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

Olivier Couvert, Stéphane Gaillard, Nicolas Savy, Pierre Mafart, Ivan Leguérinel

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

Olivier Couvert[1], Stéphane Gaillard[1], Nicolas Savy[2], Pierre Mafart[1], Ivan Leguérinel[1]*

[1] Laboratoire Universitaire de Microbiologie Appliquée de Quimper 2 rue de l'Université 29334 Quimper cedex, France Tel 33 2.98.90.02.27 Fax 33.2.98.64.03.71

[2] Institut de Recherche Mathématique de Rennes, Université de Rennes 1, 35042 Rennes Cedex, France.

*corresponding author e mail: guerinel@univ-brest.fr

Abstract

The classical D value of first order 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 "p" and scale parameter "δ", 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 parameter "p" and cannot be described by any model. On the opposite, the scale parameter "δ" 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 "δ". The slight influence of the shape parameter p variation on the goodness of fit of these models can be neglected and the simplified Weibull model with a constant p-value for given microbial population can be applied for canning process calculations.

Key words:

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), Esty and Williams 1924 on vegetative cells had confirmed this equation:

$$N = N_0 e^{-kt} \text{ Eq1}$$

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. defined the decimal reduction time that Ball and Olson (1957) symbolized by the letter D. Thus the model appears on the familiar form:

$$\log N = \log N_0 - \frac{t}{D} \text{ Eq2}$$

In this model the classical D value presents a simple biological significance: time that leads to a ten 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 was frequently 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 good accuracy either over parameterized (mechanistic models) or have parameters without any physical or biological significance (empirical models). Moreover the complexity of these models hinder their application in heat treatment process calculation.

Other authors who considered the survival curve as a cumulative form of temporary distribution of lethality event distribution, presented a probabilistic approach (Cunha et al., 1998; Fernandez et al., 1999; Peleg and Cole, 1998 Peleg, 2000; Mafart et al., 2002). The Weibull frequency distribution model (Eq3) involved to describe the time to failure in mechanical system was applied to bacterial death time.

$$f(t) = \frac{\beta}{\alpha}\left(\frac{t}{\alpha}\right)^{\beta-1} \times \exp\left(-\left(\frac{t}{\alpha}\right)^{\beta}\right) \quad \text{Eq3}$$

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

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

The cumulative distribution Weibull function is

$$F(t) = \exp\left(-\left(\frac{t}{\alpha}\right)^{\beta}\right) \quad \text{Eq 4}$$

or applied to survival kinetics curves

$$\ln S(t) = -\left(\frac{t}{\alpha}\right)^{\beta} \quad \text{Eq5}$$

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

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

$$\log N = \log N_0 - \left(\frac{t}{\delta}\right)^p \quad \text{(Eq 6)}$$

where δ is to the first reduction time that leads a ten fold reduction of survival population, and p the shape parameter β. For the traditional case where the survival curve, originated from a first order, is linear p equal 1 and the δ parameter correspond to the classical D value.

This simple and robust model can be regarded as an extension of the conventional first order equation. Like on D value, the influence of heating temperature on the δ 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’) present a prominent importance. Couvert (1999) has developed an extended Bigelow model to describe both effects of heating and recovery medium pH on the apparent bacterial spore heat resistance.

$$\log D = \log D^* - \frac{T - T^*}{z_T} - \left|\frac{pH - pH^*}{z_{pH}}\right| - \left(\frac{pH' - pH'^*}{z'_{pH}}\right)^2 \quad \text{Eq 7}$$

Where pH* and pH’* are the reference heat treatment and recovery medium pH fixed to 7. z_{pH} is a distance of pH from pH*, which leads to a ten fold reduction D-value. z_{pH} quantifies the heat medium pH influence on bacterial heat resistance. z'_{pH} is a distance of pH’ from pH’*, which leads a ten fold reduction apparent D-value. z'_{pH} characterizes the influence of the pH on the recovery of the micro-organism after a heat treatment. D* is the calculated D value corresponding to pH* and pH'* conditions. Like the Bigelow model, Couvert's model (Eq7) was suitable for the calculation of δ 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 bring arguments 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. Material and methods

2.1. Microorganism and spore production

Bacillus pumilus A40 was obtained and isolated from ingredient in a food canning industry. Spores were kept in distilled water at 4°C.

