A modified Weibull model for bacterial inactivation

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Abstract

In this paper, a modified Weibull model is proposed to fit microbial survival curves. This model can incorporate shoulder and/or tailing phenomena if they are encountered. We aim to obtain an accurate fit of the “primary” modelling of the bacterial inactivation and to provide a useful and meaningful model for biologists and food industry. A $\delta$ parameter close to the classical concept of the $D$ value, established for sterilisation processes, is used in the model. The specific parameterisation of the Weibull model is evaluated for the parameter of interest $\delta$. The goodness-of-fit of the model is compared to the one produced by the model proposed by Geeraerd et al., [Geeraerd, A.H., Herremans, C.H., Van Impe, J.F., 2000. Structural model requirements to describe microbial inactivation during a mild heat treatment. Int. J. Food Microbiol. 59, 185-209.] on experimental data. As our model provides good fits for the different types of survival curves analysed, further research can focus on the development of suitable secondary model types. In this respect, it is interesting to note that the $\delta$ parameter is close to the $D$ concept.

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1. Introduction

Modelling bacterial survival curves becomes more and more an important issue due to the increasing use of mild heat treatments for food products which have to guarantee the safety of the products and due to the increasing use of risk analyses aiming at a better control of the foodborne diseases. Whereas models have been developed during the last 15 years by predictive microbiology in order to determine microbial growth in constant and variable environment, useful models have to be proposed to deal with microbial decrease in constant and variable environment. Numerous models have been proposed yet in this scope but many of them have been developed for very specific needs and cannot deal with most typical behaviours of microbial survivor curves. An excellent review on the subject has been published by Geeraerd et al. (2000).

Most microbial survival curves have a non-log-linear behaviour. Phenomena such as shoulder (smooth initiation) and tailing (saturation) of survivor
curves are frequent. Even if a better insight of those phenomena has to be found to possibly control them, a predictive survival model must in any case incorporate those phenomena if they exist to provide good parameter estimates by an accurate fit of the experimental data. Actually, microbial survival curves have the shape of survival curves described in the literature of failure time data when distinguishing individuals (Kalbfleisch and Prentice, 1980; Lawless, 1982). Microbial survival curves are the cumulative of the individual failure distributions as mentioned previously by Peleg and Cole (1998). However, the background of bacterial inactivation is different because the individual organism failure time is unknown, the absolute population have to be described (not the population relative to the initial population) and because the possibility of a tailing phenomenon has to be incorporated.

In the context of failure time data analysis, numerous parametric models have been proposed: exponential, Weibull, log-normal, log-logistic, gamma, Pareto distributions for example. Most of those models are different from each other by their long-term behaviour but often do not have a non-zero asymptote to take into account a phenomenon of saturation.

In this paper, we propose a specific parameterisation and an extension of the Weibull model to describe microbial inactivation (Albert and Mafart, 2003) and take into account its features as mentioned previously.

This paper is organised as follows. In the next section, experimental data used for fitting the model are presented, the novel model is introduced and the methods for the parameter estimation and model evaluation are set out. Section 3 deals with the experimental data fits. On the experimental data, the new model is compared with a model proposed recently by Geeraerd et al. (2000) for bacterial inactivation and the re-parameterisation of the Weibull model is evaluated. We conclude in Section 4 by a discussion.

2. Materials and methods

2.1. Origin of experimental data used

Table 1 gives an overview of the experimental data used for the fits. Spores and vegetative bacteria are analysed. All experiments were carried out in static environmental conditions. 40 curves are analysed from four strains. The strain of Bacillus pumilus A40, isolated from eggs powder, were supplied by Saupiquet (France). The strain of Bacillus cereus Bee 1, isolated from dairy food line process, were supplied by Danone (France). The two strains were studied at Quimper University Research Department, Laboratoire Universitaire de Microbiologie Appliquée (LUMAQ). For more details on the microorganism and spore production, and the thermal treatment of spore suspension, see Mafart et al. (2002). The data sets related to Clostridium botulinum 213B originate from literature (Anderson et al., 1996). The data sets from Listeria innocua ATCC 51742 originate from LUMAQ unpublished results.

