STOCHASTIC MODELING OF THERMAL DEATH KINETICS OF A CELL POPULATION

Revisited

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Numerous biochemical processes occurring in nature or man-made systems (*e.g.*, biochemical reactors) involve randomly behaving viruses, fungi, bacteria, and plant and animal cells that are discrete and mesoscopic in size. This gives rise to incessant fluctuations in their characteristic properties, including their number concentrations (density), movements, and metabolic activities. Such fluctuations are profoundly magnified when the number concentrations of pores, spores, or cells are very low as found, for instance, in the tail end of thermal disinfection of foodstuffs.

Medical needs and public health concerns often demand that the disinfection process be complete or nearly complete. Hence, it is indeed appropriate that the notion and methodology of stochastic processes have been introduced in most of the major textbooks on biochemical engineering through analysis and modeling of the disinfection process;[1-4] the discourse in these textbooks is based on the original contributions of Fredrickson[5] and Aiba and Toda.[6] According to Ramkrishna,[7] "Fredrickson was the first to point out the importance of stochastic analysis in dealing with sterilization processes." The crux or essence of stochastic analysis and modeling is in their capability to estimate or predict inherent fluctuations of the characteristic property of a random phenomenon or process and the distribution of this fluctuating property. In these textbooks, however, only the mean (the first moment), or at most, the variance (the second moment about the mean) of the fluctuating number concentration of cells during disinfection is given. The evaluation of additional quantities defined in terms of the moments of the number concentration of cells higher than the second moment is useful and often necessary to gain insight into the stochastic, or random, nature of the phenomenon or process of interest.

In fact, mesoscopic entities in the form of bubbles, droplets, and particles are ubiquitous in many phenomena, processes, and operations taught in various courses in chemical engineering besides biochemical engineering. Some of these courses are chemical reaction engineering, transport phenomena, separation, particle technology, material science and engineering, and surface science. The phenomena, processes, and operations involving mesoscopic entities includes heterogeneous reactions,[8] gas absorption,[9] distillation, liquid-liquid extraction, adsorption,[10,11] fluidization,[12,13] filtration,[14] crystallization,[15] solids mixing,[16] and grinding and attrition.[17,18] With slight adaptation, the current contribution can

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surely be made useful for several chemical engineering courses.

The current effort aims at presenting the fluctuations in the microorganism's number concentration in the course of disinfection not only in terms of the variance but also the skewness (the third moment about the mean over the third power of the standard deviation) and the kurtosis (the fourth moment about the mean over the fourth power of the standard deviation). The results would be useful as supplementary materials to what is already available. Following the textbooks mentioned above, the example adopted is the thermal disinfection of a cell population, specifically that of *Staphylococcus aureus,*[19] where the number concentration is considered to decrease according to the first-order rate law.

Interest in stochastically analyzing and modeling bacteria, or cell populations in general, has been steadily growing in recent years in view of their importance in different areas of biochemical engineering and biotechnology. Various researchers have resorted to the master-equation and other closely related algorithms; the majority, if not all, of them have considered linear systems or processes. Tsuchiya, *et al.,*[20] reviewed their works on the growth and replication of cultures of unicellular organisms. Ramkrishna[21] wrote an informative exposition containing a variety of stochastic algorithms for modeling the dynamics of cell populations including the master-equation algorithm. Stephanopoulos and Fredrickson[22] analyzed the extinction process by the prey-predator model involving both deterministic and stochastic components. Nassar, *et al.,*[23] stochastically modeled the dynamics of a unicellular organism population; they[24] also modeled the enzymatic degradation of cellulose. Lauffenburger and Linderman[25] published a monograph based on their earlier works on receptor/ligand trafficking by the master-equation algorithm.

MODEL FORMULATION

As in any stochastic analysis and modeling, a mathematical model characterized by a random variable or variables is required for the system under consideration. It is formulated according to the procedure outlined below.[26-31]

Description of the System • The system under consideration is the population of microorganisms, or cells, that are thermally deactivated. The status, or state, of the system is specified by the number (or size) of the population, that decreases due to the death of cells that have ceased to grow, one at a time, that do not reproduce throughout the deactivation. It is assumed that each member of the cell population alive at t = 0 is independently subjected to the same risk of dying. Moreover, the current size of the cell population depends solely on the size of the immediate past population. In other words, the system possesses the so-called Markovian property, implying that only the current state of the process or system is relevant in determining its future behavior;[26-28] in fact, the system under consideration constitutes a special type of Markov processes (time-continuous Markov chains) called "the pure-death process."[27,28]

Identification of Random Variable and State Space • The number of live microorganisms, simply termed cells hereafter, at time t is taken as the random variable of the process of deactivation, N(t); a realization of N(t) is denoted by n. All the possible numbers of live cells 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 cells susceptible to thermal disinfection, *i.e.,* n at t = 0.

