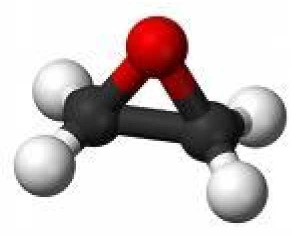

Modelling the inactivation of *Bacillus subtilis* spores by ethylene oxide processing

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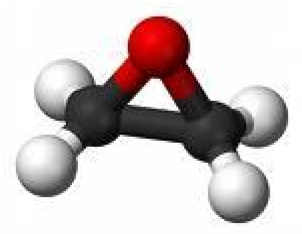
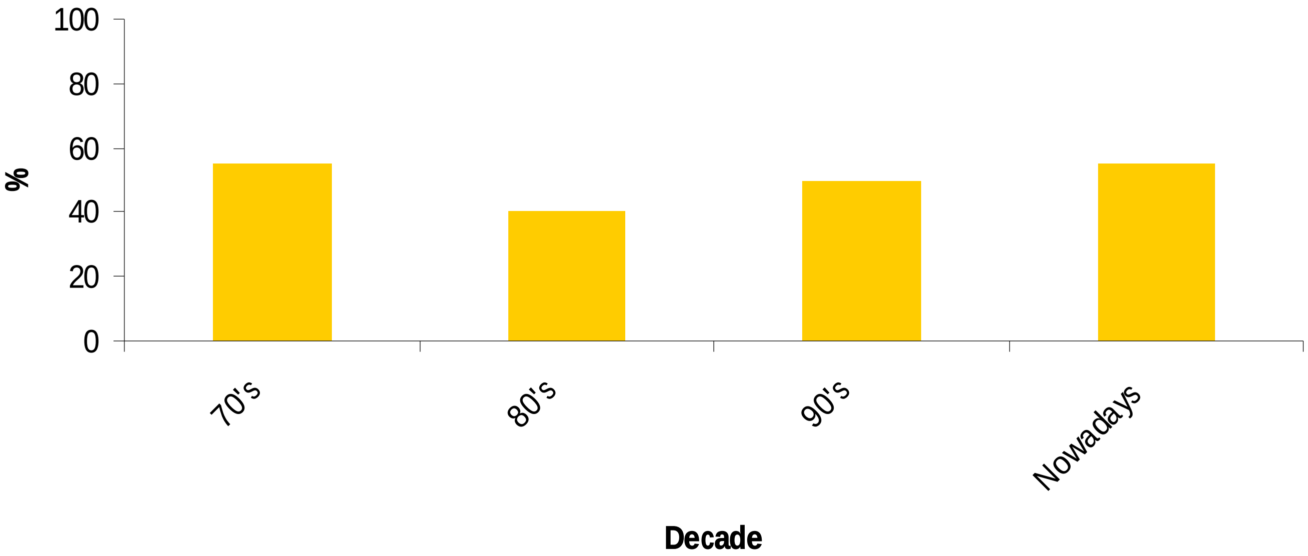
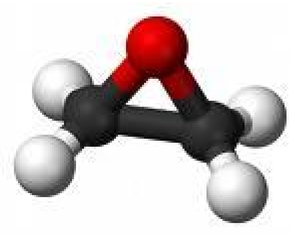


ETHYLENE OXIDE IS CURRENTLY A DOMINANT STERILIZATION AGENT USED IN MEDICAL DEVICE INDUSTRY

EO sterilization consumes for medical devices





Advantages / Disadvantages

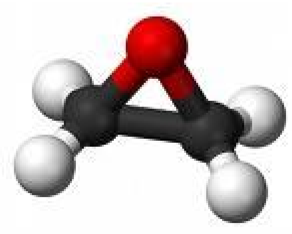
- **Advantages**

Effectiveness Diffusivity
Bactericidal, fungicidal and virucidal properties

Compatibility with most materials

Process flexibility

Low temperature sterilizer



Vantagens/Desvantagens

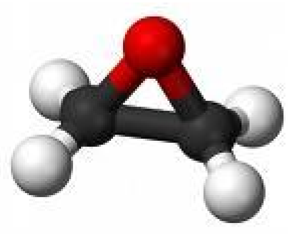
- **Disadvantages**

Toxicity of the sterilizing agent

Process complexity

Process cost

Time taken



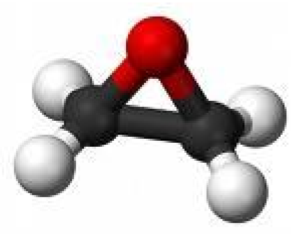
Objectives

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 the inactivation of microorganisms

