Workshop

Predictive Microbiology

Aplicações e Novas Fronteiras

Modelos para inactivação de microrganismos em alimentos

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Modelagem matemática

alguns conceitos ...

$$y = f(x, q) + e$$

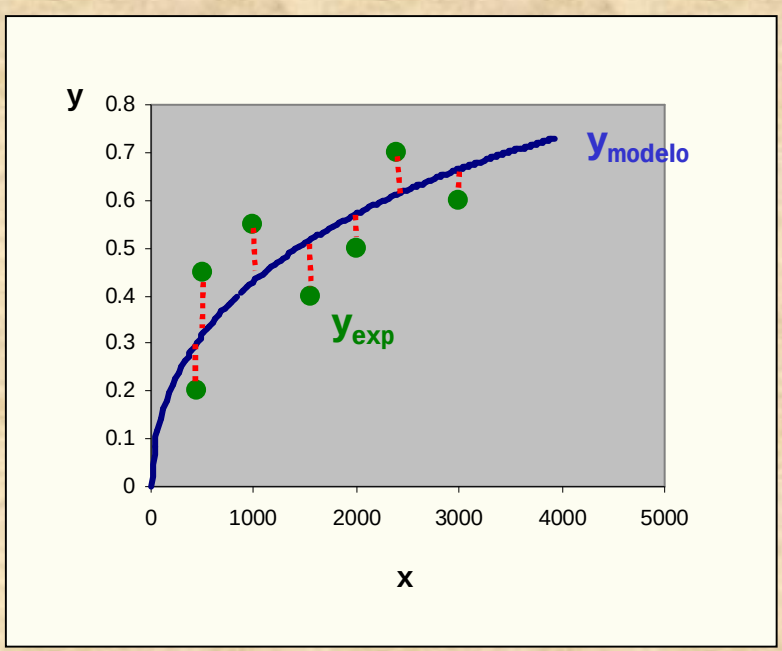
Estimativa dos parâmetros

minimização dos desvios entre os valores **experimentais** e os obtidos pelo **modelo**

q^*

Precisas ?

Exactas ?



Modelagem matemática

objectivo

descrição precisa e exacta das observações

adequabilidade do modelo

qualidade dos parâmetros

Modelagem matemática

modelos mecanísticos

- descrição fundamental dos processos físico-químicos envolvidos
- mais complexos

modelos empíricos

- *caixa preta*
- mais simples (ou não!)
- aplicação prática

ponderar **vantagens** e **desvantagens**

decisão depende do **objectivo final**

Modelagem matemática

complexidade matemática $\not\Longleftrightarrow$ **descrição adequada**

qualidade

modelo

parâmetros

vantagens

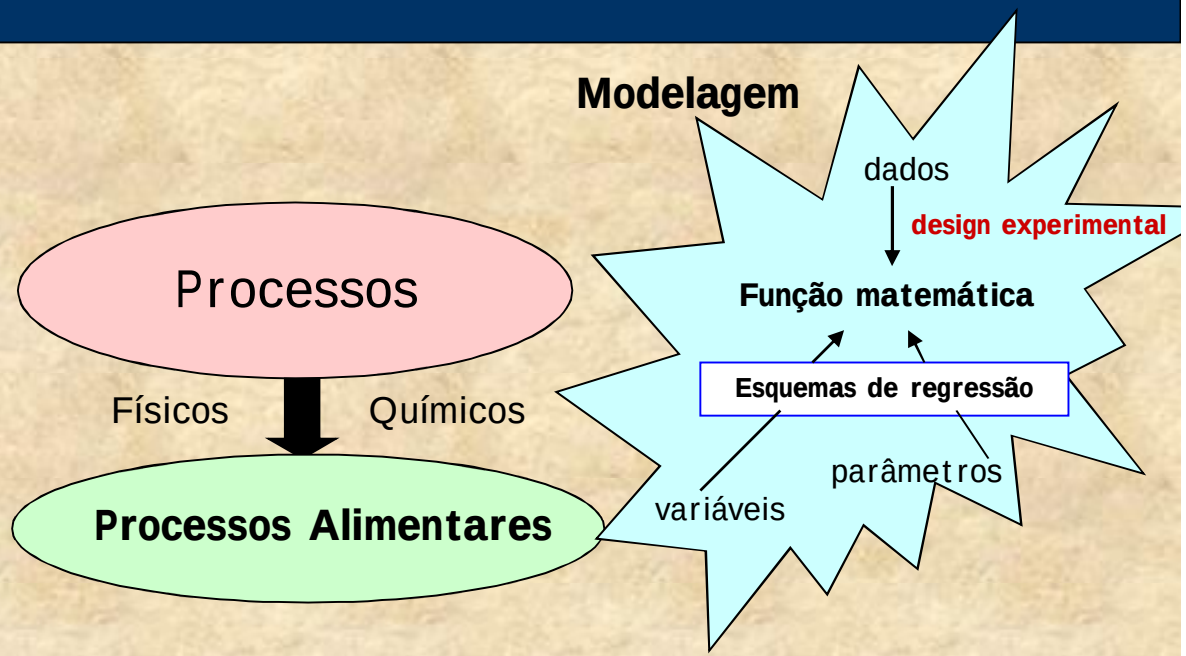
- conhecimento do processo
- efeito do processo no produto
- controlo das variáveis envolvidas

