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A mathematical model for bacterial inactivation

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Abstract

The first order kinetic model, the Buchanan model and Cerf's model, can model a linear survival curve, a survival curve with a shoulder and a survival curve with a tailing, respectively. However, they are not suitable for fitting a sigmoidal survival curve. The three models were integrated into a new model that was capable of fitting the four most commonly observed survival curves: linear curves, curves with a shoulder, curves with a tailing (biphasic curves) and sigmoidal curves. The new model was compared with the Whiting–Buchanan model using the survival curves of *Staphylococcus aureus*. The goodness-of-fit of the proposed model is practically as good as that of the Whiting–Buchanan model. Compared with the Whiting–Buchanan model, the proposed model has a more mechanistic background. Since for non-linear survival curves, such as biphasic and sigmoidal curves, the t_{m-D} value (the time required for an m-log-cycle reduction of microorganisms under a given condition) cannot be estimated accurately by the existing or traditional method, a new method is also proposed to predict accurately the t_{m-D} value for non-linear survival curves.

Keywords: Bacterial inactivation; Mathematical modelling

1. Introduction

In thermal and non-thermal inactivation of vegetative microorganisms, there are four commonly observed types of survival curves: linear curves (Fig. 1, curve A), curves with a shoulder (Fig. 1, curve B), curves with a tailing (or biphasic curves) (Fig. 1, curves C and D) and sigmoidal curves (Fig. 1, curves E and F). To model these linear and non-linear survival curves, several approaches have been proposed (Table 1). Based on the analogy of the first order chemical reaction, Chick (1908) proposed the first order kinetic model for linear survival curves. Cerf (1977) proposed a two-fraction model for describing biphasic curves, which were generally considered to represent a mix of two species or strains having different heat resistances. Kamau et al. (1990) applied three different forms of logistic equation to fit linear and non-linear survival curves for *Listeria monocytogenes* heated in a milk system. On the basis of the Kamau model (Kamau et al., 1990), Whiting and Buchanan (1992) developed a logistic equation for describing the kinetics when there was a significant shoulder and tail in survival curves. This model has been applied to non-thermal inactivation of *L. monocytogenes* and *Staphylococ-*

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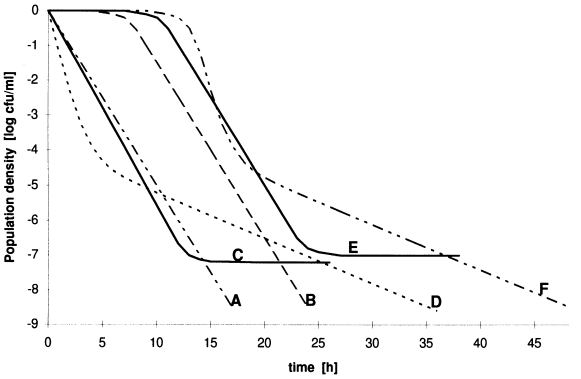

Fig. 1. Graphic representations of six different shapes of survival curves. The models fitting each of the six curves are indicated in Table 1.

cus aureus (Buchanan et al., 1994; Buchanan and Golden, 1995; Whiting et al., 1996). A vitalistic model or symmetric four parameter equation has been developed for the thermal destruction kinetics of *L. monocytogenes*, *Salmonella typhimurium* or *Yersinia enterocolitica* (Cole et al., 1993; Ellison et al., 1994; Little et al., 1994; Stephens et al., 1994). Buchanan et al. (1993) proposed a step equation to fit survival curves with a shoulder. This model has been applied in the non-thermal inactivation of *L. monocytogenes* (Buchanan et al., 1994, 1997; Buchanan and Golden, 1995). More recently, Membre et al. (1997) proposed a logistic equation to describe survival curves with a shoulder and showed that the equation can effectively model the non-thermal inactivation of *S. typhimurium* in reduced calorie mayonnaise.

Non-linear survival curves, such as sigmoidal curves (Fig. 1, curve E), have also been modelled using a modified Gompertz equation. The Gompertz equation and its modified forms have been used primarily in modelling the asymmetrical sigmoidal shape of microbial growth curves (McMeekin et al., 1993, pp. 45–55; Linton et al., 1995). Bhaduri et al. (1991) may have been the first to demonstrate that the modified Gompertz equation can model the non-linear survival curves for *L. monocytogenes* heated in liver sausage slurry, and that, for sigmoidal survival curves, it is likely to provide a more accurate estimate of a microorganism's thermal resistance than the first order kinetic model. Recently, Linton et al. (1995), (1996) successfully used the modified Gompertz equation to fit non-linear survival curves for *L. monocytogenes* Scott A and found that it was effective in modelling survival curves for both linear and those containing a shoulder and tail. However, the parameters of the modified Gompertz equation have no direct link with microbial death kinetics when the logarithmic number of microorganism cells is used. Ideally, it should be used for the cell's concentration rather than for its logarithms (Baranyi et al., 1993).

