Validation of an overall model describing the effect of three environmental factors on the apparent D-value of Bacillus cereus spores

Ivan Leguérinel, Isabelle Spegagne, Olivier Couvert, Stéphane Gaillard, Pierre Mafart

Validation of an overall model describing the effect of three environmental factors on the apparent D-value of *Bacillus cereus* spores

I. LEGUERINEL*, I. SPEGAGNE, O. COUVERT, S. GAILLARD, P. MAFART.

* Corresponding author : IUT Quimper, 2 rue de l'Université, F29334, Quimper cedex, France Tel +33 02 98 90 02 27, Fax +33 02 98 90 85 44

E mail address: guerinel@univ-brest.fr (I. Leguérinel)

ABSTRACT

Several factorial models extending the famous Bigelow model to describe the influence of the heating and recovery pH and a_w conditions on bacterial heat resistance have been developed. These models can be associated in an overall multifactorial model describing the influences of heating and recovery conditions on *D* values. For *Bacillus cereus* strain ADQP 407 the mo D el parameters characterising the environmental factor influences (pH, Temperature, aw) were evaluated. Determination of bacterial heat resistance in cream chocolate have been realised to validate these parameter values and to evaluate the level of the influence of food texture or different compounds not taken account of in the model.

KEYWORDS:

Heat resistance, pH, aw, *Bacillus cereus* , secondary model Bigelow model.

INTRODUCTION

Several environmental conditions influence bacterial heat resistance. In addition to the heating temperature, it has been recognized that pH and water activities of the heating and recovery medium are the main factors that affect the apparent heat resistance of bacteria (Esty and Bigelow, 1920; Esty and Meyer, 1922; Murrel and Scott, 1966; Cook and Gilbert, 1968; Harnulv et al., 1977; Lynch and Potter, 1988; Fernadez et al., 1996; Fernandez et al., 2002).

Different multifactorial models which describe the influence of the heating environmental condition have been published (Davey et al., 1978; Reichart, 1994; Cerf et al., 1996; Fernandez et al., 1996) Mafart and Leguérinel (1998) and Gaillard et al. (1999) have developed an extension of the linear Bigelow model to the pH and aw influence Eq. 1.

$$\log D = \log D^{*} - \left(\frac{T - T^{*}}{z_T}\right) - \left|\frac{pH - pH^{*}}{z_{pH}}\right| - \left(\frac{a_w - 1}{z_{a_w}}\right) \quad \text{Eq. 1}$$

where T^* is the reference temperature (generally, T^*=121.1°C) and pH^* is the reference pH fixed at =7, D^* is the D-value at T^*, pH^* and aw = 1, z_T is the conventional thermal z-value, z_{pH} is the difference of pH from pH^*, which leads to a ten fold reduction of D-value, z_{aw} is the difference of aw from aw^* = 1 which leads to a ten fold reduction of D-value. As the Bigelow model, this imbricate model, taking temperature, pH and water activities into account, is used to evaluate the decimal reduction ratio and the sterilization value (F- value) Mafart (2000).

However, these models assume that the heat resistance is measured at optimal recovery condition and do not take the influence of the non optimal condition of the recovery media into account. It is well known that the count of survival bacteria after heating treatment is greatly influenced by the characteristic of the recovery medium: temperature, pH, aw and composition. When the recovery condition differs from the optimal condition both a decrease in the number of heated stressed cells capable of producing colonies and a decrease in the estimate decimal reduction time, are observed (Harris, 1963; Katsui et al., 1982; Mallidis and Scholefield, 1986; Feeherry et al., 1987). Recently, according to the same approach as that adopted in the Mafart and Leguerinel model (1998), Couvert et al. (1999) and Coroller et al. (2001), have developed similar Bigelow models to describe the influence of pH and water activities respectively, of the recovery medium, on the apparent heat resistance of bacteria Eq.2-3.

$$\log D' = \log D'_{opt} - \left(\frac{pH' - pH'_{opt}}{z'_{pH}} \right)^2 \quad \text{Eq. 2}$$

$$\log D' = \log D'_{opt} - \left(\frac{a'_w - a'_{wopt}}{z'_{a_a}} \right)^2 \quad \text{Eq. 3}$$

Where *pH'* or *aw'* are the *pH* or the water activity of the recovery medium, *D'* is the apparent reduction time at *pH'* or *aw'*, pH'_{opt} and aw'_{opt} correspond to the maximal *D*'value and z'_{pH} and z'_{aw} are the distance from the pH'_{opt} or aw'_{opt} respectively, which leads to a ten fold reduction of the apparent reduction time *D'*.