Modelagem matemática

Fenómenos de Transferência
- calor
- massa
- *momentum*

Cinéticas de reacção

Propriedades



Modelagem matemática

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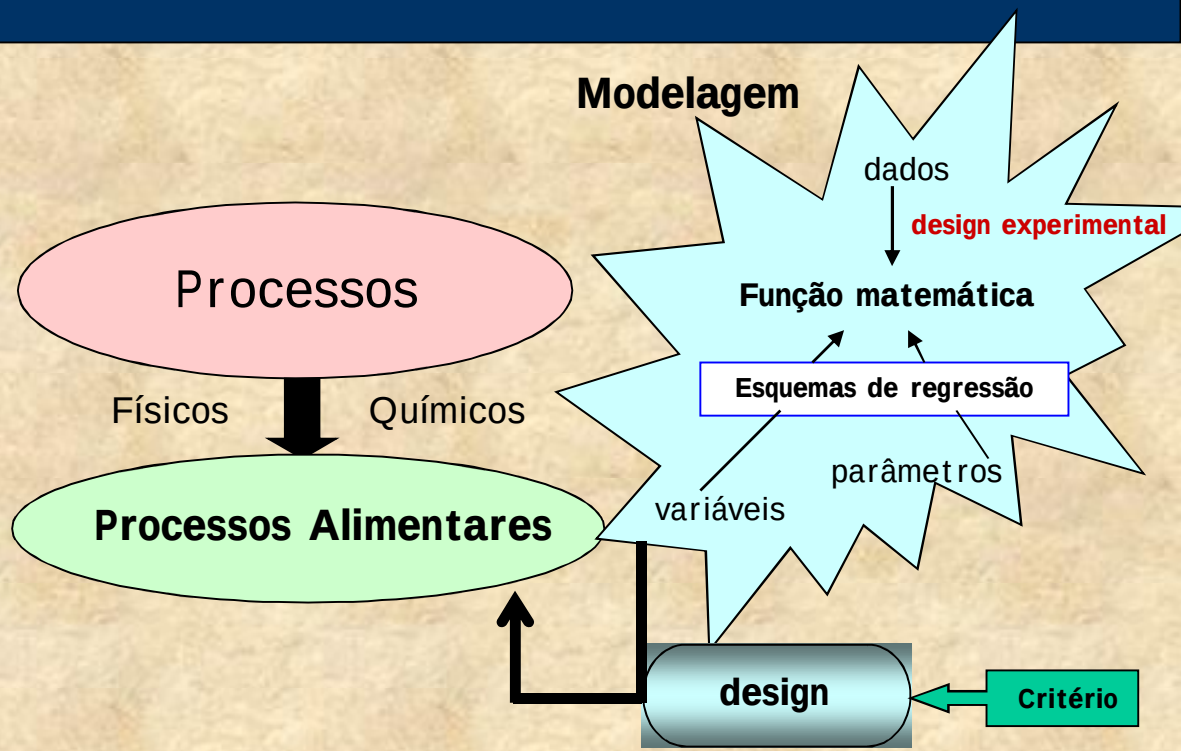

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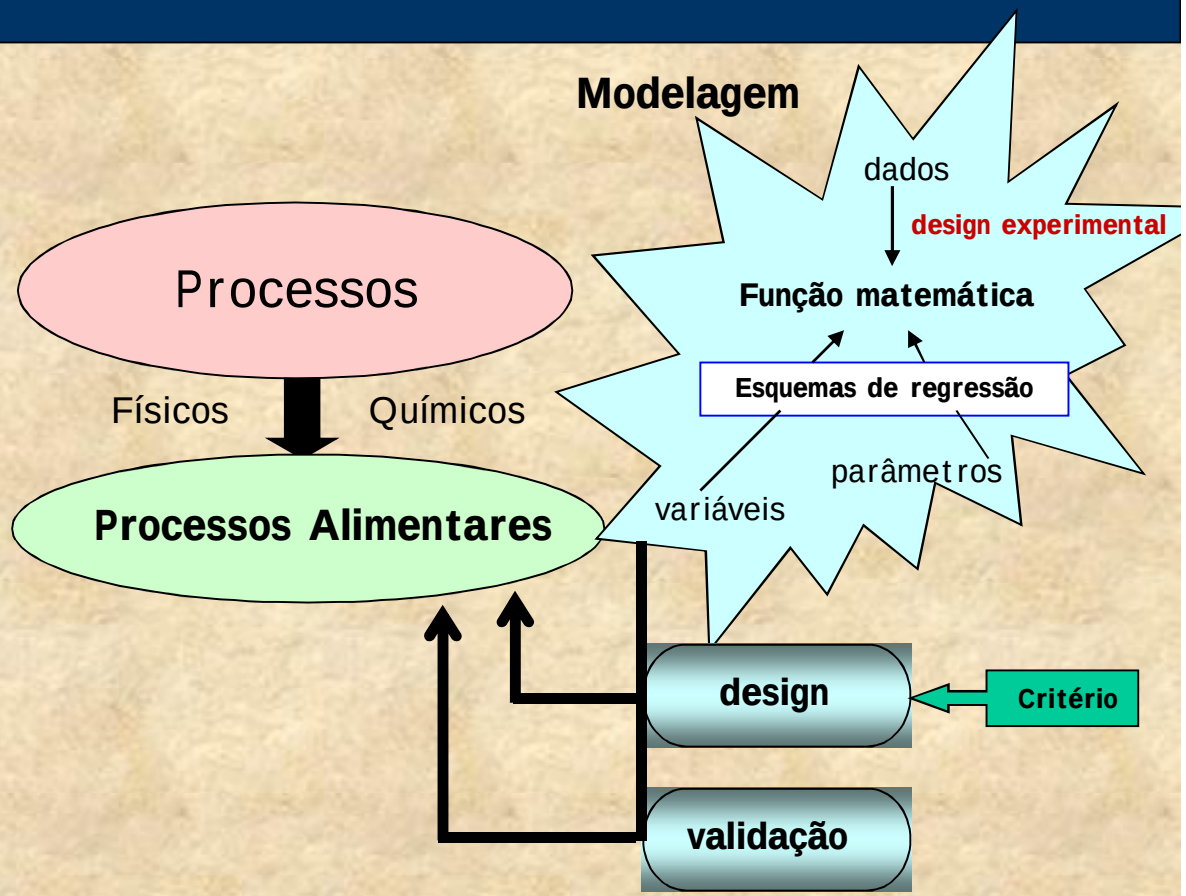

