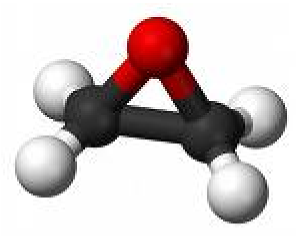

Modelling the inactivation of *Bacillus subtilis* spores by ethylene oxide processing

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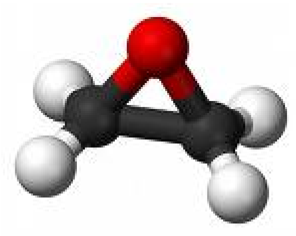
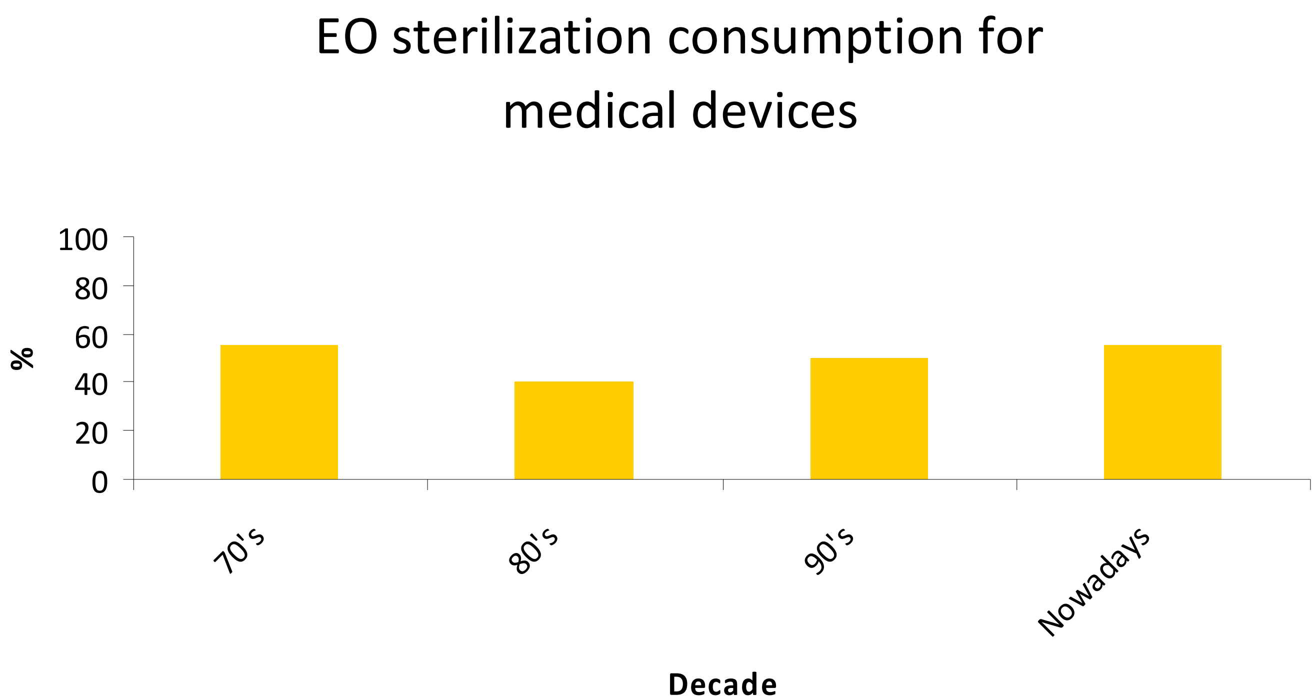
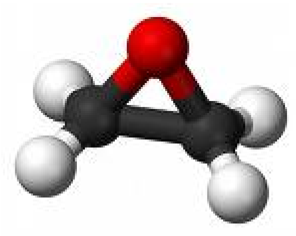