Cells were pre-cultivated at 37°C for 24 hours in Brain Heart Infusion (Difco 0037). The pre-culture was used to inoculate nutrient agar (Biokar Diagnostics, Beauvais / France) supplemented with salt ($MnSO_4$ 40mg l^{-1} and $CaCl_2$ 100 mgl^{-1}). Plates were incubated at 37°C for 5 days. Spores were then collected by scrapping the surface of the agar, suspended in sterile distilled water and washed three times by centrifugation (10000xg for 15 min) (Bioblock Scientific, model Sigma 3K30). The pellet was resuspended in 5 ml distilled water and 5 ml ethanol. The obtained suspension was kept at 4°C for 12 hours 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 (10g/l tryptone, 10g/l NaCl (Biokar)) for different pH adjusted with addition of 1M H_2SO_4, media were sterilized by filtration through 0.22µm porosity filter. 30µl of spore suspension was diluted in 3 ml of these media. Capillary tubes of 200 µl (vitrex) were filled with 100µl 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 (10g tryptone, 5g meat extract, 5g sodium chloride, 15 g agar for 1000ml water)(Biokar Diagnostic). The pH was adjusted with H_2SO_4 prior to autoclaving at 121°C for 15 min, the pH value was controlled after autoclaving.

2.3. Experimental design

To determine the thermal kinetic parameters at least ten 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 region confidence determination

To estimate Weibull parameters two fitting ways were realized. On the one hand, three parameters $\log N_0$, δ and p were estimated from each kinetic. On the other hand, two parameters $\log N_0$ and δ were estimated from each kinetic 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 ("nlinfit" 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 Lobry et al. (1991) method leads to detect possible structural correlation between model parameters. According to Beale (1960), a vector of parameter model Θ is in the confidence regions if probability α verifies the inequation:

$$SSD_{\Theta} \leq SSD_{\min}\left(1+\frac{p}{n-p}F_{p,n-p,\alpha}\right) \quad \text{Eq 8}$$

n number of data, p number of parameters, F 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. Figure 2 shows the projections of confidence region projected on three orthogonal planes. The strength shape of the projections and the high correlation coefficient associated characterize a structural correlation between model parameters. Three Weibull model parameters were estimated from each kinetic data and correlation coefficients were determined from the evaluated confidence region, for the 18 environmental conditions studied (Table 1) confirms this structural correlation between parameters for all kinetics. Thus, Weibull model parameters (log N_0, δ and p) are dependent: an error on δ 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 δ and p parameters as well as logN and p parameters (Table 1) and decreases the structural correlation between logN and δ. The Weibull model parameters become independent.

3 2 Influence of environmental factors on p-value

For each *Bacillus pumilus* survival curve, the shape parameter p values were estimated. Figure 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 pH medium on the p values for *Bacillus cereus* spores. Van Boekel (2002) used bibliography data to study the influence of heating temperature on the shape (p) and scale (δ) Weibull model parameter for 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 significantly. Constant p value means that the Weibull probability density function curves presents the same shape. Applied to the density probability distribution of inactivation death 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 p value, Fernandez et al. (2002) determines average of shape parameter determined from the different kinetics. Then, for each kinetic, the scale parameter was re-estimated from set of data with fixed p value. However, it is preferable to evaluate both single shape and scale parameter by non linear least square reduction for the whole set of data. Choosing the average value to evaluate a single p value is not suitable because the number of data in each kinetic is not equal, each kinetic have not the 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 δ-value

To evaluate the influence of fixed / free p value on the scale parameter, the corresponding δ 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 δ value confidence intervals were reduced, and δ 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 T* which leads to a ten fold reduction of the first decimal reduction time δ). Whatever the δ calculation procedure, no significant difference appears. Van Boekel (2002) has alike applied the Bigelow model to assess the heating temperature influence on the scale parameter values δ, however the Arrhenius model as well can be applied (Fernandez et al., 2002).

Like the classical D value, the scale parameter δ decreases with heating and recovery medium pH (Mafart et al., 1998; 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 δ values evaluated with the two calculation methods. Table 3 presents the parameter estimated and Figures 4 a & b compares observed and calculated values, and show a slight higher accuracy of Couvert's model when the δ values were evaluated with single p value.