These data sets were selected to explore a large range of time values (heat treatment duration from 4 to 45.25 min) and microbial population size values (from $10^3$ to $10^9$ cfu ml$^{-1}$), and for the shape of their survivor curves. Survivor curves of B. pumilus and B. cereus exhibit a “shoulder” and no tailing (downward concavity: type A curve, see Fig. 1 for example) contrary to the C. botulinum curves which have no shoulder and no tailing (upward concavity: type B curve, see Fig. 7 for example). The L. innocua survivor curves were chosen because of their sigmoidal shape (with a shoulder and a tailing effect: type C curve, see Fig. 5 for example). The average number of points by curve is 11.2. The number of observations by curve ranges from 7 to 18, and replications are sometimes available (data related to C. botulinum are all repeated two times).

2.2. The inactivation model

The kinetics of the bacterial population decrease (cfu ml$^{-1}$) versus time (min) is described by the following model:

$$
\gamma(t/\delta) = (N_0 - N_{\text{res}})10^{-(t/\delta)^p} + N_{\text{res}},
$$

where $t$ is time, $\gamma(t)$ is the bacterial concentration at time $t$, $N_0(N_0>0)$, $N_{\text{res}}(N_{\text{res}}\geq 0)$, $\delta(\delta>0)$ and $p(p>0)$ are unknown parameters which have to be estimated. $N_0$ and $N_{\text{res}}$ represent the unknown values of the
initial bacterial concentration (at time $t=0$) and the residual bacterial concentration (at the end of the observation), respectively. $N_{\text{res}}$ does not measure the value of bacterial concentration at $t=\infty$. It will be certainly zero! It has just ambition to allow the fit of the sigmoidal curve observed in the bacterial inactivation process. In the article of Geeraerd et al. (2000), possible interpretations of $N_{\text{res}}$ are given. The $\delta$ parameter represents the time of the first decimal reduction concentration for the part of the population not belonging to $N_{\text{res}}$. In Eq. (1), if $t=\delta$ we have:

$$\frac{y(\delta) - N_{\text{res}}}{N_0 - N_{\text{res}}} = \frac{1}{10}.$$  

(2)
Model (Eq. (1)) is close to the model proposed by Peleg and Cole (1998):

\[
\log \left( \frac{\gamma(t)}{N_0} \right) = -kt^p. \tag{3}
\]

Nevertheless, the model we propose is different from it because in Eq. (1), a possible tailing effect is modelled and another parameterisation of Eq. (3) is introduced with:

\[
\delta = \left( \frac{1}{k} \right)^{1/p} \tag{4}
\]

The \( p \) parameter allows to “catch” the curve concavity or convexity. If \( 0<p<1 \), the curve has no inflexion point and hence no shoulder. If \( p>1 \), the curve has an inflexion point and hence permits a shoulder effect (with a tailing if \( N_{\text{res}} \neq 0 \)). If \( p=1 \), the decrease is log-linear and it corresponds to a first-order decay reaction (if \( N_{\text{res}}=0 \)) (Chick, 1908).

Our model has unquestionable advantages:

(i) It permits the fit of most typical survivor curves (types A, B and C) because it stems from a very flexible survival model, the well-known Weibull model.

(ii) The model is parsimonious with only four parameters or three if \( N_{\text{res}} \) is not necessary (assumed to be equal to zero when no tailing).

(iii) Its parameters are meaningful. In particular, we use a \( \delta \) parameter whose meaning is close to the one of the \( D \) parameter well-known by biologists and food industry. This parameter could be the starting point of a “secondary” modelling as suggested by Bigelow (1921) (for the \( D \) value) and Mafart et al. (2001). Even if the \( p \) parameter has no direct biological interpretation, its role on the curve shape is obvious.

Finally, note that our model has the structural model requirements to describe microbial inactivation that are listed by Geeraerd et al. (2000):