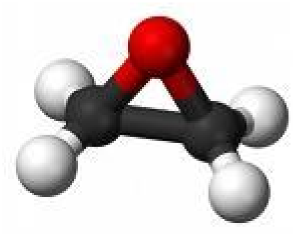
Experimental design

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)

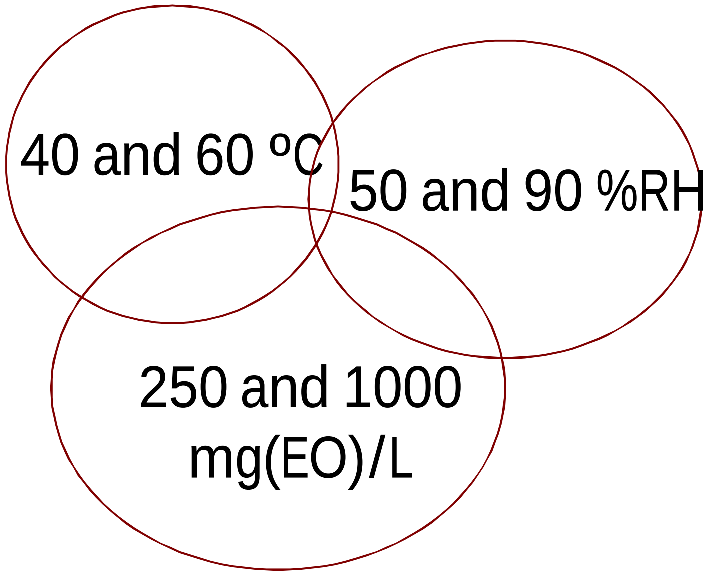
Sterilization cycles

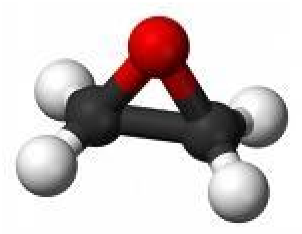


Modelling the inactivation of microorganisms

Sterilization cycles

- Target exposure conditions –

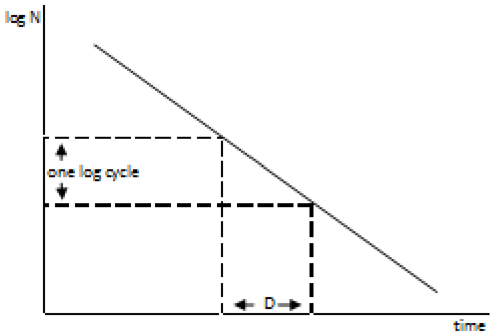