ETHYLENE OXIDE IS CURRENTLY A DOMINANT STERILIZATION AGENT USED IN MEDICAL DEVICES INDUSTRY





Advantages / Disadvantages

- **Advantages**

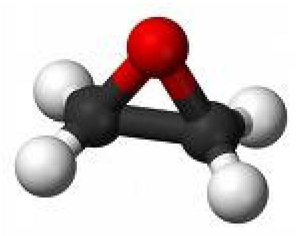
Effectiveness *Diffusivity*

Bactericidal, fungicidal and virucidal properties

Compatibility with most materials

Process flexibility

Low temperature sterilization



Advantages / Disadvantages

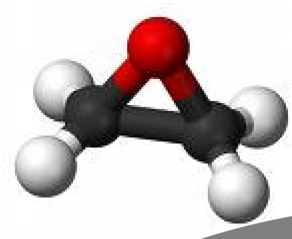
- **Disadvantages**

Toxicity of the sterilizing agent

Process complexity

Process cost

Processing time



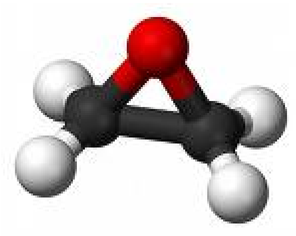
Objectives

Screen the most significant variables on *B. subtilis* inactivation by EO sterilization

Model the inactivation kinetics of *B. subtilis,* including the variables' effects

Provide a method of integrating lethality

Understanding the full dynamics of the sterilization allows design optimization / efficient control of the process - **Parametric release**



Modelling microorganisms inactivation

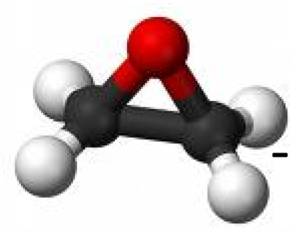
Experimental design

Bacillus subtilis, var. *niger* or *Bacillus atrophaeus* spores (ATCC 9372) inoculated in strips (biological indicators, BIs)

Matrix: Drapes

Temperature and humidity sensors
EO sensor (Infrared analyser in the sterilizer chamber headspace)

Sterilization cycles

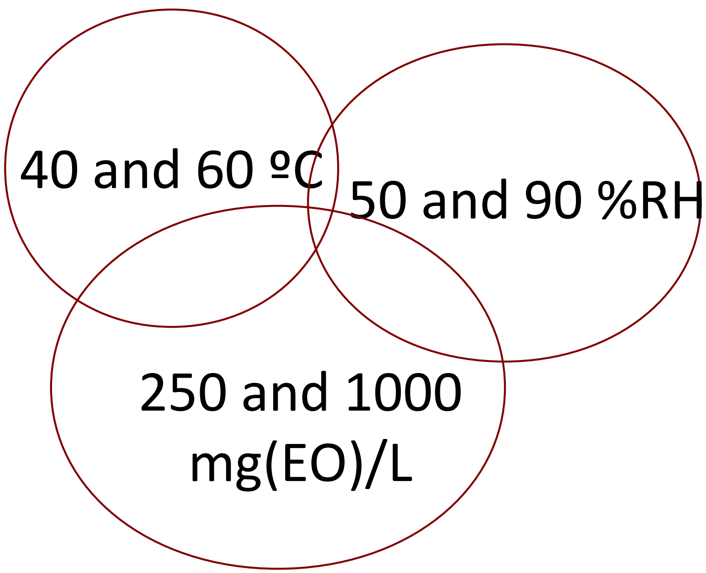


Modelling microorganisms inactivation

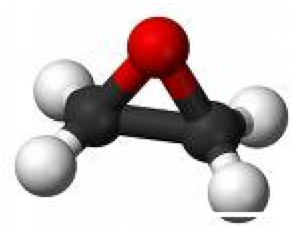
- Conditions defined according to the 2^3 factorial design -

Sterilization cycles

- Target exposure conditions –



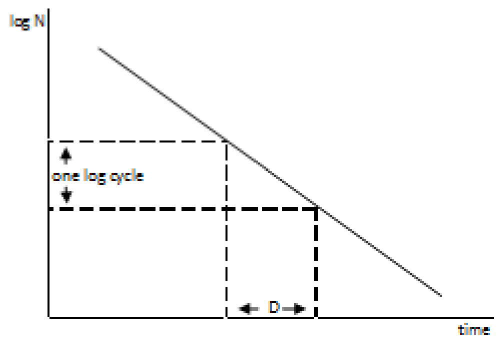
T (°C)		RH (%)		EO conc. (mg/L)	
40	(-)	50	(-)	250	(-)
40	(-)	50	(-)	1000	(+)
40	(-)	90	(+)	250	(-)
40	(-)	90	(+)	1000	(+)
60	(+)	50	(-)	250	(-)
60	(+)	50	(-)	1000	(+)
60	(+)	90	(+)	250	(-)
60	(+)	90	(+)	1000	(+)