- the model representing the bacterial population decrease as a function of time can simulate a shoulder, a tail or both and encompasses log-linear inactivation (when \( p=1 \) and \( N_{\text{res}}=0 \)).
- A model dynamic version can be written as follows:

\[
\frac{d\gamma}{dt} = -\frac{\ln(10)}{\delta^p} p t^{p-1} \left( 1 - \frac{N_{\text{res}}}{\gamma} \right)^{\gamma}. \tag{5}
\]
To fit the experimental data sets, the following statistical regression model is assumed: the observed bacterial concentration (unit: cfu ml\(^{-1}\)) at time \(t\), \(N(t)\), is written as:

\[
N(t) = \gamma(t) + \epsilon(t),
\]

where \(\gamma(t)\) describes the relationship between the observed bacterial concentration and the time \(t\) (Eq. (1)). \(\epsilon(t)\) is the discrepancy between the observation \(N(t)\) and its expectation \(\gamma(t)\). The errors \(\epsilon(t)\) (0 ≤ \(t\) ≤ \(t_n\), where \(t_n\) is the last time of observation) are centred random variables (\(E[\epsilon(t)]=0\)). The errors are assumed to be independent. Moreover, to complete the regression model, a variance model is introduced assuming that the variance of \(\epsilon(t)\), denoted by \(\sigma^2_t\), exists and equals:

\[
\sigma^2_t = \sigma^2 \gamma(t)^q,
\]

where \(\sigma^2\) and \(q\) have to be estimated. A value of \(q\) equals to 2 means that the logarithmic transformation of the responses stabilises the error variances and a value of \(q\) equals to 1 means that the square-root transformation of the responses stabilises the error variances. When the observations are counts, it is frequent to observe that the variability of the response depends on its level (Seber and Wild, 1989, pp. 68–89). This phenomenon is found in predictive microbiology. In most models, a logarithmic or square-root transformation of the responses is done to stabilise the error variances. Here, we do not choose this systematic transformation. Our variance modelling is much more flexible. It generalises the usual models. It requires the estimation of a supplementary parameter \(q\) but allows to take into account a variance heterogeneity much more adapted to the observations. A bad variance stabilisation may lead to wrong parameter estimates and parameter variances estimates.

In addition, several authors choose to model the population relative to the initial population, \(N(t)/N_0\), in place of the absolute population \(N(t)\). More precisely, they replace the unknown \(N_0\) by an estimate based on the observations in \(t=0\). Generally, the number of replications is small and therefore the estimate of \(N_0\) is very poor. As a consequence, the estimation of the parameters of the kinetics are biased and their variance is underestimated. Considering the statistical model defined by Eq. (6) overcomes these drawbacks and allows to estimate \(N_0\) with a greater precision because all the observations contribute to its estimation.

2.3. Parameter estimation and model evaluation

Nonlinear regressions were performed on the survival data using the nls2 procedure in S-PLUS (Insightful, Seattle, WA, USA) developed in our department, available on the website: http://www.inra.fr/bia/J/AB/nls2. A book is published to explain the statistical methods used and includes examples of use of nls2 (Huet et al., 2003). The important advantage of the nls2 function is that heteroscedasticity of errors can be taken into account and estimated by modelling the variance function.

To estimate the parameters, the maximum likelihood method assuming Gaussian observations is used. We chose this method to have an objective criterion, a likelihood model value, to compare our model to another non-nested model (the Geeraerd et al.’s model, see below). Other approaches are possible. The quasi-likelihood method (Huet et al., 2003) or an iterated two-stage least squares method (Seber and Wild, 1989) could be used. The use of weighted least squares is not possible because the estimators of this approach are not consistent (they are asymptotically biased) in the context of a parametric modelling of the variance.

The complete model depends on six parameters (\(\delta\), \(p\), \(N_0\), \(N_{res}\), \(q\) and \(\sigma^2\)). In nonlinear regression models, the maximum likelihood estimator values cannot be given explicitly (as well as the least squares estimator values) and are obtained by numerical computation. If the variance of the observations is constant (\(\sigma^2_0=\sigma^2\) for each value of \(t\)), the maximum likelihood estimator and the ordinary least squares estimator are the same.

For testing if the logarithmic transformation of the data can be used, the hypothesis “\(q=2\)” against “\(q\neq2\)” is tested by using the likelihood ratio test (Seber and Wild, 1989). The likelihood ratio test statistic is defined as follows:

\[
S_L = -2\log \frac{\hat{V}_A}{\hat{V}_H},
\]

where \(\hat{V}_H\) and \(\hat{V}_A\) are the maximum values of the likelihood functions under the hypotheses “\(H: q=2\)” and “\(A: q\neq2\)” respectively. The hypothesis \(H\) is
rejected if \( S_t > \lambda C \), where \( C \) is the \( 1 - \alpha \) quantile of a Chi-squared distribution with 1 degree of freedom.