Although all of these models have been successfully applied in food predictive microbiology, none except the Whiting–Buchanan model can be used to fit all of the six different shapes of survival curves shown in Fig. 1. For example, the first order kinetic model can only fit the linear survival curves (Fig. 1, curve A). The modified Gompertz equation and the Cole model are suitable mainly for survival curves like curve E shown in Fig. 1. Although the Whiting–Buchanan model can fit all of the six different shapes of survival curves given in Fig. 1, it was derived purely from an empirical logistic equation, i.e., the Kamau model for linear survival curves (Table 1), and not from the commonly used first order kinetics. The model was primarily developed for sigmoidal survival curves such as curve F (Fig. 1), and not for linear curves and those with a shoulder. The Buchanan model (Buchanan et al., 1993, 1994, 1997; Buchanan and Golden, 1995) has replaced the Whiting–Buchanan model to fit survival curves with a lag phase, which suggests that the Whiting–Buchanan model may be not good enough to fit those curves.

To assess the efficiency of inactivation treatment, it is also necessary to estimate the t_{m-D} values (such as t_{4-D} values), which is defined as the time required to achieve an m-log-cycle reduction of the bacterial population. For survival curves such as curves A and B (Fig. 1), the t_{m-D} values can easily be estimated. However, for some other survival curves, such as curves C, D, E and F (Fig. 1), estimation of the t_{m-D} values is based on the slope of the linear portion of the curves and there is no suitable method that can be used to estimate the t_{m-D} values accurately.

The purposes of this paper are: (1) to develop a model to fit all of the six different survival curves shown in Fig. 1; (2) to compare it with the Whiting–Buchanan model using the established experimental data for *S. aureus* (Whiting et al., 1996) and (3) to propose a method for the accurate estimation of the t_{m-D} values for non-linear survival curves.

Table 1
Models for survival curves

Model	Mathematical formula	Curves fitted by model	Reference
First order kinetics	$N(t)=N_0e^{-kt}$ or $\log\frac{N(t)}{N_0}=-\frac{t}{D}$	A	Chick (1908)
Cerf	$\frac{N(t)}{N_0}=fe^{-k_1t}+(1-f)e^{-k_2t}$	A, C, D	Cerf (1977)
Kamau	For linear survival curves		
	$\frac{N(t)}{N_0}=\frac{2}{1+e^{bt}}$	A	Kamau et al. (1990)
	For survival curves with a lag phase		
	$\log\frac{N(t)}{N_0}=\log(1+e^{-b_1t_{1/2}})-\log(1+e^{b_2t_{1/2}})$	B	
	For biphasic survival curves		
	$\log\frac{N(t)}{N_0}=\log\left(\frac{2f}{1+e^{b_1t}}+\frac{2(1-f)}{1+e^{b_2t}}\right)$	A, C, D	
Whiting–Buchanan	$\log\frac{N(t)}{N_0}=\log\left(\frac{f(1+e^{-b_1t_{\text{lag}}})}{1+e^{b_1(t-t_{\text{lag}})}}+\frac{(1-f)(1+e^{-b_2t_{\text{lag}}})}{1+e^{b_2(t-t_{\text{lag}})}}\right)$	A, B, C, D, E, F	Whiting and Buchanan (1992)
Gompertz	Modified Gompertz equation $\log N(t)=A-Ce^{-e^{-B(t-M)}}$ or	B, C, E	Bhaduri et al. (1991)
	$\log\frac{N(t)}{N_0}=Ce^{-e^{BM}}-Ce^{-e^{-B(t-M)}}$		Linton et al. (1995)
			Linton et al. (1996)
Cole	$\log N(t)=\alpha+\frac{\omega-\alpha}{1+e^{4\sigma(\tau-\log t)/(\omega-\sigma)}}$	B, C, E	Cole et al. (1993)
Buchanan	$\log N(t)=\begin{cases}\log N_0 & (t\le t_{\text{lag}})\\ \log N_0-\frac{t-t_{\text{lag}}}{D} & (t>t_{\text{lag}})\end{cases}$	A, B	Buchanan et al. (1993)
Membre	$\log N(t)=(1+\log N_0)-e^{kt}$	B	Membre et al. (1997)

2. Theory

2.1. Linear survival curve

The first order chemical reaction states that the rate of a chemical reaction is proportional to the product of the concentrations of the reactants (Brown and Rothery, 1993, p. 342). To apply the theory of the first order chemical reaction to the thermal destruction of microorganisms, Chick (1908) proposed the following differential equation:

$$\begin{cases}\frac{dN(t)}{dt}=-kN(t) & (0\le t<+\infty)\\ N(0)=N_0 & (N_0>0;\ t=0)\end{cases} \quad (1)$$

where $N(t)$ and N_0 are the concentrations present at time t and zero, respectively; k is the death rate

constant; $dN(t)/dt$ is the death (inactivation) rate; $[dN(t)/dt]/N(t)$ is the relative or specific death (inactivation) rate.