Mafart and Leguerinel, Gaillard et al., Couvert et al. and Coroller et al. models can be associated in an overall nested model which describes the influences of heating and recovery conditions on the estimated *D* value of bacteria. This model (eq 4) can be used like the Bigelow model to estimate the heat resistance and decimal reduction rate of bacterial population.

$$\log D = \log D^* - \left(\frac{T - T^*}{z_T} \right) - \left| \frac{pH - pH^*}{z_{pH}} \right| - \left(\frac{a_w - 1}{z_{a_w}} \right) - \left(\frac{pH' - pH'_{opt}}{z'_{pH}} \right)^2 - \left(\frac{a'_w - a'_{wopt}}{z'_{a_a}} \right)^2 \quad \text{Eq 4}$$

The aim of this paper is to obtain the model's parameters for *Bacillus cereus* spores and validate these parameter values in the food product of chocolate.

Material and methods

Micro-organism and spore production

The strain of *Bacillus cereus* ADQP407 isolated from shrimp was obtained from the ADRIA (France). Spores were kept in distilled water at 4°C.

Cells were precultivated at 37°C for 24 hrs in Brain Heart Infusion (Difco). The preculture was used to inoculate nutritive agar plates (Biokar Diagnostics BK021) added with $MnSO_4$ 40mg l^{-1} and $CaCl_2$ 100 mgl^{-1} on the surface area. Plates were incubated at 37°C for 5 days. Spores were then collected by scraping the surface of the agar and suspended in sterile distilled water and washed three times by centrifugation (10000xg for 15 min) (Bioblock Scientific, model Sigma 3K30). The final suspension (about 10^{10} spores ml^{-1}) was finally distributed in sterile Eppendorfs microtubes and kept at 4°C.

Thermal treatment of spore suspension and recovery conditions.

In basic condition the heating medium was a tryptone salt broth ($10gl^{-1}$ tryptone Biokar and $10gl^{-1}$ sodium chloride) at pH 7 with no sucrose added, the heating temperature was 100°C. The heating medium was sterilized by filtration. The influence of heating temperature was studied ranging from 95°C to 102°C, the heating pH ranging from 4.5 to 7 adjusted with HCL and the heating water activities ranging from 1 to 0.92 were adjusted using sucrose. The previous molarities of the different solutes were determined using curves from model UNIFAC-LARSEN (Achard et al. 1992). The a_w values were controlled with an aw-meter (FA-st1 GBX France Scientific Instrument).

Firstly, 30µl of spore suspension was diluted in 3 ml adjusted heating medium. Capillary tubes of 200 µl (vitrex) were filled with 100µl of sample, sealed, and submitted to a thermal treatment in a thermostated glycerol bath for different heating times. The heat treatment was stopped by cooling capillary tubes in water / ice bath. Then they 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.

The viable spores were counted by duplicate plating in nutritive agar (10g tryptone, 5g meat extract, 5g sodium chloride, 15 g agar for 1000ml water)(Biokar Diagnostic) and incubated at

37°C for 6 days. The recovery medium pH ranging from 5 to 7 was adjusted with sterile solution of HCl after autoclaving. The recovery medium water activity ranging from 1 to 0.95 was adjusted with added sucrose. To adjust a_w values, the previous molarities of the different solutes were determined using curves from model UNIFAC-LARSEN (Achard et al. 1992). Nutritive agar and sucrose solutions were sterilized separately by autoclaving to avoid the Maillard reaction. After sterilisation the two solutions were mixed, pH was adjusted to 7 and a_w value was controlled.

The validation of the heating sensibility parameters had been realized by heating *Bacillus cereus* spores scattered in “chocolate cream” (pH: 6.76 and aw: 0.968), included in capillary tubes

Experimental design and data analysis

For each environmental factor studied a monofactorial experimental design was carried out. D values were determinate by linear regression on the straight portion of curves obtained when the log number of survivors was plotted against heating time. The sensibility parameters of the models “*z*” were fitted on experimental values using Excel software.