For the *Bacillus cereus* strain, Fernandez et al. (2002), following a full factorial design, four levels of heating temperature and pH medium, evaluated Weibull scale parameter δ. The goodness of fit of Couvert's model on these data (Figure 5 & 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 Boekell 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 realized 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 δ value estimates.

.

Bibliography

Ball, C. O., and Olson, F. C. W., 1957. "Sterilization in food technology Theory, practice and calculation." McGraw-Hill Book Compagny, inc.

Bates, D.M. and Watts, D.G., 1988 “Nonlinear Regression Analysis and Its Applications”. John Wiley & Sons, NY.

Beale, E.M., 1960 Confidence region in non-linear estimation. Journal of Royal Statistic Society B22, 41-88.

Buchanan, R. L., Golden, M. A., and Phillips, J. G., 1997. Expanded models for the non-thermal inactivation of *Listeria monocytogenes*. Journal of Applied Microbiology 82, 567-577.

Cerf, O., 1977. Tailing of survival curves of bacterial spores, a review. Journal of Applied Bacteriology 42, 1-19

Chick, H., 1910. The process of disinfection by chemical agencies and hot water. Journal of Hygiene, Cambridge 10, 237-286.

Cole, M. B., Davies, K. W., Munro, G., Holyoak, C. D., and Kilsby, D. C., 1993. A vitalistic model to describe the thermal inactivation of *Listeria monocytogenes*. Journal of Industrial Microbiology 12, 232-239.

Couvert, O., Leguérinel, I., and Mafart, P., 1999. Modelling the overall effect of pH on the apparent heat resistance of *Bacillus cereus* spores. International Journal of Food Microbiology 49, 57-62.

Couvert, O., 2002. Prise en compte de l’influence du pH dans l’optimisation des traitements thermiques. These de l’Université de Bretagne Occidentale.

Cunhan, L. M., Oliveira, F. A. R., and Oliveira, J. C., 1998. Optimal experimental design foe estimating the kinetic parameters of processes described by the Weibull probability distribution function. Journal of Food Engineering 37, 175-191.

Esty, J. R., and Meyer, K. F., 1922. The heat resistance of spores of *B. botulinus* and allied anaerobes. Journal of Infectious Diseases 31, 650-663.

Esty, J. R., and Williams, C. C., 1924. Heat resistance studies. A new method for determination of heat resistance of bacterial spores. Journal of Infectious Diseases 34, 516-528.

Fernandez, A., Collado, J., Cunhan, L. M., Ocio, M. J., and Martinez, A., 2002. Empirical model building based on Weibull distribution to describe the joint effect of pH and temperature on the thermal resistance of *Bacillus cereus* in vegetable substrate. International Journal of Food Microbiology 77, 147-153.

Fernandez, A., Salmeron, C., Fernandez, P. S., and Martinez, A., 1999. Application of a frequency distribution model to describe the thermal inactivation of two strain *of Bacillus cereus*. Food Science and Technology 10, 158-162.

Geeraerd, A. H., Herremans, C. H., and Impe, J. F. V., 2000. Structural model requirements to describe microbial inactivation during a mild heat treatment. International Journal of Food Microbiology 59, 185-209.

Katzin, L. I., Sandholzer, L. A., and Strong, M. E., 1943. Application of the decimal reduction time principle to a study of the resistance of coliform bacteria to pasteurization. Journal of Bacteriology 45, 256-272.

Kilsby, D. C., Davies, K. W., McClure, P. J., Adair, C., and Anderson, W. A., 2000. Bacterial thermal death kinetics based on probability distributions: the heat destruction of *Clostridium botulinum* and *Salmonella* bedford. Journal of Food Protection 63, 1197-1203.

Linton, R. H., Carter, W. H., Pierson, M. D., and Hackney, C. R., 1995. Use of modified Gompertz equation to model nonlinear survival curves for *Listeria monocytogenes* Scott A. Journal of Food Protection 58, 946-954.

Lobry, J.R., Rosso, L., Flandrois, J.P., 1991.A FORTRAN subroutine for the determination of parameter confidence limits in non-linear model. Binary 3, 86-93.

Madsen, T., and Nyman, M. 1907. Zur theorie der desinfektion. Zeitschrift für Hygiene und Infectionskrankheiten 57, 388-404.

Mafart, P., Couvert, O., Gaillard, S., and Leguerinel, I., 2002. On calculating sterility in thermal preservation methods: application of Weilbull frequency distribution model. International Journal of Food Microbiology 72, 107-113.