Modelling microorganisms inactivation

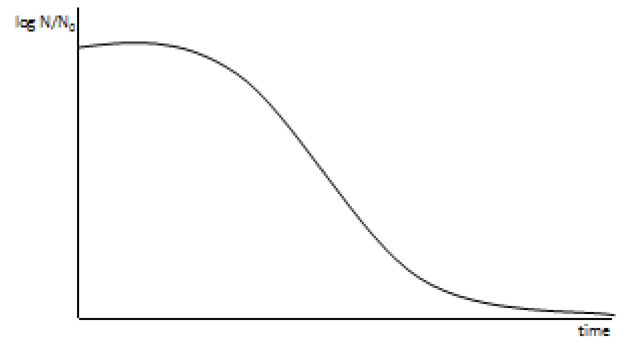
Survival curves construction

1^{st} order kinetics



$$\log N = -k.t + \log N_0$$

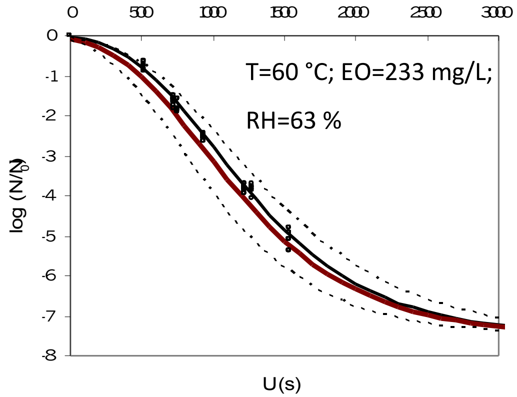
Gompertz model

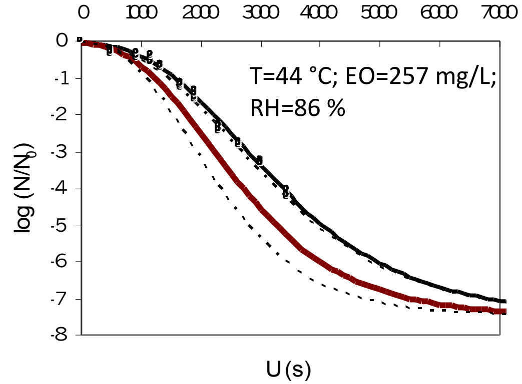


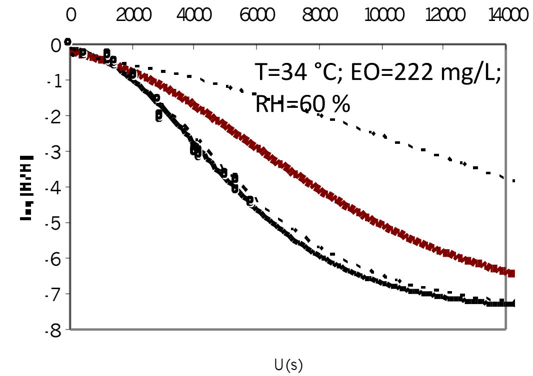
$$\log\left(\frac{N}{N_0}\right) = A \cdot \exp\left[-\exp\left\{\frac{-k_{max}e}{A}(\lambda - t) + 1\right\}\right]$$

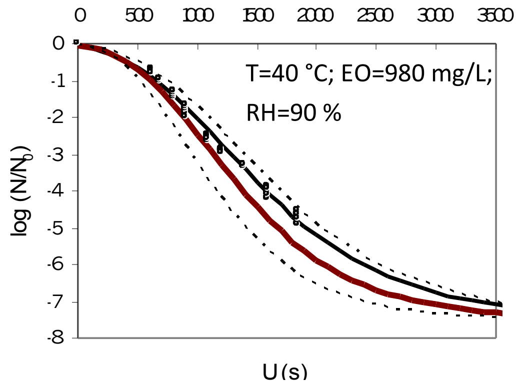
Gompertz function has the ability of modelling both linear and asymmetrical sigmoidal data

