International Journal of Food Microbiology 290 (2019) 125–131

Contents lists available at ScienceDirect

International Journal of Food Microbiology

journal homepage: www.elsevier.com/locate/ijfoodmicro

Stochastic simulation for death probability of bacterial population considering variability in individual cell inactivation time and initial number of cells

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ARTICLE INFO

Keywords:
Monte-Carlo simulation
Contour line
Poisson distribution
Weibull distribution

ABSTRACT

Decimal reduction time (*D*-value) based on the first-order survival kinetics model is not sufficient for reliable estimation of the bacterial survivors of inactivation treatment because the model does not consider inactivation curvature. However, even though doubt exists in the calculation of *D*-value, it is still widely used for risk assessment and sterilisation time estimation. This paper proposes an approach for estimating the time-to-inactivation and death probability of bacterial population that considers individual cell heterogeneity and initial number of cells via computer simulation. In the proposed approach, Weibull and Poisson distributions are respectively used to provide individual cell inactivation time variability and initial number of cells variability. Our simulation results show that the time-to-inactivation significantly depends on kinetics curvature and initial number of cells. For example, with increases in the initial number of cells, the respective variance of the time-to-inactivation of log-linear, concave downward curve, and concave upward curve remains constant, decreases, and increases, respectively. The death probability contour plot was successfully generated via our computer simulation approach without using *D*-value estimation. Further, the death probability calculated using our stochastic approach was virtually the same as that obtained using inactivation kinetics. We validated the simulation by using literature data for acid inactivation of *Salmonella* population. The results of this study indicate that inactivation curvature can replace *D*-value extrapolation to estimate the death probability of bacterial population. Further, our computer simulation facilitates realistic estimation of the time-to-inactivation of bacterial population. The R code used for the above stochastic calculation is outlined.

1. Introduction

The changes in average number of bacterial survivors are typically described using inactivation kinetics. The traditional model is log-linear inactivation kinetics and microbial inactivation is considered as a process that follows first-order-kinetics in this theory (Peleg and Cole, 1998). Decimal reduction time (*D*-value) is defined from log-linear inactivation. Further, inactivation curves with upward or downward concavity has been developed, because many inactivation behaviour show curvatures (Peleg and Cole, 1998; Xiong et al., 1999). Although microbial inactivation does not always follow the log-linear model, *D*-value is still widely used for risk assessment and sterilisation time estimation (Brown, 2002; Peleg and Normand, 2004). However, the accuracy of *D*-value calculation is in doubt (Koseki et al., 2009; Koyama et al., 2017a; Peleg, 2006). Because of the curvature of inactivation kinetics, overestimation or underestimation of bacterial survivors can occur based on *D*-value (Peleg, 2006). In addition, the death probability of bacterial population is not estimated precisely because of extrapolation of *D*-value. In fact, the worst-case scenario is generally used to estimate bacterial survival probability in order to avoid overly optimistic risk assessments (Couvert et al., 2010; FAO/WHO, 2008; Membré et al., 2006; Zwietering et al., 1996). As a result, assessment results tend to overestimate bacterial survival in worst-case scenarios. Recently, Aspridou and Koutsoumanis (2015) indicated that the time required for 1-log reduction should not be a single point, but rather a probability distribution, as the population decrease below 100 cells. The death probability of bacteria population has also been estimated using probability distribution (Koyama et al., 2017a). Thus, a probabilistic approach describing variability in bacterial behaviour is a realistic alternative to *D*-value based estimation.

Variability in bacterial behaviour is studied because risk assessment required for the probability model (Brown, 2002; Codex Alimentarius

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https://doi.org/10.1016/j.ijfoodmicro.2018.10.009
Received 31 January 2018; Received in revised form 30 August 2018; Accepted 5 October 2018
Available online 08 October 2018
0168-1605/

Commission, 2009; FAO/WHO, 2008; Ross and McMeekin, 2003; Voysey and Brown, 2000). One important source of variability is individual cell heterogeneity, which is reflected in the population inactivation curve (Koutsoumanis and Aspridou, 2016). Individual cell heterogeneity is shown as individual cell inactivation time in inactivation kinetics (Koutsoumanis and Aspridou, 2016). Thus, it is possible to describe individual cell heterogeneity as a probability distribution. Variability in initial number of cells has been described as a Poisson distribution (Aguirre et al., 2009; Koyama et al., 2017b; Nauta, 2000). Further, Poisson distribution is used to describe randomly distributed initial cells in ideal conditions (Standaert et al., 2005). One other variability source is strain (van Asselt and Zwietering, 2006). These variabilities have all been described as probability distributions. However, these variabilities have not been integrated into inactivation kinetics to overcome the conventional death probability calculation based on *D*-value.