Construction of Transition Diagram • Since no cells will be produced, the size of the population will decrease throughout disinfection. In the transition diagram of the process presented in Figure 1, the circles indicate the possible states of the system and the arrows describe transitions of the system at any moment. The figure is a typical representation of the time-homogeneous pure-death process.

Definition of Transition-Intensity Functions • The rate law adopted here for thermal deactivation (disinfection) is[1-3,5]

$$-\frac{dn}{dt} = kn \qquad (1)$$

where n is the number of cells at a specific time t; this expression is known as Chick's law.[32] The intensity of transition (intensity function) is defined as the instantaneous rate of change of the transition probability.[27-28] Hence, the intensity of death, μ_n, for the thermal disinfection under consideration can be assumed to be of the form

$$\mu_n = kn \qquad (2)$$

where k is a proportionality constant. This expression implies that the cell-death rate is proportional to the number of

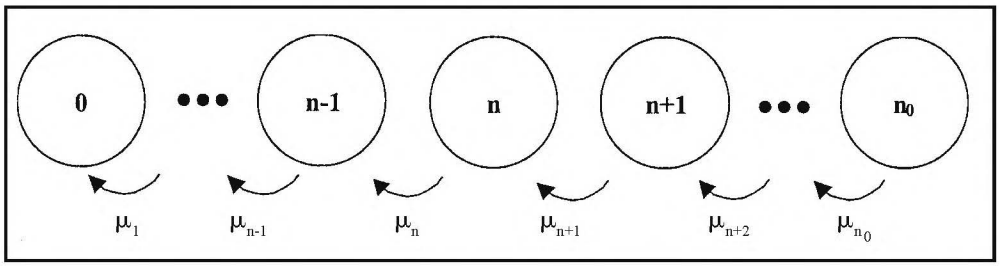

Figure 1. *Transition diagram of the pure-death process: μ_n is the intensity of death, and $n_0, n_0-1, \ldots, n+1, n, n-1, \ldots, 2, 1, 0$ are the states of the process.*

live cells. Clearly, the intensity of death, μ_n, is only a function of n and independent of time, and thus *time homogeneous.*[27,28]

Derivation of the Master Equation • The governing equation of any Markov process expresses the infinitesimal change of the transition probability, $p_{ij}(s,t)$, between state i at arbitrary time s and state j at time t.[26,27] For convenience, the governing equation is often written in terms of the state (absolute)3ô≤obability rather than the transition probability. The absolute probabilities, $p_j(t)$ and $p_i(t)$, are related through the transition probabilities, $p_{ij}(s,t)$'s, as

$$p_j(t) = \sum_i p_i(s)p_{ij}(s,t)$$

This renders it possible to transform the transition probabilities in the governing equation into the absolute probabilities, thereby yielding the gain-loss or probability-balance equation, or master equation;[28,33] for the pure-death process under consideration, it is derived as follows:

• With N(t) = n given, it is assumed that during time interval (t, t+Δt): (1) the conditional probability that a death will occur, *i.e.,* a live cell will die, is $\mu_n \Delta t + o(\Delta t)$, and (2) the conditional probability that more than one death will occur is o(Δt), which is defined such that

$$\lim_{\Delta t \to 0} \frac{o(\Delta t)}{\Delta t} = 0 \qquad (3)$$

Naturally, the conditional probability of no change in the number of live cells during this time interval is $[1 - \mu_n \Delta t - o(\Delta t)]$.

• Let the probability that exactly n cells are alive at time t be denoted as $p_n(t) = Pr[N(t) = n]$, $n = n_0, n_0 - 1,...,2,1,0$. Then, for the two adjacent time intervals, (0,t) and (t, t+Δt), the occurrence of exactly n cells being alive at time (t+Δt) can be realized in the following mutually exclusive ways:

1) With a probability of $p_{n+1}(t)[\mu_{n+1}\Delta t + o(\Delta t)]$, exactly one cell will die during the time interval (t,t+Δt), provided that exactly (n+1) cells are alive at time t.

2) With a probability of o(Δt), exactly j cells will die during the time interval (t, t+Δt), provided that exactly (n+j) cells are alive at time t, where $2 \le j \le (n_0 - n)$.

3) With a probability of $p_n(t)[1 - \mu_n \Delta t - o(\Delta t)]$, no cell will die during the time interval (t, t+Δt), provided that all n cells are alive at time t.