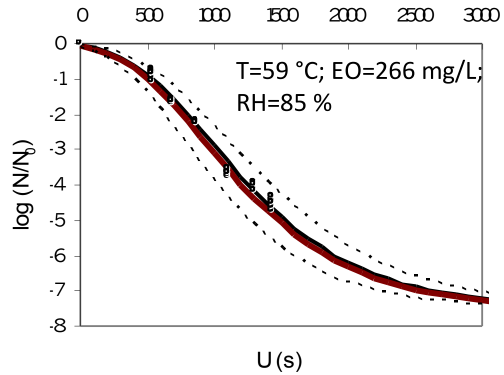
Inactivation of *B. subtilis* spores by EO sterilization
- Conditions defined according to the 2^3 factorial design -

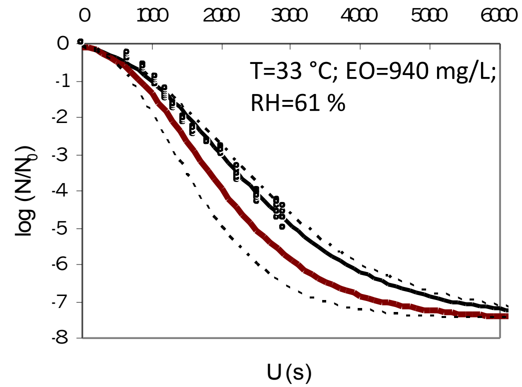


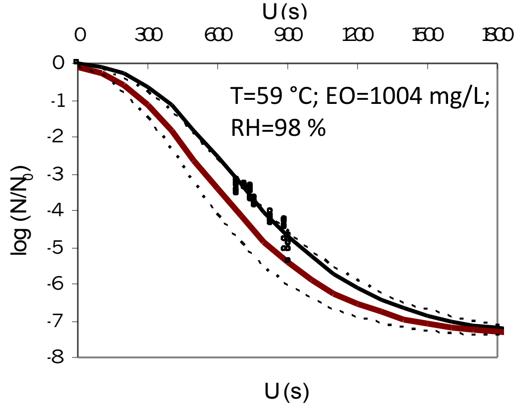












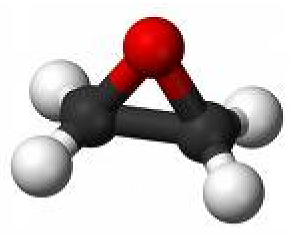
Legend

o Experimental data

___ Fitted Gompertz model

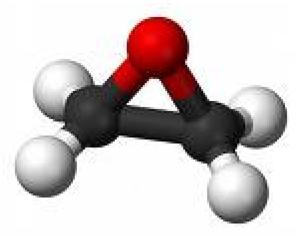
___ Predicted data

- - - Upper and lower limits of predicted data (considering the maximum fluctuations of temperature and EO concentration)



Data analysis

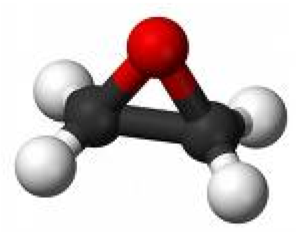
The **non-linear regression analysis** was carried in Statistica© 6.0 software (StatSoft, USA), using the Levenberg-Marquardt algorithm to minimize the sum of the squares of the differences between the predicted and experimental values.



