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

EO sterilization consumption for medical devices

Decade

%
Advantages / Disadvantages

- Advantages

  Effectiveness  Diffusivity
  Bactericidal, fungicidal and virucidal properties

  Compatibility with most materials

  Process flexibility

  Low temperature sterilization
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 –

<table>
<thead>
<tr>
<th>T ($^\circ$C)</th>
<th>RH (%)</th>
<th>EO conc. (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>40 (-)</td>
<td>50 (-)</td>
<td>250 (-)</td>
</tr>
<tr>
<td>40 (-)</td>
<td>50 (-)</td>
<td>1000 (+)</td>
</tr>
<tr>
<td>40 (-)</td>
<td>90 (+)</td>
<td>250 (-)</td>
</tr>
<tr>
<td>40 (-)</td>
<td>90 (+)</td>
<td>1000 (+)</td>
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<tr>
<td>60 (+)</td>
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</tr>
<tr>
<td>60 (+)</td>
<td>90 (+)</td>
<td>1000 (+)</td>
</tr>
</tbody>
</table>
Modelling microorganisms inactivation

Survival curves construction

1\textsuperscript{st} order kinetics

\[ \log N = -k \cdot t + \log N_0 \]

Gompertz model

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

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**
- 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 $k_{\text{max}}$ and $\lambda$ 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

Run 9 to Run 15

Legend
- Experimental data
- Fitted Gompertz model
Estimated $k_{\text{max}}$ and $l$ parameters of *B. subtilis* inactivation at the temperature, EO concentration and relative humidity conditions tested.

<table>
<thead>
<tr>
<th>Run</th>
<th>Variables</th>
<th>Parameters</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>T ($^\circ$C)</td>
<td>[EO] (mg/L)</td>
</tr>
<tr>
<td>1</td>
<td>60</td>
<td>233</td>
</tr>
<tr>
<td>2</td>
<td>44</td>
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<td>738</td>
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<tr>
<td>15</td>
<td>62</td>
<td>498</td>
</tr>
</tbody>
</table>

Meaningless value
EO concentration influence on $k_{\text{max}}$ and $\lambda$

Influence of EO concentration on $k_{\text{max}}$ at 37.0, 50.5 and 60.0 °C

Influence of EO concentration on $\lambda$ at 38.0, 50.5 and 60.0 °C
Influence of $T$ on $a_k/b_k$ and $a_\lambda/b_\lambda$ parameters

\[
a_k = 1.42 \times 10^{-4} T - 4.96 \times 10^{-3}
\]

\[
b_k = 5.54 \times 10^{-4} T + 1.25 \times 10^{-6}
\]

\[
a_\lambda = 16.34 T - 1063.61
\]

\[
b_\lambda = -124.74 T + 8227.00
\]
Data analysis

T and EO concentration have a negative effect on $\lambda$ and a positive effect on $k_{\text{max}}$:

- 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

$$\log\left( \frac{N}{N_0} \right) = (-7.5)\exp\left\{-\exp\left\{-\left[\frac{1.42 \times 10^{-4}T - 4.96 \times 10^{-3}}{5.54 \times 10^{-8}T + 1.25 \times 10^{-6}}[\text{EO}] \right]e^{-7.5} \times \left[\frac{1.63 \times 10^1T - 1.06 \times 10^3}{\ln([\text{EO}]) + \left(-1.25 \times 10^2T + 8.23 \times 10^3\right) - U} + 1 \right]\right\}\right\}$$
Inactivation of *B. subtilis* spores by EO sterilization
- Conditions defined according to the $2^3$ factorial design -

**Legend**
- Experimental data
- Fitted Gompertz model
- Predicted data
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In conclusion

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

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

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’