• Summing all these probabilities and consolidating all quantities of o(Δt) yield

$$p_n(t + \Delta t) = p_n(t)(1 - \mu_n \Delta t) + p_{n+1}(t)(\mu_{n+1}\Delta t) + o(\Delta t) \qquad (4)$$

• Rearranging this equation and taking the limit as $\Delta t \to 0$ yield the master equation of the pure-death process given below (see Figure 1):

$$\frac{d}{dt}p_n(t) = \mu_{n+1}p_{n+1}(t) - \mu_n p_n(t) \qquad n = n_0, n_0 - 1,...,2,1,0 \qquad (5)$$

For $n = n_0$, we have $\mu_{n_0+1} = 0$; thus

$$\frac{d}{dt}p_{n_0}(t) = -\mu_{n_0}p_{n_0}(t)$$

or, by virtue of Eq. (2),

$$\frac{d}{dt}p_{n_0}(t) = -kn_0p_{n_0}(t) \qquad (5a)$$

For $n = n_0 - 1, n_0 - 2,...,2,1$, Eq. (5) is

$$\frac{d}{dt}p_n(t) = \mu_{n+1}p_{n+1}(t) - \mu_n p_n(t)$$

or

$$\frac{d}{dt}p_n(t) = k(n+1)p_{n+1}(t) - knp_n(t) \qquad (5b)$$

Finally, for $n = 0$, we have $\mu_0 = 0$; thus,

$$\frac{d}{dt}p_0(t) = \mu_1 p_1(t)$$

or

$$\frac{d}{dt}p_0(t) = kp_1(t) \qquad (5c)$$

Solution of the Master Equation • As can be discerned from Eq. (2), the intensity of death, μ_n, is of linear form; as

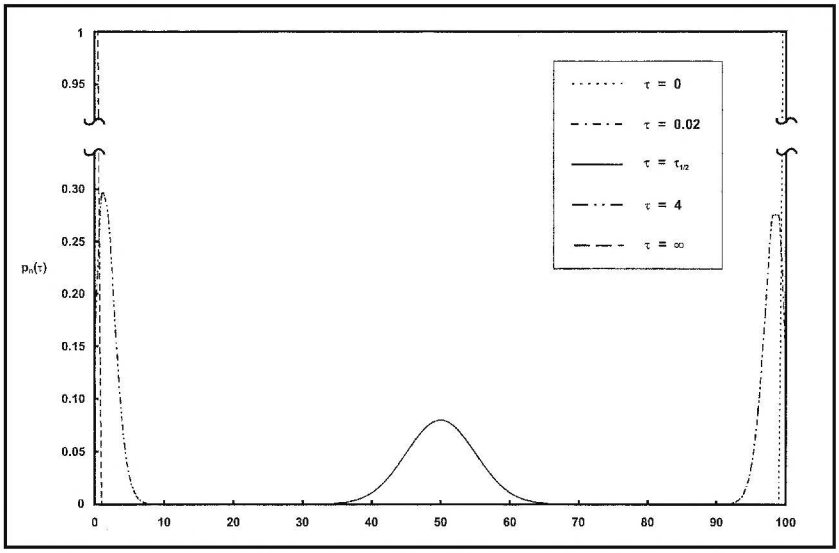

Figure 2. *Temporal evolution of the binomial distribution of the number of live cells, N(t), with* $n_0 = 100$.

a result, the master equation, Eq. (5), can be solved recursively, thereby yielding the probability that n cells will be alive at time t, $p_n(t)$, as[5,34] (see Appendix A, available at <http://www.engg.ksu.edu/CHEDEPT/fan.htm>)

$$p_n(t) = \frac{n_0!}{\left[(n_0 - n)!\, n!\right]}\left(e^{-kt}\right)^n\left(1 - e^{-kt}\right)^{n_0 - n} = \binom{n_0}{n}\left(e^{-kt}\right)^n\left(1 - e^{-kt}\right)^{n_0 - n} \quad (6)$$

The above expression indicates that the distribution of random number N(t) is binomial with two parameters. One of the parameters, n_0, representing the initial value of N(t), signifies the total number of the events that can possibly occur, and the other parameter, e^{-kt}, signifies the probability of occurrence of one event. For the thermal disinfection under consideration, n_0 is the number of cells alive at t = 0, which will eventually die, and e^{-kt} is the probability of an individual cell being alive at time t. The temporal evolution of the binomial distribution as given by Eq. (6) is illustrated in Figure 2; for simplicity, n_0 is specified to be 100 and kt is lumped as τ.