Data analysis

The experimental inactivation **data were successfully fitted with the Gompertz model**:

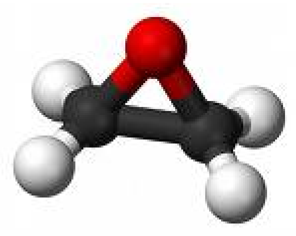
- High precision of k_{max} and λ estimates, *since low errors were attained* ($SHW_{95\%}$);
- Residuals randomness and normality;
- Coefficient of determination ($R^2>0.98$);



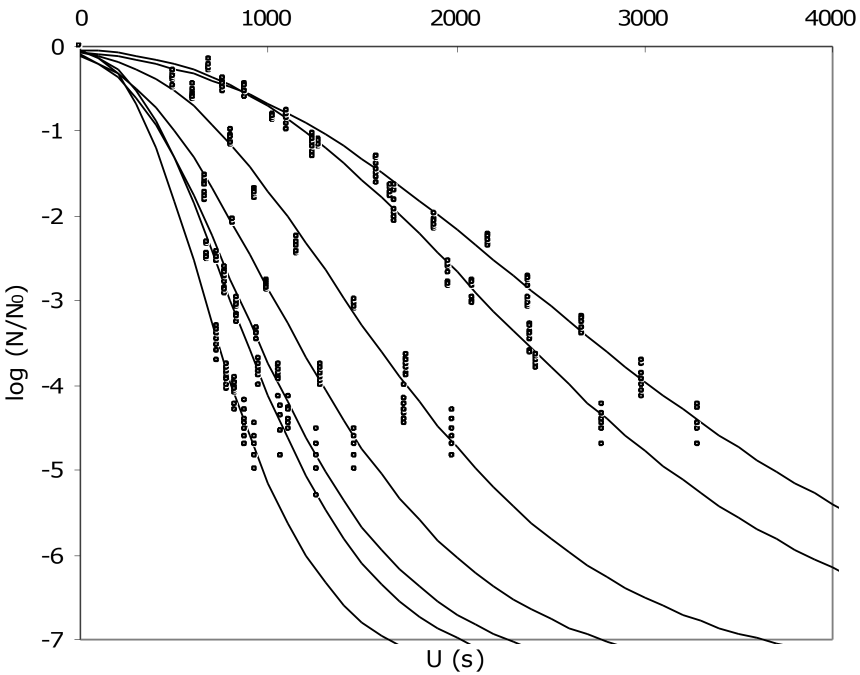
Data analysis and planning future work

The analysis of variance (ANOVA) allowed to identify the most significant parameters affecting *B. subtilis* inactivation - temperature and EO concentration

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)



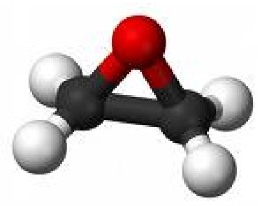
Inactivation of *B. subtilis* spores by EO sterilization at the additional experimental conditions



Legend

o Experimental data
___ Fitted Gompertz model

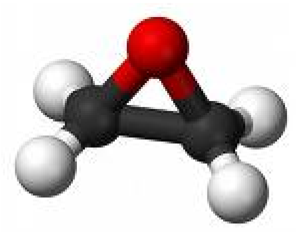
Run 9 to Run 15



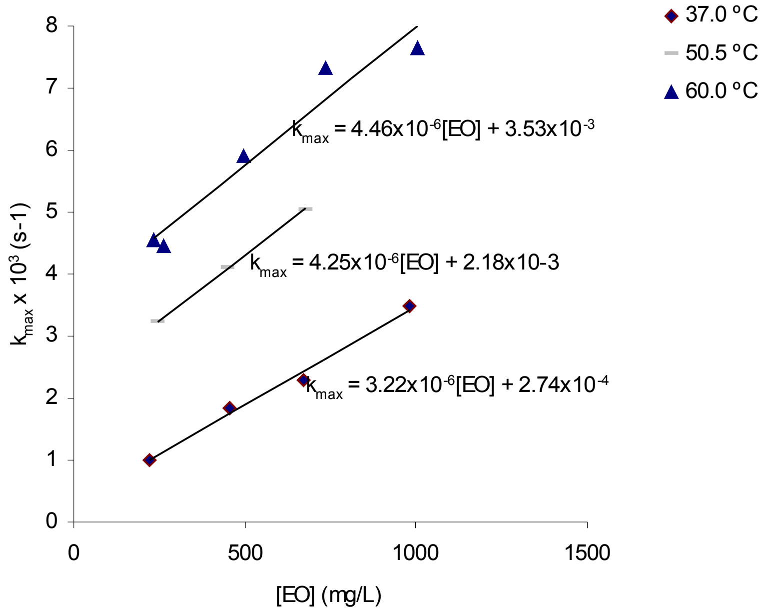
Estimated k_{max} and l parameters of *B. subtilis* inactivation at the temperature, EO concentration and relative humidity conditions tested

