Relationship between the apparent heat resistance of Bacillus cereus spores and the pH and NaCl concentration of the recovery medium

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Abstract

Conventional heat resistance data , D values, were previously established by other workers at optimal condition for spores outgrowth. However, in canned food conditions of outgrowth are generally suboptimal in term of pH, salt concentration, water activity.

Counts of survival spores after heat treatment are greatly influenced by the characteristics of the recovery medium. The selectivity of incubation conditions is enhanced by the injury of cells at sub-optimum recovery conditions. Both a decrease of number of viable cells being able to producing colonies and a decrease of the decimal reduction time is observed.

The combined effects of pH and NaCl level of the recovery medium for the D value and Z_{pH} value were studied.

Spores of *Bacillus cereus* were heated at 95°C in phospho-citric buffer media at pH7. Cells were recovered at 25°C in nutritive agar with pH ranging from 5 to 7 and 1% to 4% NaCl concentration. For each condition D' values (decimal reduction time associated with the recovery media characteristics) were determined. The results show a main influence of the recovery pH on the D' values. This effect is characterised by the z'_{pH} values, distance of recovery medium pH from optimum recovery pH* medium (6.7) which leads to ten fold reduction time of D-value. The increase of the salt concentration leads to a slight decrease of D' value. However z'_{pH} values are not significantly affected by the salt concentration.

A simple three parameter model describing the effects of pH and NaCl concentration of the recovery medium upon the heat resistance of spores is proposed

The interaction between pH and salt concentration is sufficiently low to be neglected by the model.

Key words : heat treatment, recovery, pH, sodium chloride, *Bacillus cereus*

1. Introduction

The conventional D value (decimal reduction time) which characterises the heat resistance of spores, is usually established at optimal condition of outgrowth. However, in canned foods, conditions of outgrowth are generally sub optimal in term of pH, salt concentration, water activity etc. Counts of survival spores after heat treatment are greatly influenced by the characteristics of the recovery medium. The selectivity of incubation conditions is enhanced by the injury of cells. At sub-optimum recovery conditions, both a decrease of number of viable cells able to produce colonies and a decrease of the estimated decimal reduction time is observed.
This paper aims to study quantitatively the combined effects of pH and NaCl level of the recovery medium on the D value of *Bacillus cereus* spores.

2. Material and methods

The used 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°C.
30µl of the concentrated spore suspension (10^{10} spores per ml) was diluted in phospho-citric buffer media at pH7. Capillary tubes of 25 µl (vitrex) were filled with 10µl of sample and submitted to a thermal treatment at 95°C in a temperature controlled oil bath. After heating, the tubes were cooled in a water/ice bath, washed in a solution of soap 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.
Cells were recovered at 25°C for 6 days in nutritive agar (Biokar Diagnostic) for different pH and NaCl concentrations. For each condition D' values (decimal reduction time associated to the recovery media characteristics) were determined.
An experimental design combining NaCl nutrient agar concentration level (0%, 1%, 2%, 3%, 4%) and different nutrient agar pH levels (5 to 6.7)was carried out.
The D-values were determined using linear regression (Excel software) and the model parameters were fitted by multiple linear regression (STATITCF software from « Institut Technique du Fourrage ») or by mean square error reduction using Excel software solver function.

3. Results and discussion

The effect of the pH recovery medium on the count of UFC after a heat treatment is shown in figure 1.

The large influence of the recovery medium pH on the estimated heat resistance of spores with a decrease in the D values as pH is decreasing, is in agreement with the conclusions of a number of authors (Cook and Brown 1965, Cook and Gilbert 1968, Mallidis and Sholefield 1986, Lopez et al. 1997).
After having published a model describing the effect of the pH of the heat treatment medium upon the D-value of spores (Mafart and Leguérinel, 1997), we proposed a similar model which described the influence of the recovery medium pH (noted pH') on the apparent D-value (noted D') (Couvert et al., 1999) :

$$\log D' = \log D - \left(\frac{pH' - pH'^*}{z'_{pH}} \right)^2 \qquad \text{Equation 1}$$

where D is the decimal reduction time at the optimal pH. According to the same approach as the one adopted in the early model of Mafart and Leguérinel, in order to save a parameter and obtain a more parsimonious model, it is worthwhile to fix the optimal pH (pH'*) at 7. However, taking into account the characteristics of our strain, we fixed the pH'* value at 6.7 in this set of data. The parameter z'_{pH} represents the distance of pH from pH'* which leads to a tenfold reduction in the decimal reduction time.

