Application of stochastic equations of population balances to sterilization processes

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1. Introduction

There are numerous decontamination processes where the objective is to remove most or all foreign cells. Mathemtical modeling of the expected population dynamics has aided in the understanding and design of wastewater treatment (Li \textit{et al.}, 2006; Liu \textit{et al.}, 2007), food processing (Selma \textit{et al.}, 2007; Tanaka \textit{et al.}, 2007) and disease treatment (Basse \textit{et al.}, 2004; Gaffney, 2004; Michor \textit{et al.}, 2005). However, there is an inherent uncertain timing of cellular events that leads to deviations from the expected behavior. When a large number of cells are considered, the randomness tends to average out; the population fluctuations are small compared to the population size and generally assumed to be negligible. As such, the expected population is a reasonable indicator for quantitatively describing the system. However, these deviations from this expected behavior become significant for small numbers of cells—such as those dealt with in decontamination processes—and become important for describing the state of the system. For example, the likelihood that the number of cells is below a critical threshold (the infectious dosage of \textit{E. coli} O157:H7 or \textit{Salmonella} can be as low as 10 cells, Ivnitski \textit{et al.}, 1999) or that all cells are removed may be of interest.

To answer such questions, the concept of stochastic analysis, using stochastic formulations of population balances, is adopted to describe the cell number probability distribution. Previous analyses have considered unstructured cell populations (Fredrickson, 1966) where the instantaneous birth and death properties are uniform for all cells. This allows either direct analytical solution of the cell
number probability distribution or its description by a differential equation system. Herein, this analysis is expanded to an age structure that accounts for the distribution of time elapsed between cell birth events. In the age-structured model, the timing of births and deaths is also uncertain, but these events lead to variations in the actual number density as well as the associated total number density. Previous work with such age-structured models has shown that the combination of low expected value and small variance of the number of cells can provide an indicator for when sterilization is extremely likely (Sherer et al., 2007). While this method is limited to identifying when it is likely that all cells have been removed, it illustrated the potential for quantifying the outcomes of stochastic processes. The current work expands the analysis of the properties of stochastic population balances and develops a method for describing the entire cell number probability distribution.

Before calculating the total cell number probability distribution, the relationship between the master density, which describes the probability of an actual number density, and its product densities, which allows for the calculation of the total number distribution (Ramkrishna, 2000) and its moments (Ramkrishna and Borwanker, 1974) from the total product densities, are presented in Section 2. A continuous birth model—where the mother retains its age after giving birth—is discussed in Section 3. Then, an approximation of the cell number probability distribution through its moments is introduced and shown to be in good agreement with the likely distribution. While this continuous birth model does show correlations of cell ages, it will be shown that a closure approximation does not significantly impact the accuracy of higher moments. This feature allows for a reasonable approximation of the cell number probability distribution in a tractable period of time. A mother and daughter cell birth model—where a birth event produced two cells both of age zero which may have different age-dependent birth and death properties—is given in Section 4 and the influence of the different birth rate functions between mothers and daughters are analyzed. The results and key points are then summarized in Section 5.

2. Mathematical formulation

In an unstructured cell birth and death model, the total population changes subject to a series of Bernoulli trials where each birth event increases the total population size by one and each death event decreases the size by one. Given that each cell gives birth at a rate $\Gamma$ and dies at a rate $\kappa$, the likelihood that a particular cell will undergo a birth or event in an infinitesimal time interval, $dt$, are $\Gamma dt$ and $\kappa dt$, respectively. With $N(t)$ total number of cells at time $t$, the likelihood of a birth or death event during $dt$ are $N(t)\Gamma dt$ and $N(t)\kappa dt$, respectively. So, the probability that the total population remains unchanged after $dt$ is $1 - N(t)(\Gamma + \kappa)dt$. Not only is the outcome of each cell’s Bernoulli trials uncertain, but the time at which the events occur is also random. This uncertainty in the timing and identity of events implies that the actual number of cells must be described probabilistically where the probability of having $N(t)$ total number of cells at time $t$ is given by the cell number probability distribution $P_N(t)$. So, the dynamics of the cell number probability distribution are described by a differential equation and its initial condition:

$$\frac{dP_N(t)}{dt} = \Gamma(N - 1)P_N(t) - (\Gamma + \kappa)NP_N(t) + k(N + 1)P_{N+1}(t).$$

$P_N(0)$. However, the instantaneous birth and death rates will not likely be identical for every cell in the population. For example, a newborn cell will experience a time lag before beginning birth events of its own. The timing between events can be described by transition-age probability distributions and the associated age-structured transition rates where the age, $\tau$, is the amount of time since some critical event, such as a birth, in the history of a cell. The structure of the transition rate requires accounting for age of each cell in the population via the actual number density $n(\tau, t)$. Analogous to the total number of cells in the unstructured model, the actual number density is known exactly

$$n(\tau, t) = \sum_{i=1}^{N} \delta(\tau - \tau_i),$$

where the total number of cells is found by counting the number of cells in the actual number density (see Fig. 1).

