Survival curves of heated bacterial spores: effect of environmental factors on Weibull parameters

Olivier Couvert\textsuperscript{a}, Stéphane Gaillard\textsuperscript{a}, Nicolas Savy\textsuperscript{b}, Pierre Mafart\textsuperscript{a}, Ivan Leguérinel\textsuperscript{a,}\textsuperscript{*}

\textsuperscript{a}Laboratoire Universitaire de Microbiologie, Appliquée de Quimper Pole Université, 2 rue de l’Université, 29334 Quimper cedex, France

\textsuperscript{b}Institut de Recherche Mathématique de Rennes, Université de Rennes 1, 35042 Rennes Cedex, France

Received 30 September 2003; received in revised form 28 June 2004; accepted 20 October 2004

Abstract

The classical \( D \)-value of first order inactivation kinetic is not suitable for quantifying bacterial heat resistance for non-log linear survival curves. One simple model derived from the Weibull cumulative function describes non-log linear kinetics of micro-organisms. The influences of environmental factors on Weibull model parameters, shape parameter \( \beta \) and scale parameter \( \delta \), were studied. This paper points out structural correlation between these two parameters. The environmental heating and recovery conditions do not present clear and regular influence on the shape the parameter \( \beta \) and could not be described by any model tried. Conversely, the scale parameter \( \delta \) depends on heating temperature and heating and recovery medium pH. The models established to quantify these influences on the classical \( D \) values could be applied to this parameter \( \delta \). The slight influence of the shape parameter \( \beta \) variation on the goodness of fit of these models can be neglected and the simplified Weibull model with a constant \( \beta \)-value for given microbial population can be applied for canning process calculations.

© 2004 Elsevier B.V. All rights reserved.

Keywords: Weibull distribution; Heat treatment pH; Recovery medium pH

1. Introduction

The first order kinetic model describing inactivation of micro-organisms is generally attributed to Madsen and Nyman (1907). The studies of Chick (1910), Esty and Meyer (1922) and Esty and Williams (1924) on vegetative cells had confirmed this equation:

\[
N = N_0 e^{-kt}
\]

where \( N_0 \) is the initial number of cells, \( N \) the number of surviving cells after a duration of heat treatment \( t \) and \( k \) is the first order parameter.

In 1943, Katzin et al. (1943) defined the decimal reduction time that Ball and Olson (1957) symbolized...
by the letter $D$. Thus, the model appears in the familiar form:

$$\log N_t = \log N_0 - t/D$$

(2)

In this model, the classical $D$-value presents a simple biological significance: time that leads to a 10-fold reduction of surviving population and is easily estimated from a simple linear regression. This concept still governs canning process calculation.

However, in many cases, the survival curves of heated bacteria do not present a log linear relation: a concave or upward concavity of curves has frequently been observed (Cerf, 1977).

So the bacterial heat resistance cannot be evaluated from the classical $D$ value. Consequently, many authors proposed mechanistic or purely empirical models. (Kilsby et al., 2000; Rodriguez et al., 1988; Sapru et al., 1993; Shull et al., 1963; Xiong et al., 1999; Buchanan et al., 1997; Cole et al., 1993; Geeraerd et al., 2000; Linton et al., 1995; Whiting, 1993). These models show accuracy but are either over parameterized (mechanistic models) or have parameters without any physical or biological significance (empirical models). Moreover, the complexity of these models hinders their application in heat treatment process calculations.

Other authors who considered the survival curve as a cumulative form of temporal distribution of lethality event distribution, presented a probabilistic approach (Cunhan et al., 1998; Fernandez et al., 1999; Peleg and Cole, 1998, 2000; Mafart et al., 2002). The Weibull frequency distribution model (Eq. (3)) invoked to describe the time to failure in mechanical system was applied to bacterial death time.

$$f(t) = \frac{\beta}{\alpha} \left(\frac{t}{\alpha}\right)^{\beta-1} \times \exp \left(-\left(\frac{t}{\alpha}\right)^{\beta}\right)$$

(3)

The $\beta$ parameter has a marked effect on the predicted failure rate of the Weibull distribution (Fig. 1a). According to the $\beta$ value, the distribution corresponds to a normal law ($\beta=2$), an exponential law ($\beta=1$) or an asymptotic law ($\beta<1$).

A change of the scale parameter $\alpha$, the time unit, has the same effect on the distribution as a change of the abscissae scale (Fig. 1b). If $\alpha$ increases, the distribution gets stretched out the right and its height decreases while maintaining its shape.

