_m(4)[1 - P_m(5) - P_m(5)] \ldots \ P_m(n)
\]
\[
+ [1 - P_a(1) - P_m(1)][1 - P_a(2) - P_m(2)][1 - P_m(3) - P_m(4)][1 - P_m(5)][1 - P_m(4)] - P_m(4)[1 - P_m(5)][1 - P_m(6)] \ldots \ P_m(n) - 1 - [1 - P_m(n)]
\]
\[
+ [1 - P_a(1) - P_m(1)][1 - P_a(2) - P_m(2)][1 - P_m(3) - P_m(4)][1 - P_m(5)][1 - P_m(6)] \ldots \ P_m(n - 2) - [1 - P_m(n - 1)][1 - P_m(n)]
\]
\[
\vdots
\]
\[
+ [1 - P_a(1) - P_m(1)][1 - P_m(3)][1 - P_m(4)] \ldots \ [1 - P_m(n - 2)] + [1 - P_a(1) - P_m(1)][1 - P_a(2)][1 - P_m(3)][1 - P_m(4)] \ldots \ [1 - P_m(n - 1)] + [1 - P_a(1) - P_m(1)][1 - P_m(2)][1 - P_m(3)][1 - P_m(4)] \ldots \ [1 - P_m(n - 3)] + [1 - P_m(n - 2)][1 - P_m(n - 1)][1 - P_m(n)]
\]

(7)

FIG. 5. Two Weibullian curves and one log-linear survival curve of active or activated spores generated with equation 4 as a model. Notice that a monotonically increasing mortality probability rate \( P_m(t) \) (top) produces a survival curve with downward concavity when plotted on semi-logarithmic coordinates (bottom). Decreasing mortality probability rate produces a survival curve with upward concavity. A log-linear survival curve is a special case where the mortality probability rate is constant. Also notice that the difference between the three modes of inactivation is hardly revealed when the survival curves are plotted on linear coordinates (middle).
A more realistic or accurate model will have to account for the fact that $P_a(t)$ might not be the same in the different branches and that the mortality probability rate function, $P_m(t)$, might be, and most likely is, different for dormant and activated cells. However, implementation of these considerations in the model formulation will render its mathematical expression even more cumbersome, probably to the point of total impracticality even as a tool to convey the concept.

Assigning each branch with a separate set of probabilities will also make their estimation from experimental data an almost impossible task (see below). The two curves marked with Roman numerals are different runs representing replicates. Notice that as the number of spores increases, the curve becomes smoother, and the survival pattern is more deterministic.

Figure 7 demonstrates that as the number of steps $n$ increases, so does the number of branches and with it the number of possibilities. Actually, the number of terms in equation 7 is always $n!$, and each is constructed from the probability rate values of the previous steps. For a large $n$, and low $P_a(t)$ values, this may have an impact on the computing time, which increases accordingly.

**RESULTS AND DISCUSSION**

We describe below the generation of the activation/inactivation curves of dormant spores by the models mentioned above.

The discrete model. Consider a group of 5, 10, or 50 dormant spores whose activation and inactivation follow the stochastic (discrete) model as expressed in equation 7. For the demonstration, we have assumed that the probability rate functions $P_a(t)$ and $P_m(t)$ are described by the plots shown at the top of Fig. 8; that is, the activation probability rate declines monotonically with time while the inactivation (mortality) probability rate monotonically rises. The plots shown in the middle of Fig. 8 are the simulated survival curves of groups of 5, 10, and
50 spores in a process of five time units’ duration. The three curves in each plot are a separate (repeated) run, representing hypothetical experimental replicates. The plots at the bottom of Fig. 8 show the number of activated spores of the same groups of initially dormant spores. As in pure inactivation (Fig. 6), the irregular and irreproducible activation/inactivation pattern, which is so clearly evident in very small groups of spores, became much smoother and predictable as the group’s size increased. Note that Fig. 8 shows only the simulated fate of the spores that were initially dormant. In simulations of real-life scenarios, the number of viable spores, which had already been active, has to be added. The fate of these active spores, though, is governed by the “pure survival model,” which is expressed by equation 4. According to the data of Sapru et al. (24) and Palop et al. (17) (see also Corradini and Peleg [5]), the number of dormant spores can exceed the number of active ones by about 10-fold. Examples of simulated survival curves using a smaller ratio of 5 to 1 dormant-to-active spores are shown in Fig. 9. They demonstrate that, theoretically at least, the existence of spores in two states (active and dormant) might not always be revealed, especially when only a small number of spores is present. This is due to the irregularity and irreproducibility of the survival pattern in small groups. Thus, if \( P_a(t) \) is small relative to \( P_m(t) \), the observed survival curve might be indistinguishable from that of a group of active spores only. Or, conversely, if \( P_a(t) \) is large relative to \( P_m(t) \), the observed survival curve might be indistinguishable from that of a group of dormant spores only.