In an age-structured population balance model, the probability that an event occurs in an individual cell in $dt$ depends on the age of that cell. Since each cell has a different age, the cells must be accounted for individually. This uncertainty in the timing, identity, and cell specificity of an event leads to uncertainty in the actual number density $n(\tau, t)$. So, not only is the actual total number density $N(t)$ only known probabilistically, but the age of the composite cells are also uncertain. The likelihood of having a given number density is described by the master density function $J_N$ where

$$\Pr \left\{ n(\tau, t) = \sum_{i=1}^{N} \delta(\tau - \tau_i) \right\} = J_N(\tau_1, \tau_2, ..., \tau_N; t)$$

and the probability of a given total number density is found by considering all possible age combinations of the number densities

$$\Pr(N(t)) = \prod_{r=1}^{N} \int_{0}^{\infty} d\tau J_N(\tau_1, \tau_2, ..., \tau_r, ..., \tau_N; t).$$

2.1. Product densities

Because of the combinatorics involved, the master density is rarely modeled explicitly. A common approach is to use Monte Carlo simulations to approximate the master density where each trial is one realization of the system (Shah et al., 1976; Lin et al., 2002; Mantzaris, 2006). The aggregate of many trials highlights which regions of the master density are more likely than others. Properties of the master density are also given by its product densities which are averaged properties—namely, the average of the product of actual number densities—of the master density.
The expected number density, \( n_1(\tau, t) \), whose dynamics are described by a population balance equation, is also a property of the master density. This number density is an average over all possible age combinations save one, so it is also the first order product density:

\[
n_1(\tau, t) = \mathbb{E}[n(\tau, t)] = \sum_{N=1}^{\infty} \frac{1}{(N-1)!} \prod_{r=1}^{N-1} \int_0^{\tau} \text{d} \tau_r \times J_N(\tau_1, \tau_2, \ldots, \tau_{N-1}, \tau; t),
\]

and the expected total number density is the average over all age combinations

\[
N_1(t) = \mathbb{E}[N(t)] = \int_0^{\infty} n_1(\tau, t) \text{d}\tau.
\]

Variations from the expected number density appear as correlations between cell ages. Because of the age-dependent transition rates, certain age pairing will be more prevalent and lead to variations in the actual number density. For a large number of cells, these fluctuations tend to average out, but the correlations become much more significant when the number of cells becomes small. The correlation of cell ages is given by higher order product densities of the master density which account for the ages of groups of cells. For example, the correlation in ages between a pair of cells is given by the second order product density, \( n_2(\tau, \tau', t) \), which is the expectation of the product of the actual number density of cells of age \( \tau \) with actual number density of cell with an age \( \tau' \):

\[
n_2(\tau, \tau', t) = \mathbb{E}[n(\tau, t)n(\tau', t)] = \sum_{N=2}^{\infty} \frac{1}{(N-2)!} \prod_{r=1}^{N-2} \int_0^{\tau} \text{d}\tau_r \times J_N(\tau_1, \tau_2, \ldots, \tau_{N-2}, \tau, \tau'; t).
\]

The likelihood of finding a cell in the age window \( (\tau, \tau + \text{d}\tau) \) and a second cell in the age window \( (\tau', \tau' + \text{d}\tau') \) is \( n_2(\tau, \tau', t) \text{d}\tau \text{d}\tau' \). So, the preference for age combinations is reflected in the second order product density. Likewise, higher order product densities can be written which give the pairings of multiple cell ages where the ith order product density 

\[
n_i(\tau^{(1)}, \tau^{(2)}, \ldots, \tau^{(i)}; t) = \sum_{N=i}^{\infty} \frac{1}{(N-i)!} \prod_{r=1}^{N-i} \int_0^{\tau} \text{d}\tau_r \times J_N(\tau_1, \tau_2, \ldots, \tau_{N-i}, \tau^{(1)}, \tau^{(2)}, \ldots, \tau^{(i)}; t).
\]

The dynamics of each product density are described by its product density equation. The product density equations for two different models will be derived in Sections 3 (a continuous birth model) and 4 (a mother and daughter cell model).