Peleg, M., and Cole, M. B., 1998. Reinterpretation of microbial survival curves. Critical Reviews in Food Science 38, 353-380.

Peleg, M., and Cole, M. B., 2000. Estimating the survival of Clostridium botulinum spores during heat treatment. Journal of Food Protection 63, 190-195.

Rodriguez, A. C., Smerage, G. H., Teixeira, A. A., and Busta, F. F., 1988. Kinetic effect of lethal temperature on population dynamics of bacterial spores. Transaction of the American Society of Agricultural Engineers 31, 1594-1601, 1606.

Sapru, V., Smerage, G. H., Teixeira, A. A., and Lindsay, J. A., 1993. Comparaison of predictive model for bacterial spore population resources to sterilization temperature. Journal of Food Science 58, 223-228.

Shull, J. J., Cargo, G. T., and Ernst, R. R., 1963. Kinetics of heat activation and of thermal death of bacterial spores. Applied Microbiology, 485-487.

van Boekel, M. A. J. S., 2002. On the use of the Weilbull model to describe thermal inactivation of microbial vegetative cells. International Journal of Food Microbiology 74, 139-159.

Whiting, R. C., 1993. Modeling bacterial survival in unfavorable environments. Journal of Industrial Microbiology 12, 240-246.

Xiong, R., Xie, G., Edmondson, A. E., and Sheard, M. A., 1999. A mathematical model for bacterial inactivation. International Journal of Food Microbiology 49, 45-55.

Table legend

Table 1: Correlation coefficients between Weibull model parameters evaluated from the evaluated joint confidence for the 18 environmental studied conditions.

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 3

Couvert's model parameters fitted on log δ values evaluated with multiple p values on the one hand, with single p values for *Bacillus pumilus* A40 on the other. The method used to compute the 95% confidence intervals is based on an "asymptotic normal distribution for the parameter estimate". (Bates and Watts 1988)

Table 4

Couvert's model parameters fitted on log δ values for *Bacillus cereus* INRA TZ 415 (Fernandez et al., 2002)

Figure legends

Figure 1

Simulated frequency distribution of critical inactivation time (Figures a and b) and microbial survival curves (Figures c and d) generated with the assumption that the heat resistance has a Weibull distribution.

Figures a and c: α: 5, β: 3 (—), 1(---), 0.5(····) , Figures b and d:α: 3 (—), 6(---), 9(····) ,β: 3

Figure 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)

Figure 3

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

Figures 4 a&b

Comparison of calculated and observed log δ values evaluated with multiple p values on the one hand (Figure a :□), with single p values on the other (Figure b: ○)

Figure 5

Comparison of calculated and observed log δ values. Couvert's model fitted from Fernandez et al. (2002) data

			p values estimated from each set of data			Overall p value estimated from the gathered sets of data		
T°	pH	pH’	LogNo vs δ	$\log N_0$ vs p	δ vs p	LogNo vs δ	$\log N_0$ vs p	δ 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