The fit produced by our model is compared to the one of the model proposed by Geeraerd et al. (2000):

\[
\gamma(t) = (N_0 - N_{\text{res}}) \exp(-k_{\text{max}} t) 
\times \left( \frac{1 + C_c(0)}{1 + C_c(0) \exp(-k_{\text{max}} t)} \right) + N_{\text{res}}. 
\] (9)

This model is a re-parameterisation of the Baranyi model (Baranyi et al., 1996). The same statistical model is defined to fit the data sets:

\[
\begin{align*}
N(t) &= \gamma(t) + \varepsilon(t), \\
\sigma_i^2 &= \sigma^2 \gamma(t)^q.
\end{align*}
\] (10)

The model depends on six parameters (\( C_c(0), k_{\text{max}}, N_0, N_{\text{res}}, q \) and \( \sigma^2 \)) in its complete form.

The reduced form (\( N_{\text{res}}=0 \)) of the two models (Eqs. (1) and (9)) is used for the fits according to the curve shapes: for a type A curve and a type B curve, \( N_{\text{res}} \) is assumed to be equal to 0. When possible in an estimation procedure, it is important to act like this to avoid the estimation of a parameter at the bound of its definition domain possible leading to the instability of the estimation of all other model parameters. The model proposed by Geeraerd et al. (2000) does not allow to fit upward concavity curves (type B) if \( C_c(0) > 0 \) as suggested in Geeraerd et al. (2000). Log-linear curves can be fitted when \( C_c(0)=0 \) and \( N_{\text{res}}=0 \).

The fit of the two models is compared on the type A and C curves using an Akaike model selection criterion, AIC (Akaike, 1973; McQuarrie and Tsai, 1998):

\[
\text{AIC} = -2 \times \log(\text{likelihood}) + 2 \times \text{number of parameters.} 
\] (11)

This criterion allows to compare non-nested models (as Models (Eqs. (1) and (9))) and to have parsimony considerations. This criterion is based on the log-likelihood of the model penalised by the number of parameter in the model. The model with the smallest AIC criterion is chosen. The models with more parameters are penalised even if their likelihood is greater. When the two models have the same number of parameters as in the present study, it amounts to comparing the log-likelihood of the two models.

Fig. 2. A semi-logarithmic plot of the fits from Eqs. (1) and (9) to the raw data set \( Bp3 \), shown as log\(_{10}\)cfu ml\(^{-1}\) vs. time (min).
In nonlinear regression, to calculate the confidence intervals of the parameters, we use asymptotic results which are valid when the number of observations tends to infinity. For example, the standard-error of \( \hat{\delta} \), is approximated by the quantity, say, \( \hat{S} \), and the distribution of \( \hat{T} = \hat{\delta} / \hat{S} \) is approximated by a Student’s t distribution. The theoretical results lead to the calculation of the well-known Student’s t or Gaussian confidence intervals. The quality of this approximation
depends strongly on the parameterisation of the model, especially when the number of observations is small (Ratkowsky, 1990). Because \( \delta \) is the parameter of interest for secondary subsequent analyses, we verify that the approximation of the distribution of \( \hat{\delta} \) by a Student’s \( t \) distribution is valid. For that purpose,

(i) the bootstrap distribution of \( \hat{T} \) is compared to the Student’s \( t \) or Gaussian distribution. If
these two distributions are close, the approximation of the distribution of $\hat{T}$ by a Student’s $t$ or Gaussian distribution is valid (Huet et al., 2003).

(ii) The confidence ellipsoids (obtained under hypotheses of a Gaussian distribution for each parameter) and the likelihood contours (based on the likelihood ratio test and independent of the

Fig. 7. A semi-logarithmic plot of the fit from Eq. (1) to the raw data set Cb4, shown as $\log_{10} \text{cfu ml}^{-1}$ vs. time (min) (at $t=48$ min, the two points are superposed).

Fig. 8. A semi-logarithmic plot of the fit from Eq. (1) to the raw data set Cb5, shown as $\log_{10} \text{cfu ml}^{-1}$ vs. time (min) (at $t=0.85$ min, the two points are superposed).
model parameterisation) are compared in the planes \((\delta, p)\) and \((\delta, N_0)\) to verify if the percentiles of a Gaussian distribution can be used to calculate the parameter confidence intervals (Huet et al., 2003).