For the purposes of cell number quantification, the actual total number density—which also contains the likelihood of zero cells—is ultimately of concern. Just as the integrated number density gives the expected number of cells, integrating the higher order product density gives quantities that can yield higher moments of the cell number probability distribution. The ith order total product density is given by

\[
N_i(t) = \prod_{j=1}^{i} \int_0^{\infty} \text{d}\tau_j n_j(\tau_j; t),
\]

where \( \tau_j = [\tau_1, \tau_2, \ldots, \tau_i] \) is defined as a vector containing the ages of \( i \) number of cells. If the number of initial particles is known exactly, then

\[
N_i(0) = N(0),
\]

\[
N_i(0) = \prod_{j=0}^{i-1} (N(0) - j).
\]

The solutions of the above equations for the dynamics of the total product densities can be used to describe the cell number probability distribution.

### 2.2. The cell number probability distribution and its moments

While the master density and cell number probability distribution are probabilistic quantities, their product densities and total product densities give a glimpse into the respective properties. Higher order product densities provide additional information on the correlation between particle ages, and improve the resolution of the master density. Likewise, each subsequent total product density provides additional information so that as shown in \textit{Ramkrishna} (2000), the total product densities can be used to directly calculate the probability of having \( v \) cells at time \( t \):

\[
P_v(t) = \sum_{i=v}^{\infty} \frac{(-1)^{i-v}}{v!(t-v)!} N_i(t), \quad v > 0,
\]

\[
P_v(t) = 1 - \sum_{v=1}^{\infty} P_v(t).
\]

However, several practical issues limit the application of Eq. (2). The most immediate is computational errors likely due to numerical round-off of the factorials in the summation. For example, the summation of \( P_{10}(0) \) when there are exactly 40 cells has several terms in the summation that are greater than \( 10^{16} \) and, since only 16 significant digits are retained, round-off errors lead to summation terms which do not cancel exactly. As a result, the numerical calculation of \( P_{10}(0) \) is greater than 1 and most other probabilities take impossible values that are either greater than 1 or less than 0. Another consideration is the shear number of product densities involved. If even a small birth term is considered, the higher order total product densities increase in magnitude and the number of total product densities required for the cell number probability distribution summation to converge can greatly exceed the number of cells under investigation.

Each subsequent total product density provides additional information about the cell number probability distribution. This information comes in the form of an additional moment of the distribution. These total product densities give information about the cell number probability distribution. The raw moments, \( \mu^i \), of the cell number probability distribution are found from combination of the total product densities using the Sterling numbers of the second kind. These raw moments can then be transformed to the ith central moments, \( \mu_i \), using the binomial transform:

\[
\mu_i = \sum_{j=1}^{i} \binom{i}{j} (-1)^{i-j} \mu_j^i \mu_j^{i-j}
\]

from which common properties such as the mean, \( \mu \), standard deviation, \( \sigma \), skewness, \( \gamma_1 \), and kurtosis, \( \beta_2 \), of the cell number probability can be derived:

\[
\mu = \mu_1 = N_1(t),
\]

\[
\sigma = (\mu_2)^{1/2} = (N_1(t) + N_2(t) - N_1(t)^2)^{1/2},
\]

\[
\gamma_1 = \frac{\mu_3}{(\mu_2)^{3/2}} = \frac{[2N_1(t)^2 - 2N_1(t) - 3N_2(t) + 1]N_1(t) + 3N_2(t) + N_3(t)}{[N_2(t) + N_1(t) - N_1(t)^2]^{3/2}},
\]

\[
\beta_2 = \frac{\mu_4}{(\mu_2)^2} - 3.
\]
\[
\beta_2 = \frac{\mu_0^2}{\mu_2^T} = \frac{[\mu_2(t_1) - 2N_1(t)^3 + 6N_1(t)t^2 + 6N_1(t)N_2(t) - 4N_1(t) - 12N_2(t) - 4N_3(t) + 1]N_1(t) + 7N_2(t) + 6N_3(t) + N_4(t)}{\{N_1(t) + N_2(t)\}^2}
\]

While it is difficult to entirely reconstruct a cell number probability distribution from the total product densities, these first four moments provide good qualitative indicators of the shape of the unimodal distribution. With knowledge of additional moments, a reasonable approximation of the distribution can be made as shown in Section 3.