Modelling the inactivation of microorganisms

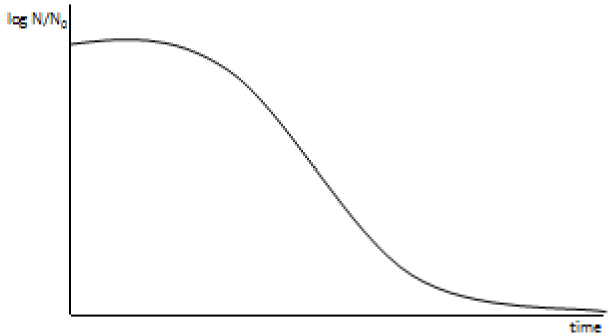
Survival curves construction

1st order kinetics



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

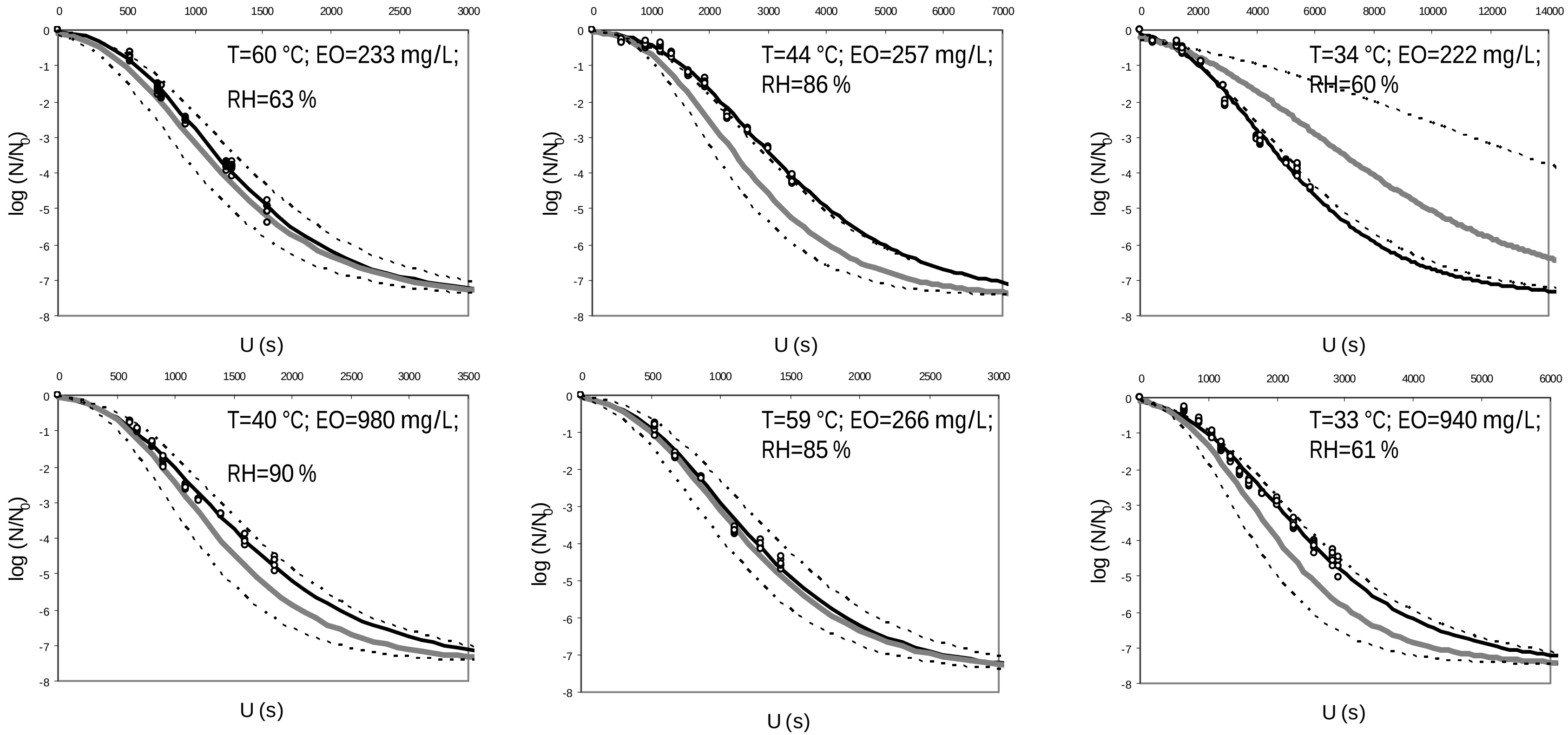
Gompertz model

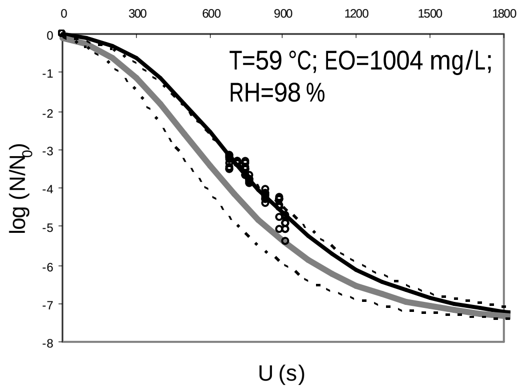


$$\log\left(\frac{N}{N_0}\right) = A \cdot \exp\left[-\exp\left\{\frac{-k_{max}e}{A}(\lambda - t) + 1\right\}\right]$$

