

# Model for Combined Effects of Temperature, pH and Water Activity on Thermal Inactivation of *Bacillus cereus* Spores

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## ABSTRACT

Initially, the effect of water activity ( $a_w$ ) on heat resistance of *Bacillus cereus* spores (decimal reduction time) was investigated. A linear relationship was found between  $\log D$  and  $1-a_w$ . The combined effects of temperature (85-105°C), pH (4.5-6.5) and water activity (0.80-1) were then studied. A four parameter model was fitted to the data. This model appeared to be parsimonious with each parameter having a biological significance. Interactions between factors were observed but they accounted for <2.4% of the total variation and they were not taken into account by the model.

**Key Words:** water activity, heat resistance, *Bacillus cereus*, modeling

## INTRODUCTION

OPTIMIZATION OF THERMAL PROCESSES OF FOOD is based on the assumed first order kinetic of microbial heat destruction:

$$N = N_0 e^{-Kt} \quad (1)$$

where  $N$  is the number of survival cells,  $t$  is the exposure time and  $K$  the death rate.

Equation (1) is often rewritten as:

$$N = N_0 10^{-(t/D)} \quad (2)$$

where  $D$ , the decimal reduction time is a specific parameter of the heat resistance of bacterial populations. Conventional models used for calculations of thermal resistance of spores account only for the temperature (Arrhenius, 1889; Bigelow et al., 1921). The effect of other factors, such as pH and water activity, has been recognized for several decades. However, few attempts have been made to develop multifactorial models for describing the thermal resistance of spores. Other several multifactorial models were developed in the field of predictive microbiology to describe growth kinetics of bacteria. Most researchers have developed quadratic polynomial models (Bratchell et al., 1989; Buchanan et al., 1989; Buchanan and Phillips, 1990; Buchanan and Klawitter, 1992; Mc Lure et al., 1993; Sutherland and Bayliss, 1994). Other types of models originated from an extension of a monofactorial model and its parameters may have a physical or biological basis (Mc Meekin et al., 1987; Davey, 1989; Adams et al., 1991; Zwietering et al., 1992; Grau and Vanderline,

1993; Wijtzes et al., 1993; Davey and Daugherty, 1995; Rosso et al., 1995).

The obvious analogy between growth and survival kinetics induced some researchers to base thermal resistance models on predictive microbiology. Fernandez et al. (1996) developed a linear and a quadratic model for describing the heat resistance of spores vs temperature and pH. Davey et al. (1978) were the first to develop a model for predicting the combined effects of process temperature and medium pH on thermal resistance of spores. Their four parameter model was explained to be an extension of the Arrhenius' equation. Mafart and Leguérinel (1998) proposed an extension of Bigelow's equation and developed a model with three parameters for describing the effects of temperature and pH. The water activity was taken into account for the first time by Reichart (1994) who derived a semi-empirical model for the death rate of *Escherichia coli*. The application of their five parameter model is difficult because it requires hydrogen and hydroxyl ion concentrations to be known. Another five parameter model was proposed by Cerf et al. (1996) from the experimental data of Reichart (1994). This model is an extension of the Davey's model that included water activity in addition to temperature and pH:

$$\ln K = C_0 + (C_1/T) + C_2 pH + C_3 pH^2 + C_4 a_w^2 \quad (3)$$

where  $T$  is the absolute temperature,  $a_w$  is the water activity and  $C_0, C_1, C_2, C_3, C_4$  are empirical coefficients without biological significance.

Our objective was to develop, using a similar approach, a new model including, in addition to temperature and pH, water activity by an extension of the first version of Mafart's model (Mafart and Leguérinel,

1998):

$$\log D = \log D^* - (1/z_T)(T - T^*) - (1/z_{pH}^2)(pH - pH^*)^2 \quad (4)$$

where  $D$  is the decimal reduction time with  $D = (\ln 10/K)$ ,  $T^*$  is the reference temperature (generally,  $T^* = 121.1^\circ\text{C}$ ) and  $pH^*$  is the pH of maximal thermal resistance (generally,  $pH^* = 7$ ).  $D^*$  is the  $D$ -value at  $T^*$  and  $pH^*$ .  $z_T$  is the conventional thermal  $z$ -value,  $z_{pH}$  is the distance of pH from  $pH^*$  which leads to a ten fold reduction of  $D$ -value.

## MATERIAL & METHODS

Microorganism and spore production

The strain of *Bacillus cereus* (CNRZ 110) was obtained from the Institut National de Recherche Agronomique (INRA France). Spores were kept in distilled water at  $4^\circ\text{C}$ .