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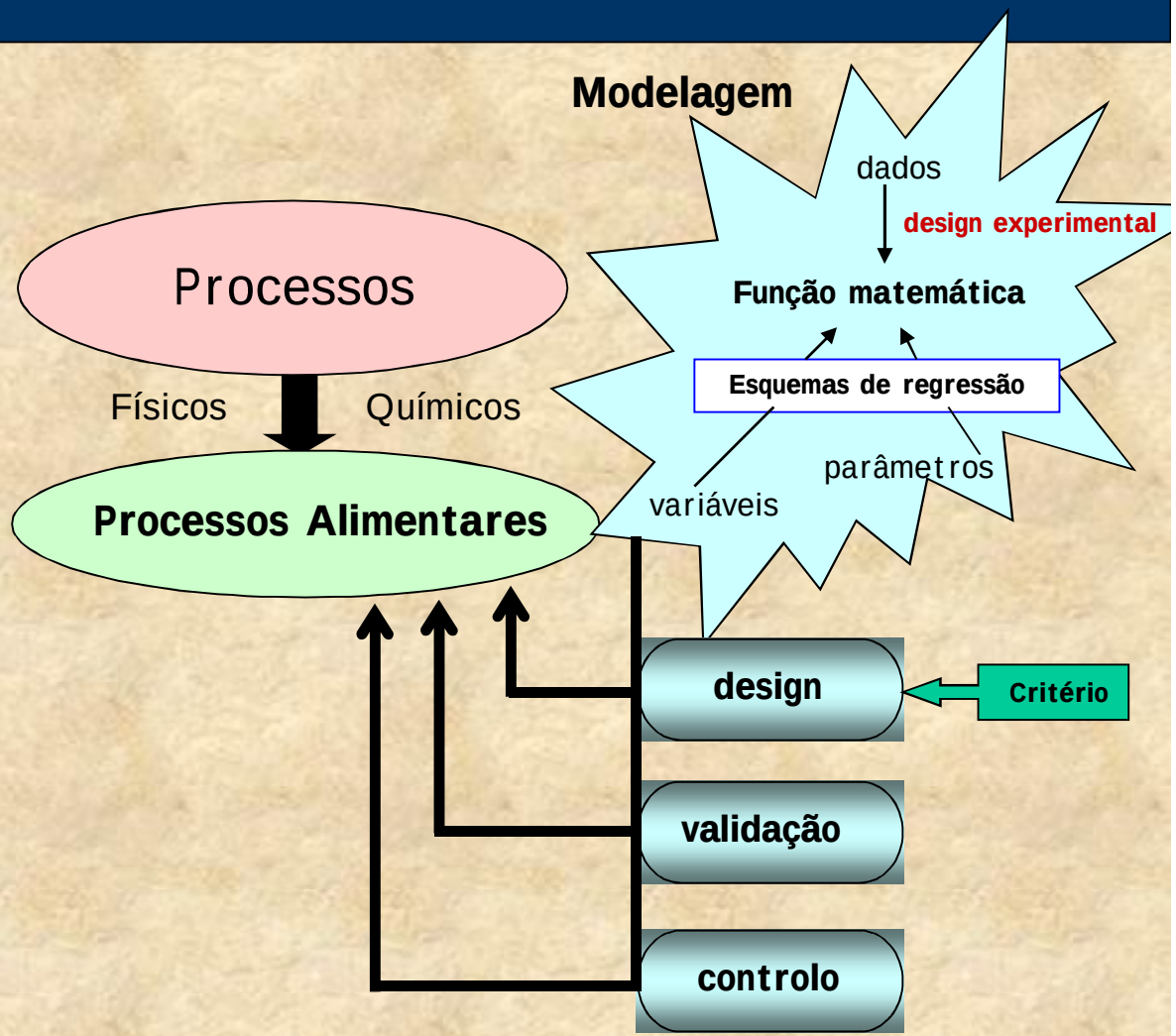

Modelagem matemática

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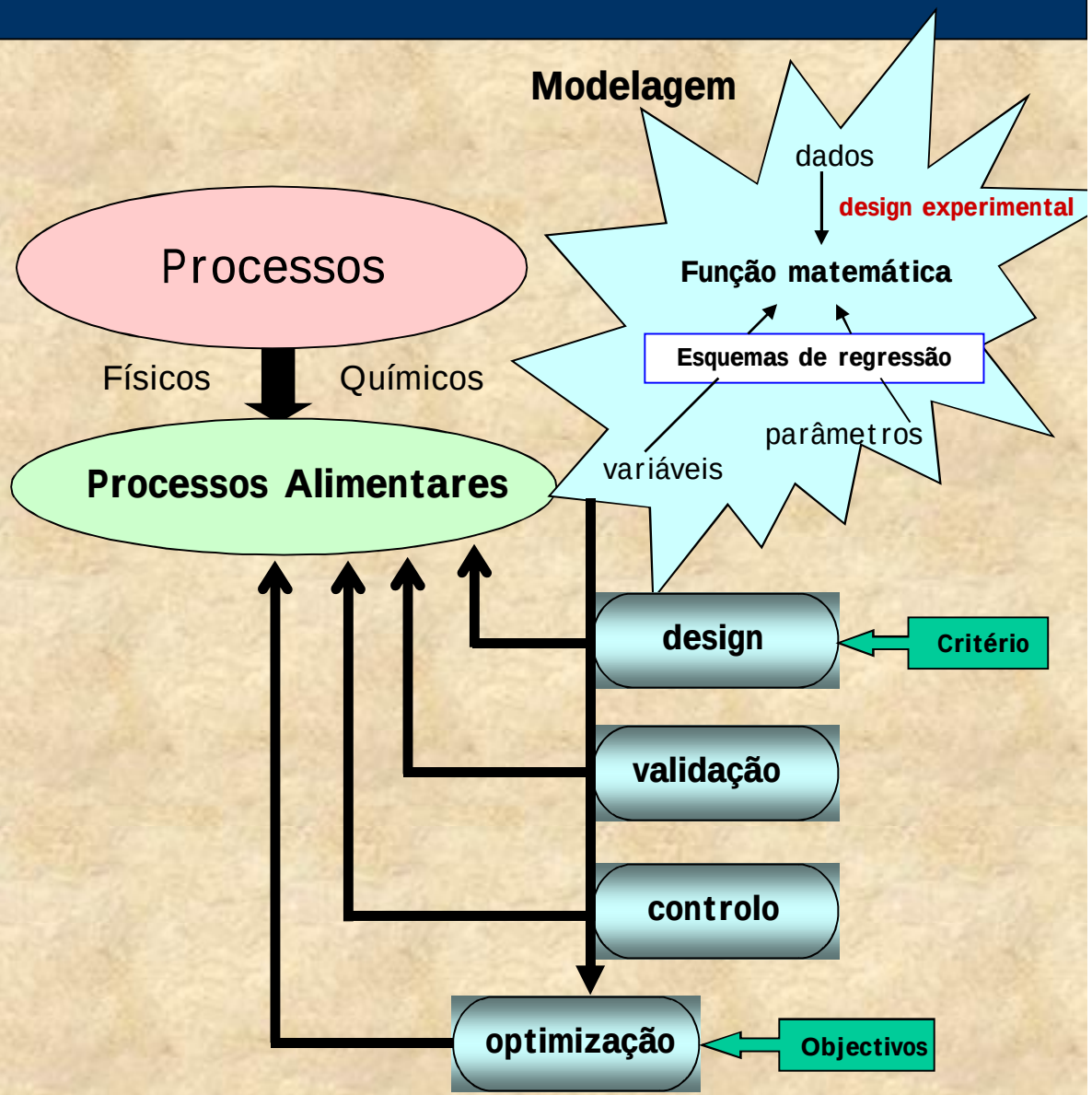