Fig. 1. Simulated frequency distribution of critical inactivation time (a and b) and microbial survival curves (c and d) generated with the assumption that the heat resistance has a Weibull distribution. a and c: $\alpha$: 5, $\beta$: 3 (---), 1 (---), 0.5 (----). Figures b and d: $\alpha$: 3 (---), 6 (---), 9 (----), $\beta$: 3.
The cumulative distribution Weibull function is

\[ F(t) = \exp \left( -\left( \frac{t}{\alpha} \right)^\beta \right) \]  

or applied to survival kinetics curves

\[ \ln S(t) = -\left( \frac{t}{\alpha} \right)^\beta \]  

where \( S(t) \) is the ratio \( N/N_0 \) at \( t \) time, \( \alpha \) and \( \beta \) are the two parameters of the Weibull probability density function.

Fig. 1c and d show the influence of these two parameters on the cumulative distribution Weibull function curves. \( \beta<1 \) corresponds to concave upward survival curves, \( \beta>1 \) to concave downward curves and \( \beta \) equal 1 to a straight line. The evolution of \( \alpha \) value modifies the slope but does not affect the curve shapes. Different forms of this model were presented in the literature; however, the decimal logarithm form (Eq. (6)), which is close to Eq. (2), seems more suitable to describe non-log linear survival curves (Mafart et al., 2002; van Boekel, 2002)

\[ \log N = \log N_0 - \left( \frac{t}{\delta} \right)^p \]  

where \( \delta \) is the first reduction time that leads to a 10-fold reduction of the surviving population and \( p \) the shape parameter \( \beta \). For the traditional case where the survival curve, is a first order, is linear \( p \) equal to one and the \( \delta \) parameter corresponding to the classical \( D \) value.

This simple and robust model can be regarded as an extension of the conventional first order equation. Like the \( D \)-value, the influence of heating temperature on the \( \delta \)-value leads a log linear relationship. The classical \( z \) value can be evaluated (Mafart et al., 2002; van Boekel, 2002) and a modified Bigelow method can be used to optimize the heat treatment for a target reduction ratio (Mafart et al., 2002).

Among environmental factors other than heating temperature, which affect the heat resistance of bacteria, the pH of the heating medium and the pH of the recovery medium (pH') are of prominence. Couvert et al. (1999) has developed an extended Bigelow model to describe both effects of heating and recovery medium pH on the apparent bacterial spore heat resistance.

\[ \log D = \log D^* - \frac{T - T^*}{Z_1} - \left| \frac{pH - pH^*}{Z_{\text{pH}}} \right| \]  

\[ - \left( \frac{pH' - pH'^*}{Z'_{\text{pH}}} \right)^2 \]  

where \( pH^* \) and \( pH'^* \) are the reference heat treatment and recovery medium pH fixed to 7. \( Z_{\text{pH}} \) quantifies the influence of plant medium pH influence on bacterial heat resistance. \( Z'_{\text{pH}} \) is a distance of pH' from pH', which leads to a 10-fold reduction in apparent D-value. \( Z'_{\text{pH}} \) characterizes the influence of the pH on the recovery of the microorganism after a heat treatment. \( D^* \) is the calculated D-value corresponding to pH* and pH'*. Conditions. Like the Bigelow model, Couvert’s model (Eq. (7)) was suitable for the calculation of \( \delta \)-values as well as for those of \( D \)-values. However, the influence of heating temperature on the \( p \)-value is not clear and variable according to several authors (Fernandez et al., 1999; Peleg and Cole, 2000; Mafart et al., 2002; van Boekel, 2002).

The aims of this paper are to derive methods to estimate a single \( p \)-value from a set of survival kinetics, whatever the heating temperature or heating and recovery medium pH for bacterial strain at a given physiology state.

2. Materials and methods

2.1. Microorganisms and spore production

\textit{Bacillus pumilus} A40 was obtained and isolated from dehydrated onion used in a food canning industry. Spores were kept in distilled water at 4 °C.

