Estimating the Survival of *Clostridium botulinum* Spores during Heat Treatments

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ABSTRACT

A recently published study of the inactivation of *Clostridium botulinum* spores at various temperatures in the range of 101 to 121°C and neutral pH revealed that their semilogarithmic survival curves all had considerable upward concavity. This finding indicated that heat inactivation of the spores under these conditions did not follow a first-order kinetics and that meaningful *D* values could not be calculated. The individual survival curves could be described by the cumulative form of the Weibull distribution, i.e., by log *S* = −*b*(T)*m(T)*, where *S* is the survival ratio and *b*(T) and *m*(T) are temperature-dependent coefficients. The fact that at all temperatures in the above range *m*(T) was smaller than 1 suggested that as time increases sensitive members of the population perish and survivors with increasing resistance remain. If damage accumulation is not a main factor, and the inactivation is path independent, then survival curves under monotonously increasing temperature can be constructed using a relatively simple model, which can be used to calculate the spores’ survival in a limiting case. This is demonstrated with computer-simulated heating curves and the experimental constants of the *C. botulinum* spores, setting the number of decades reduction to 8, 10, and 12 (the current criterion for commercial sterility).

The heat inactivation of vegetative microbial cells and bacterial spores has been traditionally described in terms of first-order kinetics (5, 7, 8, 12, 13). Often referred to as the mechanistic theory, it is based on the assumption that all the cells or spores in a population have identical heat resistance. This model has served the food industry for many years and, to date, forms the basis for the calculation of commercial thermal processes. The model is mathematically simple and can be described as follows:

\[-dN/dt = -k'N\]  

where *N* is the number of surviving spores after an exposure time *t* and *k* is a rate constant (time<sup>−1</sup> units).

Integration of equation 1 yields the following:

\[N = N_0 \exp(-k't)\]  

where *N<sub>0</sub>* is the spores’ initial number.

The survival ratio, *S* = *N/N<sub>0</sub>* is therefore

\[S = \exp(-k't)\]  

or

\[\log_e S = -k't\]  

or

\[\log_{10} S = -kt\]  

where *k* = *k'*/log<sub>10</sub> 10

According to this model, a plot of the survival ratios versus time relationship on semilogarithmic coordinates is expected to be a straight line with a slope of −*k* or −*k’*, depending on the logarithm’s base. The slope of log<sub>10</sub>*S* versus *t*, i.e., the time to accomplish 90% reduction in the population (one 10-based log cycle) is known as *D* and is considered a measure of the organism or spores heat resistance. The thermal death time, *τ*, is defined as the length of time at a given temperature necessary to completely destroy a definite concentration of cells or spores (7). In the case of the sterilization process or “botulinum cook,” it is determined by extrapolation of the log<sub>10</sub>*S* versus time curve to −12 (11):

\[\tau = 12/D\]  

There has been growing evidence that microbial survival curves, including bacterial spores of food safety concern, are not always linear, in which case assumption of the first-order mortality kinetics becomes inappropriate. Moreover, since the experimental log<sub>10</sub>*S* versus *t* relationships, which were used to determine the *D* values in the past, usually covered only five to seven decades of reduction in the sporal population, the question of whether their extrapolation to log<sub>10</sub>*S* = −12 is justified has also been raised (4). As long as the semilogarithmic survival curve is concave downward (Fig. 1), a heat treatment based on its extrapolation to log<sub>10</sub>*S* = −12 assuming linearity will result in overprocessing. However, if the log<sub>10</sub>*S* versus *t* relationship has an upward concavity (Fig. 1), then its extrapolation assuming linearity can result in underprocessing and hence in a safety risk. The risk itself can be high or low, depending on the degree of concavity and on whether the 12 decades of reduction in the spore population is a realistic criterion of sterility. This issue, although pertinent to our dis-
FIGURE 1. Schematic view of microbial survival curves generated with the cumulative Weibull distribution as a model. S is the survival ratio.

Nonlinear, semilogarithmic survival curves have commonly been treated in three ways: as an indication of a mixed population where each of its components has a first-order inactivation kinetics, as evidence that the spores (or cells) in question have an inactivation pattern governed by a higher kinetic order or a different kind of kinetics, or as evidence that the microbial population has a spectrum of heat resistances. According to the third approach the survival curve, that is the S versus t relationship, is the cumulative form of an underlying distribution of temporal lethal events, which is at least partly a manifestation of the spores’ (or cells’) biovariability. This approach was taken by Anderson et al. (1), who used a logistic distribution to model the thermal inactivation of Clostridium botulinum between 101 and 121°C. In principle, a number of different distributions, such as logistic, log normal, beta, or log beta, could serve as models to describe a population spectrum of resistances or of the inactivation events. In addition, it might be expected for the shape of the underlying distribution to change, depending on the organism and the heating conditions. In this respect, the Weibull distribution has been found to be a more convenient model (11), whose main advantages are mathematical simplicity and flexibility (see below). When applied to survival data, the cumulative form of the Weibull distribution is as follows:

\[ S = \exp - (bt^n) \]  

or

\[ \log_{10} S = -bt^n \]

where \( b = b'/\log_{10} e \).