Modelagem matemática

Fenómenos de Transferência
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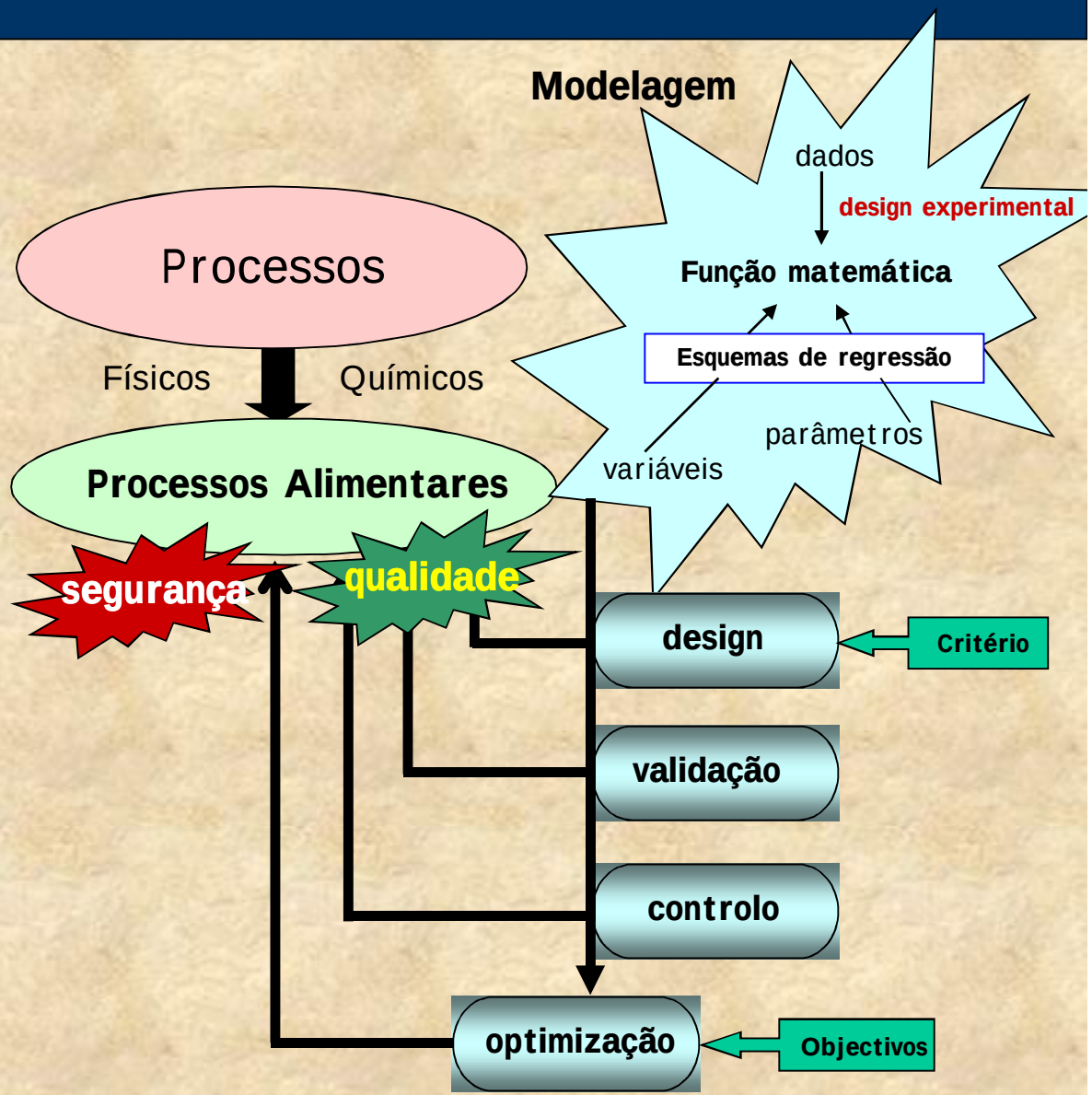


Modelagem matemática

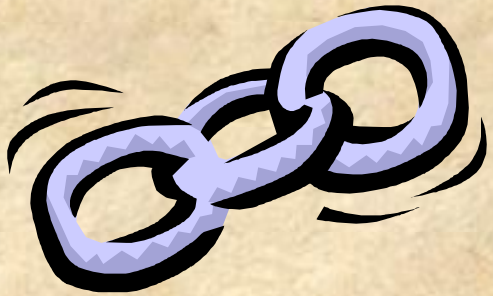
segurança

microbiologia predictiva

Modelagem matemática

microbiologia predictiva

microbiologia



estatística

matemática

aplicação

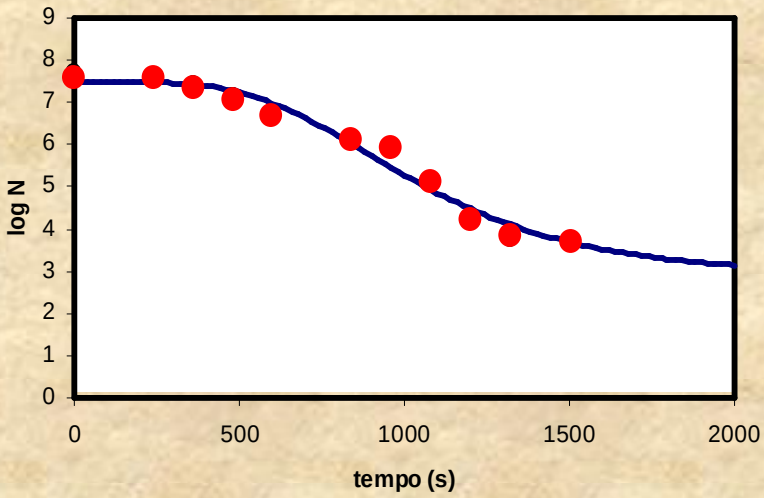
- previsão / simulação → rapidez
- desenvolvimento de processos eficazes de inactivação