T1

			p values estimated from each set of data							Single p value for the whole set of kinetics						
T°	pHt	pHr	log No	CI 95%	delta	CI 95%	p	CI 95%	SSD	log No	CI 95%	delta	CI 95%	p	CI 95%	SSD
89	7	7	4.09	0.18	49.14	4.83	2.87	0.76	0.012	4.27	0.18	42.01	3.23	1.96	0.14	0.172
92	7	7	3.83	0.18	18.74	1.97	2.50	0.67	0.028	3.93	0.18	17.04	1.32	1.96	0.14	0.097
95	7	7	4.20	0.12	9.25	0.86	2.03	0.32	0.149	4.22	0.12	9.06	0.51	1.96	0.14	0.153
98	7	7	4.04	0.16	3.88	0.40	2.26	0.51	0.103	4.10	0.16	3.64	0.25	1.96	0.14	0.138
101	7	7	3.93	0.18	1.51	0.18	2.19	0.62	0.070	3.98	0.16	1.44	0.11	1.96	0.14	0.084
104	7	7	4.04	0.21	0.57	0.08	2.16	0.49	0.094	4.10	0.20	0.53	0.04	1.96	0.14	0.109
95	4.7	7	3.66	0.26	1.91	0.56	1.10	0.36	0.543	3.35	0.17	2.70	0.23	1.96	0.14	0.689
95	5.1	7	3.61	0.20	3.77	0.57	1.75	0.44	0.727	3.54	0.16	4.00	0.28	1.96	0.14	0.737
95	5.15	7	4.20	0.29	1.66	1.19	0.59	0.46	0.008	3.84	0.28	3.22	0.47	1.96	0.14	0.352
95	5.2	7	3.95	0.25	1.73	0.50	1.08	0.26	0.151	3.54	0.17	2.85	0.21	1.96	0.14	0.544
95	5.8	7	4.03	0.22	3.67	0.61	1.78	0.42	0.051	3.96	0.18	3.90	0.28	1.96	0.14	0.066
95	6.1	7	4.13	0.28	5.54	0.95	1.99	0.62	0.115	4.14	0.26	5.50	0.49	1.96	0.14	0.115
95	7	5.27	3.89	0.24	1.12	0.25	1.32	0.32	0.280	3.60	0.17	1.48	0.11	1.96	0.14	0.563
95	7	5.55	4.05	0.24	2.62	0.43	1.95	0.55	0.052	4.05	0.22	2.63	0.20	1.96	0.14	0.052
95	7	5.82	3.79	0.16	3.72	0.35	2.60	0.61	0.084	3.93	0.16	3.28	0.23	1.96	0.14	0.209
95	7	6.04	3.99	0.17	6.00	0.63	2.33	0.55	0.017	4.09	0.15	5.57	0.41	1.96	0.14	0.063
95	7	6.26	3.83	0.20	4.54	0.60	1.95	0.45	0.177	3.82	0.17	4.55	0.31	1.96	0.14	0.177
95	7	6.52	3.98	0.18	6.12	0.78	2.09	0.62	0.033	4.01	0.16	5.96	0.48	1.96	0.14	0.037