Cells were pre-cultivated at 37 °C for 24 h in Brain Heart Infusion broth (Difco 0037). The pre-culture was used to inoculate nutrient agar (Biokar Diagnostics, Beauvais/France) supplemented with salt (MnSO\(_4\) 40 mg l\(^{-1}\) and CaCl\(_2\) 100 mg l\(^{-1}\)). Plates were incubated at 37 °C for 5 days. Spores were then collected by scraping the surface of the agar, suspended in sterile distilled water and washed three times by centrifugation (10,000×g for 15 min).
(Bioblock Scientific, model Sigma 3K30) and was resuspended in 5 ml distilled water and 5 ml ethanol. The obtained suspension was kept at 4°C for 12 h in order to reduce the number of vegetative non-sporulated bacteria, and washed again three times by centrifugation. The final suspension (about 10^10 spores ml^{-1}), containing more than 99% refractive spores and no visible vegetative cells, was finally distributed in sterile Eppendorf microtubes and kept at 4°C.

2.2. Thermal treatment of spore suspension and recovery conditions

Heating media were tryptone salt broth (10 g/l tryptone, 10 g/l NaCl (Biokar)) for different pH adjusted with addition of 1 M H_2SO_4, media were sterilized by filtration through 0.22 μm porosity filter. 30 μl of spore suspension was diluted in 3 ml of these media. Capillary tubes of 200 μl (vitrex) were filled with 100 μl of sample and submitted to a thermal treatment in a thermostated water bath. After heating, the tubes were cooled in water/ice bath. After rising, the ends were flamed with ethanol. The capillary tubes were broken at both ends and their contents poured into a tube containing 9 ml sterile tryptone salt broth (Biokar Diagnostics) by rinsing with 1 ml tryptone salt broth.

Viable spores were counted by duplicate plating in nutrient agar for different pH (10 g tryptone, 5 g meat extract, 5 g sodium chloride, 15 g agar for 1000 ml water)(Biokar Diagnostic). The pH was adjusted with H_2SO_4 prior to autoclaving at 121°C for 15 min, the pH value was adjusted after autoclaving.

2.3. Experimental design

To determine the thermal kinetic parameters at least 10 samples were counted on nutrient agar plates. For the longest heating time no colonies should be observed to detect possible sigmoid curves.

Monofactorial designs were used to evaluate the influence of heating temperature, heating and recovery medium pH. The heating temperatures investigated were 89, 92, 95, 98, 101 and 104°C (for heating and recovery media pH equal to 7), heating media pH were 7, 6.1, 5.8, 5.2, 5.15, 5.1, 4.7 and recovery media pH were 7, 6.52, 6.26, 6.04, 5.82, 5.55 and 5.27 (for temperature 95°C).

2.4. Fitting parameters and confidence interval determination

To estimate Weibull parameters two fitting methods ways were used. On the one hand, three parameters logN_0, δ and p were estimated from each kinetic. On the other hand, two parameters logN_0 and δ were estimated from each inactivation curve with only one p-value evaluated from the whole set of kinetics.

Couvert's model parameters (Eq. (7)) were estimated from these two sets of δ estimates. The parameter values and their associated confidence interval were fitted by using a non-linear module (“nlfnit” and “nlparci” Matlab 6.1, The Mathworks). “nlparci” function used to evaluate confidence interval at 95% is based on the asymptotic normal distribution for the parameter estimates (Bates and Watts, 1988). On the one hand, p-value was estimated from each set of data, and on the other hand, single p-value was evaluated from the whole set of curves. To appreciate the accuracy on the non-linear models used in this study F test and associated probability p were carried out.

3. Results and discussion

3.1. Independence of Weibull model parameters

One of the main questions to study in any regression is to check the independence of model parameters. The shape of the joint confidence region determined by using the Lobry et al. (1991) method reveals possible structural correlation between model parameters. According to Beale (1960), a vector of parameter model Θ is in the confidence regions if probability α satisfies the inequation:

$$SSD_Θ ≤ SSD_{min}(1 + \frac{p}{n - p} F_{p,n-p,\alpha})$$  \hspace{1cm} (8)

where n number of data, p number of parameters, F is the Fisher value for α at p and n-p degrees of freedom. 10,000 vectors Θ were calculated to define the joint confidence region where dimension number
is the parameter number. Fig. 2 shows the projections of confidence region projected on three orthogonal planes. The strength shape of the projections and the high correlation coefficient indicate a structural correlation between model parameters. Three Weibull model parameters were fitted to each inactivation data set and correlation coefficients were determined from the evaluated confidence region, for the 18 environmental conditions studied, Table 1 presents the estimates of the structural correlation between parameters for all kinetics. Thus, Weibull model parameters \( \log N_0, \delta \) and \( p \) are dependent: an error on \( \delta \) will be balanced by an error on \( p \) in the same way. Finally, a single \( p \)-value estimated from the whole set of kinetics eliminates the structural correlation between \( \delta \) and \( p \) parameters as well as \( \log N \) and \( p \) parameters (Table 1) and decreases the structural correlation between \( \log N \) and \( \delta \). The Weibull model parameters become independent.