Moments about the Mean • With the solution of the master equation in hand, we can proceed to calculate the mean and variance of the process that should constitute the core of any stochastic analysis and modeling. Furthermore, higher moments about the mean, such as skewness and kurtosis, are determined; they provide additional information useful for characterizing the stochastic and statistical properties of the process.[27,34-36] For illustration, the derivation of the skewness is elaborated in Appendix B (available at <http://www.engg.ksu.edu/CHEDEPT/fan.htm>)

Mean. The mean, E[N(t)] or m(t), which is the expected value (first moment) of the distribution of random variable N(t), is defined as

$$m(t) = E\left[N(t)\right] = \sum_n n p_n(t) \quad (7)$$

The mean or expected value, m(t), is the weighted sum of the realizations of the random variable where the weights are the probabilities corresponding to those realizations.[34]

For the thermal disinfection under consideration, the mean in terms of dimensionless time τ is

$$m(\tau) = n_0 e^{-\tau} \quad (8)$$

The normalized, *i.e.*, dimensionless form of the mean, $\overline{m}(\tau)$, is

$$\overline{m}(\tau) = \frac{m(\tau)}{n_0} = e^{-\tau} \quad (9)$$

Variance. The variance, Var[N(t)] or $\sigma^2(t)$, is the second moment of the distribution of random variable N(t) about the mean, m(t); thus

$$\sigma^2(t) = E\left\{\left[N(t) - E\left[N(t)\right]\right]^2\right\} = \sum\left\{n - E\left[N(t)\right]\right\}^2 p_n(t) \quad (10)$$

By expanding the above equation, $\sigma^2(t)$ can be related to the mean, m(t), as

$$\sigma^2(t) = E\left[N^2(t)\right] - m^2(t) \quad (11)$$

In the above expression, $E[N^2(t)]$ is the second moment of N(t), *i.e.*

$$E\left[N^2(t)\right] = \sum_n n^2 p_n(t) \quad (12)$$

For the thermal disinfection under consideration, the variance in terms of dimensionless time τ, $\sigma^2(\tau)$, is, from Eqs. (8) and (11)

$$\sigma^2(\tau) = n_0 e^{-\tau}\left(1 - e^{-\tau}\right) \quad (13)$$

Standard Deviation. The standard deviation, $\sigma(t)$, of the process is the square root of the variance, $\sigma^2(t)$; thus

$$\sigma(t) = \left[\sigma^2(t)\right]^{1/2} \quad (14)$$

The variance, $\sigma^2(t)$, or more specifically, the standard deviation, $\sigma(t)$, signified the fluctuations, *i.e.*, scatterings, of the values of the random variable about their mean.

For the thermal disinfection under consideration, the standard deviation in terms of dimensionless time τ, $\sigma(\tau)$, is, from Eqs. (13) and (14)

$$\sigma(\tau) = \sqrt{n_0 e^{-\tau}\left(1 - e^{-\tau}\right)} \quad (15)$$

Coefficient of Variation. The coefficient of variation, CV(t), is the quotient (or ratio) of the standard deviation, $\sigma(t)$, and the corresponding mean, m(t); thus[35]

$$CV(t) = \frac{\sigma(t)}{m(t)} \quad (16)$$

For the thermal disinfection under consideration, the coefficient of variation in terms of dimensionless time τ, $CV(\tau)$, is, from Eqs. (8) and (15),

$$CV(\tau) = \sqrt{\frac{1 - e^{-\tau}}{n_0 e^{-\tau}}} \quad (17)$$

or

$$CV(\tau) = \sqrt{\frac{1}{n_0 e^{-\tau}} - \frac{1}{n_0}} = \sqrt{\frac{1}{m(\tau)} - \frac{1}{n_0}} \quad (17a)$$

Skewness. The skewness, $\gamma(t)$, is the quotient (or ratio) of the third moment of the distribution of random variable N(t) about the mean, m(t), and the third power of the standard deviation, $\sigma(t)$; thus[36]

$$\gamma(t) = \frac{E\left\{\left[N(t) - E[N(t)]\right]^3\right\}}{\left(\sqrt{E\left\{\left[N(t) - E[N(t)]\right]^2\right\}}\right)^3}$$

$$= \frac{1}{\sigma^3(t)} \sum_n \left\{n - E[N(t)]\right\}^3 p_n(t) \qquad (18)$$

By expanding the above equation, $\gamma(t)$ can be related to the mean, m(t), and the standard deviation, $\sigma(t)$, as

$$\gamma(t) = \frac{1}{\sigma^3(t)} \left\{E\left[N^3(t)\right] - 3m(t)\sigma^2(t) - m^3(t)\right\} \qquad (19)$$

where $E[N^3(t)]$ is the third moment of N(t) defined by

$$E\left[N^3(t)\right] = \sum_n n^3 p_n(t) \qquad (20)$$

Skewness characterizes the degree of asymmetry of the distribution of random variable N(t) about the mean, m(t). Positive skewness indicates a distribution with a longer tail to the right of the mean than to the left, and negative skewness indicates a longer tail to the left of the mean than to the right. It vanishes for any symmetric distribution.

