Stochastic Modeling of Thermal Disinfection of Bacteria According to the Logistic Law

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Thermal treatment under controlled conditions results in almost complete destruction, or disinfection, of cell or bacterial populations. Medical needs and public health concerns often demand that such disinfection be as complete as possible. The bacteria, particulate and mesoscopic in size, undergo complex motion and behavior, and thus, they die off at irregular rates during disinfection. Hence, in addition to the average death rates of bacteria, the fluctuations around these average rates must be known, especially in the final period, or tail end, of disinfection where the number concentration of bacteria becomes exceedingly low, thereby magnifying the fluctuations.

It is natural and often desirable that various aspects of a process involving mesoscopic, particulate entities undergoing complex motion and behavior, such as the aforementioned bacterial disinfection, be explored on the basis of the statistical framework or a stochastic paradigm. Such aspects can range from the analysis and modeling of the particulate entities involved in the process to the optimization and control of the process itself.[1-6] Unambiguous discourses have been given as to the need of statistical or stochastic treatment of bacteria-population dynamics in disinfection.[7,8]

This note is concerned with the stochastic analysis and modeling of thermal disinfection of bacteria as a pure-death process based on a nonlinear mechanistic rate law, that is, the logistic law. Hitherto, the linear (that is, first-order) rate law has been predominantly adopted for stochastically analyzing and modeling the thermal disinfection rate of bacteria.[9-13] Nevertheless, the growth—positive or negative—of biological populations, including microbial populations, has been increasingly formulated according to nonlinear mechanistic rate laws,[14-18] such as the logistic law.[19,20] In this regard, Soboleva and Pleasants[21] incorporated the logistic law into the modeling of the growth of a biological population. In their work, the mean and variance of the process were computed numerically from the Fokker–Plank equation under the assumption that the random variable characterizing the process—the size or number density of a discrete biological population—is continuous. The adoption of the logistic law has been proposed for modeling the death rate of microorganisms in thermal disinfection[22]; apparently, no attempt has been made to incorporate this law into the stochastic formulation because of the computational complexity arising from its nonlinearity. Herein, this complexity is circumvented by resorting to the system-size expansion, a rational or logical approximation method, of the master equation of the process.[2,23,24] The numerical results are compared with the available experimental data obtained with *S. aureus*[25] as well as with those obtained with *M. paratuberculosis*.[26]

Model Formulation

The system under consideration is the population of bacteria that are being thermally deactivated. The population decreases as a result of the death of bacteria, one at a time, which have ceased to grow and do not reproduce, throughout the deactivation. The system constitutes a special class of Markov processes, the "pure-death " process.[2,13] The number of live bac-

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teria at time t, $N(t)$, is taken as the random variable of the system or process; a realization of $N(t)$ is denoted by n. All the possible numbers of bacteria are the states of the process, and the collection of these numbers, $\{n_0, n_0 - 1, \ldots, 2, 1, 0\}$, is the state space, where n_0 is the initial number of bacteria, that is, n at $t = 0$.

Master equation

For the pure-death process, the probability balance around state n leads to[2,27]

$$\frac{d}{dt}p_n(t) = \mu_{n+1}p_{n+1}(t) - \mu_n p_n(t) \qquad n = n_0, n_0 - 1, \ldots, 2, 1, 0 \quad (1)$$

which is the master equation of the process.[13,23,28] The term $p_n(t)$ in this equation denotes the probability of n bacteria being alive at time t. Based on the logistic law,[22] the intensity of death μ_n in the above expression is

$$\mu_n = -\frac{dn}{dt} = kn + k'n(n_0 - n) = (k + k'n_0)n - k'n^2 \quad (2)$$

where k and $(k'n_0)$ are proportionality constants.

Mean and variance

In evaluating the mean and the variance about the mean of the process by the aforementioned system-size expansion,[2,29] the random variable $N(t)$ is expressed as the sum of the macroscopic term $\Omega\varphi(t)$ and the fluctuation term $\Omega^{1/2}\Xi(t)$. The symbol Ω is the system's size, which is n_0 in the current work. Thus, the system-size expansion of the master equation, Eq. 1, yields the macroscopic equation in terms of $\varphi(t)$ governing the overall behavior of the process, and the equation in terms of $\Xi(t)$ governing the fluctuations of the process around the macroscopic values.[2] Integration of the macroscopic equation results in an explicit expression for $\varphi(t)$. This expression, in turn, yields the mean of $N(t)$, $E[N(t)]$ or $m(t)$, of the disinfection process as

$$m(t) = n_0\left\{\frac{(k + k'n_0)}{k\exp[(k + k'n_0)t] + k'n_0}\right\} \quad (3)$$