3.2. Influence of environmental factors of \( p \)-value

For each \( B. \) pumilus survival curve, the shape parameter, \( p \), was estimated. Fig. 3 suggests that the environmental heating and recovery conditions slightly influence the \( p \)-values. This observation is in agreement with Fernandez et al. (2002) data concerning the influence of heating temperature and heating medium pH on the \( p \)-values for \( Bacillus cereus \) spores. van Boekel (2002) used published data to study the influence of heating temperature on the shape (\( p \)) and the Weibull model parameter (\( \delta \)) for inactivation kinetics of different vegetative bacteria and yeast species survival kinetics. In most cases, the shape parameter is clearly independent of heating temperature, however, in some cases, dependencies appear significant. Constant \( p \)-value means that the Weibull probability density function curves presents the same shape. Applied to the probability density distribution of inactivation time, a single \( p \)-value leads us to consider that whatever the environmental condition, the least resistant bacteria die first and the most resistant bacteria are the last to die while maintaining proportion. For a given microbial population, at the same physiological state, if the population proportion is independent of heating and recovery conditions, the Weibull model shape parameter \( p \)-value should be constant. To estimate a single

![Fig. 2. Projection of the confident region on three orthogonal planes, from Bacillus pumilus A40 data (heating temperature: 95 °C, heating and recovery medium pH: 7).](image)
-value, Fernandez et al. (2002) determined the mean value of the shape parameter determined from the different kinetics. Then, for each kinetic, the scale parameter was re-estimated from set of data with fixed -value. However, it is preferable to evaluate both single shape and scale parameter by non linear least square minimization for the whole set of inactivation data. Choosing the average value to evaluate a single -value is not suitable because the number of data in each kinetic is not equal, each kinetic have not the

\begin{table}[h]
\centering
\begin{tabular}{ccccccc}
\hline
$T^\circ$ & pH & pH \hspace{1cm} & $p$-values estimated from each set of data & Overall $p$-value estimated from the gathered sets of data \\
\hline
 & & & Log $N_0$ vs. $\delta$ & Log $N_0$ vs. $p$ & $\delta$ vs. $p$ & Log $N_0$ vs. $\delta$ & Log $N_0$ vs. $p$ & $\delta$ vs. $p$ \\
89 & 7 & 7 & -0.78 & -0.62 & 0.92 & -0.71 & 0.15 & -0.06 \\
92 & 7 & 7 & -0.81 & -0.63 & 0.89 & -0.74 & 0.12 & -0.06 \\
95 & 7 & 7 & -0.75 & -0.59 & 0.93 & -0.67 & 0.35 & -0.12 \\
98 & 7 & 7 & -0.81 & -0.64 & 0.9 & -0.74 & 0.17 & -0.08 \\
101 & 7 & 7 & -0.84 & -0.67 & 0.89 & -0.73 & 0.12 & -0.06 \\
104 & 7 & 7 & -0.82 & -0.67 & 0.93 & -0.71 & 0.26 & -0.09 \\
95 & 4.7 & 7 & -0.84 & -0.59 & 0.73 & -0.71 & 0.09 & -0.06 \\
95 & 5.1 & 7 & -0.9 & -0.74 & 0.88 & -0.77 & 0.15 & -0.08 \\
95 & 5.15 & 7 & -0.64 & -0.11 & 0.65 & -0.55 & 0.03 & -0.06 \\
95 & 5.2 & 7 & -0.86 & -0.7 & 0.92 & -0.65 & 0.21 & -0.08 \\
95 & 5.8 & 7 & -0.81 & -0.66 & 0.93 & -0.71 & 0.22 & -0.09 \\
95 & 6.1 & 7 & -0.82 & -0.52 & 0.77 & -0.82 & 0.12 & -0.05 \\
95 & 7 & 5.27 & -0.81 & -0.632 & 0.92 & -0.75 & 0.2 & -0.11 \\
95 & 7 & 5.55 & -0.85 & -0.71 & 0.91 & -0.77 & 0.16 & -0.06 \\
95 & 7 & 5.82 & -0.82 & -0.66 & 0.91 & -0.73 & 0.19 & -0.09 \\
95 & 7 & 6.04 & -0.87 & -0.74 & 0.9 & -0.74 & 0.15 & -0.10 \\
95 & 7 & 6.26 & -0.89 & -0.73 & 0.91 & -0.79 & 0.17 & -0.08 \\
95 & 7 & 6.52 & -0.86 & -0.72 & 0.89 & -0.72 & 0.10 & -0.07 \\
\hline
\end{tabular}
\caption{Correlation coefficients between Weibull model parameters evaluated from the evaluated joint confidence for the 18 environmental studied conditions}
\end{table}