For the thermal disinfection under consideration, the skewness in terms of dimensionless time τ, $\gamma(\tau)$, is, from Eqs. (8), (15), and (19)

$$\gamma(\tau) = \frac{\left(1 - e^{-\tau}\right) - e^{-\tau}}{\sqrt{n_0 e^{-\tau}\left(1 - e^{-\tau}\right)}} \qquad (21)$$

Kurtosis (Curtosis). The kurtosis, k(t), is the quotient (or ratio) of the fourth moment of the distribution of random variable N(t) about the mean, m(t), and the fourth power of the standard deviation, $\sigma(t)$; thus[36]

$$k(t) = \frac{E\left\{\left[N(t) - E[N(t)]\right]^4\right\}}{\left(\sqrt{E\left\{\left[N(t) - E[N(t)]\right]^2\right\}}\right)^4}$$

$$= \frac{1}{\sigma^4(t)} \sum_n \left\{n - E[N(t)]\right\}^4 p_n(t) \qquad (22)$$

By expanding the above equation, k(t) can be related to the mean, m(t), the standard deviation, $\sigma(t)$, and the skewness, $\gamma(t)$, as

$$k(t) = \frac{1}{\sigma^4(t)} \left\{E\left[N^4(t)\right] - 4m(t)\gamma(t)\sigma^3(t) - m^2(t)\left[6\sigma^2(t) + m^2(t)\right]\right\} \qquad (23)$$

where $E[N^4(t)]$ is the fourth moment of N(t), *i.e.*,

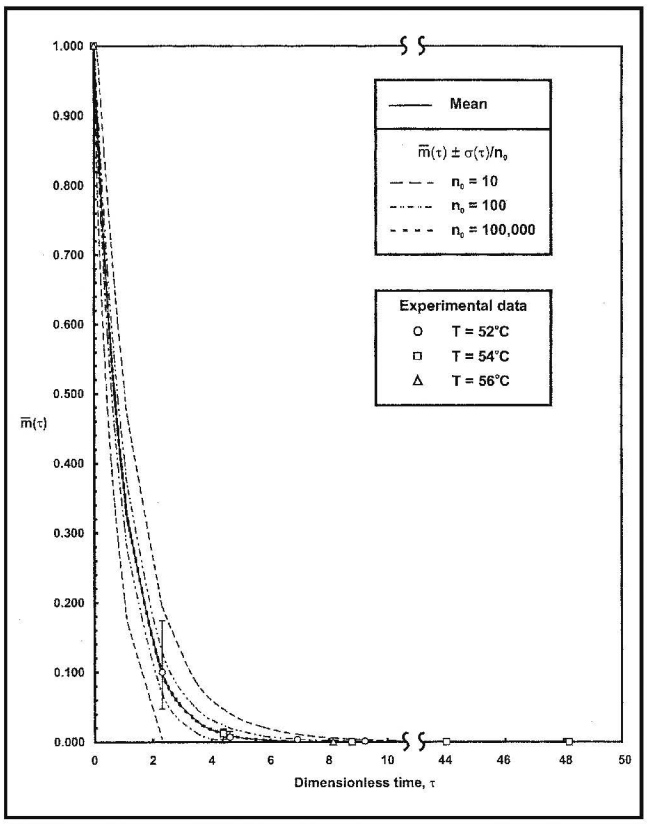

Figure 3. *Normalized mean, $\bar{m}$, and standard deviation, σ, as functions of the dimensionless time, τ, exhibiting the entire range of the number concentration of live cells.*

