DESIGNING EXPERIMENTS FOR MICROBIAL INACTIVATION KINETICS STUDIES

Gil, M.M.[a,b], Brandão, T.R.S.[a] and Silva C.L.M.[a]*

[a]Escola Superior de Biotecnologia, Universidade Católica Portuguesa, R. Dr. António Bernardino de Almeida, 4200-072 Porto, Portugal
[b]Escola Superior de Tecnologia do Mar, Instituto Politécnico de Leiria, Santuário N.ª Sra. Dos Remédios, 2524-909 Peniche, Portugal

*clsilva@esb.ucp.pt

CATÓLICA UNIVERSIDADE CATÓLICA PORTUGUESA | PORTO Escola Superior de Biotecnologia

IPL escola superior de tecnologia do mar

OBJECTIVE

Design experiments using *D*-optimal design criterion aiming at microbial inactivation kinetic parameter with maximum precision

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. Microbial inactivation can be mathematically described by a modified Gompertz model, which includes an initial shoulder (L) followed by a maximum inactivation rate (k_{max}) period. The kinetic model parameters are temperature dependent and a Ratkowsky equation or an Arrhenius-type relationship can be used to express such relationship.
- 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.
- 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.

MATHEMATICAL CONSIDERATIONS

The model

If one single temperature is chosen from a range, the microbial inactivation model assumed was the one based on modifications of the Gompertz equation:

model parameters

$$y_{inact}(t) = \log\left(\frac{N}{N_0}\right) = \log\left(\frac{N_{res}}{N_0}\right)\exp\left(-\exp\left(-\frac{k_{max}\exp(1)}{\log\left(\frac{N_{res}}{N_0}\right)}(L - t) + 1\right)\right)$$

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

If a range of temperatures is considered, the Temperature dependence of k_{max} and L may be included in the previous equation. Assuming an Arrhenius-type relationship for k_{max} and a Ratkowsky equation for L, the model a four-parameter model:

model parameters

$$y_{inact}(t) = \log\left(\frac{N_{res}}{N_0}\right)\exp\left(-\exp\left(\frac{\left(-k_{ref}\exp\left(-\frac{E_a}{R}\left(\frac{1}{T}-\frac{1}{T_{ref}}\right)\right)\right)e}{\log\left(\frac{N_{res}}{N_0}\right)}\left(\left(C_{Ratk}(T - T_{min})\right)^2 - t\right) + 1\right)\right)$$

k_{ref} – Inactivation rate at a finite reference T_{ref}; E_a – Process activation energy; C_{Ratk} and T_{min} – Ratkowsky equation parameters; R – universal gas constant

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 $|F^TF|$

Single Temperature

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

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

$$\Delta = \left|F^TF\right| = \sum_{i=1}^{n}\left(\frac{\partial y_{inact}}{\partial k_{max}}\right)_{t_i}^2 \sum_{i=1}^{n}\left(\frac{\partial y_{inact}}{\partial L}\right)_{t_i}^2 - \sum_{i=1}^{n}\left(\frac{\partial y_{inact}}{\partial k_{max}}\times\frac{\partial y_{inact}}{\partial L}\right)_{t_i}^2$$

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

- Using analysis tool packages available in Microsoft® Office Excel
- Preliminary estimates of k and L required for calculation were the ones presented in Table 1
- Six temperatures, in the range 52.5ºC to 65.0ºC, were considered and $\log(N_{res}/N_0)$ was assumed to be -5

Range of Temperatures

If four sampling conditions (i.e. temperature/sampling time) are planned (n=4) the determinant becomes:

$$\Delta = \left|F^TF\right| = \begin{vmatrix}
\sum_{i=1}^{n}\left(\frac{\partial y_{inact}}{\partial k_{ref}}\right)_i^2 & \sum_{i=1}^{n}\left(\frac{\partial y_{inact}}{\partial k_{ref}}\times\frac{\partial y_{inact}}{\partial E_a}\right)_i & \sum_{i=1}^{n}\left(\frac{\partial y_{inact}}{\partial k_{ref}}\times\frac{\partial y_{inact}}{\partial C_{Ratk}}\right)_i & \sum_{i=1}^{n}\left(\frac{\partial y_{inact}}{\partial k_{ref}}\times\frac{\partial y_{inact}}{\partial T_{min}}\right)_i \\
\sum_{i=1}^{n}\left(\frac{\partial y_{inact}}{\partial E_a}\times\frac{\partial y_{inact}}{\partial k_{ref}}\right)_i & \sum_{i=1}^{n}\left(\frac{\partial y_{inact}}{\partial E_a}\right)_i^2 & \sum_{i=1}^{n}\left(\frac{\partial y_{inact}}{\partial E_a}\times\frac{\partial y_{inact}}{\partial C_{Ratk}}\right)_i & \sum_{i=1}^{n}\left(\frac{\partial y_{inact}}{\partial E_a}\times\frac{\partial y_{inact}}{\partial T_{min}}\right)_i \\
\sum_{i=1}^{n}\left(\frac{\partial y_{inact}}{\partial C_{Ratk}}\times\frac{\partial y_{inact}}{\partial k_{ref}}\right)_i & \sum_{i=1}^{n}\left(\frac{\partial y_{inact}}{\partial C_{Ratk}}\times\frac{\partial y_{inact}}{\partial E_a}\right)_i & \sum_{i=1}^{n}\left(\frac{\partial y_{inact}}{\partial C_{Ratk}}\right)_i^2 & \sum_{i=1}^{n}\left(\frac{\partial y_{inact}}{\partial C_{Ratk}}\times\frac{\partial y_{inact}}{\partial T_{min}}\right)_i \\
\sum_{i=1}^{n}\left(\frac{\partial y_{inact}}{\partial T_{min}}\times\frac{\partial y_{inact}}{\partial k_{ref}}\right)_i & \sum_{i=1}^{n}\left(\frac{\partial y_{inact}}{\partial T_{min}}\times\frac{\partial y_{inact}}{\partial E_a}\right)_i & \sum_{i=1}^{n}\left(\frac{\partial y_{inact}}{\partial T_{min}}\times\frac{\partial y_{inact}}{\partial C_{Ratk}}\right)_i & \sum_{i=1}^{n}\left(\frac{\partial y_{inact}}{\partial T_{min}}\right)_i^2
\end{vmatrix}$$

Preliminary estimates of k_{ref}, E_a, C_{Ratk} and T_{min} were assumed to be 0.29 min^{-1}, 3.34×10^5 J mol^{-1}, 0.38 $K^{-1}min^{-0.5}$ and 337.1 K, respectively.

RESULTS AND DISCUSSION

Single Temperature

- Sampling times (t_1 and t_2), that maximise |Δ|, were temperature dependent

however…

- t_1 corresponds always to 89.09% of inactivation (i.e. $\log(N/N_0)$ = -0.96) and t_2 to 99.97% (i.e. $\log(N/N_0)$ = -3.52)

Variables used in *D*-optimal experimental design definition, and corresponding sampling conditions

Definition of the design			Optimal sampling			
T (ºC)	k_{max} (min^{-1})	L (min)	t_1 (min)	$\log(N/N_0)$	t_2 (min)	$\log(N/N_0)$
52.5	4.04×10^{-2}	69.06	91.85	-0.96	162.1	-3.52
55.0	7.56×10^{-2}	39.58	51.76	-0.96	89.31	-3.52
57.5	1.41×10^{-1}	10.82	17.36	-0.96	37.52	-3.52
60.0	4.52×10^{-1}	6.06	8.10	-0.96	14.38	-3.52
62.5	1.14×10^{0}	0.68	1.49	-0.96	3.98	-3.52
65.0	2.18×10^{0}	0.03	0.45	-0.96	1.75	-3.52

Design efficiency

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

- Since |Δ| is a measure of parameters´ precision, the ratio between |Δ| calculated with 10 sampling points equally spaced in time was compared to the one calculated for 5 replicates of each optimal t_1 and t_2.
  - The efficiency of a heuristic design was 26, 18, 33, 31, 43 and 76% for the experiments conducted at 52.5, 55.0, 57.5, 60.0, 62.5 and 65 ºC, respectively.
  - As an example, if *D*-optimal design was chosen, the confidence intervals of k and L would decrease **40%** and **59%**, respectively (at 52.5ºC), improving precision.

Range of Temperatures

If a Temperature range is considered, the Gompertz model is a four-parameter model and:

- Optimal experimental design consists of four experiments conducted within the experimental range of temperatures: one at each extreme temperature (Tmin=52.5 ºC and Tmax=65 ºC), one at the average temperature of the range tested (Tave=58.8 ºC), and the remained one at a temperature 3 % lower than the maximum extreme (T3%<Tmax=65.0 ºC).
- At each T, the sampling times corresponds to 99.95 % (Tmin), 97.49% (Tave), 99.92% (T3%<Tmax) and 99.21 % (Tmax) of inactivation.

Design efficiency

- The efficiency of a heuristic design was only 1.6 % (calculated with 18 sampling points equally spaced in time (at each one of the six T) and 27 replicates of each one of the four optimal sampling conditions).
- If *D*-optimal design was chosen, the confidence intervals of k_{ref}, E_a, C_{Ratk} and T_{min} would decrease **64, 88, 80** and **72 %** respectively, improving precision.

CONCLUSIONS

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