Before introducing the probability model, mathematical assumptions such as first-order-kinetic assumption for the log-linear model first have to be considered and defined. The log-linear model and *D*-value were developed (Peleg, 2006) and used for death probability calculation via mathematical calculation based on the concept of first-order-kinetic assumption. In the case of the probability model, the probability distribution underlying the kinetic model has to be considered. Individual cell heterogeneity is described from kinetic curvature (Koutsoumanis and Aspridou, 2016). Initial cell number is assumed under statistical aspect (Standaert et al., 2005). Defining variability as a probability distribution from a mathematical viewpoint is a first step toward probability modelling. Subsequently, bacterial inactivation should be studied based on this definition for comprehensive understanding of the process.

The objective of this study was to illustrate the time-to-inactivation and death probability of bacterial population via computer simulation considering the variability in individual cell inactivation time and initial cell number. Further, the death probability of the bacterial population was simulated with various initial cells and compared with the results calculated using inactivation kinetics. The proposed approach enables estimation of the risk of bacterial survival and selection of an appropriate bacterial population death probability without using conventional *D*-value extrapolation.

2. Material and methods

2.1. Mathematical-based assumption

In this study, stochastic population inactivation was simulated with variability in individual cell inactivation time and initial cell numbers. The following two assumptions were employed in the simulation. First, we assumed that bacterial inactivation kinetics follows the Weibull inactivation model with initial N_0 (i.e., $N_0 = 10^9$):

$$\log_{10} N(t) = -bt^n + \log_{10} N_0 \tag{1}$$

where t, $N(t)$, $\log_{10}N_0$, b, and n are the elapsed time, momentary number of survivors at time t, logarithm of number of initial cells, rate, and shape parameter of the Weibull distribution, respectively. The Weibull inactivation model is widely used for inactivation kinetics (Peleg, 2006; Peleg and Normand, 2004; van Boekel, 2002). Despite having developed a lot of survival models (Xiong et al., 1999), we picked up the Weibull model, because the Weibull model provides simply either concave upward or downward. In the case of bacterial inactivation, the individual inactivation time is reflected in the inactivation kinetics (Baranyi and Pin, 2001; Koutsoumanis and Aspridou, 2016; Peleg, 2006). According to the Weibull inactivation model, the inactivation rate of the population is changed as time elapses and inactivation of individual cell is considered to be an incidental countable event that is independent from other events. Thus, the individual cell inactivation time occurs randomly from the probability Weibull distribution.

Second, we assumed that the initial number of cells follows a Poisson distribution. If bacterial cells are randomly distributed in a liquid, then the number of bacteria in any portion of that liquid follows a Poisson distribution (El-Shaarawi et al., 1981; Standaert et al., 2005). Because when there is only a small amount of cells in liquid, the cells follow a Poisson distribution (Koyama et al., 2016), it is also conceivable that a large amount of cells also follows a Poisson distribution due to the reproductive property of the Poisson distribution. With the two assumptions outlined above, we simulated bacterial inactivation with variabilities in individual cell inactivation time and initial cell number. Third, we assumed that there is no recovery from injury cells and cells are either alive or death after the lethal treatment.

2.2. Procedure for computer simulation of bacterial inactivation

In our simulation, we referred to the methods used by Aspridou and Koutsoumanis (2015), who simulated bacterial inactivation using variability in individual cell inactivation time as individual cell heterogeneity. However, we added variability in initial cell numbers to our computer simulation, which differentiates it from their simulation. First, we determined the mean value of the initial number of cells N_0 $\{N_0 \in \mathbb{R} \mid N_0 > 0\}$. The initial number of cells in the simulation was generated from a Poisson distribution as follows:

$$N_0' \sim Poisson(N_0) \tag{2}$$

where N_0' is the initial number of cells generated from the Poisson distribution with parameter N_0. Next, we estimated the individual cell inactivation time for the N_0' cells. Using inverse transform sampling from the cumulative Weibull distribution with rate and shape parameters, we estimated the individual cell inactivation time t_i of the ith cell as follows:

$$t_i \sim Weibull(b, n),\ i = 1, 2, 3 \ldots N_0' \tag{3}$$

For example, when the initial number of cells $N_0' = 1000$, random sampling is generated 1000 times for individual cell inactivation time t_i of the ith cell. Then, we determined whether the ith cell survives or dies over time as follows:

$$\begin{aligned} t < t_i \quad & S_i(t) = 1 \ \text{(survival)} \\ t \geq t_i \quad & S_i(t) = 0 \ \text{(death)} \end{aligned} \tag{4}$$

where $S_i(t)$ is survival or death of the ith cell. Thus, the number of survivors $N(t)$ at time t is described as follows:

$$N(t) = \sum_{1}^{N_0'} S_i(t) \tag{5}$$

Eq. (5) describes one simulated survival curve. To replicate the above the simulation, stochastic bacterial inactivation was conducted via Monte Carlo simulation. In this study, we changed replication used in the simulation according to the purpose of the estimation. The R code used for the above stochastic calculation is outlined at R code viewer (Appendix A).

2.3. Trend of time-to-inactivation of bacterial population

To visualise the stochastic inactivation processes and time-to-inactivation, we simulated bacterial inactivation with different parameters of Weibull distribution and initial number of cells. Various parameters such as inactivation rate, and type of inactivation curve and number of initial cells were examined in the bacterial inactivation simulation (Table 1). In one condition, 100 replicates of bacterial inactivation were simulated. In each simulation, the maximum time of t_i became a random variable of the time-to-inactivation of the bacterial population. The time-to-inactivation of the bacterial population was illustrated as a histogram. Furthermore, we calculated the variance of the time-to-inactivation of the bacterial population with the initial

Table 1
Rate parameter b and shape parameters n of Weibull distribution in inactivation kinetics ($log_{10}N(t) = -bt^n + log_{10}N_0$) discussed in this work.

Inactivation rate	High		Middle		Low	
Parameter	b	n	b	n	b	n
Linear survival curve	0.9000	1.0	0.2000	1.0	0.1000	1.0
Concave downward curve	0.0400	2.0	0.0050	2.0	0.0013	2.0
Concave upward curve	2.2000	0.5	1.3500	0.5	1.0000	0.5

number of cells between 10^0 and 10^5 cells.

2.4. Death probability of the bacterial population

To estimate the death probability of the bacterial population, we simulated 10^6 replications of bacterial inactivation with initial number of cells between 10^0 and 10^5 cells. Then, we obtained a random variable for time-to-inactivation from 10^6 replicate simulations. The obtained time-to-inactivation were then sorted from highest to lowest, and the $(10^5 + 1)th$, $(10^4 + 1)th$, $(10^3 + 1)th$, $101th$, and $11th$ selected to calculate the certainty level of bacterial inactivation for five levels of death probabilities of the bacterial population, ranging from 90 to 99.999%. To arrange death probability of the various numbers of initial cells, contour lines of death probability were described. In addition, the death probability of the bacterial population was calculated directly from the inactivation kinetics curvature. Death probability calculation using the inactivation kinetic curvature is analogous to extrapolation of D-value. Extrapolation of D-value methods uses the survival numbers of bacterial population as survival probability of bacterial population, when the number of survivors is below one. Thus, the death probability of bacterial population P is described as $\{P = 1 - N(t) \mid N(t) \leq 1\}$. Inactivation time is described from Eq. (1) as follows:

$$t_{Inact}(P) = \sqrt[n]{\frac{1}{b} log_{10}\left(\frac{N_0}{1-P}\right)},\ P = 0.9,0.99,0.999,0.9999,0.99999 \quad (6)$$

where $t_{Inact}(P)$ is the inactivation time for the death probability of bacterial population P. The representative death probability of the bacterial population estimated from the kinetics model is illustrated in Fig. 2. Subsequently, the death probabilities given by the kinetic and stochastic models were compared.

2.5. Validation of bacterial inactivation behaviour

To validate our simulation, we compared our simulation with experimental data by referring the previous study (Aspridou and Koutsoumanis, 2015). Acid inactivation of *Salmonella enterica* ser. Agona with initial cell concentration about 3.4 $\log_{10}$CFU/ml and 2.3 $\log_{10}$CFU/ml has been conducted in acidified tryptone soy broth with lactic acid (pH 3.5). The inactivation experiments of *Salmonella* population are 9 independent trials at both initial cell levels. We used 3 trials for the computer simulation with Weibull model and 6 trial for validation. We fixed initial cell level N_0 as mean value of 3 trials data and fitted the experimental data by Weibull model (Eq. (1)) to minimise root mean square errors (RMSE). We simulated inactivation of *Salmonella enterica* ser. Agona populations with a mean initial level of validation data, 2926 CFU/ml and 197 CFU/ml, with Poisson distribution for initial cell number and the fitted Weibull model for individual cell inactivation time. Then, we compared our computer simulation with experimental data. All statistical analyses conducted using R statistical software (Version 3.4.2 for Mac OS X; http://ww.r-project.org).