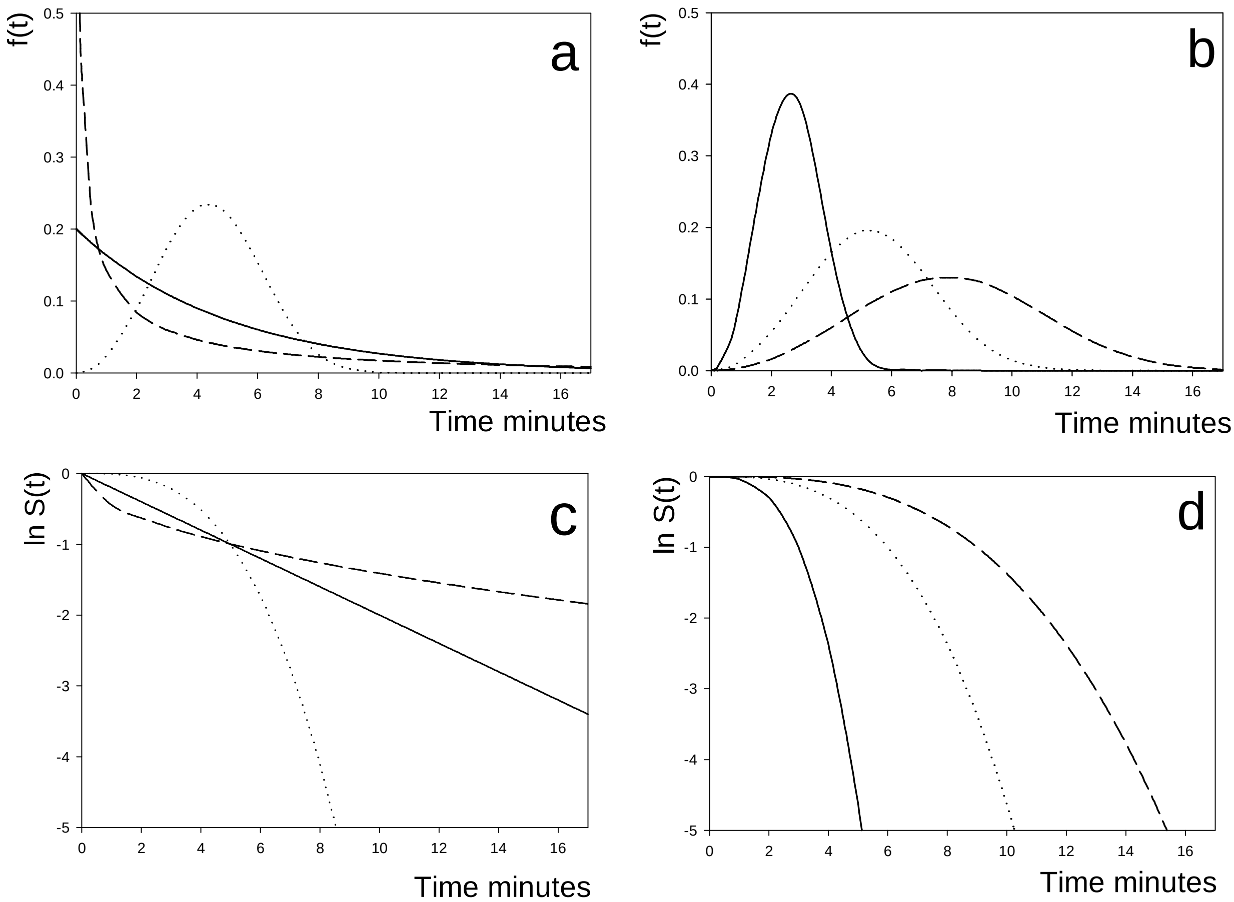
T2

	p values determined for each kinetic		Single p value for the whole set of kinetics	
	Values	CI 95%	Values	CI 95%
$\mathrm{Log}\delta_{121.1}$*	-2.38	0.44	-2.36	0.26
z_T	7.90	1.08	8.06	0.66
z_{pH}	3.37	0.77	5.09	1.03
z'_{pH}	1.92	0.23	2.06	0.17
F test	5.42		5.57	
p value	0.0084		0.0076	

T3

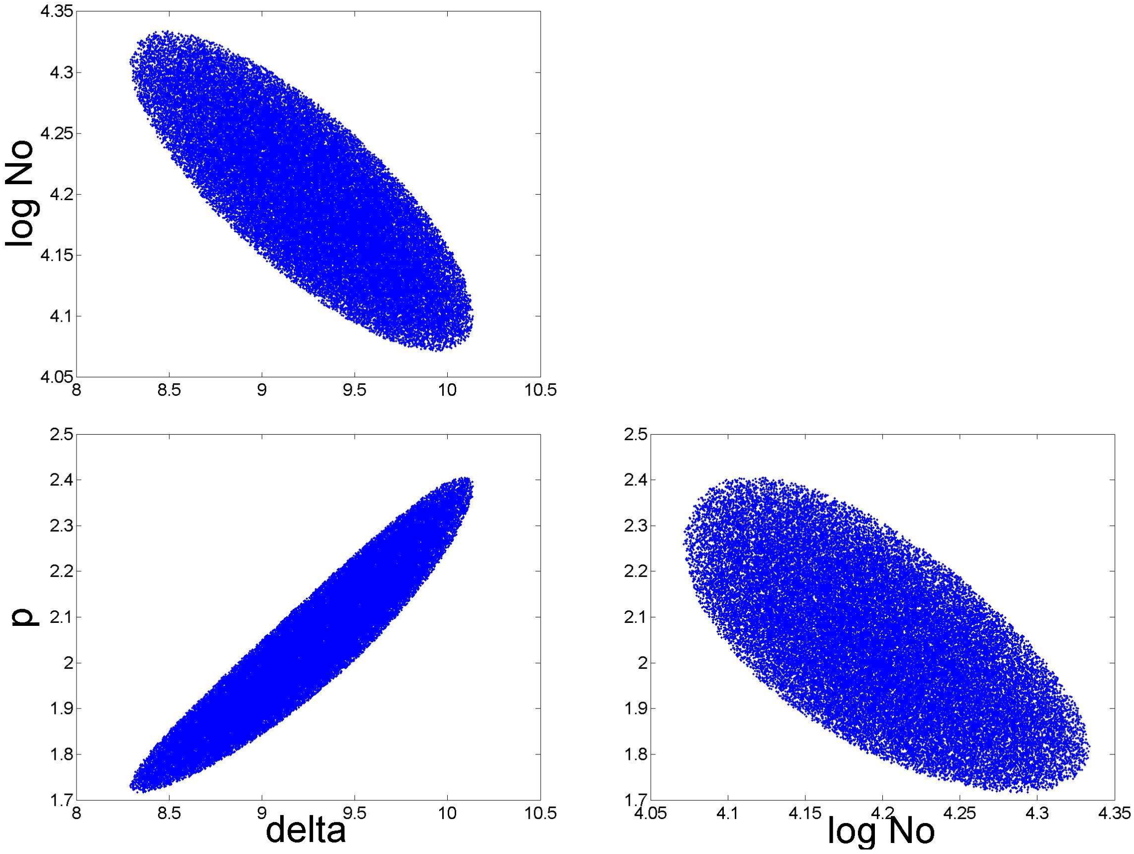
	Values	CI 95%
$\log \delta_{121.1°C}$*	-3.48	0.21
z_T	7.71	0.34
z_{pH}	3.26	0.59
F test	7.46	
p value	0.0021	