3. Evaluation of a continuously budding model

Section 2.2 introduced a description of the cell number probability through its total product densities which are governed by the product density equations representation of the stochastic nature of the population balance model. This section utilizes this description in introducing and analyzing a methodology to approximate the cell number probability distribution for a continuous birth model. While an approximation, this method is a first step towards timely quantification of a structured model. In addition, the quality of the approximation can be adjusted by the level of detail of the master density via additional product density equations and total product densities.

In this model a cell continuously produces new cells of age zero at a rate \(\Gamma(t)\), but the age of the original cell continually increases (see Fig. 2). The ith order product density equation is given by

\[
\frac{\partial n_i(t, \tau)}{\partial t} + \sum_{j=1}^{i} \frac{\partial n_j(t, \tau)}{\partial \tau_j} = \sum_{j=1}^{i} k(t, \tau_j)n_j(t, \tau),
\]

subject to the initial and boundary conditions

\[
n_i(t_{\tau}, 0),
\]

\[
n_i(t_{\tau-1}, 0, t_{\tau+1}, \ldots, t_{\tau}, t)
\]

\[
= \int_0^\infty \Gamma(t) n_i(t_{\tau}, t) \, dt
\]

\[
+ \sum_{j=1}^{i-1} \Gamma(t) n_{i-1}(t_{\tau-1}, t_{\tau+1}, \ldots, t_{\tau-j}, t),
\]

respectively.

The expected number density can be separated into the total expected number density, \(N_1(t)\), and the expected number density distribution, \(\rho_1(\tau)\).

\[
n_1(t_{\tau}, t) = N_1(t)\rho_1(\tau).
\]

Under the special condition of balanced growth, this expected number density distribution, \(\rho^b_1(\tau)\), can be solved for iteratively

\[
\rho_1(\tau) = \frac{\exp[-\int_0^\tau (\Gamma(t) - k(t)) \, dt]}{\int_0^\infty \exp[-\int_0^\tau (\Gamma(t') - k(t')) \, dt'] \, dt'}
\]

If initial independence of cells and balanced growth are assumed, the initial condition is

\[
n_i(t_{\tau}, 0) = \prod_{j=0}^{i-1} n_i(t_{\tau-1}, 0, t_{\tau+1}, \ldots, t_{\tau-j}, t),
\]

utilizing the age initial and boundary conditions gives

\[
\frac{dN_1(t)}{dt} = \sum_{j=1}^{i} \int_0^\infty \Gamma(t) n_i(t_{\tau}, t) \, dt
\]

\[
- \sum_{j=1}^{i} \int_0^\infty \Gamma(t) n_{i-1}(t_{\tau-1}, t_{\tau+1}, \ldots, t_{\tau-j}, t) \, dt,
\]

which reduces to

\[
\frac{dN_1(t)}{dt} = \sum_{j=1}^{i} \int_0^\infty \Gamma(t) n_j(t_{\tau}, t) \, dt
\]

\[
- \sum_{j=1}^{i} \int_0^\infty \Gamma(t) n_{j-1}(t_{\tau-1}, t_{\tau+1}, \ldots, t_{\tau-j}, t) \, dt,
\]

are the ith order specific birth and death rates, respectively.

These rates can be used to calculate the total product densities via Eq. (5) and ultimately the moments of the cell number probability distribution. In addition, the cell number probability distribution becomes less sensitive as the order of the moment increases. That is, variations in the mean and standard deviation may be noticeable, but alterations in such properties as the skewness and kurtosis need be relatively larger to be reflected in the distribution. As such, a closure approximation is proposed that allows for the approximation of higher order total product densities based on lower order densities.
When specific rates up to the $J$th order are calculated, all specific division rates of order greater than $J$ are set equal to the $J$th order rate, so the $i$th order specific division rate is

$$G_i(t) = G_J(t) \quad \text{for } i > J.$$  \hfill (6)

### 3.1. Age-structured death rates

With age-structured transition rates, the corresponding specific rates depend on the nature of the age structure and the resulting product density. As such, the product densities are calculated and integrated to find the total product densities. In Figs. 3 and 4, the continuously increasing birth rate with a death rate that is equal to twice the birth rate (as shown in Fig. 5a) model is applied to an initial population of $10^9$ independent cells. The birth rate corresponds with a normalized Gaussian distribution, for the time interval until the first birth event, with a mean of 12 h and a standard deviation of 3 h:

$$G(t) = \frac{2\exp(-((t-12)^2/2(3)^2))}{\sqrt{2\pi}\sigma}\text{erfc}(t-12/\sqrt{2}(3)).$$  \hfill (7)

This scenario results in the exponential population decline seen in Fig. 5b. For the expected total population dynamics ($\mu(t)$) shown in Fig. 5b, the number densities in Figs. 5c and d describe the likelihood of finding a cell in an infinitesimally small age window at $t = 0$ and 500 h, respectively. While the number density distribution does not change with time, the value of the $y$-axis continually decreases as the expected total number of cells declines. The probabilistic nature of the expected number density is clear at $t = 500$ h when there may or may not even be a single cell.

