Study of the influence of ethylene oxide sterilization variables on *Bacillus subtilis* inactivation

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ABSTRACT

Nowadays, ethylene oxide is a dominant sterilization agent used in medical device industry due to its effectiveness and compatibility with most materials, together with the technical and technological advances that allow overlapping difficulties associated to this agent.

This work intends to study the influence of the process variables, e.g. temperature (T), ethylene oxide (EO) concentration and relative humidity (RH), on *Bacillus subtilis* spores inactivation, aiming to provide a model for lethality prediction.

MATERIALS AND METHODS

Experiments were carried out in an EO sterilizer with controlled T, EO concentration and RH, using a full factorial experimental design at two levels (2^3 factorial design). The limit target exposure conditions for EO concentration, T and RH were 250 - 1000 mg EO/L, 40 - 60 °C and 50 - 90 % (runs 1 to 8 in Table 1).

The sporicidal activity of a specific EO sterilization cycle was assessed by recovering and enumeration of bacterial viable spores from *B. subtilis* biological indicators, and the Gompertz model was applied in data fitting of the inactivation data:

$$\log\left(\frac{N}{N_0}\right) = A \cdot \exp\left[-\exp\left\{\frac{-k_{max} e}{A}(\lambda - t) + 1\right\}\right]$$

where N is the microbial load at a particular process time t (the index 0 is related to initial microbial load), A is a model parameter, k_{max} is the maximum inactivation rate, and λ the shoulder period.

Non-linear regression analysis was carried out in Statistica© 6.0 software (StatSoft, USA), using the Levenberg-Marquardt algorithm to minimize the sum of the squares of the differences between the predicted and experimental values.

An analysis of variance (ANOVA) allowed to identify the most significant parameters affecting *B. subtilis* inactivation. Additional experiments, considering intermediate conditions of these parameters, were defined in order to model their effects and combined effects on the lethality (runs 9 to 15 in Table 1).

RESULTS

Results showed that T and EO concentration were the most significant factors affecting the inactivation kinetics of *Bacillus subtilis* spores.

Mathematical relations describing T and EO concentration influence on k_{max} and λ were successfully developed and included in the Gompertz kinetic model:

$$\log\left(\frac{N}{N_0}\right) = (-7.5)\exp\left\{-\exp\left[\frac{-\left[\left(1.42\times10^{-4}T - 4.96\times10^{-3}\right) + \left(5.54\times10^{-8}T + 1.25\times10^{-6}\right)[EO]\right]e}{-7.5} \times \left[\left(\left(1.63\times10^{1}T - 1.06\times10^{3}\right)\ln([EO]) + \left(-1.25\times10^{2}T + 8.23\times10^{3}\right) - t\right] + 1\right]\right\}$$

The predictive ability of this integrated model was assessed, and its adequacy in predicting *B. subtilis* inactivation was proven (Figure 1, 2 and Table 1).

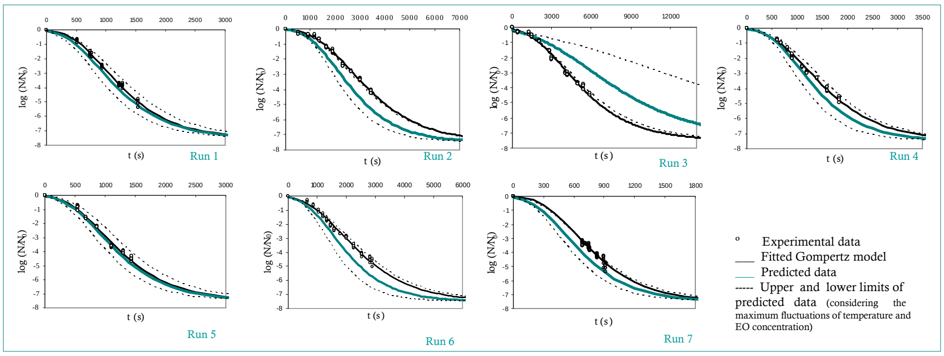

Figure 1. Inactivation of *B. subtilis* spores by EO sterilization at conditions defined according to the 2^3 factorial design

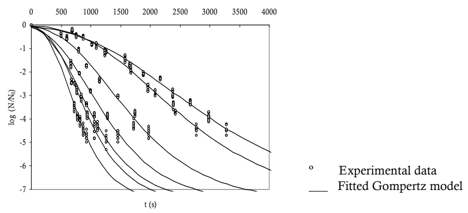

Figure 2. Inactivation of *B. subtilis* spores by EO sterilization at the additional experimental conditions (runs 9 to 15)

Table 1. Estimated k_{max} and λ parameters of *B. subtilis* spores inactivation at the tested temperature, EO concentration and relative humidity conditions

Run	Variables			Parameters				Regression analysis
	T (°C)	[EO] (mg/L)	RH (%)	k_{max} x10^3 (s^{-1})	$SHW_{95\%}$	λ (s)	$SHW_{95\%}$	R^2
1	60 (+)	233 (-)	63 (-)	4.56	3.01	391.22	5.24	0.992
2	44 (-)	257 (-)	86 (+)	1.78	2.42	1079.26	3.25	0.993
3	34 (-)	222 (-)	60 (-)	0.989	2.93	1178.85	7.45	0.988
4	40 (-)	980 (+)	90 (+)	3.49	2.66	417.73	5.26	0.991
5	59 (+)	266 (-)	83 (+)	4.46	3.56	353.18	7.19	0.991
6	33 (-)	940 (+)	61 (-)	2.16	2.50	605.12	5.63	0.989
7	59 (+)	1004 (+)	98 (+)	7.65	8.59	265.92	16.75	0.983
8	60 (+)	977 (+)	46 (-)	10.00	*	0.00	*	*
9	37	674	73	2.28	2.72	831.31	4.05	0.991
10	37	456	80	1.83	2.57	821.44	4.68	0.991
11	51	247	80	3.23	3.60	481.61	7.10	0.985
12	51	447	67	4.09	3.37	300.04	8.67	0.994
13	50	675	72	5.04	4.94	256.72	14.53	0.992
14	60	738	71	7.33	8.01	254.67	17.83	0.994
15	62	498	77	5.89	6.25	291.40	12.25	0.988

* Meaningless value
SHW – Standardized half-width

CONCLUSIONS

A model that describes *B. subtilis* spores inactivation kinetics in terms of the relevant process variables (temperature and EO concentration) was achieved. The adequacy of this integrated model in predicting the microorganism inactivation was assessed.

The results of this work are certainly a contribution for an efficient control, design and optimization of EO sterilization processes.

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