3. Results

3.1. Variability in time-to-inactivation of bacterial population

In all the tested conditions, the stochastic inactivation process and the time-to-inactivation of bacterial population were successfully illustrated (Figs. 3 and 4). The trend in the time-to-inactivation of the bacterial population varied depending on the initial number of cells and the shape and rate parameters of the Weibull distribution. As the inactivation rate decreased, the variance of the time-to-inactivation increased regardless of the type of curve (Fig. 5). In contrast, the variance of the time-to-inactivation depended on the type of inactivation curvature with different initial number of cells. As the number of initial cells increased, the variance of the time-to-inactivation remained virtually constant in the case of the log-linear model. In the case of downward and upward concavity, the variance of the time-to-inactivation decreased and increased as the number of initial cells increased (Fig. 5).

3.2. Descriptions for the death probability of the bacterial population

To summarise the death probability of various initial cells, we described the death probability of the bacterial population as an interface (Fig. 6). The target number of cells was described as a negative value of the initial number of cells (Figs. 6 and 7). The death probability contour line for the log-linear model exhibited a linear relationship between time and targeted initial cell reduction (Fig. 6). In contrast, the death probability contour line for curvature with downward or upward concavity did not show a linear relationship between time and targeted initial cell reduction (Fig. 6). The trend in the contour line of the death probability had the same curvature as inactivation kinetics (Figs. 1 and 6). We compared the death probability of the bacterial population calculated via inactivation kinetics with that given by the stochastic model developed in this present study. The contour line of death probability was virtually congruent with the inactivation kinetics calculation (Fig. 7). This result indicates that the death probability of the bacterial population can be estimated from the kinetic model, as in Fig. 2.

3.3. Validation of bacterial inactivation behaviour

The best fit parameters for 3.4 $\log_{10}$CFU/ml level are $b = 0.064$ and

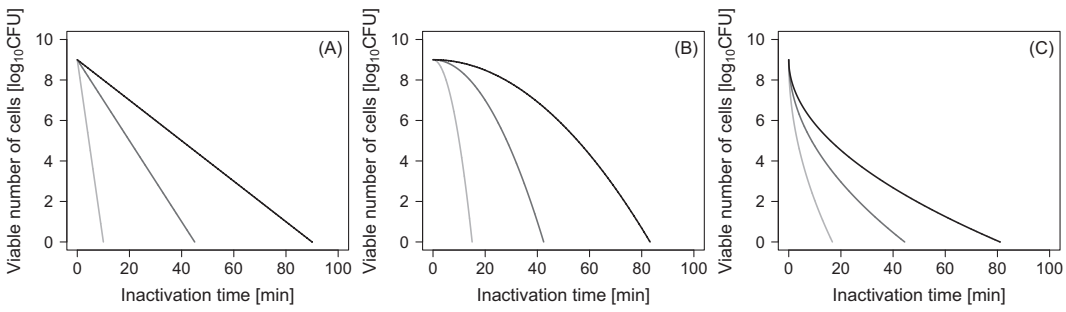

Fig. 1. Log-Weibull models of linear (A), downward concavity (B), and upward concavity (C) with initial number of cells $N_0 = 10^9$.

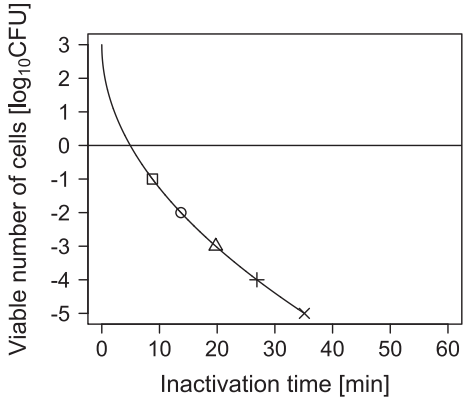

Fig. 2. Death probability estimation using inactivation kinetics model ($N_0 = 10^3$, $b = 1.35$, $n = 0.5$). The point P of 0.9 (90% probability of death), 0.99, 0.999, 0.9999, and 0.99999 is indicated by a square, circle, triangle, plus, and cross, respectively.