Fitted parameters obtained at 1% NaCl concentration are respectively D'*=2.16 minutes, z'_{pH}=1.74, correlation coefficient : r = 0.973, (Figure 2)

Sodium chloride also shows a clear inactivation effect on the recovery of heat injured spores (Figure 3). An increase in the salt level from 1% to 4% leads to a decrease in apparent spore heat resistance.
This effect was described for *Bacillus stearothermophilus*, (Cook and Gilbert 1969, Briggs and Yazdany 1970, Feeherry et al. 1987), *Clostridium sporogenes* (Roberts et al. 1966) *Clostridium botulinum* (Hutton et al. 1991 and *Bacillus cereus* Gonzalez et al. 1997).

From our own data and those of other investigators (Hutton et al. 1991; Gonzalez et al. 1997) a linear relation between D' and recovery medium NaCl concentration can be observed.(Figure 4)

Regarding our data, at each value of NaCl concentration of the recovery medium z'_{pH} and D'* values were calculated. These values and the corresponding correlation coefficient of fitting are shown in table 1.

For the NaCl concentrations equal to or higher than 1% it can be seen that the NaCl concentration did not affect z'_{pH} values but has a slight decreasing effect on D values (Decimal reduction time at optimum pH). These results seem to indicate the absence of interaction between the NaCl concentration and the pH of the recovery medium, regarding their influence on the thermal resistance of *Bacillus cereus* spores. Consequently, these two effects can be modelled by a simple combination of equations 1 and linear relation between D-value and NaCl concentration.

$$\log D' = \log D - \frac{\left(pH' - pH'^{*}\right)^2}{z'^{2}_{pH}} + \log\left(1 - k\left(\left[NaCl\right] - \left[NaCl^{*}\right]\right)\right) \quad \text{Equation 2}$$

where [NaCl*] is the optimal NaCl recovery medium concentration (1%) and k a constant .
The parameters fitted to our data are shown in Table 2

The observed and calculated data were shown respectively in figures 5 and 6.

At 0% recovery medium NaCl concentration, an interaction between pH and NaCl appears. The absence of NaCl increases the pH recovery influence on the apparent D-value ; the z'_{pH} value is lower than z'_{pH}-value at NaCl concentrations equal to or higher than 1%. This observation can be explained by the lower osmotic pressure in the medium than in spores. During germination the acidified water entry into the cells modified the inner pH and enhanced the pH effect in the recovery of the heating spores. A specific study of the effect of NaCl concentration from 0% to 1% should allow to determine exactly the optimum concentration of maximum D-value and to take into account these concentrations in an extended model.

4. References

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Table 1 :

Rate coefficient related to Eq. 1

NaCl concentration	0%	1%	2%	3%	4%
Number of data	9	13	14	14	14
Correlation coefficient	0.977	0.973	0.937	0.947	0.974
z'_{pH}	1.09	1.74	1.77	1.75	1.67
D*	1.98	2.17	2.04	1.88	1.77

Table 2 :

Rate coefficient related to Eq. 2

Number of data	36
R^2	0.917
D'* min	2.187
pH'*	6.7
NaCl'* %	1
z'_{pH}	1.738
k	0.075

Legends

Figure 1
log N *Bacillus cereus* vs time (minutes) for heat treatment at 95°C, and recovery medium at pH 5.9 (○)and 5.25 (□)

Figure 2
Calculated (straight line) and observed log D' values (circles) were plotted *vs* (pH'-pH'*)², recovery medium (1% NaCl w/w) for *Bacillus cereus* CNRZ 110 heated at 95°C

Figure 3
log N *Bacillus cereus* vs time (minutes) for heat treatment at 95°C, and NaCl concentration of recovery medium at 1% w/w(■)and 4% w/w (●)

Figure 4
D values minutes vs NaCl concentration for : *Bacillus cereus* strain ATCC 4342 ○ heating at 100°C and pH7: Gonzalez et al. data , *Bacillus cereus* CNRZ 110 □heating at 95°C and pH 6.9 : Couvert et al . data, and *Clostridium sporogenes* PA3679 Δ heating at 112.8°C and pH 7 : Hutton et al. data.