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 -





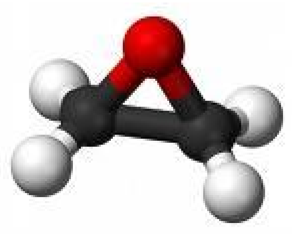
Legend

o Experimental data

___ Fitted Gompertz model

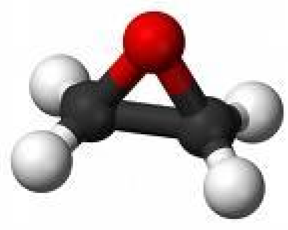
___ Predicted data

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



Data analysis

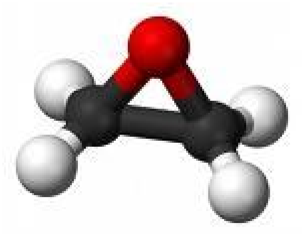
The non-linear regression analysis was carried in Statistica© 6.0 software (StatSoft, USA), using the Levenberg-Marquardt algorithm to minimize the sum of the squares of the differences between the predicted and experimental values.



Data analysis

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

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

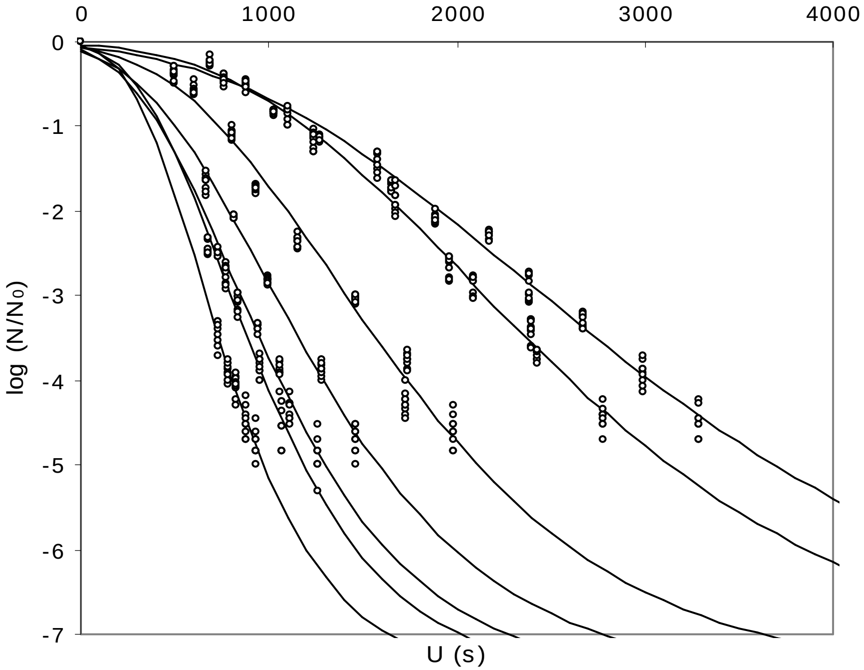


Data analysis and planning future work

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: T=37 °C; EO=674 mg/L; RH=73%