In terms of the base ten logarithm, the solution of Eq. (1) can be written as:

$$\log \frac{N(t)}{N_0} = -\frac{t}{D} \quad (t \geq 0) \tag{2}$$

where D is the D value or the decimal reduction time, namely $D = \ln(10)/k = 2.303/k$. From Eq. (2), $\log [N(t)/N_0]$ versus time t is a straight line on a semi-log plot, so Eq. (2) can be used to fit linear survival curves such as curve A in Fig. 1.

2.2. Survival curve with a shoulder

A number of survival curves start with a shoulder followed by a linear decline curve (Fig. 1, curve B) (Kamau et al., 1990; Bhaduri et al., 1991; Linton et al., 1995, 1996). There are several reasons for the shoulder. If clumps of microorganisms exist in the suspension, all cells in the clump need to be inactivated before the colony forming ability of the clump is inactivated (Adams and Moss, 1997, p. 62), and the lag phase will be observed. If the lag phase represents a period in which the cells are able to resynthesize a vital component, death ensues only when the rate of destruction exceeds the rate of resynthesis (Mossel et al., 1995, p. 84–85). If thermal inactivation of microorganisms is cumulative rather than instantly lethal, or there are multiple target sites for thermal inactivation (Adams and Moss, 1997, p. 63), the lag phase may also be observed.

In the lag phase, the change in the concentration of microorganisms is very small and, in the linear phase, the specific death rate is constant or almost constant. To model curve B in Fig. 1, it is assumed that the concentration of microorganisms remains the same in the lag phase and the inactivation of microorganisms follows first order kinetics in the linear phase, which can be expressed as:

$$\begin{cases} \dfrac{dN(t)}{dt} = -kN(t) & (t \geq t_{lag}) \\ N(0) = N_0 & (N_0 > 0;\ 0 \leq t \leq t_{\mathrm{lag}}) \end{cases} \tag{3}$$

where t_{lag} is the lag time duration, which is defined as the mean time required for the initial inactivation of microorganisms. The solution of Eq. (3) can be arranged as:

$$\log \frac{N(t)}{N_0} = \begin{cases} 0 & (0 \leq t \leq t_{\mathrm{lag}}) \\ -\dfrac{t - t_{\mathrm{lag}}}{D} & (t \geq t_{\mathrm{lag}}) \end{cases} \tag{4}$$

where parameters D and t_{lag} can be determined by a non-linear regression procedure. It is interesting to find that Eq. (4) is identical to the model proposed by Buchanan et al. (1993). In this paper, therefore, Eq. (4) is referred to as the Buchanan model. When t_{lag} equals zero, Eq. (4) becomes Eq. (2), the first order kinetic model.

The lag time duration and death rate constant are determined traditionally by drawing a straight line through the linear phase. This model provides a mathematical means of doing so. It has been applied to the survival curves with a lag phase for *L. monocytogenes* (Buchanan et al., 1993, 1994, 1997; Buchanan and Golden, 1995).

2.3. Survival curve with a tail

Many survival curves also contain a ‘tailing’. There are also many explanations for it. If some microorganisms are intrinsically more resistant than others or protected by various factors (such as dead cells, the products of their destruction, localised sites with low water activity a_{w}, inactivation of the microbicidal agent, oxygen depletion, etc.) (Cerf, 1977), they can survive under testing conditions or the reduction rate of their population will be slowed down. Fig. 1 shows that there are two different shapes of tailing (curves C and D). In Fig. 1, curve C has a level tailing (a zero slope of tailing), while curve D has a slope tailing (a small slope of tailing). These two shapes of curves, called biphasic curves, are generally held to represent a mix of two fractions or sub-populations of different heat resistance (Cerf, 1977; Kamau et al., 1990; Whiting and Buchanan, 1992). The first straight portion of the curve mainly describes death of the less resistant microorganisms and the second portion describes the death of the more resistant ones. Cerf (1977) reviewed a number of publications and proposed a two-fraction model. The two-fraction model, called Cerf’s model, supposes the existence of two sub-populations or fractions and, for each sub-population or fraction, the inactivation rate is constant. Two fractions of micro-

organisms are further assumed to be inactivated independently and irreversibly and following first order kinetics. The corresponding differential equations, therefore, could be expressed as:

$$\begin{cases} \dfrac{dN_1(t)}{dt} = -k_1 N_1(t) & (t \geq 0) \\ N_1(0) = N_{01} & (N_{01} > 0;\ t = 0) \end{cases} \quad (5a)$$

$$\begin{cases} \dfrac{dN_2(t)}{dt} = -k_2 N_2(t) & (t \geq 0) \\ N_2(0) = N_{02} & (N_{02} > 0;\ t = 0) \end{cases} \quad (5b)$$

$$\begin{cases} N(t) = N_1(t) + N_2(t) \\ N_0 = N_{01} + N_{02} \end{cases} \quad (5c)$$

where $N(t)$, $N_1(t)$ and $N_2(t)$ are the concentrations of the whole population, and of the less resistant and more resistant fractions at any time, respectively. The parameters k_1 and $k_2 (k_1 > k_2 \geq 0)$ are the death rate constants for $N_1(t)$ and $N_2(t)$, respectively. The solution of Eq. (5a)–(5c) can be given by:

$$N(t) = N_0(fe^{-k_1 t} + (1-f)e^{-k_2 t}) \quad (6)$$

where f and $(1-f)$ are the initial proportion in the less resistant fraction and the more resistant fraction, respectively, i.e. $f = N_{01}/N_0$ and $(1-f) = N_{02}/N_0$. e is the Naperian base. This model can be used to model survival curves A, C and D given in Fig. 1.