The normalized, or dimensionless, form of the mean, $\bar{m}(\tau)$, is obtained from this expression as

$$\bar{m}(\tau) = \frac{m(\tau)}{n_0} = \frac{1}{1 + \alpha[\exp(\tau) - 1]} \quad (4)$$

where $\tau = [(k + k'n_0)t]$ is dimensionless time and $\alpha = k/(k + k'n_0)$. Similarly, from the equation governing the fluctuations the variance of $N(t)$, $\mathrm{Var}[N(t)]$ or $\sigma^2(t)$, of the disinfection process is obtained as

$$\sigma^2(t) = n_0\frac{k(k + k'n_0)\exp[(k + k'n_0)t]}{\{k\exp[(k + k'n_0)t] + k'n_0\}^4} \times \{k^2\exp[2(k + k'n_0)t] - (k'n_0)^2 + (k + k'n_0) \times [k'n_0 + k(2k'n_0t - 1)]\exp[(k + k'n_0)t]\} \quad (5)$$

or in terms of α and τ,

$$\sigma^2(\tau) = n_0\{\alpha\exp(\tau)\{1 + \alpha[\exp(\tau) - 1]\}^{-4}(2\alpha - 1 + \exp(\tau) \times \{1 - 2\alpha[1 + (\alpha - 1)\tau] + 2\alpha^2\sinh(\tau)\})\} \quad (6)$$

The standard deviation $\sigma(\tau)$ is the square root of the variance $\sigma^2(\tau)$. Its normalized form, $\bar{\sigma}(\tau)$, is

$$\bar{\sigma}(\tau) = \frac{\sigma(\tau)}{n_0} = n_0^{-1/2}\{1 + \alpha[\exp(\tau) - 1]\}^{-2}\times \sqrt{\alpha\exp(\tau)(2\alpha - 1 + \exp(\tau)\{1 - 2\alpha[1 + (\alpha - 1)\tau] + 2\alpha^2\sinh(\tau)\})} \quad (7)$$

Results and Discussion

The model formulated has been fitted to the experimental data in the final period, or tail end, of the process in terms of the temporal mean expressed in Eq. 3 for the thermal disinfection of *S. aureus*[25] obtained at the temperatures of 325.15 K (52°C), 327.15 K (54°C), and 329.15 K (56°C), and for the thermal disinfection of *M. paratuberculosis*[26] at the temperature of 336.65 K (63.5°C). The distinct final period, or tail end, of thermal disinfection can be identified from the plots of the experimental data at each temperature; the time at the very outset of the final period is taken as zero. By resorting to the Levenberg–Marquardt method,[30] a gradient-based method, the nonlinear regression of the data has been implemented by SAS version 9.1 for Windows, resulting in the values of the two adjustable parameters, k and $(k'n_0)$, both in the unit of inverse time, that is, frequency. Because of the profound stochasticity and nonlinearity of the bacteria's interactions in the final period of disinfection, the parameters determined involve significant uncertainty. With these values in hand, it is possible to compute the dimensionless time τ, that is, $[(k + k'n_0)t]$, the parameter α, and the normalized mean $\bar{m}(\tau)$, from Eq. 4. For illustration, the values of $\bar{m}(\tau)$ for *S. aureus*[25] at 327.15 K (54°C) and those for *M. paratuberculosis*[26] at 336.65 K (63.5°C) are plotted in Figure 1; the experimental data,[25,26] given as the number of bacteria at dimensionless time τ relative to the initial number of bacteria, that is, n/n_0 or $n(\tau)$, are also superimposed in the figure for comparison. Note that $\bar{m}(\tau)$ is in accord with the experimental data's decaying trend in both cases.

The standard deviation σ signifies the deviations attributable to the internal or characteristic noises of the process as predicted by the stochastic model. As evident from Eq. 7, the expression for $\bar{\sigma}(\tau)$ depends on the initial number of bacteria