Run 10: T=37 °C; EO=456 mg/L; RH=80%

Run 11: T=51 °C; EO=247 mg/L; RH=80%

Run 12: T=51 °C; EO=447 mg/L; RH=67%

Run 13: T=50 °C; EO=675 mg/L; RH=72%

Run 14: T=60 °C; EO=738 mg/L; RH=71%

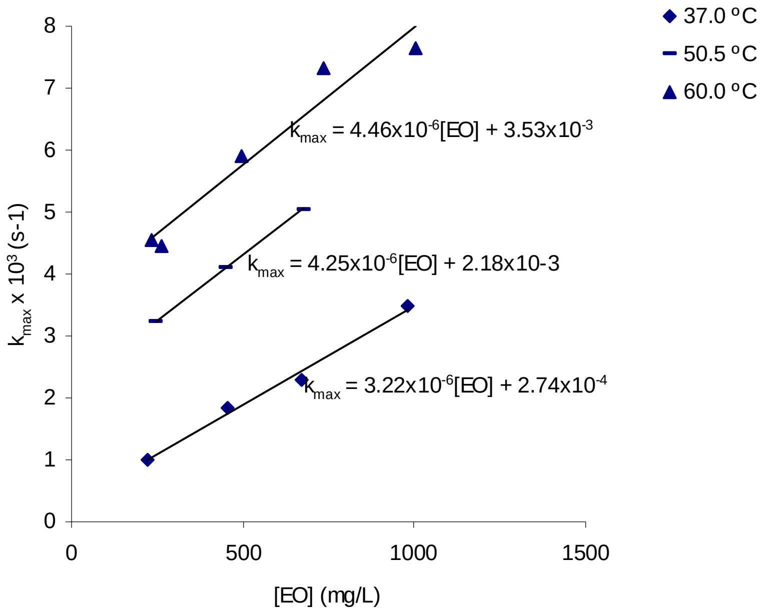
Run 15: T=62 °C; EO=498 mg/L; RH=77%

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

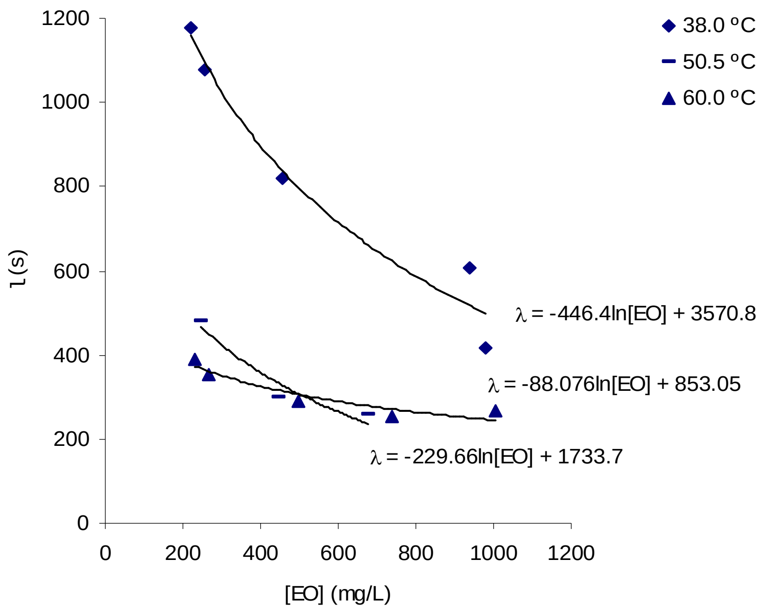
Run	Variables						Parameters				Regression analysis
	T (°C)		[EO] (mg/L)		RH (%)		k_{max} x10^2 (s^{-1})	$SHW_{95\%}$	λ (s)	$SHW_{95\%}$	R^2
1	60	(+)	233	(-)	63	(-)	4.56	3.01	391.22	5.24	0.992
2	44	(-)	257	(-)	86	(+)	1.78	2.42	1079.26	3.25	0.993
3	34	(-)	222	(-)	60	(-)	0.989	2.93	1178.85	7.45	0.988
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 l