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

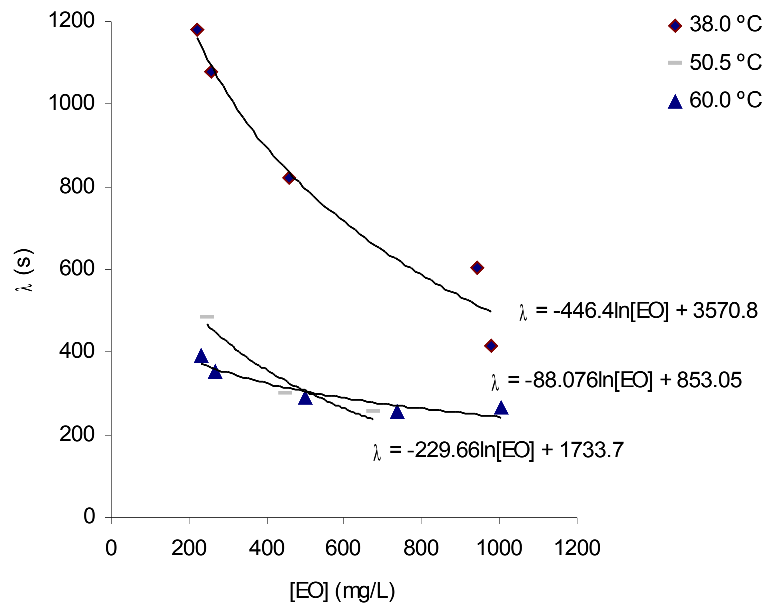
* Meaningless value



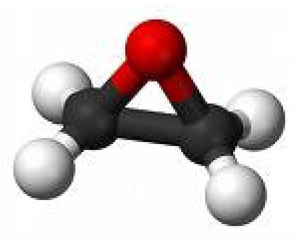
EO concentration influence on k_{max} and λ



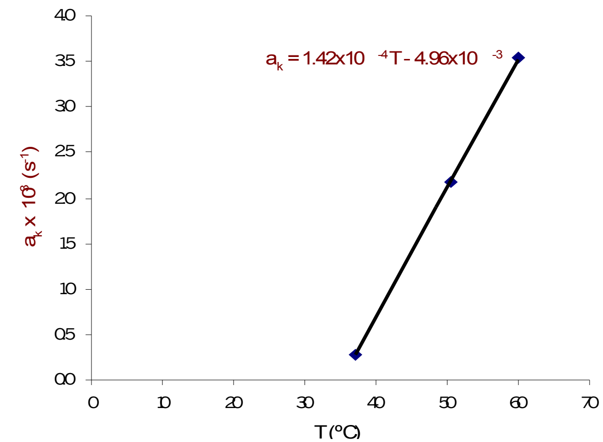
Influence of EO concentration on k_{max} at 37.0, 50.5 e 60.0 °C