Figures 5&6
Observed and calculated D values vs pH and % NaCl (1% to 4%)

Figure 1

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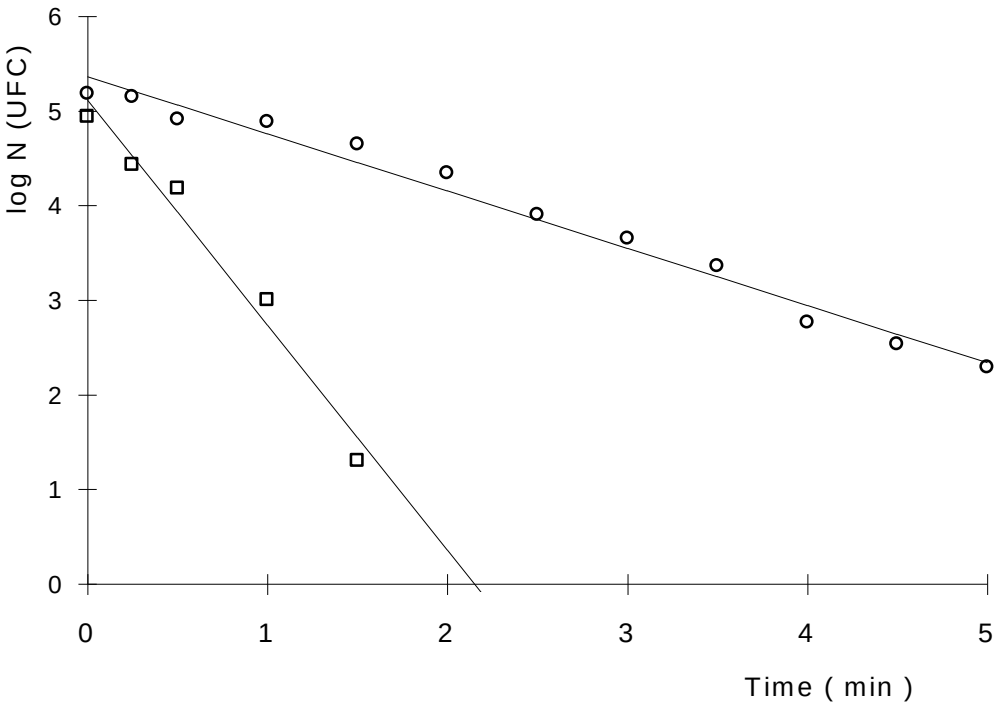

Figure 2

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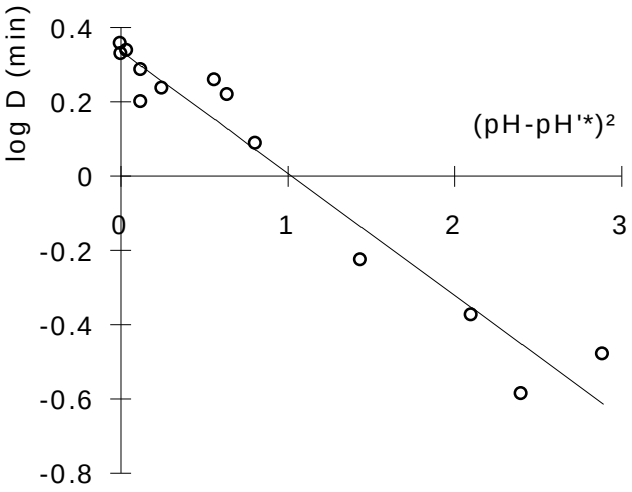