The semicontinuous model. Examples of activation/survival curves of dormant spores using the fully deterministic semicontinuous version of the probabilistic model are given in Fig. 10. (The curves were generated by performing Mathematica’s Interpolation operation on the discrete sets of probabilities calculated with equation 6 as a model.) Since for large populations the survival ratio value \( S(t) \) is equal to \( P(t) \), the curves could be calculated directly with the model’s equation and plotted. Figure 10 demonstrates how the activation and inactivation (mortality) probability rate functions, \( P_a(t) \) and \( P_m(t) \), affect the shape of the activation/survival pattern of dormant spores. As shown in Fig. 10, the model can produce semilogarithmic survival curves with an activation shoulder, which are visually very similar to those reported in the literature, for example, by Sapru et al. (25), which are shown in Fig. 2. The survival ratio’s postpeak drop, which is also shown in Fig. 10, need not be log linear, again in agreement with the experimental observations by Sapru et al. (25) and others. In principle, the model can be used to estimate the parameters of the underlying probability rate functions \( P_a(t) \) and \( P_m(t) \) from experimental activation/survival data by regression or other minimization methods. But because \( P_a(t) \) and \( P_m(t) \) must have at least two (temperature-dependent) parameters each and because the ratio between the initially active and dormant spores is usually unknown a priori, the experimental survival data for extracting these functions must be dense and with very low scatter for such methods to succeed. Thus, for describing isothermal survival curves, empirical four-parameter models like equations 1 and 2 have obvious practical advantages. And because they are identified as empirical from the outset, no kinetic or mechanistic assumptions are required for estimating their parameters. Moreover, since such models need not be unique, as demonstrated in Fig. 2, they can be chosen in light of purely pragmatic considerations. However, as stated earlier, at least theoretically, such empirical models can also be used to predict dynamic activation/inactivation patterns (20) and, at least in principle, to estimate the ratio of dormant to initially active spores (5). Therefore, the main value of the presented stochastic model is that it provides theoretical support to the notion that the first-order kinetics assumption is unnecessary to explain or quantify the activation shoulder observation and that there must be cases where this assumption would be unwarranted on theoretical grounds. According to the model, the observed curvature in the semilogarithmic survival curves at its
"postshoulder" stage is not an experimental artifact but a consequence of the manner in which the spores are inactivated (4). Yet the model does not preclude the existence of a postpeak log-linear region in the survival curve. In fact, it would even predict it if, after a sufficiently long time, \( P_a(t) \) becomes or approaches zero and \( P_m(t) \) assumes a constant value. But while one can take for granted that the first condition \([P_a(t) \to 0]\) might almost always be satisfied, this need not be the case with \( P_m(t) \) approaching a constant value. It would be much more likely that when the temperature is sufficiently high, \( P_m(t) \) will progressively rise as increased damage accumulation will show its effect. This is manifested in the recorded survival curve’s downward concavity, as can also be seen in Fig. 2, which shows no sign of disappearing, at least within the experiment’s duration. However, none of the above excludes the theoretical possibility that the survival curve’s postpeak continuation will have upward concavity (tailing). If a significant fraction of the surviving spores are heat resistant at the particular temperature, their \( P_m(t) \) values will decrease with time, and the drop of the survival ratio will progressively slow down. Thus, to make a case for the first-order kinetics’ universality, i.e., to claim that the mortality probability rate always becomes a constant, one must come with compelling experimental evidence and convincing theoretical argument that this indeed must happen.

For similar reasons, the stochastic model questions the utility of the tangent extrapolation method as a means to estimate the number of dormant spores, as presented in several food microbiology textbooks. Even if the survival curve does end up in a straight line, which according to the above is possible, the relation between this line’s slope and intercept and the initial number of dormant spores would have to be explained. At this time, experimental evidence of the mere existence of such a relation is still absent, and it is doubtful that it will be forthcoming any time soon.

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**FIG. 8.** Simulated survival and activation curves of groups of 5, 10, and 50 dormant spores generated with the discrete version of the activation/survival model (equation 6). The three curves marked with Roman numerals are different runs representing replicates. Notice that as the number of spores increases, the curves become smoother, and the patterns are more deterministic.
Estimation of the activation and inactivation probability rate parameters from experimental data. The probabilistic activation/inactivation model, equation 7, differs from models developed from growth/mortality (10) or inactivation only (4) in that it is not expressed as an algebraic equation. Consequently, its parameters cannot be retrieved from experimental activation/inactivation data by standard nonlinear regression methods. Moreover, the initial number of dormant spores can only be roughly estimated from the survival curve, which records only the number of the active spores at any given time (5). However, one can determine the total initial number of spores directly by microscopy, for example, and, separately, culture and count those that have been already active at time zero. The difference between the two counts can then be considered the initial number of dormant spores. Based on the assumption that all the dormant spores were viable at the beginning of the experiment. Estimates reached by this method indicate that dormant *Bacillus stearothermophilus* spores can outnumber the initially active ones by a factor as large as 10 to 14 (17, 24); i.e., they can constitute 90 to 93% of the total. In light of this observation, and for the sake of simplicity, we shall assume that their contribution to the total viable spores’ counts had most likely been overshadowed by the experimental scatter (see below). We’ll also assume that the probability rate functions $P_a(t)$ and $P_m(t)$ were similar to those used in the simulations shown in Fig. 9, i.e., that $P_a(t)$ decreased in a logistic fashion while $P_m(t)$ monotonically rose. These patterns can be described with three- and two-parameter models, respectively, whose values can be estimated from simulated or actual experimental data by minimizing the mean squared distance between the data points, and the curve can be generated with the semicontinuous version of equation 7 as a model. This was done with a minimization program based on the Simplex algorithm especially written for the purpose in Mathematica. The initial number of dormant spores ($32.7 \times 10^8$) was taken from Sapru et al. (24). (The set of suitable initial parameters estimates [“guessed values”] was found by matching the experimental data with a curve created by the model’s equation with coefficients adjusted by moving sliders on the screen using Mathematica’s Manipulate function.)