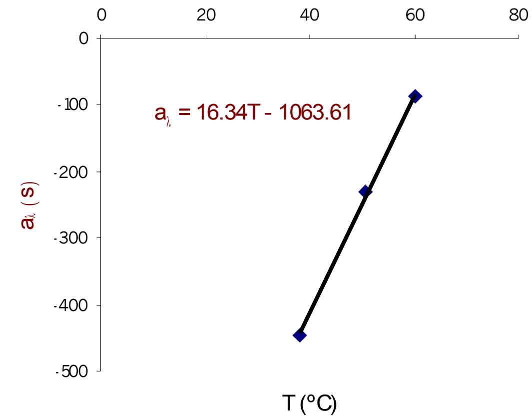


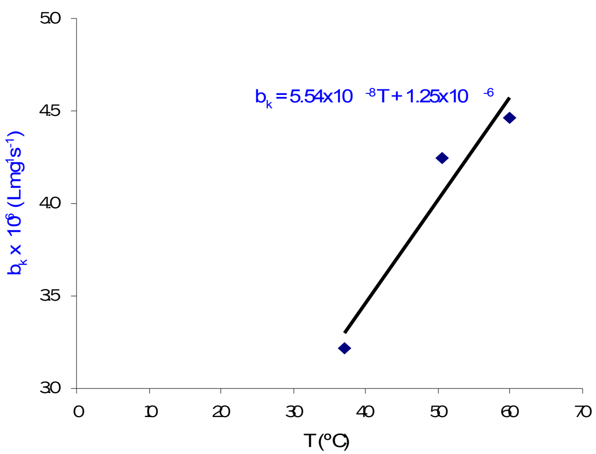
Influence of EO concentration on λ at 38.0, 50.5 and 60.0 °C

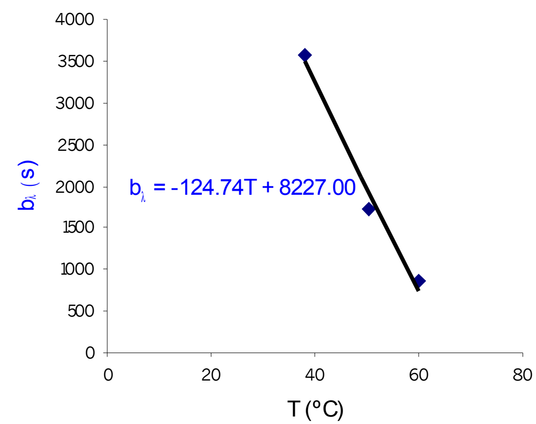


T influence on parameters a_k/b_k and a_λ/b_λ



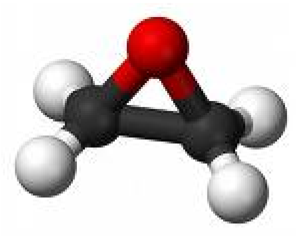






Influence of T on a_k e b_k parameters

Influence of T on a_k e b_k parameters

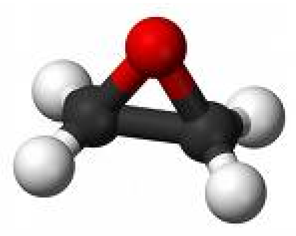


Data analysis

T and EO concentration have a negative effect on λ and a positive effect on k_{max}:

- Higher temperatures and EO concentration imply narrow shoulder times and higher inactivation rates;
- Lower inactivation rates and more evident shoulder phases

  were observed at the lowest temperature and EO concentration;