2.4. Survival curve with a shoulder and a tailing

In some cases, survival curves contain both a shoulder and a tailing, i.e. sigmoidal curves. Because of the two different shapes of tailings, there are two corresponding sigmoidal curves, i.e. curves E and F (Fig. 1). To model curve F, a new differential equation, which combines Eqs. (3), (5a)–(5c), can be obtained:

$$\begin{cases} \dfrac{dN_1(t)}{dt} = -k_1 N_1(t) & (t \geq t_{1\text{lag}}) \\ N_1(0) = N_{01} & (N_{01} > 0;\ t \leq t_{1\text{lag}}) \end{cases} \quad (7a)$$

$$\begin{cases} \dfrac{dN_2(t)}{dt} = -k_2 N_2(t) & (t \geq t_{2\text{lag}}) \\ N_2(0) = N_{02} & (N_{02} > 0;\ t \leq t_{2\text{lag}}) \end{cases} \quad (7b)$$

$$\begin{cases} N(t) = N_1(t) + N_2(t) \\ N_0 = N_{01} + N_{02} \end{cases} \quad (7c)$$

where $t_{1\text{lag}}$ and $t_{2\text{lag}}$ are the lag time durations for the two fractions, respectively.

If $t_{1\text{lag}} \geq t_{2\text{lag}}$, the solution of Eqs. (7a)–(7c) is given by:

$$N(t) = \begin{cases} N_0 & (0 \leq t \leq t_{1\text{lag}}) \\ N_0(fe^{-k_1(t-t_{1\text{lag}})} + (1-f)) & (t_{1\text{lag}} < t \leq t_{2\text{lag}}) \\ N_0(fe^{-k_1(t-t_{1\text{lag}})} + (1-f)e^{-k_1(t-t_{2\text{lag}})}) & (t > t_{2\text{lag}}) \end{cases} \quad (8a)$$

If $t_{1\text{lag}} \leq t_{2\text{lag}}$, the solution of Eqs. (7a)–(7c) is given by:

$$N(t) = \begin{cases} N_0 & (0 \leq t \leq t_{2\text{lag}}) \\ N_0(f + (1-f)e^{-k_2(t-t_{2\text{lag}})}) & (t_{2\text{lag}} < t \leq t_{1\text{lag}}) \\ N_0(fe^{-k_1(t-t_{1\text{lag}})} + (1-f)e^{-k_2(t-t_{2\text{lag}})}) & (t > t_{1\text{lag}}) \end{cases} \quad (8b)$$

In order to simplify eq. Eqs. (8a) and (8b), assumption is made that the two fractions of microorganisms undergo the same lag phase. Therefore, Eqs. (8a) and (8b) becomes:

$$\log \frac{N(t)}{N_0} = \begin{cases} 0 & (t \leq t_{\text{lag}}) \\ \log(fe^{-k_1(t-t_{\text{lag}})} + (1-f)e^{-k_2(t-t_{\text{lag}})}) & (t \geq t_{\text{lag}}) \end{cases} \quad (9)$$

Although this four-parameter model is derived for the survival curve F, it can also be used to model the other five different shapes of survival curves, i.e. curves A ($k_2 = t_{\text{lag}} = 0$ and $f = 1$), B ($k_2 = 0$ and $f = 1$), C ($k_2 = t_{\text{lag}} = 0$), D ($t_{\text{lag}} = 0$) and E ($k_2 = 0$). Eqs. (2) and (4) or Eq. (6) is a special case of Eq. (9).

2.5. t_{m-D} estimation

The t_{m-D} value is the time required for an m-log-cycle reduction of bacteria under a given condition and can be expressed by Eq. (10):

$$\log \frac{N(t_{m-D})}{N_0} = -m \quad (10)$$

where $N(t_{m-D})$ is the concentration of microorganisms present at time t_{m-D} ; m is the number of log reductions (or D values) required, usually $m = 4$.

Combining Eqs. (9) and (10), the following equation for the t_{m-D} value can be obtained if the effect of the more resistant fraction on the t_{m-D} value is ignored:

$$t_{m-D} = t_{\text{lag}} + mD_1 \tag{11}$$

where $D_1 = \ln(10)/k_1 = 2.303/k_1$ is the D value for the less resistant fraction at a reference condition (temperature, pH, NaCl, etc.).