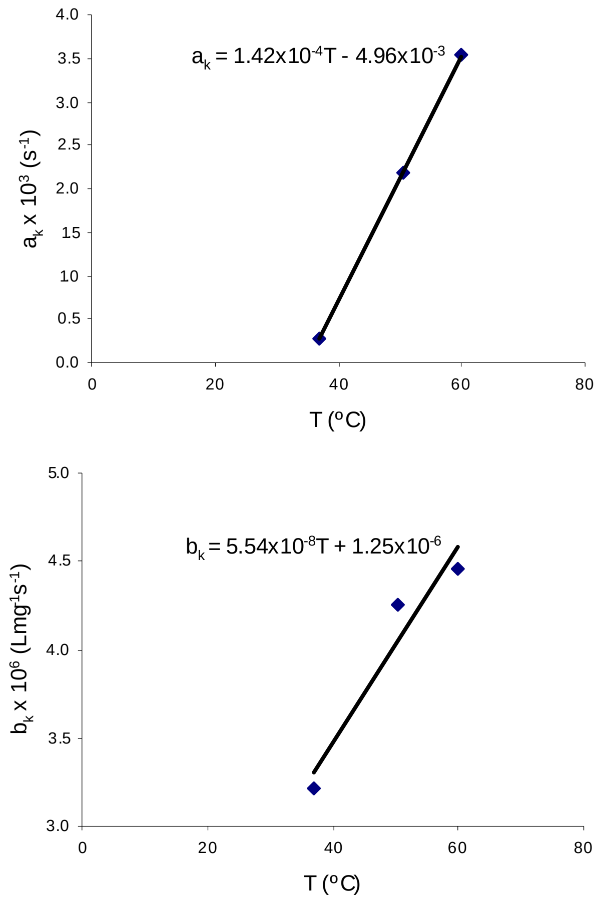


Influence of EO concentration on k_{max} at 37.0, 50.5 e 60.0 °C

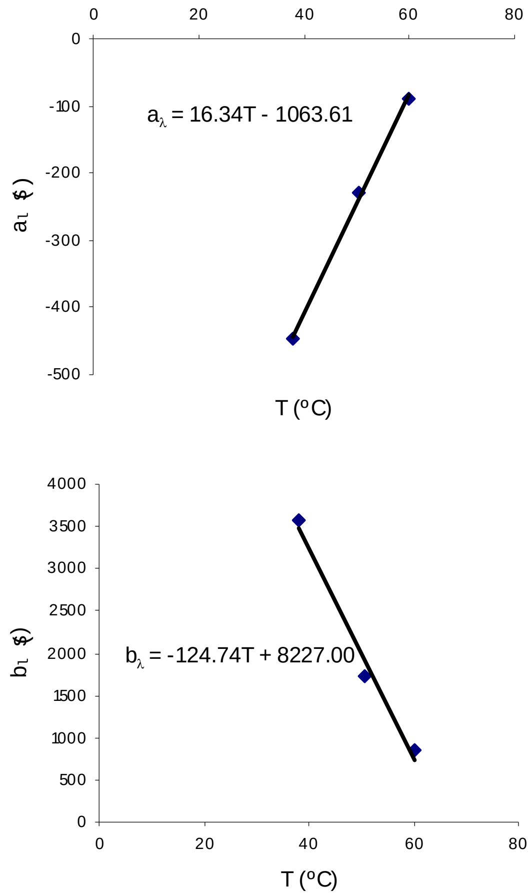


Influence of EO concentration on l at 38.0, 50.5 and 60.0 °C

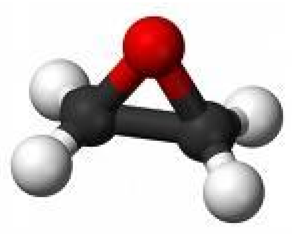
T influence on parameters a_k/b_k and $a_ι/b_ι$



Influence of T on a_k e b_k parameters



Influence of T on a_k e b_k parameters



Data analysis

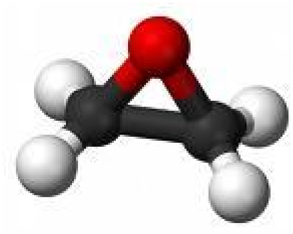
T and EO concentration have a negative effect on l and a positive effect on k_{max}:

- 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

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

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



Thanks’