Cells were pre-cultivated at  $37^\circ\text{C}$  for 24h in Brain Heart Infusion (Difco). The preculture was used to inoculate nutrient agar (Biokar Diagnostics BK021) supplemented with sporulation salt added ( $\text{MnSO}_4$  40  $\text{mgL}^{-1}$  and  $\text{CaCl}_2$  100  $\text{mgL}^{-1}$ ). Plates were incubated at  $37^\circ\text{C}$  for 5 days. Spores were then collected by scraping the surface of the agar, suspending in sterile distilled water and washed three times by centrifugation (10,000  $\times g$  for 15 min) (Bioblock Scientific, model Sigma 3K30). The pellet was resuspended in 5 mL distilled water and 5 mL ethanol. The suspension was kept at  $4^\circ\text{C}$  during 12h in order to eliminate vegetative bacteria, and washed again three times by centrifugation. The final suspension (about  $10^{10}$  spores  $\text{mL}^{-1}$ ) containing more than 99% refractile spores and no visible vegetative cells was at last distributed in sterile Eppendorfs microtubes and kept at  $4^\circ\text{C}$ .

Thermal treatment of spore suspension

$D$ -values in citrate-phosphate buffers adjusted to 4.5, 5.5 and 6.5 and to water activity levels ranging from 0.8 to 1 were determined for temperatures of 85, 95 and  $105^\circ\text{C}$ .

Water activity was adjusted by adding glucose. Appropriate amounts of glucose were estimated from published curves of the UNIFAC-LARSEN model at  $25^\circ\text{C}$  (Achard et al., 1992). and checked by direct measurement by an  $a_w$ -meter (FA-st/1 from GBX

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Table 1—D values as related to temperature, pH and water activity

T°C	a <sub>w</sub>	D minutes		
		pH 6.5	pH5.5	pH 4.5
105	1.00	0.151	0.093	0.084
105	0.98	0.192	0.105	0.129
105	0.95	0.278	0.126	0.150
105	0.92	0.455	0.224	0.221
105	0.86	1.282	0.808	0.475
95	1.00	2.386	1.040	0.511
95	1.00		0.978	
95	0.98	2.870	1.658	0.892
95	0.98		1.462	
95	0.95	5.010	2.848	1.409
95	0.95		2.523	
95	0.92	7.495	4.332	2.640
95	0.92		3.679	
95	0.89		7.932	
95	0.89		7.647	
95	0.86	13.842	14.513	7.776
95	0.86		8.346	
95	0.83		22.871	
95	0.83		13.605	
95	0.80		29.236	
95	0.80		17.014	
85	1.00	63.398	13.085	5.042
85	0.98	63.298	18.821	9.647
85	0.95	72.253	30.540	16.286
85	0.92	102.745	40.275	23.323
85	0.86	68.909	91.540	33.910

Table 2—Fitted parameters of set of data (85-105°C, pH 4.5-6.5, a<sub>w</sub> 0.80-1).

D*	0.676 min
z <sub>T</sub>	9.28°C
z <sub>pH</sub>	4.08
z <sub>aw</sub>	0.164
number of data	56
R <sup>2</sup>	0.976
mean square error	0.0331

Table 3—z<sub>pH</sub> values as related to temperature and water activity

Temp	Water activity	z <sub>pH</sub>
85°C	1	2.75
	0.98	3.20
	0.95	3.58
	0.92	3.60
95°C	0.86	4.80
	1	3.54
	0.98	4.04
	0.95	3.87
105°C	0.92	4.32
	0.86	5.05
	1	5.82
	0.98	7.55
	0.95	5.92
	0.92	5.32
	0.86	4.34

values of bacterial spores. (Murrel and Scott 1966, Harnulv et al. 1977) In order to study the effects of water activity alone in the range 0.8 to 1, temperature and pH were first fixed at 95°C and 5.5 respectively. A linear relationship (Fig. 1), between the heat resistance of spores (D<sub>95°C</sub>-value) and log (1-a<sub>w</sub>) was demonstrated.

The whole experimental set of data, monofactorial design associated to the multifactorial design (Table 1) was fitted according to the following generalized model:

$$\log D = \log D^* - (1/z_T)(T - T^*) - (1/z_{pH}^2)(pH - pH^*)^2 - (1/z_{aw})(a_w - 1)$$

As the thermal resistance of *Bacillus cereus* is relatively low, we adopted the standard temperature T\* = 100°C instead of 121.1°C. However, a preliminary experiment showed that the pH of maximal thermal resistance related to the strain studied was close to 7.5 (data not shown). The model was then fitted with pH\* = 7.5 obvious (Table 2) The goodness of fit of the model was determined (Fig. 2).

Statistical criteria point out a fair goodness of fit of the model which was however clearly less than expected when only 1 of 3 factors varied separately. This relatively poor fit could be explained by interactions between factors that are not taken into account in the model. Table 3 presents z<sub>pH</sub> values at constant temperature levels and decreasing water activities. However, our z<sub>T</sub> values were at constant pH levels and decreasing water activities (Table 4). Interactions between temperature and pH which had been previously noted (Gaillard et al. 1998) were confirmed. At pH 4.5 (mean z<sub>T</sub> value of 10.48°C), the thermal resistance of spores was less sensitive to variations of temperature than at pH 6.5 (mean z<sub>T</sub> value of 8.78°C). Similarly, at

105°C (mean z<sub>pH</sub> value of 5.79), the heat resistance of spores was less sensitive to variations of pH than at 85°C (mean z<sub>pH</sub> value of 3.95).