↓

contribuição para **segurança**

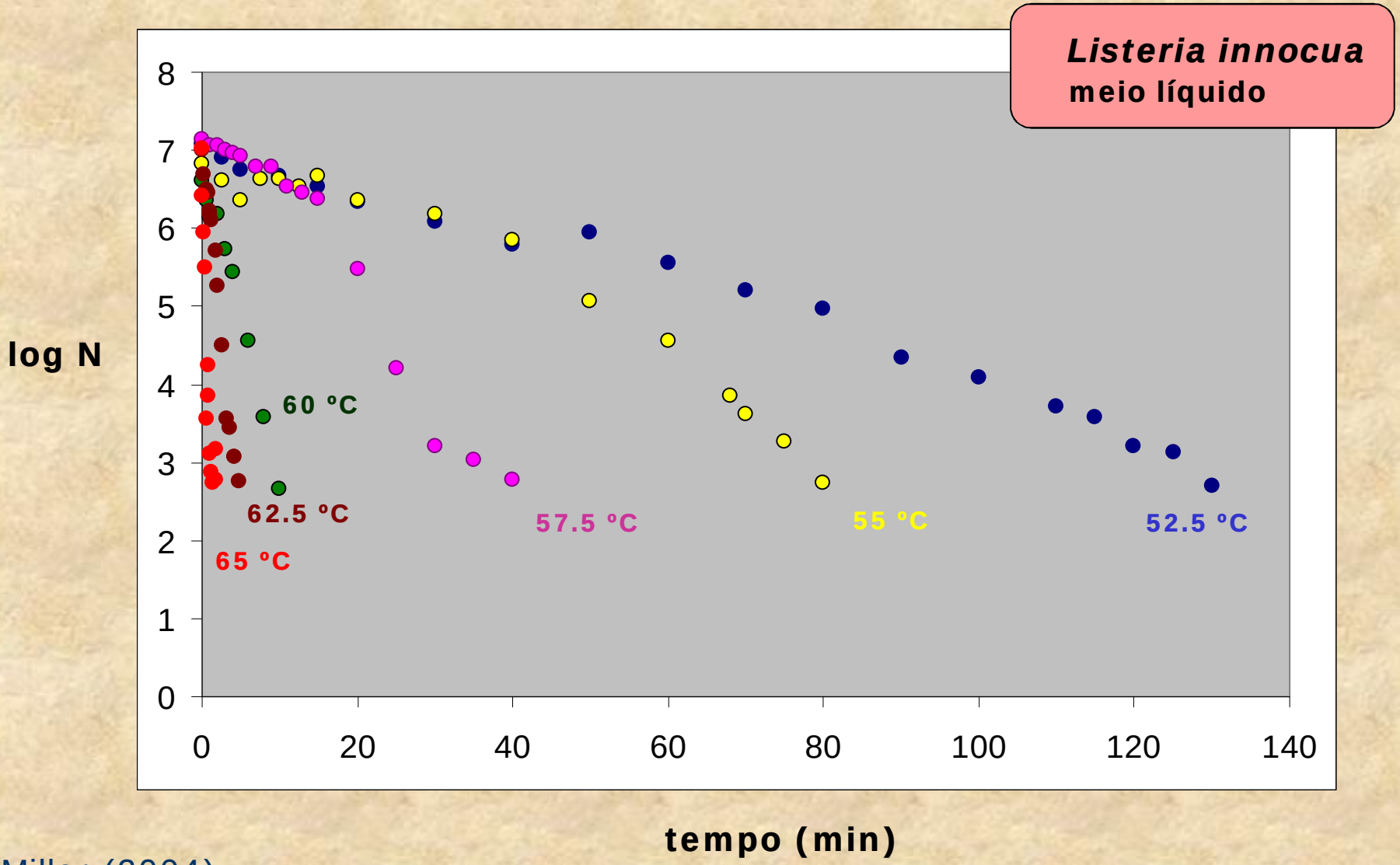
inactivação

comportamento sigmoidal



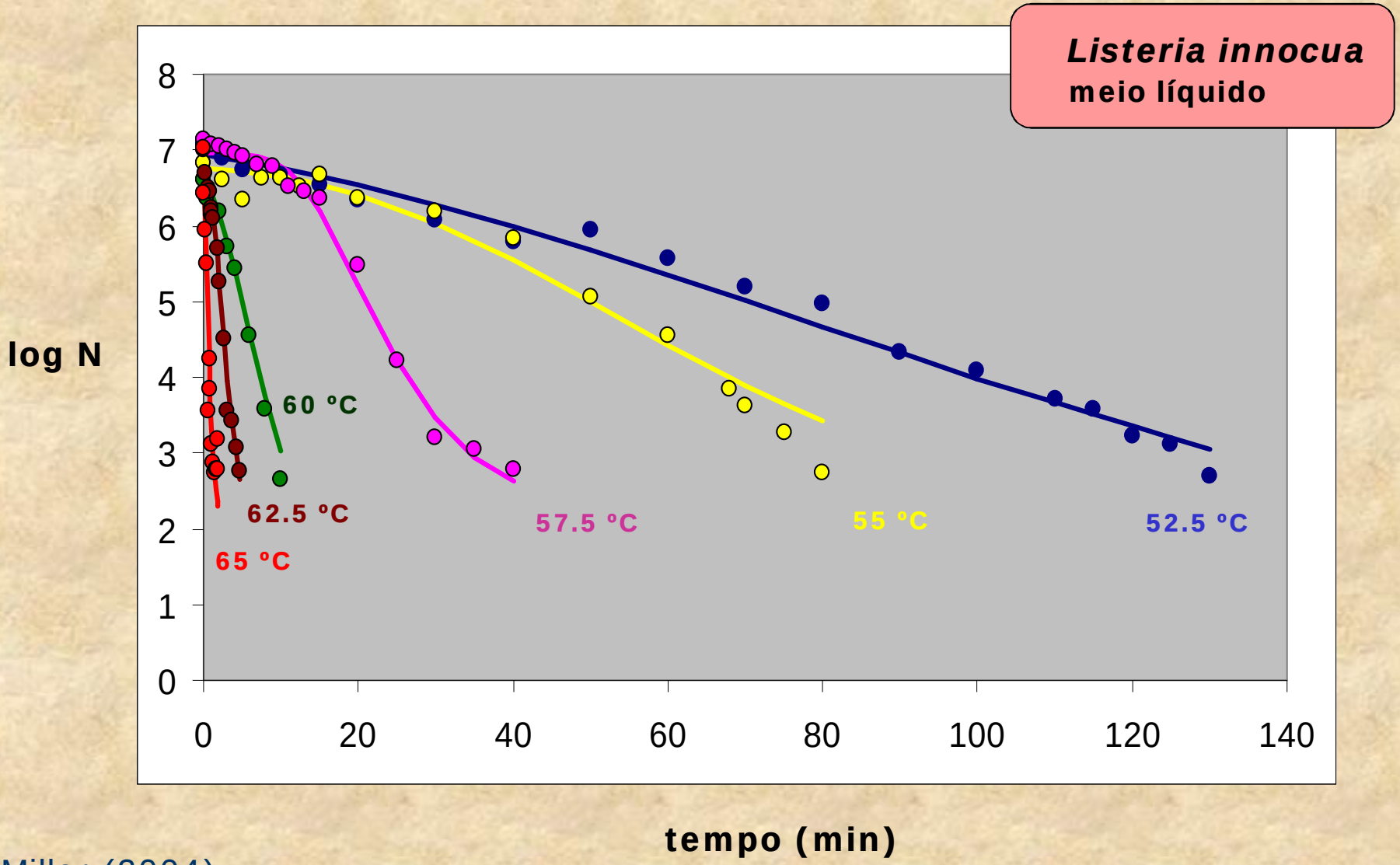
reflecte a presença de *agregados* de