The fit of the semicontinuous version of equation 7 to activation/inactivation data sets obtained at two temperatures is shown in Fig. 11. This figure also shows the corresponding estimated $P_a(t)$ and $P_m(t)$. It demonstrates that it is possible, at least in principle, to estimate these probability rate functions from experimental survival data, even with a considerable scatter, provided that the initial number of dormant spores has been determined independently. When different mathematical
expressions having similar characteristics were used to describe \( P_a(t) \) and \( P_m(t) \), the method rendered similar underlying probability rate functions (4, 10). Once estimated in this way, such functions can be used to simulate the fate of small groups of spores and assess their potential food safety implications or role in spoilage. How reproducible the activation patterns at a given temperature are and whether the temperature effect on the activation and mortality rate function shown in Fig. 11 is typical are questions that can be answered only on the basis of a larger experimental base than the one available to us. Experimental procedures that will reduce the data scatter would be very helpful. If the scatter could be reduced, the described method could be also used to quantify variations in the activation and inactivation patterns and to determine how they might be affected not only by temperature but also by the medium, its pH, the spores’ history, etc.

We should mention that even with suitable initial estimates of the model parameters, application of the Simplex algorithm could take a few minutes to render a fit with a personal computer of the current generation. In contrast (Fig. 2), empirical models with only four adjustable parameters could fit semilogarithmic survival curves and perform this task much faster. Thus, once the temperature dependence of such an empirical model’s parameters has been determined, it can be used to estimate the efficacy of dynamic heat processes, at least theoretically (18, 20). The probabilistic model, therefore, is not an effective tool to calculate thermal sterility in industrial processes where Bacillus spores are the target—and it is not intended for this purpose. Its main value is primarily conceptual: it explains the activation shoulder’s emergence without resorting to the traditional kinetic assumptions. It also demonstrates that the postpeak part of the survival curve can be log linear or approximately log linear, as has been reported in the literature, but it does not have to. The probabilistic model’s potential utility could be in simulations of the fate of single spores.

FIG. 10. Simulated activation/inactivation curves of 100 dormant spores generated with the semicontinuous version of the model (equation 7) plotted on linear and semilogarithmic coordinates. Notice that a very similar survival pattern would be observed had a fraction of initially active spores been added.
or small groups of spores, which the traditional kinetic models do not address.

**Isothermal versus dynamic conditions.** Most primary models of spore inactivation (including equations 1 and 2) are in the form of an algebraic equation. Commonly, this equation is used as a model to fit a set of isothermal survival data by regression in order to calculate the organism’s or spore’s survival parameters and their temperature dependence. The latter is described by what is known as “secondary models,” like the traditional Arrhenius equation or an ad hoc empirical relationship (18, 19). The secondary models are incorporated into a rate model (differential) equation in order to simulate or predict the survival pattern under dynamic conditions, i.e., when the temperature varies with time. Because of the way the probabilistic model is constructed, it has the same form for isothermal and dynamic conditions. This is true for both its discrete and continuous versions. The only change is that the probability rate functions become nested terms: i.e., $P_a(t) = P_a[T(t)]$ and $P_m(t) = P_m[T(t)]$, where $T(t)$ is the momentary temperature during the heat treatment. This is similar to a conventional kinetic survival model when it is expressed as a rate equation for isothermal conditions too. However, since the presented probabilistic model in both forms has been developed as a concept rather than as a replacement for current methods to calculate sterility, we have not elaborated on this aspect. Nonetheless, the probabilistic model can be used to quantify the effect of temperature on the activation and inactivation pattern by modifying the probability rate functions, $P_a(t)$ and $P_m(t)$, and hence the ratio between them.

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**REFERENCES**


FIG. 11. Activation/survival of *B. stearothermophilus* spores at 105 and 110°C described by the semicontinuous version of equation 7 (top) and the corresponding activation and mortality rate functions $P_a(t)$ and $P_m(t)$, respectively (bottom). The original data are from Sapru et al. (24).