### 3. Results

Figs. 1 and 2 give examples of fits of \(B. \text{pumilus}\) curves (type A) using our model and the Geeraerd et al. (2000) model. Figs. 3 and 4 give examples of fits of \(B. \text{cereus}\) curves (type A) with the two models. Figs. 5 and 6 give examples of fits of \(L. \text{innocua}\) curves (type C). Figs. 7 and 8 give fits of type B curves (\(C. \text{botulinum}\)) using our model. Modified Weibull model’s parameter estimates and their 95% Gaussian confidence intervals are given in Table 2 for \(B. \text{pumilus}\) curves, Table 3 for \(B. \text{cereus}\) curves, Table 4 for \(L. \text{innocua}\) and Table 5 for \(C. \text{botulinum}\).

On all the experimental data, the hypothesis ‘\(q\) equals 2’ is tested by likelihood ratio tests. This hypothesis is rejected with asymptotic level 5%: 2 times out of 9 on \(B. \text{pumilus}\) curves (data set number: \(Bp1, Bp9\)), 3 times out of 8 on \(B. \text{cereus}\) curves (data set number: \(Bc1, Bc7, Bc8\)), 2 times out of 18 on \(L. \text{innocua}\) curves (data set number: \(Li4, Li6\)) and 1 time out of 5 on \(C. \text{botulinum}\) curves (data set number: \(Cb1\)). Generally, the parameters of the kinetics are estimated considering the data after a logarithmic transformation. This is equivalent in some way to the case \(q=2\), because if the variance of \(N(t)\) is proportional to \(\gamma(t)^2\), then the variance of \(\log N(t)\) is approximately constant. To evaluate the difference between our results and this approach, we compare the estimated parameters obtained after the logarithmic transformation to the 95% confidence intervals calculated under our model. We observe 1 time out of 2 that the estimates of \(\delta, p\) and \(N_0\) when the data are transformed, are not in the 95% Gaussian confidence intervals of \(\delta, p\) and \(N_0\) calculated when there is no data transformation and the parameter \(q\) is estimated. For example: for the data set \(Bc7\) on \(B. \text{cereus}\), when \(q=2, \delta=8.92, p=1.58\) and \(N_0=1.9 \times 10^6\), values which are not in the Gaussian intervals given in Table 3; for

### Table 2

Parameter estimates (mean) and their 95% Gaussian confidence intervals on \(Bacillus \text{pumilus}\) curves

<table>
<thead>
<tr>
<th>Data set number</th>
<th>(\delta)</th>
<th>(p)</th>
<th>(N_0)</th>
<th>(N_{\text{res}})</th>
<th>(q)</th>
<th>(\sigma^2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Bp1)</td>
<td>4.00 [3.89;4.12]</td>
<td>2.56 [2.29;2.83]</td>
<td>10 162 [9 \times 10^5;11 \times 10^5]</td>
<td>–</td>
<td>1.27 [1.16;1.38]</td>
<td>4.07</td>
</tr>
<tr>
<td>(Bp2)</td>
<td>3.80 [3.64;3.95]</td>
<td>2.79 [2.43;3.15]</td>
<td>5 826 [5 \times 10^5;6 \times 10^5]</td>
<td>–</td>
<td>1.65 [1.53;1.76]</td>
<td>0.35</td>
</tr>
<tr>
<td>(Bp3)</td>
<td>6.00 [5.85;6.15]</td>
<td>2.32 [2.19;2.46]</td>
<td>9 886 [8 \times 10^5;10 \times 10^5]</td>
<td>–</td>
<td>2.01 [1.90;2.11]</td>
<td>0.006</td>
</tr>
<tr>
<td>(Bp4)</td>
<td>18.77 [18.08;19.45]</td>
<td>2.51 [2.27;2.76]</td>
<td>6 757 [5 \times 10^5;7 \times 10^5]</td>
<td>–</td>
<td>1.95 [1.82;2.08]</td>
<td>0.02</td>
</tr>
<tr>
<td>(Bp5)</td>
<td>9.24 [8.78;9.69]</td>
<td>2.02 [1.84;2.20]</td>
<td>16 120 [13 \times 10^5;18 \times 10^5]</td>
<td>–</td>
<td>1.84 [1.75;1.92]</td>
<td>0.13</td>
</tr>
<tr>
<td>(Bp6)</td>
<td>4.72 [4.24;5.20]</td>
<td>2.10 [1.71;2.49]</td>
<td>6 190 [3 \times 10^5;8 \times 10^5]</td>
<td>–</td>
<td>2.11 [1.99;2.25]</td>
<td>0.04</td>
</tr>
<tr>
<td>(Bp7)</td>
<td>7.42 [6.91;7.92]</td>
<td>1.67 [1.42;1.91]</td>
<td>9 661 [8 \times 10^5;11 \times 10^5]</td>
<td>–</td>
<td>1.37 [1.25;1.48]</td>
<td>3.65</td>
</tr>
<tr>
<td>(Bp8)</td>
<td>9.14 [8.67;9.62]</td>
<td>2.46 [2.15;2.77]</td>
<td>7 614 [6 \times 10^5;9 \times 10^5]</td>
<td>–</td>
<td>1.72 [1.60;1.83]</td>
<td>0.2</td>
</tr>
<tr>
<td>(Bp9)</td>
<td>6.46 [6.18;6.74]</td>
<td>3.83 [3.44;4.23]</td>
<td>3 643 [2 \times 10^5;5 \times 10^5]</td>
<td>–</td>
<td>2.89 [2.73;3.05]</td>
<td>0.0002</td>
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### Table 3