microrganismos ou sub populações mais **resistentes** à **temperatura** (ou outro **factor adverso**)

Exemplos



Miller (2004)

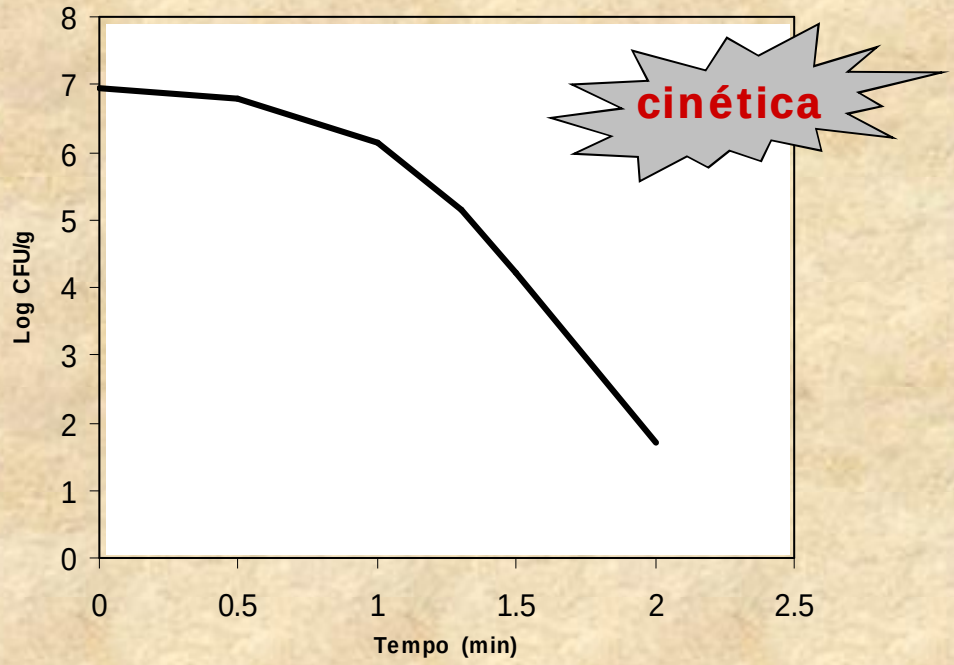
Exemplos



Miller (2004)