This is a traditional method used to estimate the t_{m-D} value. However, this method can give a good approximation of the 'real t_{m-D} value' only when the effect of the more resistant fraction on the t_{m-D} value is negligible. In order to estimate the t_{m-D} value accurately, the following function, referred to as the t_{m-D} calculation function, is proposed:

$$r(t) = \log\frac{N(t)}{N_0} + m \tag{12}$$

where $\log [N(t)/N_0]$ can be the proposed model (Eq. (9)) or any one of the models listed in Table 1. When $t = t_{m-D}$, Eq. (12) becomes Eq. (10). The solution of Eq. (12) can be obtained by following the steps below:

1. initialise t using $t_i = t_1$ (where t_1 is the initial time);
2. calculate $r(t_i)$. If $r(t_i) = 0$, go to step 5; otherwise, go to step 3;
3. let $t_{i+1} = t_i + \Delta t$ if $r(t_i) > 0$ [let $t_{i+1} = t_i - \Delta t$ if $r(t_i) < 0$] ($i = 1, 2, 3, 4,...$), and then calculate $r(t_{i+1})$;
4. repeat step 3 until $r(t_{i+1}) \le 0$ [$r(t_{i+1}) \ge 0$];
5. calculate the t_{m-D} value using Eq. (13).

$$t_{m-D} = \begin{cases} t^0 & (\text{if } r(t) = 0) \\ t^+ + \dfrac{r(t^+)}{r(t^+) - r(t^-)}\Delta t & (\text{if } r(t) \neq 0) \end{cases} \tag{13}$$

where t^-, t^0 and t^+ are the time at which the function $r(t)$ is less than, equal to and greater than zero, respectively; Δt is the time increment, i.e. $\Delta t = t_{i+1} - t_i = t^- - t^+$. The smaller the time increment Δt, the more accurate the estimated t_{m-D} value. A Δt value of one or below is recommended for accurate estimation. For survival curves C and E (Fig. 1), however, the solution of Eq. (10) or Eq. (12) may not exist if $m > -\log(N_\infty/N_0)$ [where N_∞ ($\neq 0$) is the number of microorganisms present at time $t = \infty$]. In this case, the number of microorganisms will not be reduced to the required number under testing conditions.

3. Results and discussion

The proposed model (Eq. (9)) was evaluated using the first 20 survival curves from the established experimental data for *S. aureus* (Whiting et al., 1996). The code numbers used by Whiting et al. (1996) in the experiment are listed in Tables 2 and 3. As a comparison, the data were also fitted using the Whiting–Buchanan model (Whiting and Buchanan, 1992; Whiting et al., 1996), which is shown below:

$$\log\frac{N(t)}{N_0} = \log\left(\frac{f(1 + e^{-b_1 t_{\text{lag}}})}{1 + e^{b_1(t - t_{\text{lag}})}} + \frac{(1-f)(1 + e^{-b_2 t_{\text{lag}}})}{1 + e^{b_2(t - t_{\text{lag}})}}\right) \tag{14}$$

$$t_{4-D} = t_{\text{lag}} + \frac{\ln\left(\dfrac{1 + e^{-b_1 t_{\text{lag}}}}{0.0001} - 1\right)}{b_1} \tag{15}$$

where $b_1 = 2.3/D_1$ is the inactivation rate of the major population group (or the first fraction); $b_2 = 2.3/D_2$ is the inactivation rate of the minor population group (or the second fraction); D_1 and D_2 are the D values for the two population groups, respectively.

The experimental survival curves were fitted to the proposed model and the Whiting–Buchanan model using the non-linear regression procedure in the SAS System for Windows (Release 12.6; SAS Institute, Cary, NC, USA) with the Gauss–Newton estimation method. The t_{4-D} values were calculated using Microsoft Excel for Windows (Version 5a).

The goodness of the fit of the models was assessed using the root mean square error (RMSE) and correlation coefficient (R^2) between the experimental and predicted values. The model or kinetic parameters [such as k_1, k_2, t_{lag} and $(1-f)$], standard deviation, RMSE, R^2 and t_{4-D} for both the proposed and Whiting–Buchanan models are summarised in Tables 2 and 3, respectively. It is found that both the proposed model (Eq. (9)) and the Whiting–Buchanan model (Eq. (14)) are effective in modelling the

Table 2
Estimates of the model parameters, standard deviation, R^2, RMSE and t_{4-D} values for the proposed model

