APPLICATION OF OPTIMAL EXPERIMENTAL DESIGN CONCEPT IN MICROBIAL INACTIVATION STUDIES

Gil, M.M., Brandão, T.R.S. and Silva C.L.M.*

Escola Superior de Biotecnologia, Universidade Católica Portuguesa, R. Dr. António Bernardino de Almeida, 4200-072 Porto, Portugal *clsilva@esb.ucp.pt

OBJECTIVE

Determination of sampling times for improved microbial inactivation prediction using *D*-optimal design concept

INTRODUCTION

- Predictive microbiology is gaining considerably 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 used approach seeking parameter precision (Brandão *et al.*, 2001).
- Precision increases with the number of experimental points. But in many situations, when replicates of a number of experimental points equal to the number of model parameters is considered, maximum precision is attained.

METHODOLOGY

The microbial inactivation model assumed is the one based on the Gompertz equation

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

maximum inactivation rate

Temperature dependent

lag parameter

N - microbial load at time t; No – initial microbial load; Nres – Residual microbial load; t - time

D-Optimal experimental design criterion

Precision

Minimisation of parameters´ variance → Minimisation of the determinant of the variance – covariance matrix of parameters $|F^TF|^{-1}$

mathematically corresponds to…

Maximisation of the determinant $|F^TF|$

For a two - parameter model, the simplest design corresponds to an isothermal experiment, with two samplings at time t_1 and t_2

Partial derivatives of the Gompertz model in order to the parameters – evaluated at all experimental conditions

$$\Delta \equiv \left|F^TF\right| \equiv \begin{vmatrix} \left.\frac{\partial\left(\log\frac{N}{N_0}\right)}{\partial k}\right|_{t_1} & \left.\frac{\partial\left(\log\frac{N}{N_0}\right)}{\partial L}\right|_{t_1} \\ \left.\frac{\partial\left(\log\frac{N}{N_0}\right)}{\partial k}\right|_{t_2} & \left.\frac{\partial\left(\log\frac{N}{N_0}\right)}{\partial L}\right|_{t_2} \end{vmatrix}^2$$

The two sampling times that maximise the determinant $|\Delta|$ were calculated:

- Using 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 (Gil *et al.*, 2003)
- N_0 and N_{res} were assumed to be 10^7 and 10^3 (cfu/ml)
- Six temperatures, in the range 52.5ºC to 65.0ºC, were considered

RESULTS AND DISCUSSION

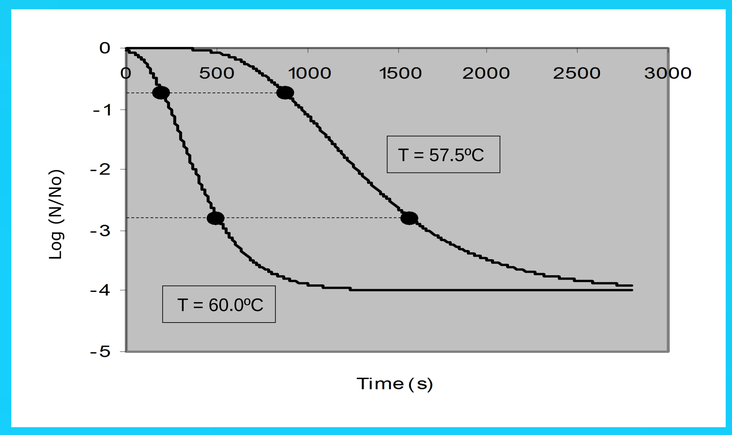
- Sampling times (t_1 and t_2), that maximise $|\Delta|$, were temperature dependent

however…

- 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. $\log(N/N_0) = -2.81$)

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



Listeria innocua inactivation (• optimal sampling)

Design efficiency

The *D*-optimal experimental design was compared to a heuristic design in terms of parameters´ precision.

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.

For T = 57.5ºC:

- The efficiency of a heuristic design with samples spaced in 250 s for a total of 2500 s, was only 33%
- If *D*-optimal design was chosen, the confidence intervals of k and L would decrease 28% and 45%, respectively, improving precision

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 models 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.