A linear \( \log_{10} S \) versus \( t \) relationship, the basis of the first-order kinetic interpretation, is only a special case of equation 8, with \( n = 1 \) and \( k = k(11) \). According to equation 8, concave upward \( \log_{10} S \) versus \( t \) curves are produced when the underlying distribution has \( n < 1 \) and concave downward when \( n > 1 \) (Fig. 1). The reader will notice that external conditions, such as temperature, pH, and the presence of a preservative, etc., that influence the spores’ resistances also affect the Weibull distribution’s parameters \( b \) and \( n \). It is conceivable, and there is evidence to support this notion, that survival curves can change their concavity direction as a result of changes in the external conditions (10). One can expect that under “mild” conditions, “moderate” temperature and high pH, for example, the \( \log_{10} S \) versus \( t \) relationship will have an upward concavity, reflecting the progressive elimination of sensitive members of the population and the increased sturdiness of the survivors. In contrast, under harsh conditions, extremely high temperature and low pH for example, the semilogarithmic survival curve can be concave downward, an indication that the survivors sensitivity may increase as a result of damage accumulation.

Theoretically, fitting survival data with equation 7 or 8 should yield the same constants \( b \) and \( n \). In practice,
however, because of inevitable data scatter, the values may differ somewhat as a result of the different relative weight that is assigned to the different parts of the survival curves. It can be shown that under such circumstances equation 8 gives a more faithful account of the distribution’s tail and hence is a more practical model. This is because the safety of food products is primarily determined by the number of survivors and to a lesser extent by the number of spores or organisms initially destroyed (10).

In the laboratory, it is possible to subject microorganisms to almost ideal heating conditions. The methods used are designed to minimize the time for temperature equilibration, or “come-up time,” to ensure that, as near as possible, the whole sporal or cell population experiences the same heating conditions and to optimize the cooling and recovery conditions. In contrast, most commercial thermal processes do not achieve rapid heating, rapid cooling, or isothermal conditions. Over the years, a considerable expertise has been developed in the field of thermal processing (13). It enables the formulation of effective commercial processing based on taking into account the complex heat transfer dynamics within the actual food. Although considerable effort has been focused on the engineering aspects of thermal processing, relatively little effort has been invested in attempts to gain a better understanding of what happens to the microbial target (which is, after all, the purpose of most thermal processes). Thus, most, if not all, thermal process calculations are based on first-order inactivation kinetics (5, 7, 13) without verification that it indeed represents the inactivation process. For cases where the deviation from a first-order kinetics is clearly evident, it has been proposed that the survival curve be divided into regions that could be dealt with separately using a linear approximation (14) or other methods based on nonlinear destruction kinetics (8). Any general method of estimating microbial survival, which is explicitly based on the principle that the semilogarithmic survival curves are nonlinear, will require a solution of a rather difficult differential equation. If, however, the semilogarithmic curves are all concave upward, $n(T) < 1$ over the whole temperature range,
and it can safely be assumed that damage accumulation is not a major factor, i.e., that the degree of inactivation is path independent, then a much simpler model can be used to estimate survival under a monotonously increasing temperature.

The objectives of this work are to demonstrate the use of this special model with recently published data on the thermal inactivation of C. botulinum spores at different temperatures and to illustrate how it can be used to assess the efficacy of the heating stage in a sterilization process.

MATERIALS AND METHODS

Survival data for C. botulinum spores at different temperatures, previously reported by Anderson et al. (1), were fitted with equation 8 as a model. Since the heat resistance of spores is temperature dependent, the equation’s parameters, b and n, are also expected to change with temperature. The magnitude of the change can be expressed algebraically by fitting the experimental b(T) and n(T) values to any number of empirical models primarily selected for their simplicity and convenience. The temperature history of the coldest point in the food, T(t), hypothetical or measured, can also be expressed algebraically by a variety of mathematical models. If the degree of survival only depends on T and t, then when T(t) is known and b(T) and n(T) determined, the degree of survival can be calculated from the following (10):

\[
\log_{10} S = -b(T(t))e^{n(T(t))}
\]  

(9)

This relationship can be plotted and used to estimate the time needed to accomplish a given number of decades of reduction in the microbial of sporal population t_{kill}. In the syntax of Mathematica, the program used in this work, it is calculated as follows:

\[
t_{kill} = \text{FindRoot}[LS[t] = -d, \{t, t_1\}]
\]

(10)

where LS[t] is \(\log_{10} S\), as defined by equation 9, d is the number of log cycle reductions, and \(t_1\) is an initial value to start the iterations. According to this model, conventional commercial sterility is reached when \(d = 12\), but other values can be selected if deemed appropriate.

It was previously demonstrated that equations 9 and 10 can be used to assess the efficacy of the “heating” stage of a variety of thermal and nonthermal preservation processes (10). The reader will notice that the described procedure will also apply to survival curves expressed by alternative models, provided that the assumptions previously stated hold.