For times less than 200 h, when the expected number of cells is above $10^3$, the scaling of the standard deviation roughly as the square root of the expected population is characteristic of a Poisson process. When the expected population is large, the Poisson distribution can be approximated as a Gaussian distribution and corresponding small skewness indicates the symmetry of the normal curve. The Poisson-like behavior is due to the averaging of birth and death Bernoulli trials of individual cells when there are a large number of cells. In this case, each cell can essentially be treated as identical with no regard for the age-structure. However, when the population size decreases to less than $10^3$ ($>200$ h) the individual behaviors begin to have an impact and the Poisson approximation breaks down. The transition to cell dependence is seen in changes in the higher order product density distributions (Figs. 3 and 4). Since the division rate continually increases with age, correlations begin to develop between the older and younger cells. For example, in Fig. 4d, a young cell ($\tau = 0$ h) is more likely than average to be paired with an older cell (likely a progenitor cell) or another younger cell (likely a sister cell from the same progenitor). As a cell ages ($\tau = 5$ h), it is most likely paired with another cell of its own age. Once the cell reaches ages when it produces significant birth events ($\tau = 10$ or 15 h), pairing with younger cells are likely. Such correlations are reflected in changes in the specific division and death rates away from the expected rates (see Fig. 6a).

However, the changes in the specific division rates are surprising small. When the death rate is twice the birth rate (Fig. 6a), the correlation of cell ages change the second order specific division rate by less than 1% until there are $<10$ cells with a maximum difference less than 4%. The maximum difference between the first order and second order specific rates increases as the death rate increases (Fig. 6b), but this difference is still $<10\%$ when the death rate is $\times 10$ larger than the birth rate. However, the expected population size at which this divergence occurs continually decreases as the death rate increases (Fig. 6c). So, while the difference in specific rates is $\sim 10\%$ when the death rate is $\times 10$ the birth rate, the divergence between...
Fig. 4. Slices of the second order product density distributions associated with the model of Fig. 2 and dynamics of Fig. 5b. The age of one cell is fixed and the probability of a paired cell’s age is plotted. (a) The ages of cells in a pairing nearly independent at $t = 200\, h$. (b) After 250\, h, and even with only 100 cells remaining, the ages of cell in a pairing are almost completely independent. (c) Correlations in ages are present at $t = 300\, h$ when less than 10 cells are expected. (d) The correlations are strengthened at $t = 350\, h$ as the expected population continues to decline below 1 cell.

Fig. 5. The expected number density at the beginning and near the end of a simulation of the continuous budding yeast model is described in Section 3. Under model assumptions of (i) balanced growth conditions, (ii) the age-structured birth rate shown in Fig. 2a, (iii) a death rate that is twice the birth rate, and (iv) $10^9$ initial cells, the time-invariant transition rates imply a time-invariant expected number density distribution. (a) Rate at which buds are formed as a function of the amount of time since the mother cell was born. The birth rate corresponds with a normalized Gaussian distribution for the age at which the first birth occurs. (b) The expected population dynamics ($N(t)$), its standard deviation ($\sigma(t)$), and skewness ($\gamma_1(t)$). (c) The number density at the beginning of the simulation, $t = 0\, h$. (d) The number density at a low number of expected cells, $t = 500\, h$. 
these rates do not occur until the expected population is 10 cells. With such a dominating death rate there are relatively fewer births than deaths as the population moves from 10 cells to 0 cells, so there is less of an opportunity for the errors due to differences in specific birth rates to propagate in the calculations of the total product densities when the closure approximation is applied. The second and third order rates are even more similar. In fact, if the first order rate is used to approximate the third and fourth order specific rates, when the initial population is $10^3$ cells rather than $10^9$, the resulting skewness and kurtosis are nearly identical to the exact values (Fig. 7).