Code	k_1 [log cfu/ml/h]	k_2 [log cfu/ml/h]	t_{lag} [h]	$1-f$	R^2	RMSE	t^a_{4-D} [h]	t^b_{4-D} [h]
1	0.2725±0.0635	0.0227±0.0102	41.15±4.97	0.000044±7.51E−05	0.983	0.370	74.95	75.78
2	0.4984±0.1580	0.0672±0.0062	4.80±1.02	0.000977±0.000552	0.997	0.135	23.28	38.75
2N	2.9065±1.9762	0.3077±0.0400	0.00±1.39	0.000142±7.59E−05	0.998	0.098	3.17	3.54
3	0.0390±0.0038	0.0064±0.0037	114.75±24.26	3.20E−06±7.20E−06	0.989	0.273	351.19	351.37
4	0.0151±0.0009	0.0076±0.0186	0.00±23.65	0.000138±0.003143	0.981	0.296	608.45	609.32
5	0.2644±0.5295	0.0221±0.0020	3.17±4.80	0.060734±0.036095	0.953	0.411	38.01	292.97
5N	0.7944±0.1842	0.0422±0.0585	3.25±0.47	4.37E−07±1.10E−06	1.000	0.065	14.84	14.84
6	0.0660±0.0151	0.0150±0.0036	0.00±14.67	0.000318±0.000530	0.971	0.429	139.52	146.15
6R	0.0774±0.0153	0.0035±0.0005	0.00±7.82	0.000878±0.000465	0.963	0.371	119.03	628.88
7	0.2065±0.5038	0.0036±0.0002	0.00±12.80	0.107624±0.024552	0.970	0.207	44.61	1944.85
8	0.0283±0.0022	0.0000±0.0000	0.00±25.71	0.000000±1.03E−07	0.963	0.467	325.50	325.50
9	0.0302±0.0022	0.0122±0.0184	24.74±16.49	0.000059±0.000682	0.983	0.296	329.46	329.93
10	0.0176±0.0010	0.0054±0.0011	0.00±21.77	0.000202±0.000266	0.985	0.261	523.79	530.73
11	0.8514±0.5022	0.0016±0.0002	22.11±0.00	0.194232±0.032527	0.914	0.169	32.93	4675.02
12	0.0760±0.0903	0.0051±0.0002	0.00±15.96	0.200553±0.057597	0.969	0.283	121.15	1495.28
13	0.6382±0.7130	0.0060±0.0004	0.00±0.00	0.085121±0.034218	0.949	0.438	14.43	1122.33
14	0.1210±0.0215	0.0000±0.0000	0.00±12.98	0.000000±0.000000	0.980	0.396	76.11	76.11
15	0.0333±0.0057	0.0159±0.0007	240.00±9.57	0.097731±0.040398	0.997	0.130	516.30	673.68
16	0.2926±0.0000	0.0090±0.0000	0.00±0.00	2.14E−07±0.000000	1.000	0.000	31.47	31.48
17	0.1368±0.0000	0.0575±0.0000	10.50±0.00	0.002192±0.000000	1.000	0.000	77.85	81.26

[a] The t_{4-D} value was estimated by the traditional method.
[b] The t_{4-D} value was estimated by the proposed method.

Table 3
Estimates of the model parameters, standard deviation, R^2, RMSE and t_{4-D} values for the Whiting–Buchanan model

Code	b_1 [log cfu/ml/h]	b_2 [log cfu/ml/h]	t_{lag} [h]	$1-f$	R^2	RMSE	t^a_{4-D} [h]	t^b_{4-D} [h]
1	0.2786±0.0679	0.0233±0.0096	41.92±5.52	3.55E−05±5.94E−05	0.983	0.369	74.98	75.58
2	0.4925±0.0787	0.0675±0.0054	5.71±1.51	0.000574±0.000292	0.998	0.121	24.53	37.67
2N	3.2786±4.5078	0.3221±0.0546	0.00±5.54	9.94E−05±5.77E−05	0.997	0.121	3.02	3.39
3	0.0385±0.0037	0.0065±0.0035	111.35±24.86	2.40E−06±5.40E−06	0.989	0.269	350.92	351.09
4	0.0165±0.0021	0.0101±0.0073	0.00±93.10	0.002229±0.019213	0.980	0.306	599.66	605.70
5	0.3359±0.6701	0.0228±0.0020	6.63±8.18	0.038636±0.023312	0.954	0.402	34.35	294.84
5N	1.0010±0.2123	0.0632±0.0108	3.94±0.40	6.37E−07±2.00E−07	1.000	0.049	13.16	13.17
6	0.1202±0.3212	0.0184±0.0029	0.00±130.51	0.000826±0.001250	0.965	0.473	82.41	148.91
6R	0.0874±0.0245	0.0037±0.0005	0.00±30.65	0.000643±0.000323	0.963	0.371	113.27	670.90
7	0.1393±0.1389	0.0037±0.0002	0.00±43.35	0.065316±0.015167	0.969	0.209	71.11	1939.98
8	0.0298±0.0034	0.0000±0.0000	0.00±83.55	9.20E−08±0.000000	0.962	0.473	332.00	332.00
9	0.0321±0.0029	0.0140±0.0124	32.68±28.33	0.000113±0.000856	0.985	0.278	328.76	329.66
10	0.0190±0.0018	0.0056±0.0011	0.00±74.63	0.000131±0.000175	0.980	0.297	520.53	527.84
11	0.4268±0.9489	0.0019±0.0003	21.82±19.25	0.169124±0.033031	0.922	0.161	43.40	4227.27
12	0.0696±0.1010	0.0052±0.0003	0.00±108.18	0.119714±0.037886	0.970	0.278	142.21	1498.13
13	0.1396±0.2678	0.0061±0.0004	0.00±86.87	0.047514±0.023513	0.939	0.476	70.93	1127.77
14	0.1271±0.0279	0.0000±0.0000	0.00±33.23	0.000000±0.000000	0.968	0.498	77.93	77.93
15	0.0461±0.0125	0.0162±0.0006	268.67±14.65	0.074852±0.027050	0.996	0.150	468.56	678.19
16	0.3215±0.0000	0.0254±0.0000	0.00±0.00	1.18E−06±0.000000	1.000	0.000	30.80	30.80
17	0.1433±0.0000	0.0632±0.0000	10.71±0.00	0.003072±0.000000	1.000	0.000	76.35	81.49