Results and discussion

For the strain of *Bacillus cereus* ADQP 407 studied, all survival curves present a log linear relation between the number of colony forming units and heating time. The classical D values were determined by linear regression. One example is presented Fig1.The whole set of data values is presented table 1.

The fitting of Bigelow model, related to the heating temperature, on the experimental D values (Fig 2), gives a z_T value equal to 7.1°C, which corresponds to the values currently given for *Bacillus cereus* spores (Bergere and Cerf, 1992).

The decrease of heating and recovery medium pH values reduces the apparent bacterial heat resistance. (Fig 3-4). The fitting of parameters (eq 1-2) and associated correlation coefficients were computed Table 2. The decrease in recovery pH medium (z'_{pH}: 2.18) appears to have more influence on the apparent heat resistance than a decrease in heating medium pH (z_{pH} 3.45).

The dominating influence of recovery pH medium has been observed for other bacterial species (Couvert et al., 1999; Couvert thesis 2002)

Regarding the water activity influences, a decrease in aw value leads to a thermo-protective effect (Fig 5). In the recovery medium heated spores show an apparent maximum heat resistance at an optimum close to 0.985. Under this optimal value, an increase in sucrose concentration reduces the bacterial heat resistance (Fig 6). The aw decrease of recovery medium (z_{aw}' 0.092) presents a more pronounced effect than the protective effect of heating medium (z_{aw} 0.156).

These values correspond to the parameters determined for other *Bacillus cereus* strain with sucrose as the same water depressor (Coroller et al., 2001). For the different models the D^* or $D'opt$ correspond to the D value evaluated to the reference or the optimal conditions respectively, and could not be considered the same. To get one and only D value, the overall equation (Eq 4) was fitted on the whole set of data . The D^* value correspond closely to those determined at heating condition: T^*: 121.1°C, pH^*: 7, aw^*: 1 and the evaluated optimal recovery conditions pH'_{opt} and aw'_{opt}. The heat resistance parameters; z_T, z_{pH}, z'_{pH}, z_{aw} and z'_{aw} obtained in Table 3, show the parameter values determined from each monofactorial design. Fig 7 illustrates the relationship between experimental and calculated D values

In food product the main factors that influence the apparent heat resistance are the temperature, pH and water activities. Moreover different compounds or food textures can influence the bacterial heat resistance. However, previous studies, not published, have shown that these secondary factors had a low influence on the sensibility parameters “*z*”. The part of these factors is evaluated by the ratio between the experimental heat resistance determined in food and the corresponding calculated values.

This comparison is made on the heat treatment only on the one hand and, on the overall apparent heat resistance, heating and recovery on the other hand. Fig 8 shows the comparison of the experimental and calculated *Bacillus cereus* death kinetics determined in chocolate cream (pH 6.76 and aw:0.968). The figure and ratio 1.73, higher than 1, shown that the model taking temperature, pH and water activities into account, underestimates the bacterial heat resistance. However, on the overall apparent heat resistance heating and recovery, the experimental result confirms the calculated forecast concerning the overall apparent heat resistance (Table 4); after heat treatment at 100°C for 35 minutes no growth was observed in cream chocolate incubated at 37°C for 7 days.

The confrontation between the validation ratio and the food texture and composition could bring to the fore new factors or compounds that affect apparent heat resistance.

Acknowledgement

This work was carried out with financial support from the French Research Ministry and Sym’Prévius programme 01 T 0389.

REFERENCES

Bergère, J.-L., and Cerf, O. 1992. Heat resistance of *Bacillus cereus* spores. Bulletin of the International Dairy Federation 275, 23-25.

Cerf, O., Davey, K. R., and Sadoudi, A. K. 1996. Thermal inactivation of bacteria - a new predictive model for the combined effect of three environmental factors: temperature, pH and water activity. Food Research International 29, 219-226.

Cook, A. M., and Gilbert, R. J. 1968. Factors affecting the heat resistance of *Bacillus stearothermophilus* spores I. The effect of recovery condition on colony count of unheated and heated spores. Journal of Food Technology. 3, 285-293.