$n = 0.970$ with RMSE 0.172 and those for 2.3 $\log_{10}$CFU/ml level are $b = 0.087$ and $n = 0.914$ with RMSE 0.151. The result of validation is shown in Fig. 8. Observed data (●) are shown inside of simulation, where number of survivors is expected. No survivor in 6 trials (×) are mostly shown after the time simulation lines finished, when no survivor is expected. Thus, our simulation could describe the variability in the inactivation behaviour of the population.

4. Discussion

In this study, we simulated bacterial inactivation based on variability in individual cell inactivation time and initial number of cells. In all the tested conditions, the time-to-inactivation of the bacterial population was successfully described as histogram (Figs. 3 and 4). The variance of the time-to-inactivation was found to depend on the type of inactivation curve (Fig. 5). In addition, we successfully presented a contour line of the death probability of bacterial population (Fig. 6) that is virtually the same as that obtained via kinetics calculation (Fig. 7). The stochastic model proposed in this study indicates that the conventional inactivation kinetic model enables estimation of the death probability of the bacterial population (Fig. 2). Further we validated our computer simulation by using experimental data from literature (Fig. 8). Thus, we could estimate the death probability of the bacterial population based on inactivation kinetics curvature instead of extrapolation of D-value.

Time-to-inactivation has not been estimated as probability distribution via the conventional kinetic model. In this study, variability in time-to-inactivation was described using histograms. Further, the trend in time-to-inactivation was visualised using various Weibull distribution parameters and initial number cells (Figs. 3 and 4). The shape of the inactivation curvature and inactivation speed was shown to significantly influence the time-to-inactivation (Figs. 3 and 4). For example, low inactivation rate causes the bacteria to remain longer with higher variance than in high inactivation rate regardless of type of curvature (Fig. 3). With high initial number of cells, bacteria survive longer with high variance for concave upward curve (Fig. 4). These trends cannot be estimated from calculation using extrapolation of D-value. The above information is useful for determining the risk of bacterial survivors after the inactivation process.

As one of the variabilities, the initial number of cells was described as a Poisson distribution in this study (Eq. (2)). The initial number of cells should be described with a probability distribution in risk assessment (Nauta, 2002). Poisson distribution was used to express variability in the initial number of cells (Nauta, 2000). We simulated stochastic inactivation of the bacterial population, in which the initial number of cells follows a Poisson distribution. The number of survival cells was also described by a Poisson distribution under the assumption that the initial cells following the Poisson distribution randomly die (Aguirre et al., 2009). In our model, individual cell inactivation time is independent (Eq. (3)). Thus, the number of survivors simulated in this study follows a Poisson distribution, regardless of the parameters of the Weibull distribution and initial number cells. In addition, Poisson distribution allow us to set initial cell number as real number, in case without Poisson distribution initial cell number is limited to integer. The approach proposed in this study can estimate not only the death probability of bacterial populations but also the number of survivors,