RESULTS AND DISCUSSION

Characterization of the survival patterns of C. botulinum spores. The regression parameters of the spores survival data are listed in Table 1, and the actual fit of equation 8 is demonstrated in Figure 2. All the experimental semilogarithmic survival curves had a noticeable upward concavity and, at least by statistical criteria, could adequately be described by equation 8 as a model with \(n < 1\). The relationship’s \(b(T)\) and \(n(T)\) are plotted in Figure 3.

In the corresponding temperature range, they could be described by the empirical equations:

For \(T \geq 100^\circ C\)

\[
b(T) = -14.1 + 0.0057T^{1.73}
\]

\((r^2 = 0.997)\)

(11)

and

For \(T \geq 100^\circ C\)

\[
n(T) = 1 - (T - 100)/(0.696 + 1.446(T - 100))
\]

\((r^2 = 0.991)\)

(12)

Since \(b(T)\) cannot assume negative values, the exact value where it becomes zero was calculated by solving numerically the first (upper) part of equation 11. These two models were selected for the good fit that they provide and, hence, can only formally be used safely for interpolation. Although it is doubtful that they will become totally inadequate at temperatures a few degrees higher than those that were used to determine their coefficients. They are also not the most convenient models, and their format can almost certainly be improved. The fit of equations 11 and 12 is demonstrated in Figure 3. Since survival data below 101°C were unavailable and since even at that temperature mortality was not intensive, it was assumed for the purpose of
FIGURE 5. Simulated heating processes and the corresponding survival patterns of C. botulinum spores. The simulation parameters are listed in Table 2. The corresponding two-dimensional survival curves are shown in Figure 5.

FIGURE 6. A three-dimensional view of the survival patterns of C. botulinum spores in simulated heating processes. The simulation parameters are listed in Table 2. The reader will notice that if the previously stated conditions are satisfied, sporal survival during any heating process, at which the temperature monotonically increases, will be represented by a “sliding path” down the three-dimensional surfaces that are shown in the figure. The reader will also notice that since the calculated surface is constructed by interpolation, its shape is independent of the mathematical model used.

Simulation of heating process with a monotonously increasing temperature. Let us describe a heating-cooling cycle by the following empirical equation (10):

$$T(t) = T_0 + t(c_1 + c_2t)$$  \hspace{1cm} (13)

where $T_0$ is the initial temperature and the $c$’s constants. (Having two constants, this model has more flexibility than the model based on conduction.) Again, this model was only selected for its convenience and the realistic curves that it produces (see below). The maximum possible temperature according to this model is regulated by $c_2$, i.e., as $t \to \infty \Rightarrow T \to T_0 + 1/c_2$. The heating rate is primarily determined by $c_1$, i.e., the larger the $c_1$, the slower it is.

Once $b(T)$ and $n(T)$ have been determined from the experimental isothermal survival curves, the survival pattern of C. botulinum (equations 11 and 12) during a heating process characterized by equation 13, or any other suitable model, could be generated with equation 9.
mathematical expression $\log_{10} S(t)$ is rather elaborate, it can be handled by modern mathematical software. Mathematica included. Examples of calculated survival curves under different heating conditions created with the experimental parameters of $C. \text{botulinum}$ are shown in Figures 5 and 6. The characteristics of the heating curves, expressed in terms of the parameters of equation 13, are given in Table 2. The figures and table demonstrate how the efficacy of the different heat treatments could be assessed using the actual parameters of the targeted organism, in our case $C. \text{botulinum}$ spores. The time needed to reduce the sporal population by 8, 10, or 12 decades, $t_{\text{kill}}$, is also listed in Table 2. It was calculated by numerically solving equation 9 using the command shown as equation 10. Obviously, the same values can be obtained from the plot of the corresponding survival curve. When the process is inadequate, the desired level of decades reduction cannot be achieved within the assigned heating time. Since the generation of any thermal history using the described procedure and the calculation of the corresponding $t_{\text{kill}}$ values are performed quickly, the method can potentially be a convenient tool to assess the efficacy of new thermal processes where spores of $C. \text{botulinum}$ are the target. It can also facilitate the evaluation of existing processes once their thermal history is described by equation 13 or a similar expression. The examples given above serve to illustrate the ease with which this approach can be used to estimate the efficacy of a given thermal process, provided that the condition of path independence is fulfilled or that the simulation is treated as a limiting case. Whether this is truly the case ought to be established independently, since the shapes of the survival curves alone are insufficient to exclude partial weakening of the surviving spores. However, since spores are almost certainly not being strengthened by a heat treatment at lethal temperatures, the assessment reached by the described procedure would give a higher number of survivors than the actual if damage is indeed accumulated. Neither the model presented in this work nor the one used by Anderson et al. (1) can indicate whether failure to reach a 12-log reduction means that the process is unsafe. The issue of whether the 12D is a realistic criterion of sterility remains to be resolved, regardless of the method used to calculate the process. It is likely that the guidelines of the International Commission for the Microbiological Specifications for Foods (6) could be useful in determining more realistic risk-based criteria for processed foods. Whether these criteria are actually met in a given process could easily be assessed by the described method.

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