T4

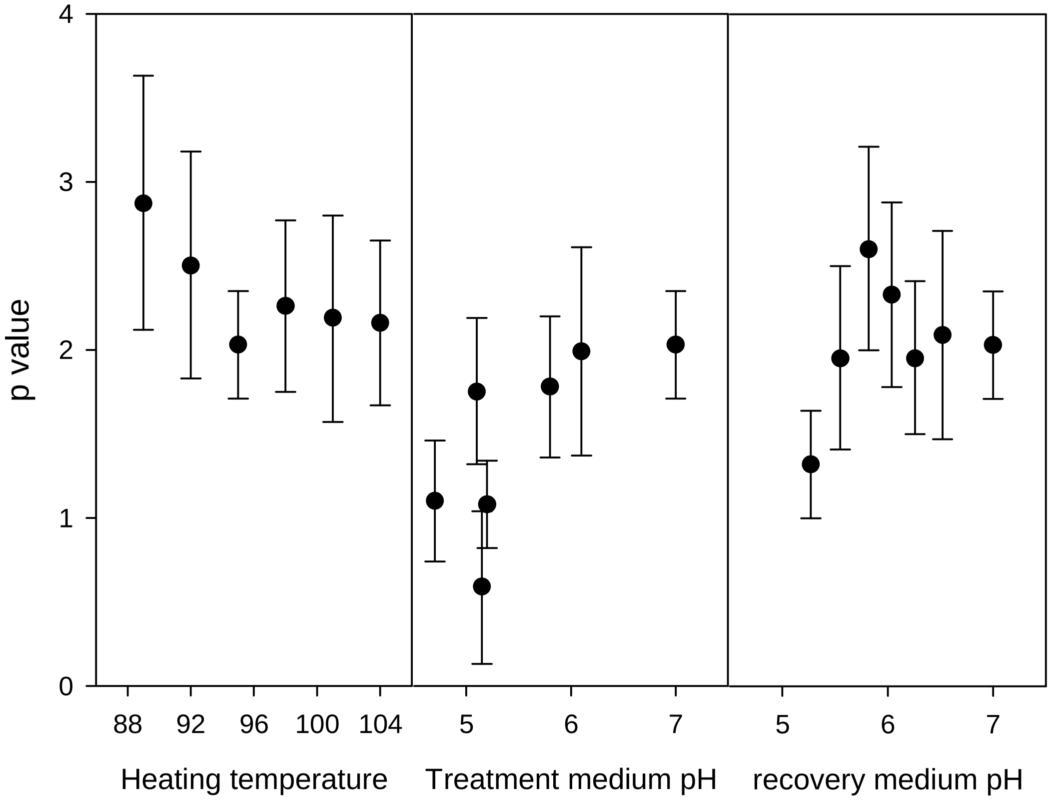


f(t)
a
b
ln S(t)
c
d
Time minutes
Time minutes
Time minutes
Time minutes

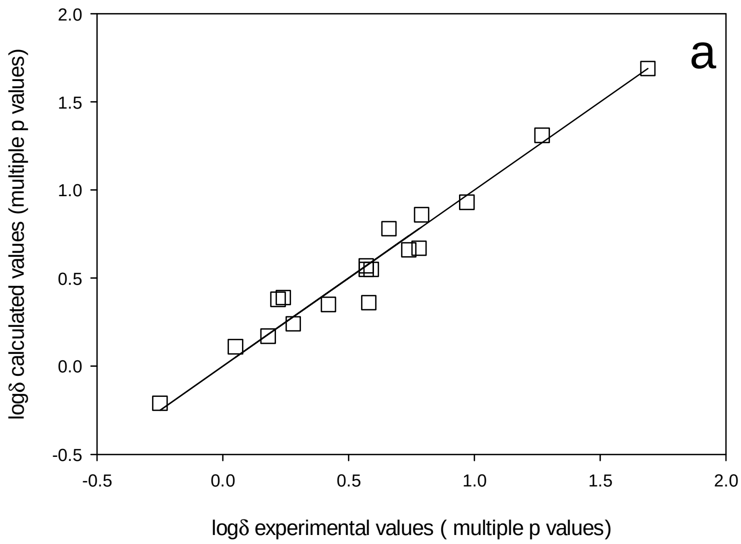
F1

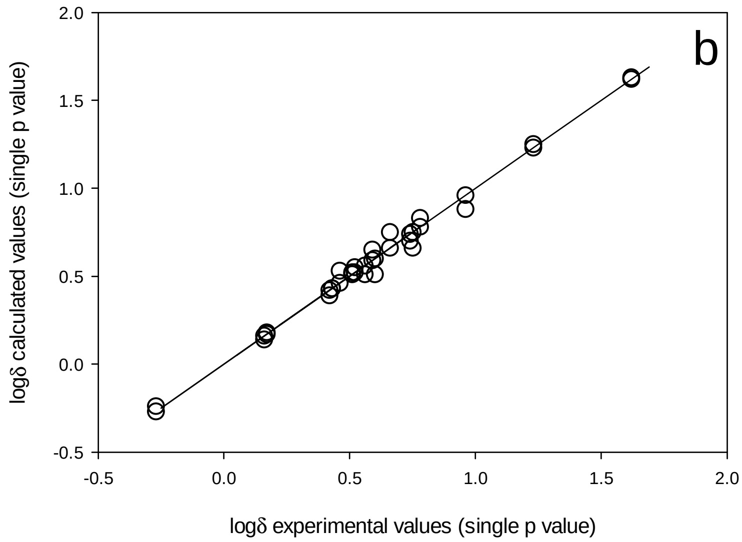


F2

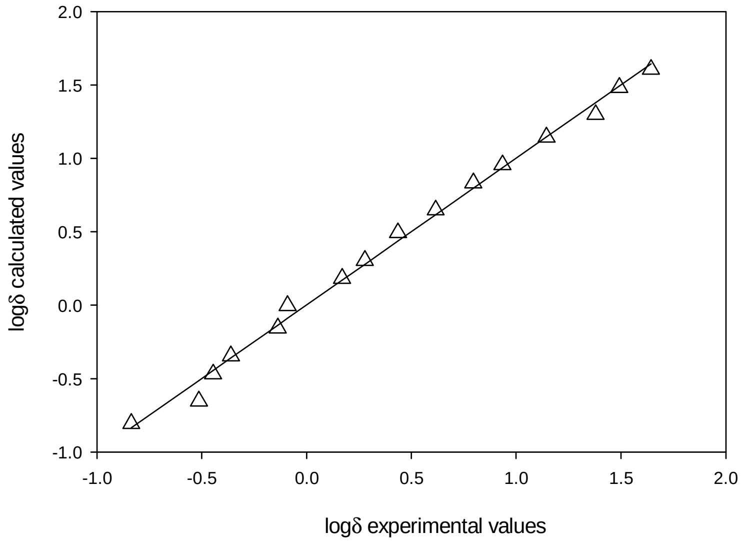

p value
0
1
2
3
4
88
92
96
100
104
5
6
7
5
6
7
Heating temperature
Treatment medium pH
recovery medium pH

F3


a
2.0
1.5
1.0
0.5
0.0
-0.5
-0.5
0.0
0.5
1.0
1.5
2.0
logδ calculated values (multiple p values)
logδ experimental values (multiple p values)


b
2.0
1.5
1.0
0.5
0.0
-0.5
-0.5
0.0
0.5
1.0
1.5
2.0
logδ calculated values (single p value)
logδ experimental values (single p value)

F4 a&b


2.0
1.5
1.0
0.5
0.0
-0.5
-1.0
logδ calculated values
-1.0
-0.5
0.0
0.5
1.0
1.5
2.0
logδ experimental values

F5