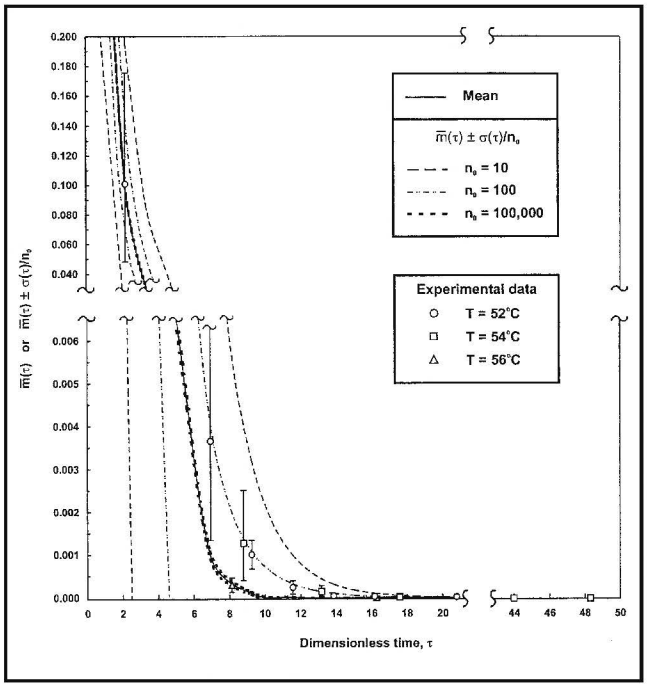

Figure 4. *Normalized mean, $\bar{m}$, and standard deviation, σ, as functions of the dimensionless time, τ, for the low range of the number concentration of live cells.*

$$E\left[N^4(t)\right]=\sum_n n^4 p_n(t) \qquad (24)$$

Kurtosis is a measure of how outlier-prone, or peaked, the distribution of random variable N(t) is. The kurtosis of a distribution that is less outlier-prone than the normal distribution tends to be greater than 3, which is the kurtosis of the normal distribution, and the opposite is the case for a distribution that is more outlier-prone.

For the thermal disinfection under consideration, the kurtosis in terms of dimensionless time τ, is, from Eqs. (8), (15), (21), and (23)

$$k(\tau)=\frac{1-6e^{-\tau}\left(1-e^{-\tau}\right)}{n_0 e^{-\tau}\left(1-e^{-\tau}\right)}+3 \qquad (25)$$

NUMERICAL SOLUTION

The mean, variance, skewness, and kurtosis of N(t) have been computed from their corresponding analytical expressions, Eqs. (8), (13), (21), and (25), respectively. They are functions of both the size of the initial cell population, n_0, and the time, t. The value of the proportionality constant, k, in the transition intensity, Eq. (2), can be recovered through the least-square fitting of the expression for the mean, Eq. (8), to the available experimental data of the thermal disinfection by means of a nonlinear minimization method, *e.g.*, the Levenberg-Marquant method.[37]

RESULTS AND DISCUSSION

The model derived is illustrated with the same set of the available experimental data for the thermal death of *S. aureus* strain S-1 in neutral phosphate buffer[19] as those adopted by the major textbooks in biochemical engineering.[1-4] These data have been obtained at the temperatures of 325.15 K (52°C), 327.15 (54°C), and 329.15 (56°C), thereby yielding the values of k as 0.0192 s^{-1} (1.15 min^{-1}), 0.0362 s^{-1} (2.17 min^{-1}), and 0.0678 s^{-1} (4.07 min^{-1}), respectively. With these values of k, the mean, as well as the variance or standard deviation, skewness, and kurtosis, of the number concentration of live cells have been computed. These quantities are graphically presented and their significance is discussed.

Mean • The mean, $m(\tau)$, and the normalized mean, $\overline{m}(\tau)$, have been computed according to Eqs. (8) and (9), respectively. Only the latter, which is the exponential decay function independent of any parameters, is graphically plotted in Figures 3 and 4 as a function of dimensionless time τ. The former exhibits the entire range of $\overline{m}(\tau)$ and the latter, the low range. Naturally, Eq. (8) or (9) as well as $\overline{m}(\tau)$ are in accord with those given in the available textbooks[1-4] as well as in the original contributions.[5,6] The experimental data[19] are also superimposed in both figures for comparison.

Variance, Standard Deviation and Coefficient of Variation • The expression for variance, σ^2, Eq. (13), is given by Blakebrough[1] and Fredrickson.[5] The variance is a measure of the variability, spread, or dispersion of the values of a random variable. Naturally, the larger the value of the variance, the greater the dispersion of the values of the random variable about their mean.

The standard deviation, σ, is obviously the square root of the variance, σ^2. The value of $\sigma(\tau)$ as given by Eq. (15) varies from 0 at $\tau=0$, reaches its maximum at $\tau_{1/2}$, or $\tau=\ell n 2$, where $\overline{m}(\tau)=1/2$, and eventually vanishes as $\tau\to\infty$, as expected. This trend of the $\sigma(\tau)$'s variation in terms of $\overline{m}(\tau)\pm\sigma(\tau)/n_0$ for three values of n_0 is also illustrated in Figures 3 and 4. Note that in these figures, especially in the latter, the deviations of the majority, if not all, of the available experimental data are substantially more pronounced than those of the deviations predicted by the model in view of the reported n_0 between 7.5×10^6 and 19×10^6 in obtaining the experimental data.[19] This is almost always the case: the overall deviations of the experimental data include not only those attributable to the internal or characteristic noises of the process as predicted by the stochastic model, but also to the external noises due to instrumental deficiencies and errors that can never be totally eliminated.