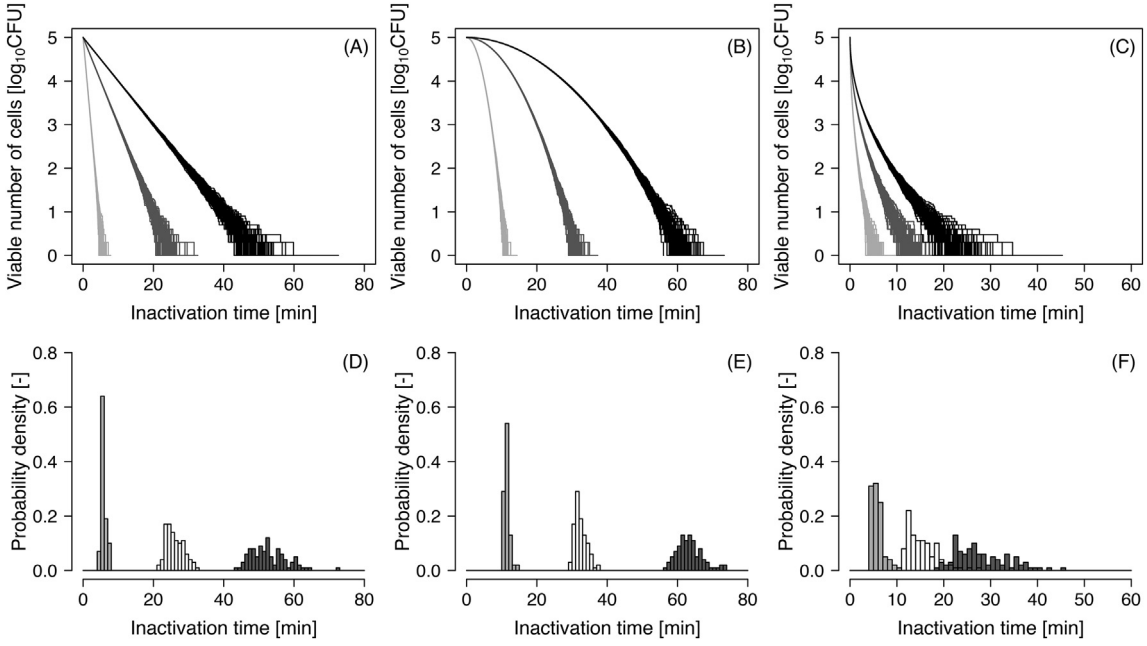

Fig. 3. (Top row) Monte Carlo simulation results (100 simulations) for the inactivation curve of linear (A), downward concavity (B), and upward concavity (C) with initial cells ($N_0 = 10^5$). (Bottom row) Histograms showing time-to-inactivation of bacterial population for the inactivation curve for linear (D), downward concavity (E), and upward concavity (F).

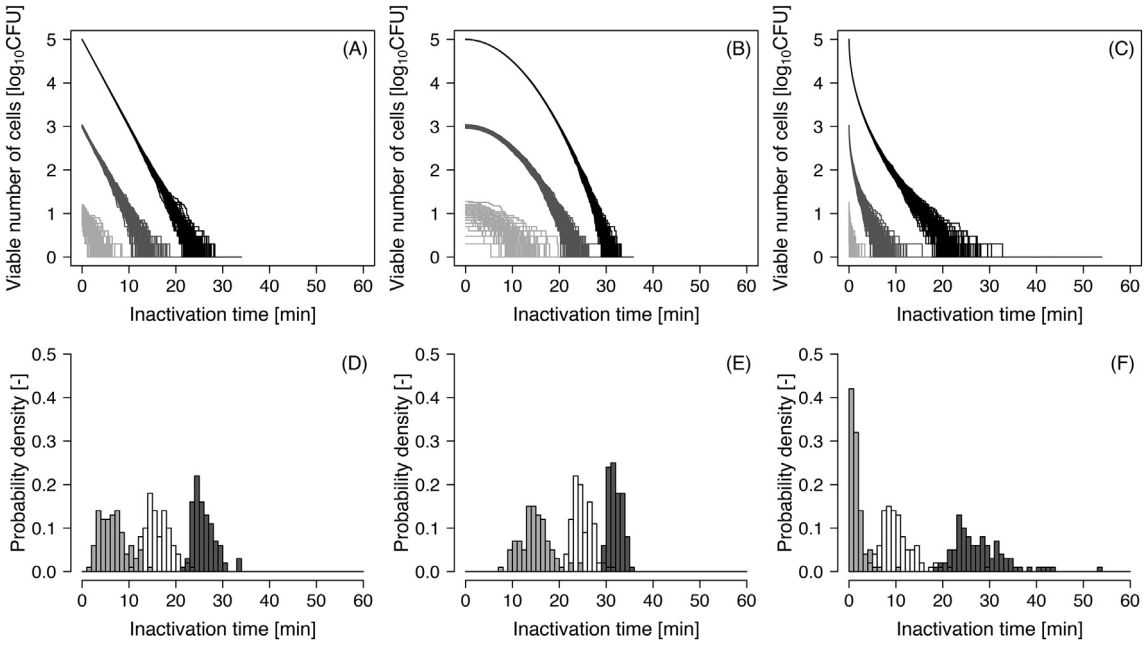

Fig. 4. (Top row) Monte Carlo simulation results (100 simulations) for the inactivation curve of linear ($b = 0.2$, $n = 1$) (A), downward concavity ($b = 0.005$, $n = 2$) (B), and upward concavity ($b = 1$, $n = 0.5$) (C) with different initial cells ($N_0 = 10$, 10^3, 10^5). (Bottom row) Histograms showing time-to-inactivation of bacterial population for the inactivation curve for linear (D), downward concavity (E), and upward concavity (F).