Interactions were also shown between a<sub>w</sub> and temperature (Table 3), and between a<sub>w</sub> and pH (Table 4). At 85°C and 95°C a decrease of sensitivity of spores to pH variations was observed when the water activity decreased. On the contrary, at 105°C and in the range of low water activities, an increase of the pH sensitivity of spores was observed with decreasing water activities. The effect of a<sub>w</sub> on z<sub>T</sub> values seemed to be more complex. At pH 4.5 and 5.5, a maximum sensitivity of spores to variations of temperature was reached at a<sub>w</sub> = 0.95. At pH 6.5, the maximum heat sensitivity was reached at a<sub>w</sub> = 1 while z<sub>T</sub> values clearly increased with decreasing a<sub>w</sub>.

Clearly interactions between the three factors, which are not taken into account in the model, limit either its goodness of fit or its range of validity. However, it can be deduced from the R<sup>2</sup> values that interactions between factors accounted for <2.4% of the total variation.

France Scientific Instrument).

The spore suspension (30 µL) was diluted in 3 mL citrate buffer added with glucose. Capillary tubes of 25 µL (vitrex) were filled with 10 µL of sample and submitted to a thermal treatment in a temperature controlled oil bath. After heating, the tubes were cooled in water/ice bath, washed in a soap solution and rinsed with sterile distilled water. The capillary tubes were broken and their contents poured into a tube containing 9 mL sterile tryptone salt broth (Biokar Diagnostics) by rinsing with 1 mL tryptone salt broth contained in a syringe equipped with a needle.

Viable spore count

Viable spores were counted by duplicate plating in nutrient agar (Biokar Diagnostic) and incubating at 30°C for 48h.

Experimental design

A monofactorial experimental design, 8 water activity levels (1, 0.98, 0.95, 0.92, 0.89, 0.86, 0.83, 0.8), at pH 5.5 and 95°C, was first carried out in duplicate.

In the second step, a multifactorial experimental design combining temperature (85, 95 and 105°C), pH (4.5, 5.5, and 6.5) and water activity (5 levels ranging from 0.86 to 1) was carried out

Data analysis

Multiple linear regressions used to fit the model were carried out with the STAT-ITCF software (Institut Technique des Céréales et du Fourrage France).

RESULTS & DISCUSSION

IN THE RANGE OF USUAL A<sub>w</sub> VALUES OF food products a decrease of a<sub>w</sub> increases D-

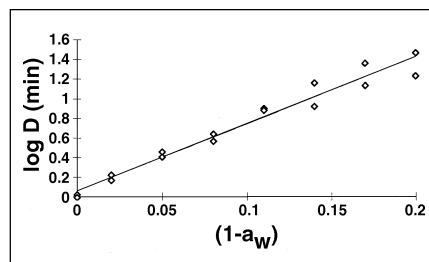


Fig. 1—Log D vs 1-a<sub>w</sub> at 95°C and pH 5.5.

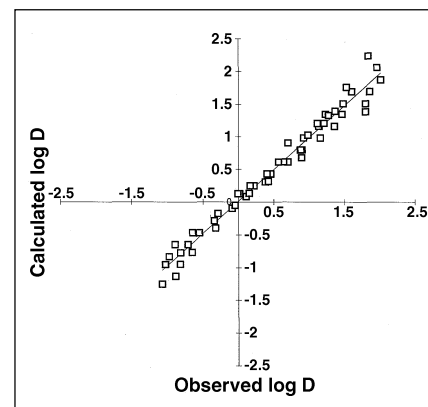


Fig. 2—Comparison of calculated and observed Log D values related to the whole set of data (85-105°C, pH 4.5-6.5, a<sub>w</sub> 0.80-1).

Table 4— $z_T$  values as related to pH and water activity

pH	Water activity	$z_{T(C)}$
4.5	1	11.25
	0.98	10.67
	0.95	9.83
	0.92	9.88
5.5	0.86	10.79
	1	9.31
	0.98	8.88
	0.95	8.39
6.5	0.92	8.87
	0.86	9.74
	1	7.63
	0.98	7.94
	0.95	8.28
	0.92	8.50
	0.86	11.56

Mafart and Leguérinel (1998) extended the concept of F-value and the biological destruction value by incorporating pH in models. They defined the sterilization unit by the equivalent effect of heat treatment at 121.1°C for 1 min in a pH 7 medium. The biological destruction value was defined as

$$L(T, \text{pH}) = 10 [(T - T^*)/zT] + (\text{pH} - \text{pH}^*)/z\text{pH}$$

We could extend again the generalization of such concept by incorporating water activity. However further works would be needed to validate the model on other species than *Bacillus cereus* and to check that  $z_{aw}$  values of main types of spores were in the same

magnitude, i.e., close to that of *Clostridium botulinum*.

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