Modelos matemáticos

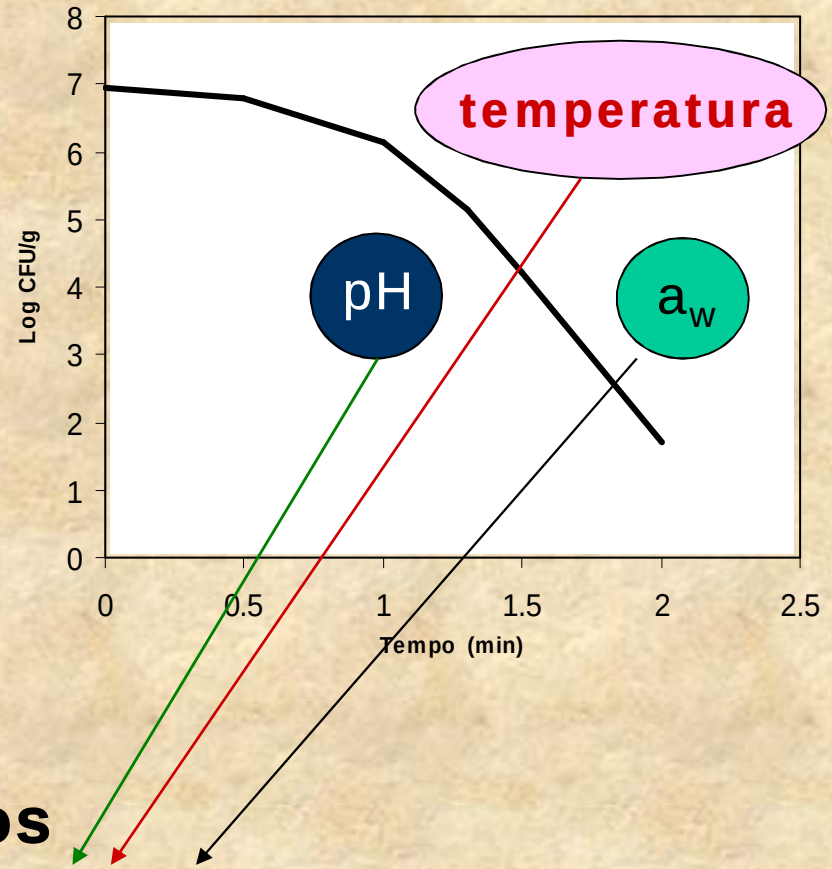
- **primários**



parâmetros

Modelos matemáticos

- **primários**

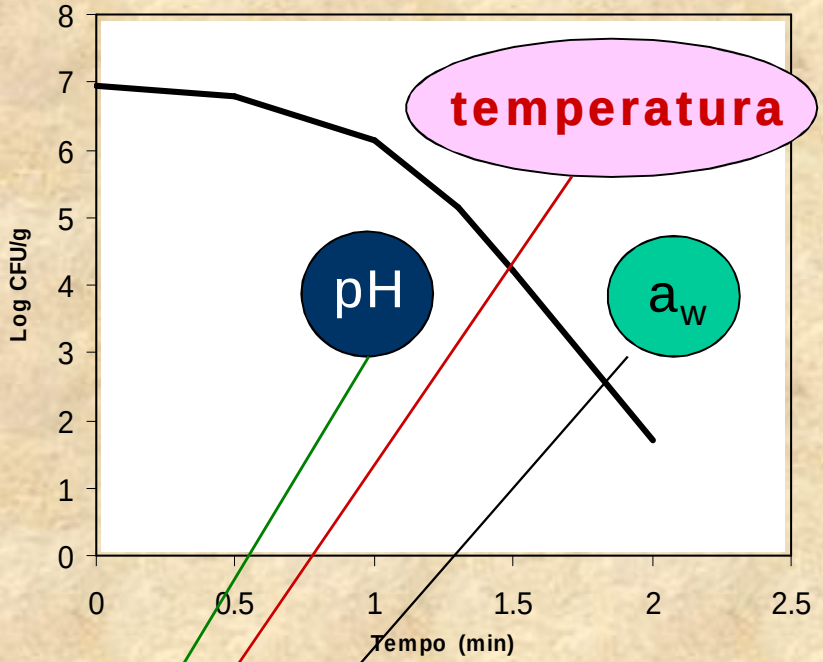


- **secundários**

  parâmetros

Modelos matemáticos

- **primários**



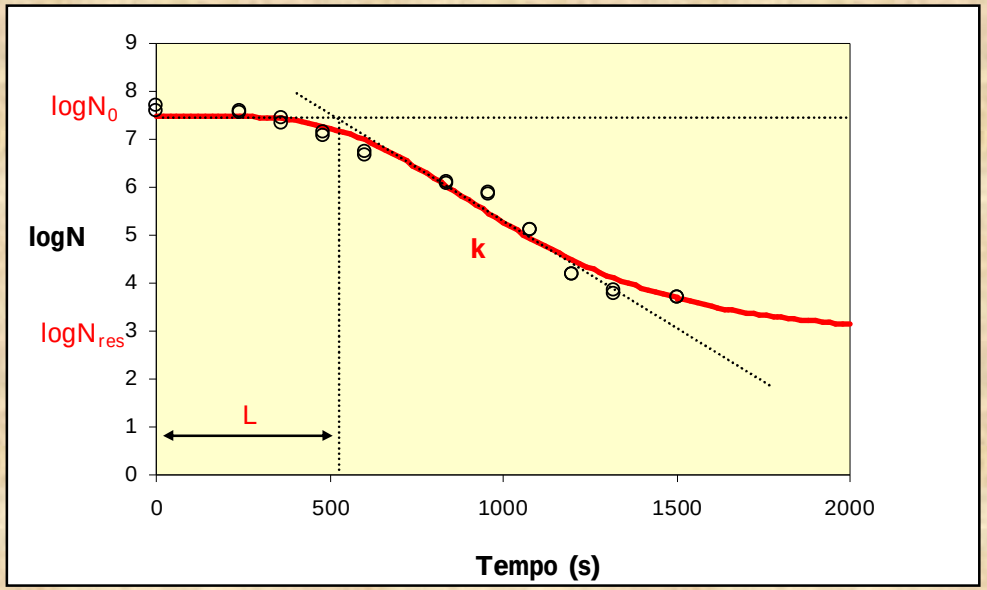
- **secundários**

  parâmetros

- **terciários -** integração dos modelos anteriores - **software**

Modelos de inactivação

- **primários**

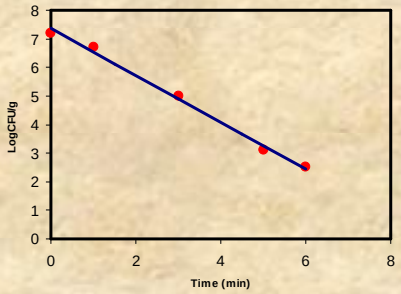


empíricos

fundamentais

Modelos de inactivação

❖ primários



$$N = N_0 \exp(-kt)$$

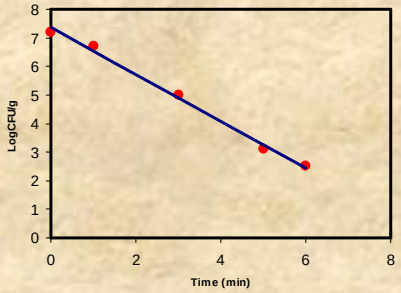
$$\log N = \log N_0 - \frac{t}{D}$$

1ª ordem

D – tempo de redução decimal

Modelos de inactivação

❖ primários



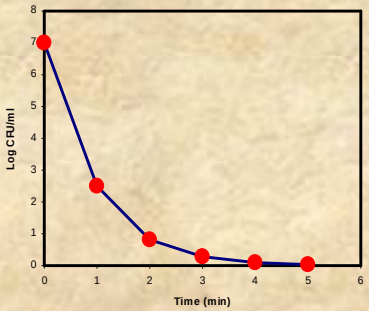
$$N = N_0 \exp(-kt) \Leftrightarrow \log N = \log N_0 - \frac{t}{D}$$