The coefficient of variation, $CV(\tau)$, is defined to provide a meaningful relative measure of the variability, spread, or dispersion of the values of a random variable about their mean. Note that $CV(\tau)$ expresses the random variable's dispersion as a fraction of the mean, or frequently, as a percentage.

For the thermal disinfection under consideration, $CV(\tau)$, specifically, the relative variation of size of the cell population about its mean, at any dimensionless time τ is inversely proportional to the square root of the initial cell population size, n_0, as indicated by Eq. (17) or (17a). By evaluating $CV(\tau)$ for various values of n_0, $m(\tau)$, and their combinations, it can be readily shown that

- At any τ, the larger the n_0, the smaller the $CV(\tau)$, or the relative extent of the fluctuations
- For any n_0, $CV(\tau)$ is initially zero and increases monotonically with τ, and the smaller the $m(\tau)$, the larger the $CV(\tau)$
- For any $m(\tau)$, $CV(\tau)$ increases monotonically with the increase in n_0 and asymptotically approaches a constant value, and the smaller the n_0, the smaller the $CV(\tau)$.

Skewness • Skewness $\gamma(t)$, as defined by Eq. (18), measures the extent of asymmetry of the distribution of random variable N(t) relative to its extent of deviation or dispersion. Thus, it is indeed a meaningful measure of asymmetry; nevertheless, $\gamma(t)$ depends on parameter n_0.

For the thermal disinfection under consideration, $\gamma(\tau)$ has been evaluated according to Eq. (21) for n_0 of 10, 100, and 100,000; the values of $\gamma(\tau)$ obtained are illustrated in Figure

5. The configurations of the resultant curves can be discerned at least qualitatively from that of the probability distribution of N(t) presented in Figure 2; it is binomial in nature. Note that when τ is nearly 0, N(τ) can assume only those values immediately to the left of n_0; thus, its distribution is highly skewed to the left and consequently, gives rise to $\gamma(\tau)$ with an appreciable negative value. The opposite is the case as $\tau \to \infty$. Naturally, in between these two extremes, $\gamma(\tau)$ approaches from either direction to 0, which is the value of $\gamma(\tau)$ for the normal distribution, at $\tau = \tau_{1/2} = \ell n2$.

Kurtosis • Kurtosis k(t), as defined by Eq. (22), measures the degree of peakedness of the distribution of random variable N(t) relative to its extent of deviation or dispersion. Thus, it is indeed a meaningful measure of peakedness; nevertheless, as for $\gamma(t)$, k(t) depends on parameter n_0.

For the thermal disinfection under consideration, the values of $k(\tau)$ have been computed by Eq. (25) for n_0 of 10, 100, and 100,000 and plotted in Figure 6. Similar to $\gamma(\tau)$ presented in Figure 5, the configuration of the resultant curves can be readily interpreted in the light of Figure 2. When τ is nearly 0, N(τ) can assume only those values in the immediate vicinity of n_0, and thus its distribution is highly peaked, giving rise to $k(\tau)$ with a large positive value; the same is the case at $\tau \to \infty$ when the values of N(τ) are nearly 0. In between these two extremes, $k(\tau)$ approaches from either direction to 3, which is the value of $k(\tau)$ for the normal distribution, at $\tau = \tau_{1/2} = \ell n2$.

CONCLUSIONS

A stochastic model for the thermal death kinetics of a cell population as a pure-death process has been derived based on the first-order rate law. The solution of the governing differential equation of the model, termed the master equation, yields the probability distribution of the number concentration (density) of live cells during disinfection, which is regarded as the random variable of the process; the resultant distribution is the binomial distribution whose two parameters are the number of cells initially alive, which will eventually die, and the probability of an individual cell being alive, expressed as the exponential decay function. In addition to the mean of live cells, various higher moments about the mean have been derived to characterize this distribution. These higher moments include variance (second moment about the mean), skewness (third moment about the mean over the third power of the standard deviation), and kurtosis (fourth moment about the mean over the fourth power of the standard deviation). Only the expressions for the mean and variance are available in one or more of the existing major textbooks in biochemical engineering. Naturally, augmenting them with the skewness and kurtosis would better characterize the distribution, thereby deepening the understanding of stochastic, *i.e.*, temporally varying probabilistic, nature of thermal death of cells during their disinfection.