While a large number of total product densities are required to exactly calculate the cell number probability distribution, a reasonable approximation can be made based on only a handful of properties when the expected number is low. This is especially true since the variance of the distribution is small for $> 10^3$ expected number of cells after which deviations begin to arise. As such, the cell number probability density, resulting from $10^3$ initially independent cells with $\lambda(t) = 2 \times \Gamma(t)$, is fit to the first 10 central moments, calculated from Eqs. (5) and (6) and using a linear combination of three backward integrated normal distributions (means, standard deviations, weighting variables, and $P_0(t)$ are fit) and the Matlab local search routine “fminsearch”. As seen in Fig. 8, this approximation matches the shape of an approximation based on 1000 age-structured Monte Carlo simulations. However, the moment approximation holds a definite advantage in the computation time: $\sim 15$ m versus $\sim 50$ h on a Pentium 4 personal computer. This method allows for a rapid quantification of potential sterilizing treatments (e.g., heating, freezing, UV light, radiation, chemotherapy, or other potential interventions).

However, the resolution of the quantification is limited by the number of product densities that can be calculated, the number of total product densities used to approximate the distribution, and the approximation method itself. While for the scenarios examined, 2–3 product densities and 10 total product densities give sufficient information about the distribution, methods to best fit these moments have not been thoroughly investigated herein. The accuracy and precision of the estimates could likely be increased by improving the method for approximating the cell number probability distribution from its moments.

3.2. Uniform death rates

A common assumption for cell death behavior is that cells of all ages are equally susceptible and die at the same, age-independent, rate. In this case, for younger cells, the death rate may exceed the birth rate while the rate of birth is more rapid for older cells. The behavior of the age-importance is similar to that of the age-structured death rates. The processes can again be approximated as Poisson until less than $10^3$ expected cells when correlations begin to appear and the corresponding alterations in the specific division rate are observed (Fig. 9a). In this case, the second order specific rates increase relative to the first order specific rates (Fig. 9b) with percentages greater than the 4–10% seen with age-dependent death (Fig. 9c). But as seen previously, the difference in specific rates decreases as the order of the specific rate increases. This implies that the effect of the closure moment order decreases as the order increases. In the current scenario, the second order specific division rate provides a
Fig. 7. Approximation of higher order moments of the cell number probability distribution using a closure approximation for the specific division rates: all specific rates of order > 1 equal \( \Gamma_i(t) \). (a) Skewness and (b) kurtosis.

Fig. 8. Comparison of approximations of the cell number probability distribution; 1000 Monte Carlo simulations versus fitting the first 10 central moments with normalized Gaussian distributions. (a) The cell number probability distribution at \( t = 80 \) h. (b) The likelihood of removing all cells and the likelihood of having 10 or less cells.

reasonable approximation of all higher order specific rates. This is shown in the agreement in the quantification of the cell number probability distribution between Monte Carlo simulations and fitting the first 10 moments after applying the closure approximation (Fig. 9d). If the fourth order specific division rate was to be used for the closure approximation, the moments would be more accurate, but again, the moment approximation method is likely limited by the methodology used to approximate the moments.

4. Mother and daughter cell model

The results in Section 3 show that the concepts introduced in Section 2 can be used to approximate the cell number probability distribution. This section applies the methodology to cell birth model that explicitly accounts for the time between birth events. In this model, each birth event produces one mother cell and one daughter cell; both cells are of age zero immediately after the birth event. However, the age-structured birth and death rates may be different depending on whether a cell is a mother or daughter. The mother cells birth and death rates are denoted as \( I_{m}^{\text{m}}(t) \) and \( k_{m}^{\text{m}}(t) \), respectively, and the daughter cells follow \( I_{d}^{\text{d}}(t) \) and \( k_{d}^{\text{d}}(t) \) (see Fig. 10). It is assumed that the birth behavior does not vary with time and the death rate affects all cells of the same type equally.

The first order product density equation for mother–daughter model of

\[
\frac{d n_1(\tau, t)}{dt} + \frac{d n_1(\tau, t)}{d\tau} = D_1^{\text{in}}(\tau, t) n_1(\tau, t),
\]

\[
n_1(\tau, 0) = n_{1,0},
\]

\[
n_1(0, t) = \int_0^\infty D_1^{\text{in}}(\tau) n_1(\tau, t) d\tau,
\]

\[
n_1(\tau, t) = \begin{bmatrix} n_{1,0} \end{bmatrix} e^{D_1^{\text{in}}(\tau, t)t}.
\]
Fig. 9. Analysis of the continuous birth model with an age-independent death rate. (a) The mean, standard deviation, and skewness of the cell number probability distribution as calculated from the product density equations. (b) First three specific division rates when the death rate is 0.30 h\(^{-1}\). (c) Maximum difference between the second and first order specific division rates with the death rate. (d) Comparison of approximations of the likelihood of removing all cells and the likelihood of having 10 or less cells as calculated from the product density equations.