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure3}
\caption{Graph of the shape parameter $p$ and 95% confidence interval associated as function of heating temperature, treatment and recovery medium pH for Bacillus pumilus A40.}
\end{figure}
same weight on the \( p \)-value evaluation. On the other hand, evaluating \( p \)-value by estimating process on the whole set of data consider that each data have the same weight in the \( p \)-value evaluation.

### 3.3. Influence of environmental factors on \( \delta \)-value

To evaluate the influence of fixed or free \( p \)-value on the scale parameter, the corresponding \( \delta \)-values were compared (Table 2). The results show clearly that the accuracy of the Weibull model, characterized by \( F \) test and associated probability, is lower when a single \( p \)-value is evaluated. However, the \( \delta \)-value confidence intervals were reduced, and \( \delta \) parameter could be described by the Bigelow model and the classical \( z_T \) value can be evaluated (Table 3) (\( z_T \) is the distance of temperature from the \( T^* \) which leads to a 10-fold reduction of the first decimal reduction time \( \delta \)). Whatever the \( \delta \) calculation procedure, no significant difference appears. Similarly, van Boekel (2002) has applied the Bigelow model to assess the heating temperature influence on the scale parameter values \( \delta \); however, the Arrhenius model as well can be applied (Fernandez et al., 2002).

#### Table 2

Weibull model parameters definite with associated \( p \)-value determined for each kinetic for one part, for the other with single \( p \)-value evaluated for the whole set of kinetics for *Bacillus pumilus* A40

<table>
<thead>
<tr>
<th>( T^* )</th>
<th>( \text{pH}_{t} )</th>
<th>( \text{pH}_{r} )</th>
<th>( p )-values estimated from each set of data</th>
<th>Single ( p )-value for the whole set of kinetics</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \log N_0 )</td>
<td>CI 95%</td>
<td>( \delta )</td>
<td>CI 95%</td>
<td>( p )</td>
</tr>
<tr>
<td>89</td>
<td>7</td>
<td>7</td>
<td>4.09</td>
<td>0.18</td>
</tr>
<tr>
<td>92</td>
<td>7</td>
<td>7</td>
<td>3.83</td>
<td>0.18</td>
</tr>
<tr>
<td>95</td>
<td>7</td>
<td>7</td>
<td>4.20</td>
<td>0.12</td>
</tr>
<tr>
<td>98</td>
<td>7</td>
<td>7</td>
<td>4.04</td>
<td>0.16</td>
</tr>
<tr>
<td>101</td>
<td>7</td>
<td>7</td>
<td>3.93</td>
<td>0.18</td>
</tr>
<tr>
<td>104</td>
<td>7</td>
<td>7</td>
<td>4.04</td>
<td>0.21</td>
</tr>
<tr>
<td>95</td>
<td>4.7</td>
<td>7</td>
<td>3.66</td>
<td>0.26</td>
</tr>
<tr>
<td>95</td>
<td>5.1</td>
<td>7</td>
<td>3.61</td>
<td>0.20</td>
</tr>
<tr>
<td>95</td>
<td>5.15</td>
<td>7</td>
<td>4.20</td>
<td>0.29</td>
</tr>
<tr>
<td>95</td>
<td>5.2</td>
<td>7</td>
<td>3.95</td>
<td>0.25</td>
</tr>
<tr>
<td>95</td>
<td>5.8</td>
<td>7</td>
<td>4.03</td>
<td>0.22</td>
</tr>
<tr>
<td>95</td>
<td>6.1</td>
<td>7</td>
<td>4.13</td>
<td>0.28</td>
</tr>
<tr>
<td>96</td>
<td>7</td>
<td>5.27</td>
<td>3.89</td>
<td>0.24</td>
</tr>
<tr>
<td>95</td>
<td>5.55</td>
<td>4.05</td>
<td>0.24</td>
<td>2.62</td>
</tr>
<tr>
<td>95</td>
<td>7</td>
<td>5.82</td>
<td>3.79</td>
<td>0.16</td>
</tr>
<tr>
<td>95</td>
<td>7</td>
<td>6.04</td>
<td>3.99</td>
<td>0.17</td>
</tr>
<tr>
<td>95</td>
<td>7</td>
<td>6.26</td>
<td>3.83</td>
<td>0.20</td>
</tr>
<tr>
<td>95</td>
<td>7</td>
<td>6.52</td>
<td>3.98</td>
<td>0.18</td>
</tr>
</tbody>
</table>