Parameter estimates (mean) and their 95% Gaussian confidence intervals on \(Bacillus \text{cereus}\) curves

<table>
<thead>
<tr>
<th>Data set number</th>
<th>(\delta)</th>
<th>(p)</th>
<th>(N_0)</th>
<th>(N_{\text{res}})</th>
<th>(q)</th>
<th>(\sigma^2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Bc1)</td>
<td>12.83 [11.42;14.24]</td>
<td>1.30 [1.10;1.50]</td>
<td>6.5 \times 10^7 [5 \times 10^7;8 \times 10^7]</td>
<td>–</td>
<td>1.54 [1.49;1.60]</td>
<td>92</td>
</tr>
<tr>
<td>(Bc2)</td>
<td>2.01 [1.80;2.21]</td>
<td>1.88 [1.60;2.15]</td>
<td>10 \times 10^5 [7 \times 10^5;13 \times 10^5]</td>
<td>–</td>
<td>1.97 [1.90;2.04]</td>
<td>0.12</td>
</tr>
<tr>
<td>(Bc3)</td>
<td>11.35 [9.89;12.82]</td>
<td>1.59 [1.34;1.86]</td>
<td>5 \times 10^5 [3 \times 10^5;7 \times 10^5]</td>
<td>–</td>
<td>1.79 [1.71;1.88]</td>
<td>1.02</td>
</tr>
<tr>
<td>(Bc4)</td>
<td>1.67 [1.34;1.99]</td>
<td>1.20 [0.97;1.43]</td>
<td>7.5 \times 10^5 [5 \times 10^5;10 \times 10^5]</td>
<td>–</td>
<td>1.67 [1.59;175]</td>
<td>5.72</td>
</tr>
<tr>
<td>(Bc5)</td>
<td>23.01 [20.79;25.22]</td>
<td>2.37 [2.01;2.73]</td>
<td>15 \times 10^5 [9 \times 10^5;21 \times 10^5]</td>
<td>–</td>
<td>1.83 [1.76;191]</td>
<td>1.65</td>
</tr>
<tr>
<td>(Bc6)</td>
<td>11.27 [10.06;12.48]</td>
<td>1.74 [1.50;1.98]</td>
<td>6.5 \times 10^6 [4 \times 10^6;9 \times 10^6]</td>
<td>–</td>
<td>1.95 [1.87;2.04]</td>
<td>0.09</td>
</tr>
<tr>
<td>(Bc7)</td>
<td>10.02 [9.83;10.22]</td>
<td>2.00 [1.90;2.10]</td>
<td>1.65 \times 10^6 [1.6 \times 10^6;1.7 \times 10^6]</td>
<td>–</td>
<td>1.06 [0.96;1.16]</td>
<td>263.41</td>
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</table>
the data set Bc8, the behaviour is similar: \( \delta = 11.07 \), 
\( p = 1.48 \) and \( N_0 = 4.9 \times 10^7 \) when \( q = 2 \).