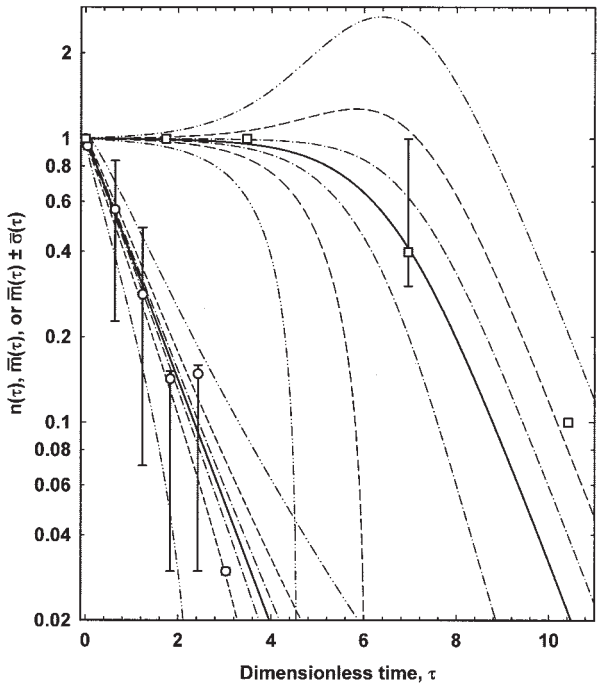

Figure 1. Normalized mean $\bar{m}(\tau)$ (—) in the final period of thermal disinfection of *S. aureus*[25] at 327.15 K (54°C) and *M. paratuberculosis*[26] at 336.65 K (63.5°C).

The values of normalized standard deviation $\bar{\sigma}(\tau)$ are plotted about $\bar{m}(\tau)$ [that is, $\bar{m}(\tau) \pm \bar{\sigma}(\tau)$], for n_0 values of 1000 (– · –), 100 (– – –), and 10 (– ·· –). The experimentally measured values of the number concentration of bacteria, n/n_0, or $n(\tau)$, are superimposed for comparison: *S. aureus* (○); *M. paratuberculosis* (□).

n_0; thus, the values of $\bar{\sigma}(\tau)$ have been computed according to Eq. 7 for n_0 values of 10, 100, and 1000 for *S. aureus*[25] at 327.15 K (54°C) as well as for *M. paratuberculosis*[26] at 336.65 K (63.5°C). These values are plotted around $\bar{m}(\tau)$ [that is, $\bar{m}(\tau) \pm \bar{\sigma}(\tau)$] in Figure 1. Note that in this figure, the deviations, or fluctuations, of the experimental data are substantially more pronounced than those predicted by the model; this is almost always the case: The overall deviations of the experimental data include those attributable not only to the internal noises of the process, as predicted by the model, but also to the external noises arising from inevitable measurement errors and instrumental deficiencies that can never be totally eliminated.

It is worth noting that when the value of $(k'n_0)$ is negligible compared with that of k, the expression for the mean, $m(t)$, in Eq. 3, and that for the variance, $\sigma^2(t)$, in Eq. 5, reduce to the corresponding expressions for the linear model of thermal disinfection, $m(t) = n_0\exp(-kt)$ and $\sigma^2(t) = n_0\exp(-kt)[1 - \exp(-kt)]$, respectively.[9,10,13]

Naturally, k as well as $(k + k'n_0)$ in Eq. 5 have the connotation of kinetic constants, thus obeying the Arrhenius law. For thermal disinfection of *S. aureus*,[25] however, these kinetic constants obey the Arrhenius law only approximately, which is attributable to the severe stochasticity and nonlinearity of the experimental data[25] in the final period of disinfection. The activation energy calculated on the basis of k is 247.7 kJ mol^{-1}, and that calculated on the basis of $(k + k'n_0)$ is 284.3 kJ mol^{-1}. The small difference between these two values implies that the effect of $(k'n_0)$ is relatively feeble on the rate of thermal disinfection at the tail end. For comparison, a value of 224.0 kJ mol^{-1} was reported for the activation energy of thermal disinfection of *S. aureus* in milk.[31]

Conclusions

A stochastic model for the thermal disinfection rate of bacteria as a pure-death process has been derived based on a nonlinear mechanistic rate law, specifically, the logistic law. The complexity in solving the resultant master—that is, governing—equation of the process has been circumvented by resorting to a rational approximation method, system-size expansion. The mean and variance of the number concentrations of bacteria, *S. aureus* and *M. paratuberculosis*, at the final period, or tail end, of thermal disinfection have been computed from the master equation. The mean value of the model is in accord with the available experimental data. As expected, fluctuations of the data around their mean are large when compared with those predicted by the model. Moreover, it has been observed that the model's kinetic constants for *S. aureus* approximately obey the Arrhenius law.

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Supporting Information Available. Three appendixes (Appendix A: Expansion of the Master Equation; Appendix B: Derivation of Mean and Variance; Appendix C: Additional Plots) can be viewed or downloaded from the corresponding author's web site at http://www.engg.ksu.edu/CHEDEPT/fan.htm, or can be obtained as hard copies by writing to the author.

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