#### Table 3

Couvert’s model parameters fitted on log \( \delta \)-values evaluated with multiple \( p \)-values on the one hand, with single \( p \)-values for *Bacillus pumilus* A40 on the other

<table>
<thead>
<tr>
<th>( p )-values determined for each kinetic</th>
<th>Single ( p )-value for the whole set of kinetics</th>
</tr>
</thead>
<tbody>
<tr>
<td>Values</td>
<td>CI 95%</td>
</tr>
<tr>
<td>Log ( \delta_{121.1}^* )</td>
<td>–2.58</td>
</tr>
<tr>
<td>( z_T )</td>
<td>7.90</td>
</tr>
<tr>
<td>( z_{\text{pH}} )</td>
<td>3.37</td>
</tr>
<tr>
<td>( z_{\text{VpH}} )</td>
<td>1.92</td>
</tr>
<tr>
<td>( F ) test</td>
<td>5.42</td>
</tr>
<tr>
<td>( p )-value</td>
<td>0.0084</td>
</tr>
</tbody>
</table>

The method used to compute the 95% confidence intervals is based on an “asymptotic normal distribution for the parameter estimate” (Bates and Watts, 1988).

Like the classical \( D \)-value, the scale parameter \( \delta \) decreases with heating and recovery medium pH (Marfart and Leguerinel, 1997; Couvert et al., 1999; Couvert, 2002). Couvert’s model (Eq. (7)), including the dependence temperature and heating and recovery medium pH, was fitted on the \( \delta \)-values evaluated with the two calculation methods. Table 3 presents the parameters estimated and Fig. 4a and b compare observed and calculated values, and show a slightly
higher accuracy of Couvert’s model when the \( \delta \)-values were evaluated with single \( p \)-value.

For the \( B. \) cereus strain, Fernandez et al. (2002), following a full factorial design, four levels of heating temperature and pH medium, evaluated Weibull scale parameter \( \delta \). The goodness of fit of Couvert’s model on these data (Fig. 5 and Table 4) confirms the adequacy of this model on the scale parameter estimated with a single shape parameter value \( p \).

These results confirm that single \( p \)-value evaluated from a set of survival kinetics is sufficient to describe the survival kinetics and the effect of external factors on bacterial heat resistance. Furthermore, the evolution of \( p \)-values, determined for each kinetics according to environmental conditions, are too irregular to be described by any constant model (van Boekel, 2002).

The Weibull model is suitable for describing log linear, or not, heat survival curves. However, a simplification of this model consisting in getting a single overall estimation of \( p \)-value per strain, regardless of environmental conditions of heat treatment and recovery, seems to be enough for bacterial food predictive modeling and canning process calculation (Mafart et al., 2002). Moreover, despite a slight loss of goodness of fit, this modification leads to an improvement of the robustness of the model. However, the cell physiology states seem to influence the density function; as a result, the \( p \)-values are likely to change. Further works should be performed to assess the influence of spore age and environmental sporulation or germination conditions on the Weibull shape parameter value.

As expected, the secondary model developed to describe the heating and recovery environmental influence on the classical \( D \)-values remains suitable for \( \delta \)-value estimates.

Table 4
Couvert’s model parameters fitted on log \( \delta \)-values for \( B. \) cereus INRA TZ 415 (Fernandez et al., 2002)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Values</th>
<th>CI 95%</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \log \delta_{21.1} ) ( e^a )</td>
<td>-3.48</td>
<td>0.21</td>
</tr>
<tr>
<td>( z_T )</td>
<td>7.71</td>
<td>0.34</td>
</tr>
<tr>
<td>( z_{pH} )</td>
<td>3.26</td>
<td>0.59</td>
</tr>
<tr>
<td>( F ) test</td>
<td>7.46</td>
<td></td>
</tr>
<tr>
<td>( p )-value</td>
<td>0.0021</td>
<td></td>
</tr>
</tbody>
</table>
References