Coroller, L., Leguérinel, I., and Mafart, P. 2001. Effect of water activities of heating and recovery media on apparent heat resistance of *Bacillus cereus* spores. Applied and Environmental Microbiology 67, 317-322.

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

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.

Davey, K., Lin, S., and Wood, D. 1978. The effect of pH on continuous high-temperature / short time sterilization of liquid foods. American Institut Chemistry Engineering Journal 24, 537-540.

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.

Feeherry, F. E., Munsey, D. T., and Rowley, D. B. 1987. Thermal inactivation and injury of *Bacillus stearothermophilus* spores. Applied and Environmental Microbiology 53, 365-370.

Fernandez, P. S., Ocio, M. J., Rodrigo, F., Rodrigo, M., and Martinez, A. 1996. Mathematical model for the combined effect of temperature and pH on thermal resistance of *Bacillus stearothermophilus* and *Clostridium sporogenes* spores. International journal of food microbiology 32, 225-233.

Fernandez, A., Collado, J.,Cunha, 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.

Gaillard, S., Leguérinel, I., and Mafart, P. 1998. Model for combined effects of temperature, pH and water activity on thermal inactivation of *Bacillus cereus* spores. Journal of Food Science. 63, 887-889.

Harris, N. D. 1963. The influence of the recovery medium and the incubation temperature on the survival damaged bacteria. Journal of Applied Bacteriology. 26, 387-397.

Härnulv, B. G., Johansson, M., and Snygg, B. G. 1977. Heat resistance of *Bacillus stearothermophilus* spores at different water activities. Journal of Food Science. 42, 91-93.

Katsui, N., T.Tsuchido, M.Takano, and Shibasaki, I. 1982. Viability of heat-stressed cells of micro-organisms as influenced by pre-incubation and post-incubation temperature. Journal of Applied Bacteriology. 53, 103-108.

Lynch, D. J., and Potter, N. N. 1988. Effects of organic acids on thermal inactivation of *Bacillus stearothermophilus* and *Bacillus coagulans* spores in frankfurter emulsion slurry. Journal of Food Protection. 51, 475-480.

Mafart, P. 2002. taking injury of surviving bacteria into account for optimising heat treatment. International Journal of Food Microbiology. 55, 175-179.

Mafart, P., and Leguérinel, I. 1998. Modelling combined effect of temperature and pH on the heat resistance of spores by a non-linear Bigelow equation. Journal of Food Science. 63, 6-8.

Mallidis, C. G., and Scholefield, J. 1986. Evaluation of recovery media for heated spores of *Bacillus stearothermophilus*. Journal of applied Bacteriology. 61, 517-523.

Murrel, G. W., and Scott, W. J. 1966. The heat resistance of bacterial spores at various water activities. Journal of General Microbiology 43, 411-425.

Reichart, O., and Mohacsi-Farkas, C. 1994. Mathematical modelling of combined effect of water activity, pH and redox potential on the heat destruction. International Journal of Food Microbiology 24, 103-112.

Legend of figure

Fig 1: log N versus heating time

Fig 2: Log D versus heating temperature

Fig 3: Log D versus heating medium pH

Fig 4: Log D versus recovery medium pH

Fig 5: Log D versus heating medium aw

Fig 6: Log D versus recovery medium aw

Fig 7 : correlation between experimentally log D values and theoretically log D values calculated from the overall model

Fig 8: comparison of the experimental (—) and calculated (---) *Bacillus cereus* death kinetics, heating in chocolate cream and recovery in nutritive agar pH7, aw1.

