APPLICATION OF OPTIMAL EXPERIMENTAL DESIGN CONCEPT IN MICROBIAL INACTIVATION STUDIES

Maria M. Gil, Teresa R. S. Brandão and Cristina L. M. Silva
Escola Superior de Biotecnologia, UCP, R. Dr. António Bernardino de Almeida, 4200 – 072 Porto, Portugal
Fax: + 351225090351, clsilva@esb.ucp.pt

Keywords: D-optimal design, predictive microbiology, thermal inactivation

Introduction. *Predictive microbiology* is gaining considerable importance in the food processing domain, particularly in the design of efficient and safe inactivation treatments. This terminology designates the use of mathematical models in the description of microbial responses to environmental stressing factors, such as temperature, pH or water activity. Those models should predict the microbial behaviour accurately and precisely, which depends mutually on the adequacy of the model and on parameters' quality. If a mathematical model is properly chosen and the prime objective is to improve parameter estimation, underlying statistical theories can be applied. The criterion aiming at minimisation of parameters' variance, nominated as *D*-optimal design, is an appropriate and common used approach seeking parameter precision[1]. Even so, it was never applied in food microbiology studies. Precision increases with the number of experimental points. But in many situations, when replicates of a number of experimental points (n) equal to the number of model parameters (p) is considered, maximum precision is attained. The objective of this work was to determine sampling times, supported by *D*-optimal design concept, for microbial inactivation at isothermal conditions.

Methodology. The microbial inactivation model assumed is the one based on the *Gompertz* equation[2]:

$$y = \log\left(\frac{N}{N_0}\right) = \log\left(\frac{Nres}{N_0}\right)\exp\left(-\exp\left(-\frac{k\exp(1)}{\log\left(\frac{N_{res}}{N_0}\right)}(L-t)+1\right)\right) \qquad (1)$$

N - microbial load at time t; 0 and res indexes denote initial and residual, respectively.

This is a two-parameter model, being k the maximum inactivation rate and L the lag parameter. *D*-optimal experiments were planned by minimisation of parameters' variance, which corresponds mathematically to the minimisation of the determinant of the variance-covariance matrix $\mathbf{[F^TF]^{-1}}$(pxp) (or maximisation of $|\mathbf{F^TF}|$)[3]. The elements of **[F]**(nxp) are the partial derivatives of the model (eq.1) in order to the parameters, evaluated at all experimental conditions. For n=p=2, $\mathbf{[F^TF]}$ is a square matrix and the corresponding determinant simplifies to:

$$\Delta = \left|F^T F\right| = \left(\sum_{i=1}^{n}\left(\frac{\partial y}{\partial k}\right)_{t_i}^2 \sum_{i=1}^{n}\left(\frac{\partial y}{\partial L}\right)_{t_i}^2 - \sum_{i=1}^{n}\left(\frac{\partial y}{\partial k}\times\frac{\partial y}{\partial L}\right)_{t_i}^2\right) \qquad (2)$$

The two sampling times that maximise $|\Delta|$ were calculated numerically, using the analysis tool packages available in Microsoft® Office Excel. Preliminary estimates of k and L required for calculation were the ones obtained for *Listeria innocua* thermal inactivation[2]. N_0 and N_{res} were assumed to be 10^7 and 10^3 (cfu/ml), respectively. Six temperatures, in the range 52.5 ºC to 65.0 ºC, were considered.

Results and Discussion. Results showed that the sampling times (t_1 and t_2) that maximise $|\Delta|$ were temperature dependent (*see Table 1, Figure 1)*. Curiously, t_1 corresponds always to 83.0% of inactivation (i.e. $\log(N/N_0)$=-0.77) and t_2 to 99.8% (i.e. $\log(N/N_0)$=-2.81). Since $|\Delta|$ is a measure of parameters´ precision, the ratio between $|\Delta|$ calculated with 5 replicates of each optimal t_1 and t_2 was compared to the one calculated for 10 sampling points equally spaced in time, thus being a measurement of design efficiency. For 57.5ºC, the efficiency of a heuristic design with samples spaced in 250s for a total of 2500s, was only 33%. If *D*-optimal design was chosen, the confidence intervals of k and L would decrease 28% and 45%, respectively, improving precision.

Table 1. Variables used in D-optimal experimental design definition, and corresponding sampling conditions

Variables			Optimal sampling			
T (ºC)	k (s^{-1})	L (s)	t_1 (s)	$\log(N/N_0)$	t_2 (s)	$\log(N/N_0)$
52.5	2.96×10^{-4}	1677	4165	-0.77	11838	-2.81
55.0	9.85×10^{-4}	779	1527	-0.77	3833	-2.81
57.5	3.30×10^{-3}	669	893	-0.77	1581	-2.81
60.0	7.54×10^{-3}	112	209	-0.77	510	-2.81
62.5	2.11×10^{-2}	38	73	-0.77	181	-2.81
65.0	6.22×10^{-2}	11	23	-0.77	59	-2.81

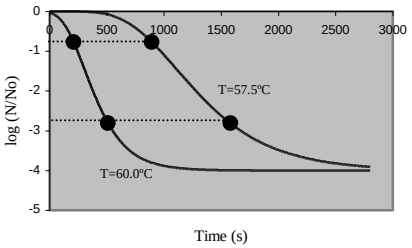

Fig.1. Listeria innocua inactivation (● optimal sampling)

Conclusions. Application of *D*-optimal design concept to microbial inactivation processes may considerably improve parameters´ precision, when compared to commonly use heuristic designs.

References

1. Brandão, T.R.S., Oliveira, F.A.R. and Cunha, L.M. (2001). Design of experiences for improving the precision in the estimation of diffusion parameters under isothermal and non-isothermal conditions. *Int J Food Sci Tech.* 36: 291-301.
2. Gil, M.M., Miller, F.A., Brandão, T.R.S. and Silva, C.L.M. (2003). Mathematical model to predict thermal inactivation kinetics of *Listeria innocua* 10528. Poster presented at: "X Congresso Nacional de Biotecnologia (BIOTEC´2003), Lisboa, Portugal, 6 to 8 December.
3. Atkinson, A.C. (1982). Developments in the design of experiments. *Int Stat Rev.* 50: 161-177.