[a] The t_{4-D} value was estimated by the traditional method.
[b] The t_{4-D} value was estimated by the proposed method.

different shapes of the survival curves, but neither of them consistently produces the best fit to all the experimental survival curves. In terms of the RMSE and R^2 values, the Whiting–Buchanan model fits better in nine of the total 20 survival curves, while the proposed model does better in nine, and for the other two survival curves, both models have the same RMSE values. From Tables 2 and 3, it is observed that, between the two models, the differences in RMSE values for the individual survival curves are not very significant and the estimated kinetic parameters are similar. Two reasons for this are (1) the structural similarity between the proposed model and the Whiting–Buchanan one and (2) the small difference between the first order kinetic model, which the proposed model is based on and the Kamau model for linear curves (Table 1), which the Whiting–Buchanan model is derived from. Some examples of fitted survival curves are presented in Fig. 2. The figure shows that the survival curves are non-linear with different shapes and both models produce very similar fitting curves.

There are significant differences between the proposed model and the Whiting–Buchanan model. The proposed model, derived from some assumptions based upon the observations, has a more mechanistic background than the Whiting–Buchanan model, which is purely empirical. The well-known first order kinetic model can be derived from the proposed model, but not from the Whiting–Buchanan model. Both the Buchanan model and the Cerf's model are also a special case of the proposed model. The kinetic parameters k_1 and k_2 in the proposed model are the death rate constant but the parameters b_1 and b_2 in the Whiting–Buchanan model are not. Consequently, the D values can be derived directly from the inverse of k_1 and k_2, i.e. $D=2.303/k$, but not from the inverse of b_1 and b_2, i.e. $D\neq 2.303/b$. It is not correct that D values, such as D_1 and D_2, are defined by the Whiting–Buchanan model as 2.303 times the inverse of the parameter b_1 and b_2, respectively. It is also inappropriate that the parameters b_1 and b_2 are termed the inactivation rate because the death (inactivation) rate is theoretically meant as the decrease in cell concentration per unit time.

For the linear phase of the survival curves, the Whiting–Buchanan model shows a slight curvature, while the proposed model produces a straight line. Comparing the fittings in Fig. 2, it is found that, in the linear phase, the predicted curve from the Whiting–Buchanan model is slightly higher in the first-half portion of the curve and slightly lower in the second-half portion than those predicted using the proposed model. The reason for this may be that the specific death rate in the Whiting–Buchanan model changes with time. This also implies that the 'D values' estimated by the Whiting–Buchanan model are not the real D values.

The lag phase is described differently by the two models. The proposed model assumes that the population concentration of microorganisms in the lag phase remains the same and that the lag time duration is the mean time required for the initial inactivation of microorganisms, while the Whiting–Buchanan model does not have a clear definition for both the lag phase and the lag time duration. Traditionally, the lag time duration has been determined by drawing a line through the linear phase of the curve. The proposed model into which the Buchanan model (Buchanan et al., 1993) is incorporated provides a mathematical means for the traditional estimation of the lag time duration. Considering that there is no generally accepted definition for the lag phase in the inactivation process, the quantitative definition for the boundary between the lag and linear phases assumed by the proposed model appears reasonable and justifiable. From Tables 2 and 3, it is found that, in most cases, the lag time duration estimated by the proposed model is slightly lower than that from the Whiting–Buchanan model.

The t_{4-D} values obtained by the two models are similar. In most cases, the t_{4-D} values estimated using the proposed model are slightly larger than those from the Whiting–Buchanan model. However, the t_{4-D} values estimated using the traditional method (Eq. (11) or Eq. (15)) are the same as or smaller than those from the proposed method (Eq. (13)). This is because the effect of the less resistant fraction on the t_{4-D} values is ignored in the traditional method. From Tables 2 and 3, it is found that when $(1-f)$ is close or equal to zero, the t_{4-D} values estimated using the traditional method are the same or almost the same as those from the proposed method. However, the traditional method produces much smaller t_{4-D} values when $(1-f)$ moves away from zero. The smaller the value of $(1-f)$, the smaller the difference in the t_{4-D} values between the