Table of legends

Table 1 Effects of heating temperature, heating and recovery medium pH and aw on D-values (min) of *Bacillus cereus*

Table 2: models parameters

Table 3: fitting parameters on the whole set of data

Table 4: comparison of the experimental and calculated *Bacillus cereus* growth in capillary tube after heating and recovery in chocolate cream for different heating times

Fig1

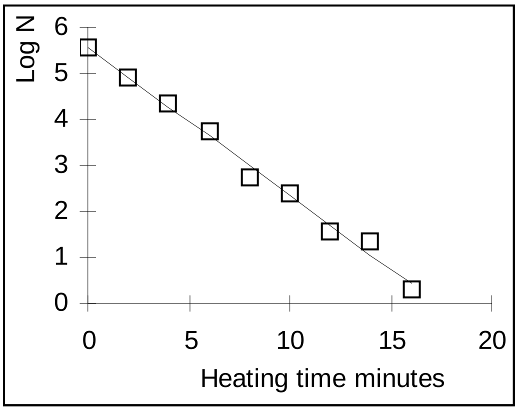


Fig2

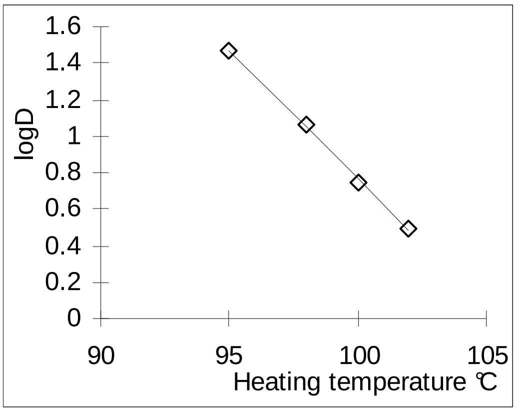


Fig3

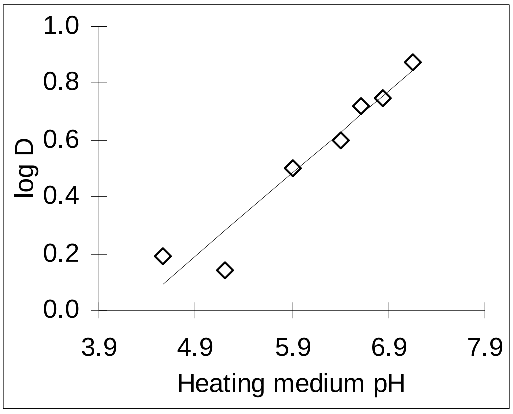


Fig4

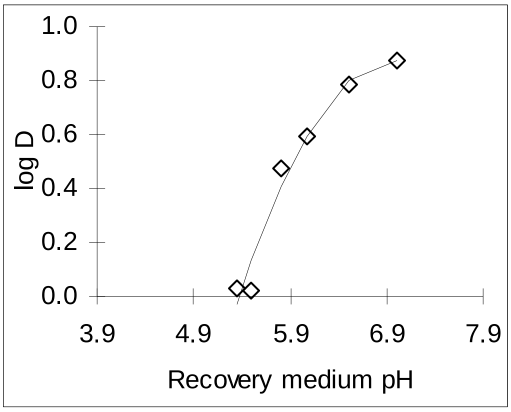


Fig5

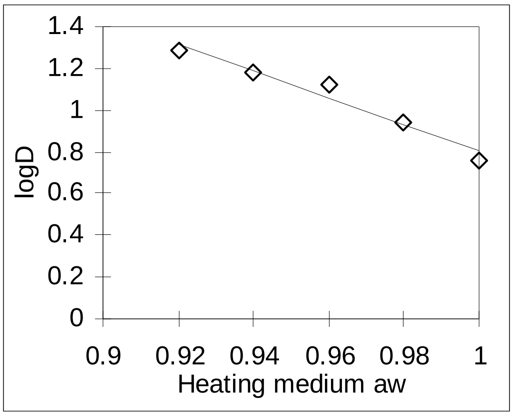


Fig6

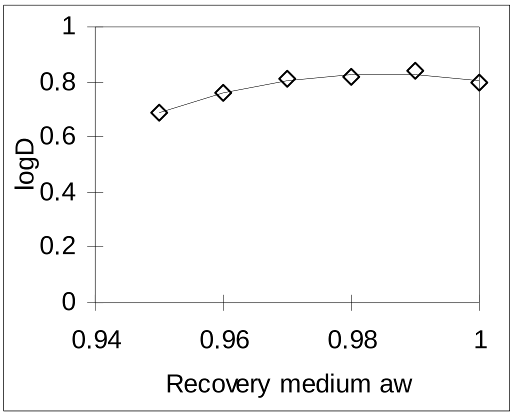


Fig7

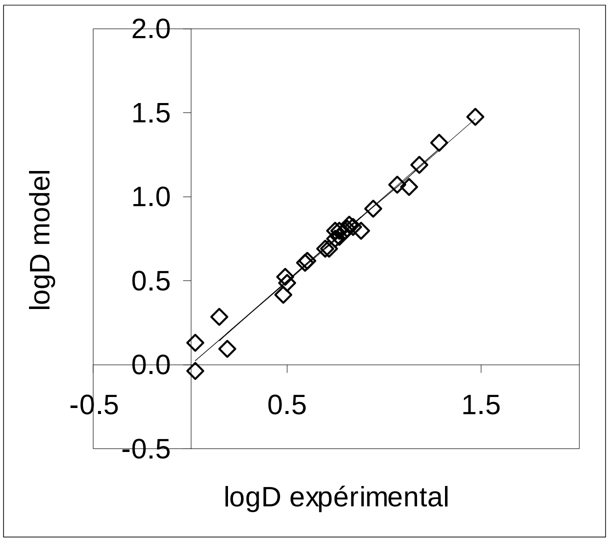
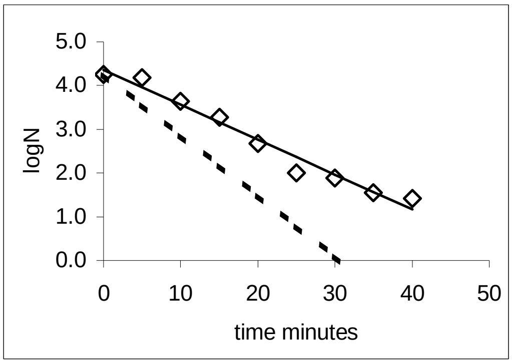


Fig8



Heating temperature °C	Heating medium pH	Recovery medium pH'	Heating medium aw	Recovery medium aw'	D value minutes	Confidence interval
95	7	7	1	1	29.52	± 2.31
98	7	7	1	1	11.54	±0.78
100	7	7	1	1	5.61	±0.46
102	7	7	1	1	3.11	±0.19
100	6.85	7	1	1	5.57	±0.57