Table 4 gives the AIC criterion value of each model for type A and C curves. The AIC values are smallest for the modified Weibull model on 5 curves on 9 for B. pumilus, on 6 curves on 8 for B. cereus and on 8 curves on 18 for L. innocua. According to this criterion, the modified Weibull model is preferable for these curves (when it is the smallest). In fact, the observed values of the criterion are often very close between the two models which shows that the two model fits are equivalent on these two types of curves. This result can be also confirmed by the visualisation of the two model fits on the experimental data (see Figs. 1–6). Often the curves are very close. The shoulder effect might be more marked with the Geeraert et al. model. But the model proposed by Geeraert et al. does not allow to fit upward concavity curves (type B) as ours.

On the experimental data, the parameterisation of the model is evaluated. Note that in nonlinear models, the model parameterisation is an important point because it can cause curvature effects (local minima can appear) and problems of parameter identifiability (for example, we could have difficulties to estimate the \( \delta \) parameter if it is too close to zero). We need to assess the parametric nonlinearity of the model. Particularly, the parameter of interest \( \delta \) is studied. A weak parametric nonlinearity of this parameter will allow to calculate the confidence intervals of the parameter from the Normal or Student’s \( t \) percentiles. From each data set, the bootstrap distribution of \( \hat{T} = \delta - \hat{\delta} \hat{S} \) is calculated from 1000 bootstrap simulations. It can be done with nls2. For example, Fig. 9

<table>
<thead>
<tr>
<th>Data set number</th>
<th>( \delta )</th>
<th>( p )</th>
<th>( N_0 )</th>
<th>( N_{res} )</th>
<th>( q )</th>
<th>( \sigma^2 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Li1</td>
<td>5.7</td>
<td>5.17</td>
<td>6.24</td>
<td>2.27</td>
<td>1.93</td>
<td>1.45</td>
</tr>
<tr>
<td>Li2</td>
<td>11.59</td>
<td>10.52</td>
<td>12.67</td>
<td>3.76</td>
<td>2.79</td>
<td>4.72</td>
</tr>
<tr>
<td>Li3</td>
<td>11.94</td>
<td>10.89</td>
<td>12.98</td>
<td>1.84</td>
<td>1.55</td>
<td>1.23</td>
</tr>
<tr>
<td>Li4</td>
<td>5.02</td>
<td>4.69</td>
<td>5.35</td>
<td>2.81</td>
<td>2.42</td>
<td>3.19</td>
</tr>
<tr>
<td>Li5</td>
<td>6.58</td>
<td>5.53</td>
<td>7.58</td>
<td>1.49</td>
<td>1.29</td>
<td>1.88</td>
</tr>
<tr>
<td>Li6</td>
<td>4.02</td>
<td>3.11</td>
<td>4.92</td>
<td>1.26</td>
<td>1.07</td>
<td>1.45</td>
</tr>
<tr>
<td>Li7</td>
<td>6.55</td>
<td>5.26</td>
<td>6.59</td>
<td>1.46</td>
<td>1.28</td>
<td>1.65</td>
</tr>
<tr>
<td>Li8</td>
<td>4.71</td>
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<td>5.15</td>
<td>1.49</td>
<td>1.35</td>
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<tr>
<td>Li9</td>
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<td>2.37</td>
<td>3.31</td>
<td>1.74</td>
<td>1.51</td>
<td>1.97</td>
</tr>
<tr>
<td>Li10</td>
<td>5.41</td>
<td>4.65</td>
<td>5.18</td>
<td>1.42</td>
<td>1.25</td>
<td>1.58</td>
</tr>
</tbody>
</table>