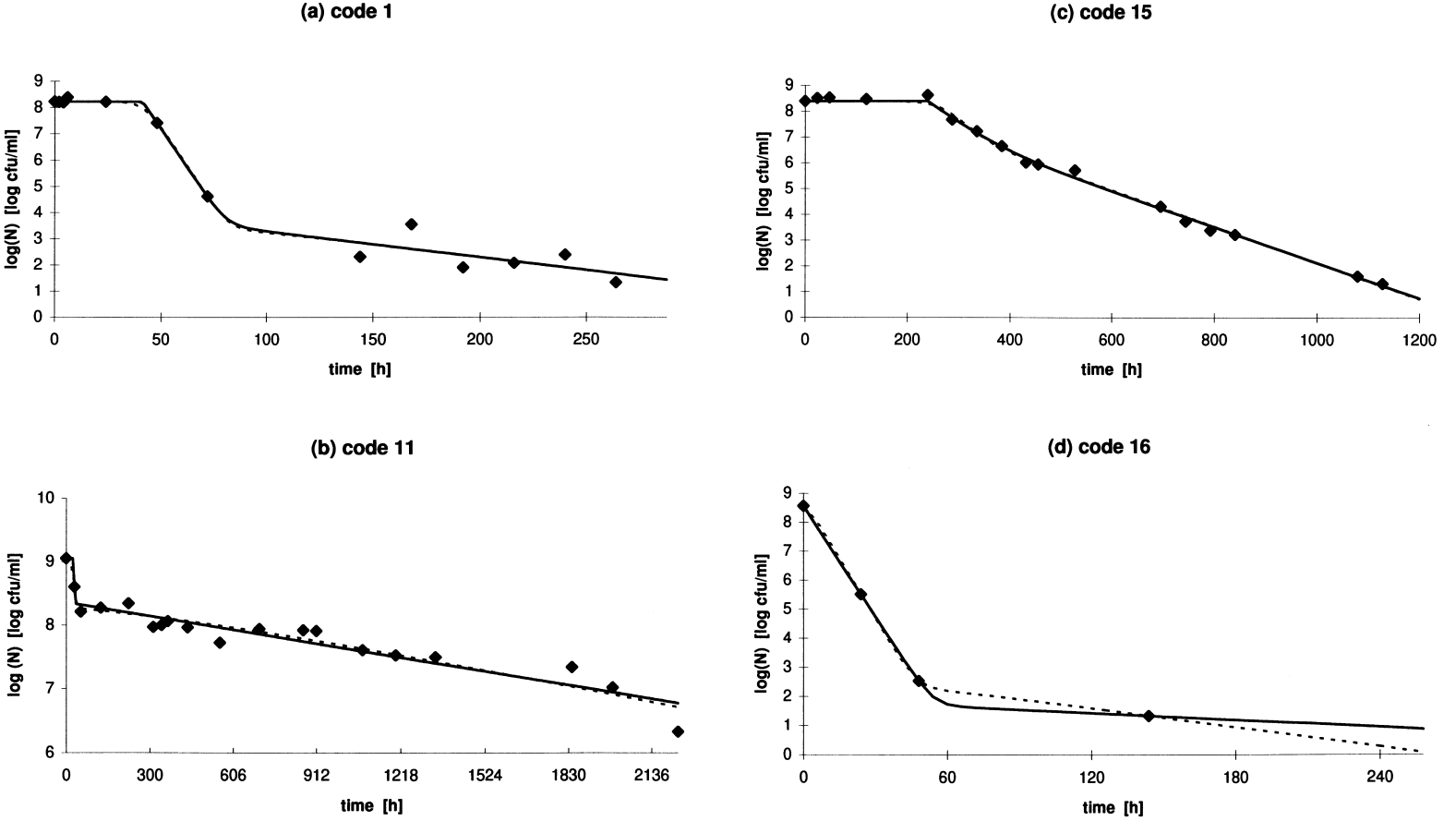

Fig. 2. Comparison of survival curve fitting for *Staphylococcus aureus*. Observed data (♦); predicted data by the proposed model (——) and by the Whiting-Buchanan model (---).

two methods. The biggest difference is observed for the treatment of Code 11 (Tables 2 and 3). For the proposed model, for example, the traditional method (Eq. (11)) estimated a t_{4-D} value of 32.93 h, while the new method (Eq. (13)) predicted a t_{4-D} value of 4675.02 h. In this treatment, the number of microorganisms was 9.05 (log cfu/ml) initially, and 6.33 (log cfu/ml) at the end of the treatment for 2232 h. From Fig. 2b, the t_{4-D} value from the proposed method agrees well with the 'visual' observation. This reflects the fact that the traditional method cannot give an accurate prediction of the t_{4-D} values if the value of $(1-f)$ is not negligible. For food safety concerns, the proposed method should be used to predict the t_{4-D} values for non-linear survival curves.

4. Conclusion

The first order kinetic model, the Buchanan model and Cerf's model were integrated into a proposed model that is capable of fitting the four commonly observed types of survival curves: linear curves, curves with a shoulder, biphasic curves and sigmoidal curves. The proposed model has practically the same goodness-of-fit as the Whiting–Buchanan model, and a more mechanistic background than the Whiting–Buchanan model. A new method is also proposed to predict accurately the t_{4-D} values for non-linear survival curves.

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