Figure 3

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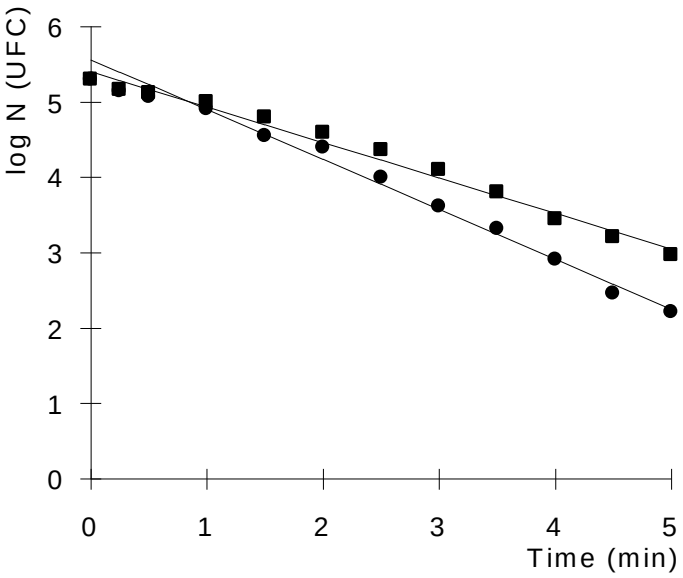

Figure 4

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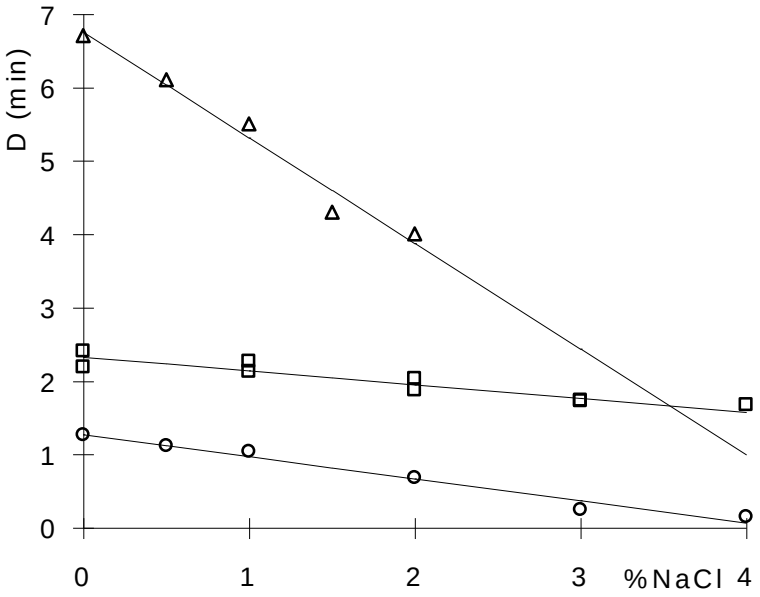

Figure 5

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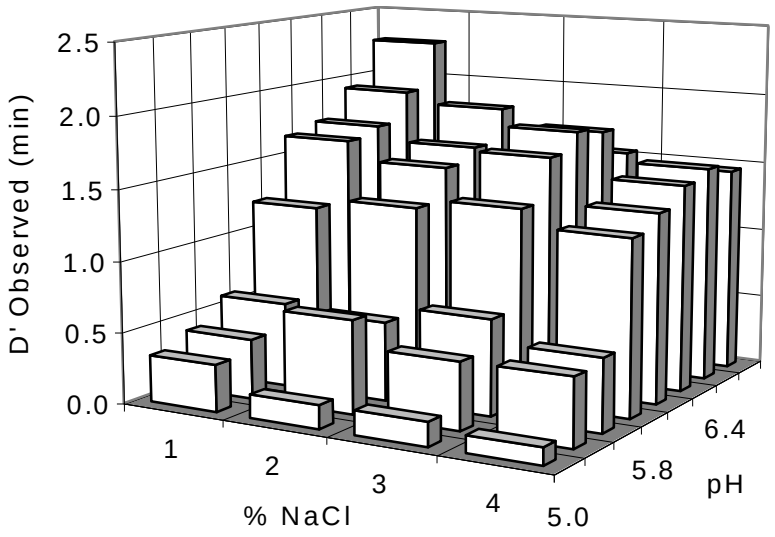

Figure 6

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