(b) First three specific division rates when the death rate is 0.30 h\(^{-1}\):

- \(d_1\) where the death rate is 0.30 h\(^{-1}\):
- \(d_2\) where the death rate is 0.30 h\(^{-1}\):
- \(d_3\) where the death rate is 0.30 h\(^{-1}\):

Mother and daughter cell model. Each birth event creates one new mother cell of age zero and one new daughter cell of age zero. The birth and death rates may vary depending on whether a cell is a mother or a daughter.

\[
D_{100}^{out}(t,t) = \begin{bmatrix}
-I^m(t) - k^m(t) & 0 \\
0 & -I^d(t) - k^d(t)
\end{bmatrix}
\]

\[
D_{200}^{out}(t,t) = \begin{bmatrix}
-I^m(t) - I^m(t') - 2k^m(t) & 0 & 0 \\
0 & -I^m(t) - I^d(t') - k^m(t) - k^d(t) & 0 \\
0 & 0 & -I^d(t) - I^d(t') - 2k^d(t)
\end{bmatrix}
\]

\[
D_{200}^{in}(t) = \begin{bmatrix}
I^m(t) & 0 & I^d(t) \\
0 & I^m(t) & 0 \\
0 & 0 & I^d(t)
\end{bmatrix}
\]

\[
\Gamma_m(t) = \int_0^\infty \frac{\partial n_2(t,t')}{\partial t} dt + \delta(t') \Gamma_{100}^{in}(t)n_1(t,t),
\]

where

- \(n_2(t,t')\) is the second order product density equation for

- \(\Gamma_0(t)\) is the first order specific division rates with the death rate.

\[
\Gamma_m(t) = \int_0^\infty \frac{\partial n_2(t,t')}{\partial t} dt + \delta(t') \Gamma_{100}^{in}(t)n_1(t,t),
\]

\[
\Gamma_0(t) = \begin{bmatrix}
-I^m(t) & 0 & 0 \\
0 & -I^m(t) & 0 \\
0 & 0 & -I^d(t)
\end{bmatrix}
\]

\[
\Gamma_1(t) = \begin{bmatrix}
-I^m(t) & 0 & I^d(t) \\
0 & I^m(t) & 0 \\
0 & 0 & I^d(t)
\end{bmatrix}
\]

\[
\Gamma_2(t) = \begin{bmatrix}
-I^m(t) & 0 & 0 \\
0 & I^m(t) & 0 \\
0 & 0 & I^d(t)
\end{bmatrix}
\]

The second order product density equation for

\[
n_2(t,t') = \begin{bmatrix}
n_2^{in}(t,t',t) \\
n_2^{in}(t,t',t) \\
n_2^{in}(t,t',t)
\end{bmatrix}
\]

\[
is \frac{\partial n_2(t,t',t)}{\partial t} + \frac{\partial n_2(t,t',t)}{\partial t'} + \frac{\partial n_2(t,t',t)}{\partial t'}
\]

\[
= D_2^{out}(t,t')n_2(t,t'),
\]

\[
n_2(0, t', 0) = n_2|_{t'=0},
\]

\[
n_2(0, t', 0) = \int_0^\infty D_2^{in}(t)n_2(t, t', 0) dt + \delta(t') \Gamma_{100}^{in}(t)n_1(t, 0),
\]

where

- \(n_2(t, t', 0)\) is the second order product density equation for

- \(\Gamma_0(t)\) is the first order specific division rates with the death rate.

\[
\Gamma_0(t) = \begin{bmatrix}
-I^m(t) & 0 & 0 \\
0 & -I^m(t) & 0 \\
0 & 0 & -I^d(t)
\end{bmatrix}
\]

\[
\Gamma_1(t) = \begin{bmatrix}
-I^m(t) & 0 & I^d(t) \\
0 & I^m(t) & 0 \\
0 & 0 & I^d(t)
\end{bmatrix}
\]

\[
\Gamma_2(t) = \begin{bmatrix}
-I^m(t) & 0 & 0 \\
0 & I^m(t) & 0 \\
0 & 0 & I^d(t)
\end{bmatrix}
\]
Population size mean and standard deviation: identical mother and daughter cells

$$\mu_1(t) = \gamma_1^{(m)}(t) = \gamma_1^{(d)}(t).$$

Higher order product density equations can be written in a similar manner.