* According to the Gaussian confidence interval, in these cases, \( N_{res} \) can be considered to be equal to 0 (equivalent to a Wald test).
gives the histogram of this distribution on the data set Bp1 with superposed Normal and Student’s t centred and scaled distributions. We observe that the distributions are very close. The 95% bootstrap confidence interval of $\delta$ is 

$$I_B = [\bar{\delta} - b_{1.96} \bar{S}; \bar{\delta} + b_{1.96} \bar{S}]$$

where $b_{1.96}$ is the 95 percentile of the bootstrap distribution of $\bar{T}$, equals to [3.84;4.17]. They are almost similar. Moreover, the likelihood contours (based on the likelihood ratio test and independent of the model parameterisation) and the confidence ellipses (assuming Normal distributions) of $\delta$ among the $p$ and $N_0$ parameters (Figs. 10 and 11, respectively, for the data set Bp1) are similar. The likelihood contours are close to the ellipses. This result indicates a weak parametric nonlinearity of the $\delta$ parameter. The 95% confidence interval of $\delta$ based on the likelihood contours equals to [3.86;4.12]. Same results are found on all the experimental data. So, the specific parameterisation of the Peleg and Cole’s model with $d = \frac{k}{C_0/C_1}$ is satisfactory. The confidence intervals of the $\delta$ parameter calculated from the Normal or Student’s t percentiles are acceptable and very close to the intervals based on the likelihood contours.

### 4. Discussion

We have demonstrated the capacities of our model to model a large range of inactivation curves: curves with or without shoulder, with or without tailing. A good fit of the inactivation curve is essential to obtain good estimates of the model parameters. It is an important point for the “secondary” modelling which uses the “primary” parameter estimates. The goodness-of-fit of the “primary” modelling guarantees that the parameter values are a good summary of the “primary” modelling. An accurate fit is essential to obtain reliable predictions (for example, reliable decontamination times). Before the fits, we verified using the sensitivity functions (Huet et al., 2003) if the experimental designs (the chosen time values) permit an accurate estimation of all the model parameters. Those functions are a first approach to deal with this. The results (not shown here) are often conclusive. Perhaps, some reserves can be done concerning the $N_{res}$ parameter which could be better evaluated with longer times of observation.

On the experimental data curves A and C fits, our model fits are similar globally to the fits produced by the model of Geeraerd et al. (2000). Moreover, our model is more flexible because it permits curve B fits. Note that we have sometimes encountered difficulties to estimate the Geeraerd et al. model (difficulties to fix
initial parameter values for the numerical computation. The values of the $C_c(0)$ parameter are very variable from curve to curve. The confidence intervals of this parameter are very large. The authors of this model have recently developed a little software tool called GlnaFit which aims at fitting a re-parameterised

Fig. 9. Histogram of the centred and scaled bootstrap distribution of $\delta$, t.b, with superposed Gaussian and Student’s $t$ distributions on the data set $Bp1$.

Fig. 10. The confidence ellipses and likelihood contours for the parameters $(\delta, p)$ at levels 90%, 95% and 99% on the data set $Bp1$. 

The parameterisation of the model we propose seems to be satisfactory for the $d$ parameter. For the $q$ and $p$ parameters, no estimation difficulty has been encountered. We can only note that the Gaussian confidence intervals for the $q$ parameter are a little bit narrower compared to the likelihood ratio test (often they did not contain the 2 value whereas the hypothesis $q=2$ is not rejected by the likelihood ratio test). Confidence intervals based on the likelihood contours will be in accordance with the test. For the $p$ parameter, we tested by likelihood ratio tests if it can be considered constant by strain. For the four types of bacteria strains studied, the hypothesis is rejected. More investigations concerning this scope have to be done. It should be interesting to study the behaviour of $p$ according to environmental factors (temperature, pH, aw, possibly other factors and possibly their interactions).

The parameterisation proposed here is very interesting because it introduces a $d$ parameter close to the $D$ value established for sterilisation processes. $D$ is yet a starting point of “secondary” modelling (Bigelow, 1921) and $d$ (when estimating without $N_{res}$) has been modelled according to environmental factors (Mafart et al., 2001). An important work has to be done in this domain, especially to find the better function linking the $d$ value to the environmental factors. In failure time data analysis, authors have introduced covariates directly in the Weibull model. This possibility has to be studied. The direct introduction of covariates in the “primary” model would avoid a two-stage estimation which is embarrassing for parameter estimation because the response of the “secondary” model, for example the $d$ parameter, is not an observation but an estimation (from the “primary” modelling). In addition, a Bayesian approach allows a single-step evaluation (Pouillot et al., 2003).

References


Fig. 11. The confidence ellipses and likelihood contours for the parameters $(d, N_0)$ at levels 90%, 95% and 99% on the data set Bp1.