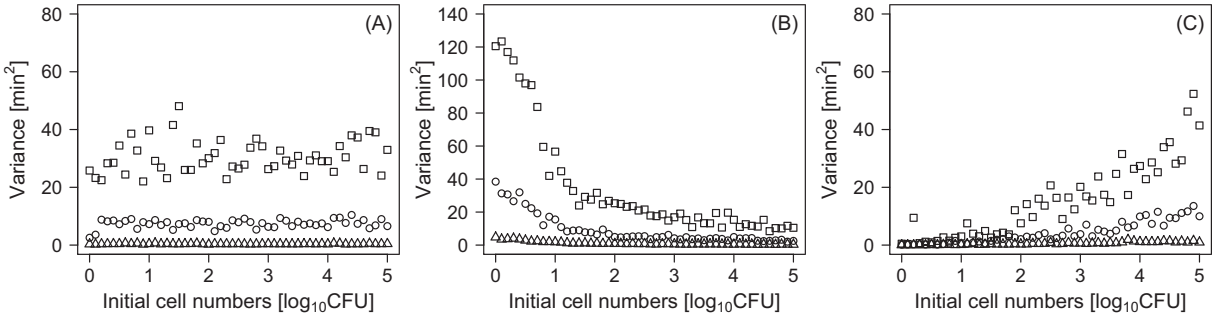

Fig. 5. Variance of time-to-inactivation of bacterial population (100 simulations) with various numbers of initial cells for log-linear (A), downward concavity (B), and upward concavity (C). The inactivation rate of kinetics in low, middle, and high is indicated by a square, circle, and triangle, respectively.

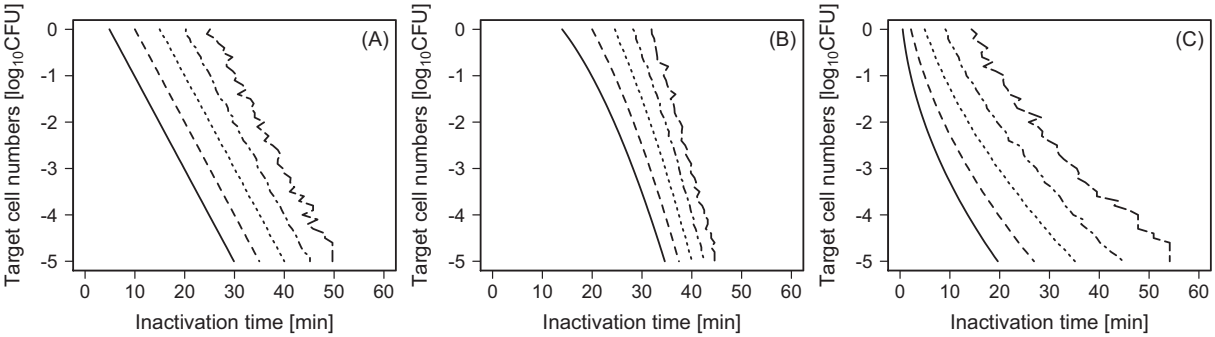

Fig. 6. Representation of the death probability of bacterial population for log-linear ($b = 0.2$, $n = 1$) (A), downward concavity ($b = 0.005$, $n = 2$) (B), and upward concavity ($b = 1.35$, $n = 0.5$) (C) calculated via our stochastic approach. The interface P of 0.9 (90% probability of death), 0.99, 0.999, 0.9999, and 0.99999 is indicated by solid, dashed, dotted, dot-dashed, and long-dashed lines, respectively.

which are both required for exposure assessment (Brown, 2002). Further, flexible setting of number of initial cell not only as Poisson distribution but also another distribution would enable to expand variation of computer simulation for risk assessment.

The procedure for calculating D-value ignores the inactivation curvature (Peleg, 2006). This is one of the reasons why D-value cannot precisely estimate the death probability of a bacterial population. Considering the inactivation curvature, we calculated the death probability of bacterial population from the aspect of probability approach and inactivation curvature (Figs. 2 and 6). The results indicated that the death probability of the bacterial population enables estimation from the inactivation curvature (Fig. 7). Thus, conventional death kinetics