In the mother–daughter model the first order product density is a combination of the individual mother and daughter cell product densities

$$n_1(t, t) = n_1^{(m)}(t, t) + n_1^{(d)}(t, t),$$

where Eq. (1) can be applied to both the individual and overall product densities to give the respective total product densities. Higher order product densities examine all combinations of mother and daughter cells; for example the second order product density is

$$n_2(t, t', t') = n_2^{m,m}(t, t', t') + n_2^{m,d}(t, t', t')$$

$$+ n_2^{d,m}(t, t', t') + n_2^{d,d}(t, t', t').$$

The $i$th order product density can be found by summing the appropriate $2^i$ individual product densities.

For the mother and daughter cell model, the overall specific rates are partitioned between the two newborn cells. Eq. (1) cannot be applied to find the overall specific birth rate as no overall age-structured division rate exists. Rather, as each of the individual product densities change with time and the overall specific rate is a combination of the changes to the specific birth rates of the mother and daughter cells. For example, the first specific birth rate is

$$\Gamma_1(t) = \frac{\gamma_1^{(m)}(t) N_1^{(m)}(t) + \gamma_1^{(d)}(t) N_1^{(d)}(t)}{N_1(t)}.$$
mother cells divide more readily than daughter cells \( (m(t) = 1.25f(t) \) and \( m'(t) = 1.25f'(t) \)) the second order specific division changes by <2% (see Figs. 1c and d). This small change is in spite of the variable specific division rates between the mother and daughter cells. This change is also less than the 3% when the expected division rate of the continuously budding model predicted the entire cell number probability distribution.

5. Summary

The ultimate goal of a decontamination processes is the eradication of all or most of the foreign cells, but this condition cannot be guaranteed due to the uncertain nature of the timing of cell divisions and deaths. These random behaviors lead to deviations from the expected behavior, so the expected number of cells is only a first indicator of sterilizer progression. What is truly of interest is the likelihood that all cells have been removed. To this end, the properties of this cell number probability density are studied through the master density. While the master density itself is evasive, its properties can be found through its product densities and corresponding total product densities. The derivation of these equations is presented and their properties discussed. In particular, how the moments of the cell number probability distribution can be formed from the total product densities. The full cell number probability distribution can then be approximated from these moments. Integration of the product density partial differential equations results in a series of ordinary differential equations for the total product densities. When an age-structured birth term is added, the age-averaged division rate will vary with the product density order.

Two models were evaluated to examine the trends of these specific rates and their application towards quantifying the cell number probability distribution. In the continuous birth model, the behavior of the model subject to both age-structured and age-unstructured death rates was examined. In both cases, variations in the specific rates were not seen for greater than \( 10^2 \) cells and the properties of the first three moments of the cell number probability distribution indicate that it follows a nearly Gaussian distribution until this threshold. This behavior is indicative of a Poisson process approximated by the Bernoulli trial-like birth and death behavior of individual cells which averages out for such large populations. However, as the population decreases, the behavior of individual cells becomes important (though the expected population threshold at which this transition occurs decreases as the death rate increases) and higher order specific rates should be considered. While including the correlation effects are important, the marginal change and importance in the specific rate decreases with increasing order so that a closure approximation can be applied for use in approximating higher order total product densities. This closure approximation is validated for the skewness and kurtosis using only the first specific rate before approximating the first 10 central moments of the cell number probability distribution. The full cell number probability distribution can be approximated from these moments (while the methods used were crude, the predictions are in excellent agreement with Monte Carlo simulations), and quantitative indicators extracted from the resulting distribution. This methodology is shown to apply to a mother and daughter cell model. The approximations are likely even more accurate for this model as the age-correlations observed are weaker than in the continuous birth model.

While the existence of correlations is demonstrated for small number of cells, the impact on the cell number probability distribution was negligible for the cases studied. The higher order specific division rates nearly exactly match the expected division rate until there are few cells and there is insufficient time for the deviations to significantly influence the model predictions. Starting with 1000 cells, the first order product density gives an excellent prediction of the properties of the cell number probability distribution; for \( 10^3 \) initial cells, the first two product densities provide good quantitative predictions. While this scenario allows for extremely efficient approximations of the cell number probability distribution, the types of models investigated was limited and models and scenarios likely exist where the correlation effects do play a significant role. The presented methodology is general to any such systems where additional product densities can be considered until the approximation of the total number densities is sufficiently good.

References