Thermal disinfection has long been regarded as a suitable or useful instructional example for illustrating stochastic analysis and modeling of biochemical phenomena or processes, the majority of which deal with discrete mesoscopic entities that are neither microscopic nor macroscopic. To enhance its usefulness, the example has been substantially elaborated in the current exposition. As indicated at the outset of this article, various chemical engineering courses are richly populated with subjects involving mesoscopic entities, such as bubbles, droplets, and particles including nanoparticles. Thus, these subjects would be suitable examples for

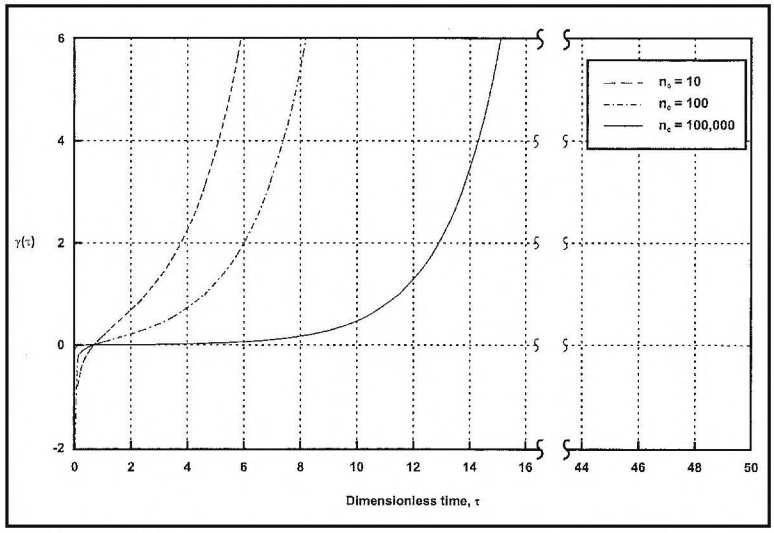

Figure 5. *Skewness as a function of the dimensionless time, τ, and the initial size of cell population, n_0, as the parameter.*

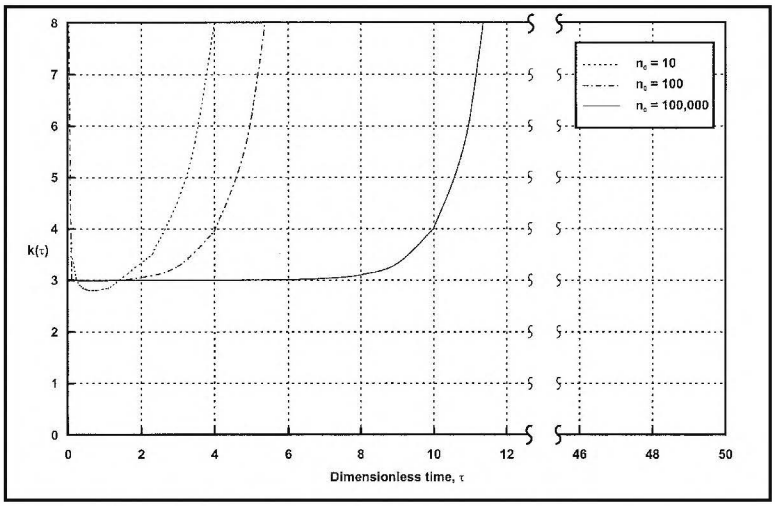

Figure 6. *Kurtosis as a function of the dimensionless time, τ, and the initial size of cell population, n_0, as the parameter.*

introducing the application of stochastic processes in these courses, similar to the thermal destruction of microorganisms for biochemical engineering.

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NOMENCLATURE

E[N(t)]	mean, expected value, or the first moment of the random variable, N(t)
$E[N^2(t)]$	second moment of the random variable, N(t)
$E[N^3(t)]$	third moment of the random variable, N(t)
$E[N^4(t)]$	fourth moment of the random variable, N(t)
k	proportionality constant in the intensity of death, Eq. (2), min^{-1}
k(t)	kurtosis of the random variable, N(t)
N(t)	random variable
m(t)	E[N(t)]
n	realization of the random variable, N(t)
n_0	number of live cells at t = 0
$p_n(t)$	probability that the process will be in state n at time t
t	time
$\gamma(t)$	skewness of the random variable, N(t)
μ_n	intensity of death for the pure-death process in state n
$\sigma^2(t)$	Var[N(t)]
$\sigma(t)$	standard deviation of the random variable, N(t), as defined in Eq. (14)

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