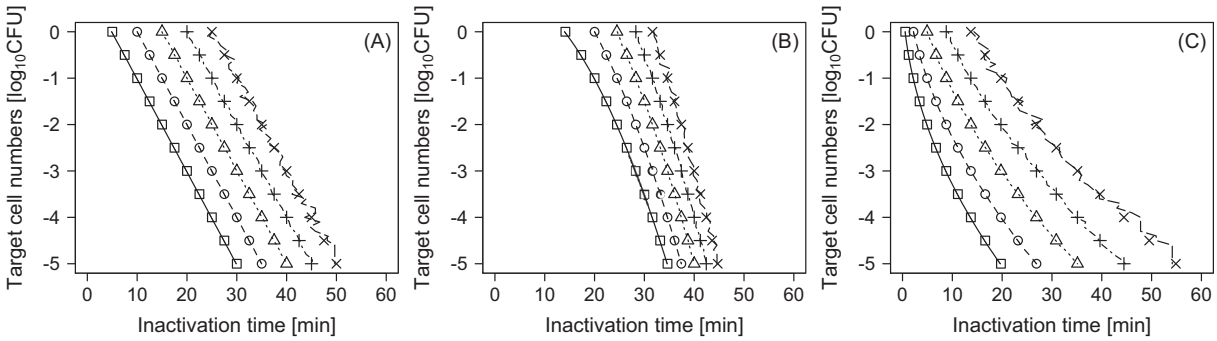

Fig. 7. Comparison of death probability of bacterial population between probability calculation and inactivation kinetic calculation for log-linear (A), downward concavity (B), and upward concavity (C). The lines show the probability calculation and the plot shows the kinetics calculation. The point P of 0.9 (90% probability of death), 0.99, 0.999, 0.9999, and 0.99999, indicated by a square, circle, triangle, plus, and cross, was plotted on Fig. 6.

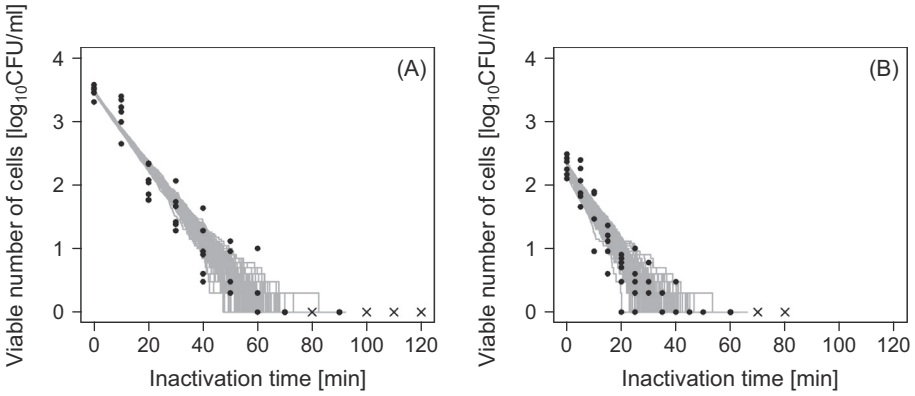

Fig. 8. Comparison between observed and simulated inactivation of *Salmonella enterica* ser. Agona populations with a mean initial level of $N_0 = 2926$ CFU/ml (A) and $N_0 = 197$ CFU/ml (B). Inactivation was calculated with Monte Carlo simulation (100 simulations) based on Eq. (5) and Poisson distribution for initial number of cells and Weibull distribution with parameter $b = 0.064$ and $n = 0.970$ (A) and $b = 0.087$ and $n = 0.914$ (B) for individual cell inactivation time. Observed data and no survivor in 6 trials are described as solid circle and cross. Experimental data is taken from reference Aspridou and Koutsoumanis (2015).

studies have so far reported that time can be applied to death probability calculation. While the previous probability model is needed for new and additional experimental data (Koyama et al., 2017a) or complex calculation, the calculation procedure presented in this study does not require a complicated calculation process (Eq. (6)). Thus, application to probabilistic evaluation for databases such as ComBase is easier because the only thing we need is the kinetic model.

In this study, the time-to-inactivation and death probability of the bacterial population were described via computer simulation based on a mathematical approach. The approach enables us to discuss the death probability of the bacterial population based on probability theory, instead of traditional calculation using extrapolation of deterministic approaches. To transform the deterministic approach into a probability or stochastic model, a mathematical approach is important. In addition, computer simulation is important for visualising the complex situation described by stochastic formulation. The stochastic growth model and growth/no growth model can also be discussed in terms of a combination of mathematical approaches and computer simulation from the aspect of probability theory. For example, variability in growth behaviour can be calculated and simulated using exponential growth kinetics. Our stochastic simulation for the death probability of bacterial population can help to estimate the risk of bacterial survivors without need for the *D*-value concept.

Acknowledgements

This work was supported by the Japan Society for the Promotion of Science (JSPS) KAKENHI Grants-in-Aid for JSPS Fellows [grant number 17J04163]. We thank Konstantinos P. Koutsoumanis and Zafiro Aspridou for giving advice on validation and providing us with inactivation trial data for *Salmonella* populations.

Appendix A. Supplementary data

The R code used for the stochastic calculation is outlined at 'R-File1 here'. Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijfoodmicro.2018.10.009.

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