100	6.62	7	1	1	5.22	±0.60
100	6.41	7	1	1	3.99	±0.32
100	5.92	7	1	1	3.14	±0.22
100	5.21	7	1	1	1.61	±0.19
100	4.56	7	1	1	1.89	±0.16
100	7	7	1	1	7.53	±0.73
100	7	6.52	1	1	6.06	±0.54
100	7	6.07	1	1	3.92	±0.44
100	7	5.8	1	1	2.98	±0.33
100	7	5.5	1	1	3.59	±0.37
100	7	5.35	1	1	1.04	±0.14
100	7	7	1	1	5.78	±0.48
100	7	7	0.98	1	8.71	±1.39
100	7	7	0.96	1	13.32	±1.67
100	7	7	0.94	1	15.07	±1.66
100	7	7	0.92	1	19.10	±2.31
100	7	7	1	1	6.55	±0.53
100	7	7	1	0.99	6.93	±0.61
100	7	7	1	0.98	6.58	±0.49
100	7	7	1	0.97	6.44	±0.60
100	7	7	1	0.96	5.79	±0.38
100	7	7	1	0.95	4.90	±0.47

Table1

	Values	r
z_T°C	7.09	0.999
z_{pH}	3.44	0.967
z'_{pH}	2.18	0.920
pH'_{opt}	6.96	
z_{aw}	0.156	0.979
z'_{aw}	0.092	0.989
aw'_{opt}	0.985	

Table 2

	Fitting on the whole set of data
D* minutes	0.009
logD*	-2.02
z_T °C	7.32
z_{pH}	3.48
z'_{pH}	1.55
z_{aw}	0.153
z'_{aw}	0.088
pH'opt	6.78
aw'opt	0.983
r	0.990

Table3

Heating time minutes	Tubes with observed growth	Calculated N value in tube
20 min	2 / 2	54
25 min	2 / 2	11
30 min	1 / 2	2
35 min	1 / 2	0
40 min	0 / 2	0
45 min	0 / 2	0

Table4