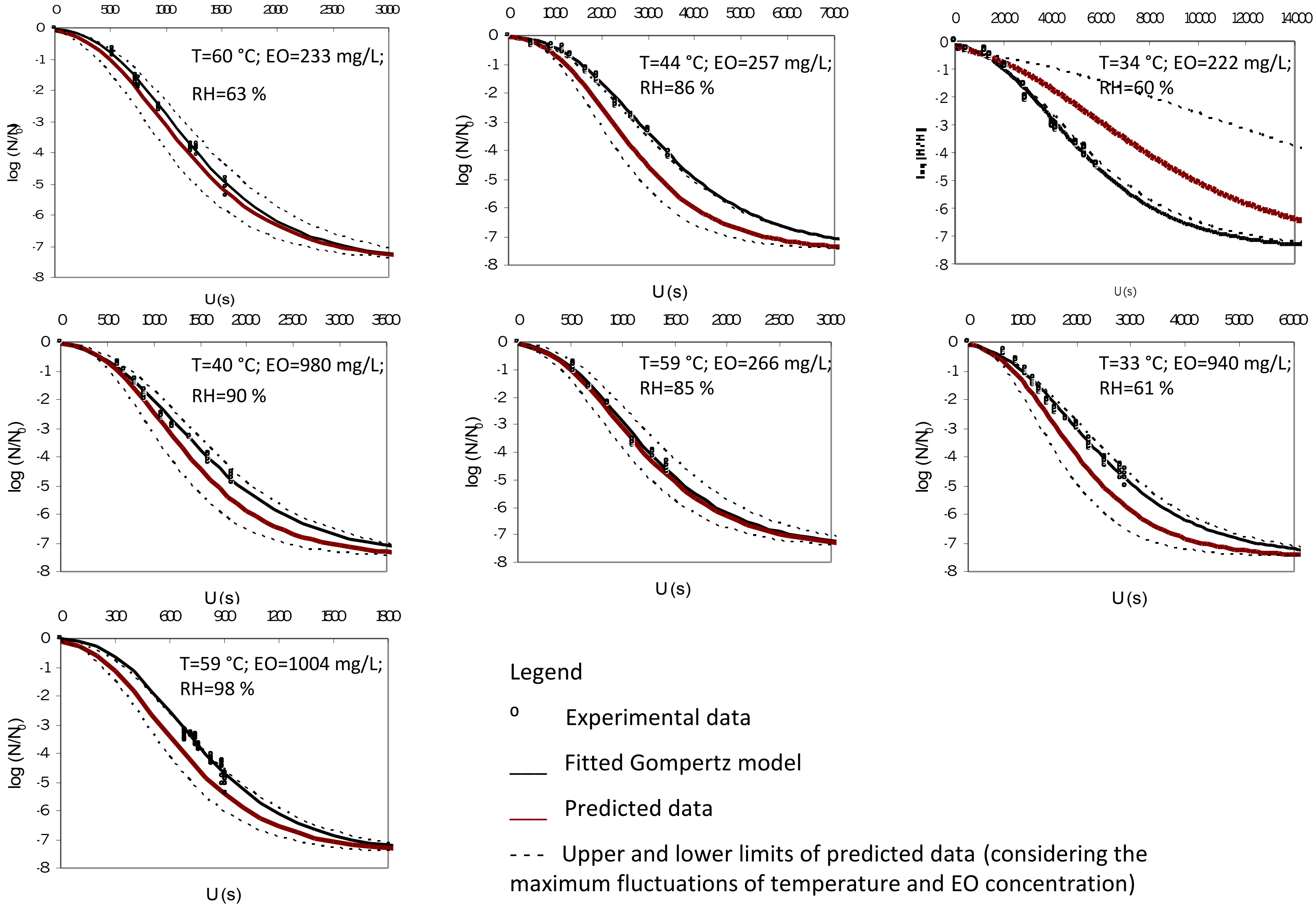


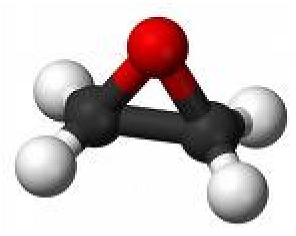
Mathematical model resulting from the integration of the T and EO concentration parameters for lethality calculation of the EO sterilization process

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

$$\left.\left.\times\left[\left[\left(1.63\times10^{1}T-1.06\times10^{3}\right)\ln([EO])+\left(-1.25\times10^{2}T+8.23\times10^{3}\right)-U\right]+1\right]\right]\right\}$$

Inactivation of *B. subtilis* spores by EO sterilization
- Conditions defined according to the 2^3 factorial design -



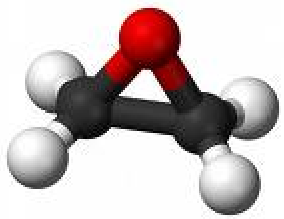


In conclusion

A mathematical inactivation model expressed only in terms of the relevant process variables (T and EO concentration) was achieved.

$$\log\left(\frac{N}{N_0}\right) = (-7.5)\exp\left\{-\exp\left[\frac{-\left[\left(1.42\times 10^{-4}T - 4.96\times 10^{-3}\right) + \left(5.54\times 10^{-8}T + 1.25\times 10^{-6}\right)[EO]\right]e}{-7.5}\times\left[\left(1.63\times 10^{1}T - 1.06\times 10^{3}\right)\ln([EO]) + \left(-1.25\times 10^{2}T + 8.23\times 10^{3}\right) - U\right] + 1\right]\right\}$$

The conventional design of EO sterilization cycles usually involves a significant amount of experimental work, which is time consuming and also expensive. The results of this work are certainly a contribution for an **efficient control**, **design** and **optimization** of the **EO sterilization process**.



Thanks’