1ª ordem

D – tempo de redução decimal

$$\frac{N}{N_0} = F_1 \exp(-k_1 t) + (1 - F_1)\exp(-k_2 t)$$

Cerf (1977)



bifásicos

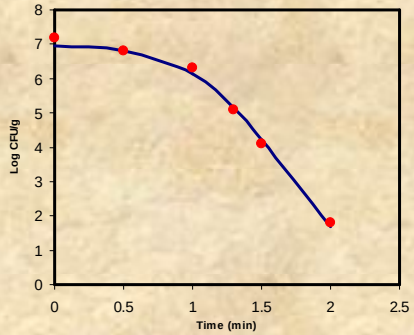
F_1 – fracção de microrganismos inactivados
k_1 e k_2– constantes cinéticas

$$\log \frac{N}{N_0} = \log\left(\frac{2F_1}{1 + \exp(k_1 t)} + \frac{2(1 - F_1)}{1 + \exp(k_2 t)}\right)$$

Kamau et al. (1990)

Modelos de inactivação

- **primários**



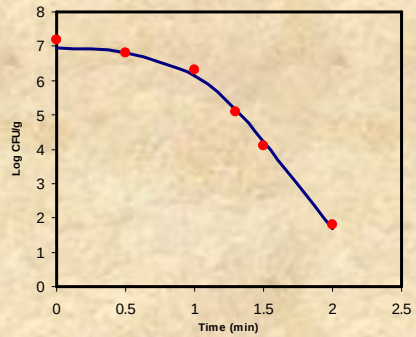
Whiting & Buchanan (1992)

$$\log\frac{N}{N_0} = \log\left(\frac{F_1\left(1+\exp\left(-k_1L\right)\right)}{1+\exp\left(k_1\left(t-L\right)\right)} + \frac{\left(1-F_1\right)\left(1+\exp\left(-k_2L\right)\right)}{1+\exp\left(k_2\left(t-L\right)\right)}\right)$$

L – atraso

Modelos de inactivação

❖ **primários**



Whiting & Buchanan (1992)

$$\log \frac{N}{N_0} = \log\left(\frac{F_1(1+\exp(-k_1 L))}{1+\exp(k_1(t-L))} + \frac{(1-F_1)(1+\exp(-k_2 L))}{1+\exp(k_2(t-L))}\right)$$

L – atraso

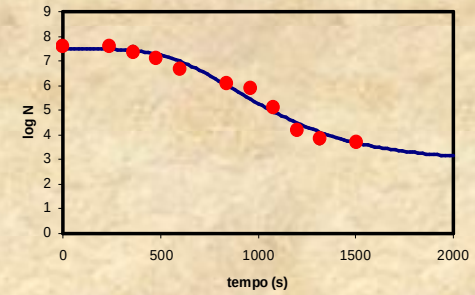
Cole et al. (1993)

$$\log N = \alpha + \frac{w-\alpha}{1+\exp\left(\frac{4\sigma(\lambda-\log t)}{w-\sigma}\right)}$$

Distribuição da sensibilidade ao calor da população dos microrganismos

Modelos de inactivação

❖ **primários**



Baranyi et al. (1993)

$$\frac{dN}{dt} = -k\,\alpha(t)\,\beta(t)\,N$$

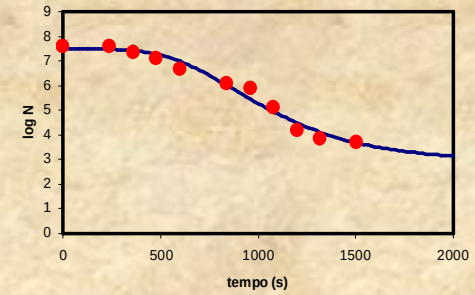
$$N(t=0) = N_0$$

função 'cauda'

função 'atraso'

Modelos de inactivação

❖ **primários**



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função 'cauda'

função 'atraso'

Baranyi et al. (1993)

Geeraerd et al. (2000)

$$\frac{dN}{dt} = -k_{max}\,k_Q(Q)\,N$$

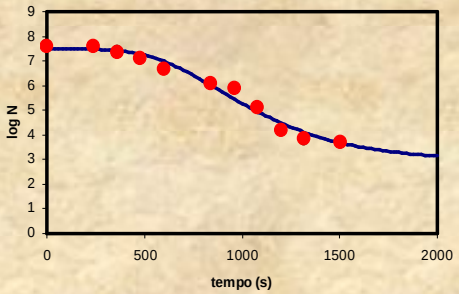
$$\frac{dQ}{dt} = -k_{max}\,Q$$

$$\log\left(\frac{N}{N_0}\right) = \log\left(\exp\left(-k_{max}t\right)\right)\frac{1+Q(0)}{1+Q(0)\exp\left(-k_{max}t\right)}$$

Q – variável relacionada com o estado fisiológico dos microrganismos

Modelos de inactivação

❖ **primários**



Gompertz

Bhaduri et al (1991)
Linton et al. (1995, 1996)
Xiong et al. (1999)

↓

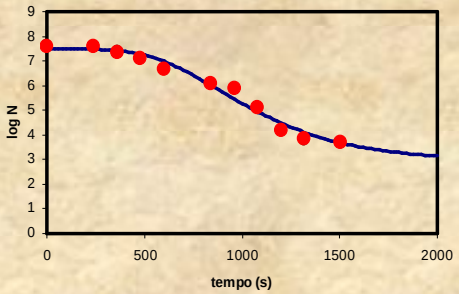
Listeria monocytogenes

$$\log N = \log N_0 - \log\left(\frac{N_0}{N_{res}}\right)\exp\left(-\exp\left(\frac{k\,e}{\log\left(\frac{N_0}{N_{res}}\right)}(L - t) + 1\right)\right)$$

reparameterização para inactivação com base em Zwitering (1990)

Modelos de inactivação

❖ primários



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reparameterização para inactivação com base em Zwitering et al. (1990)

Logística

$$\log N = \frac{c}{1 + \exp(k(t - L))}$$

c – constante

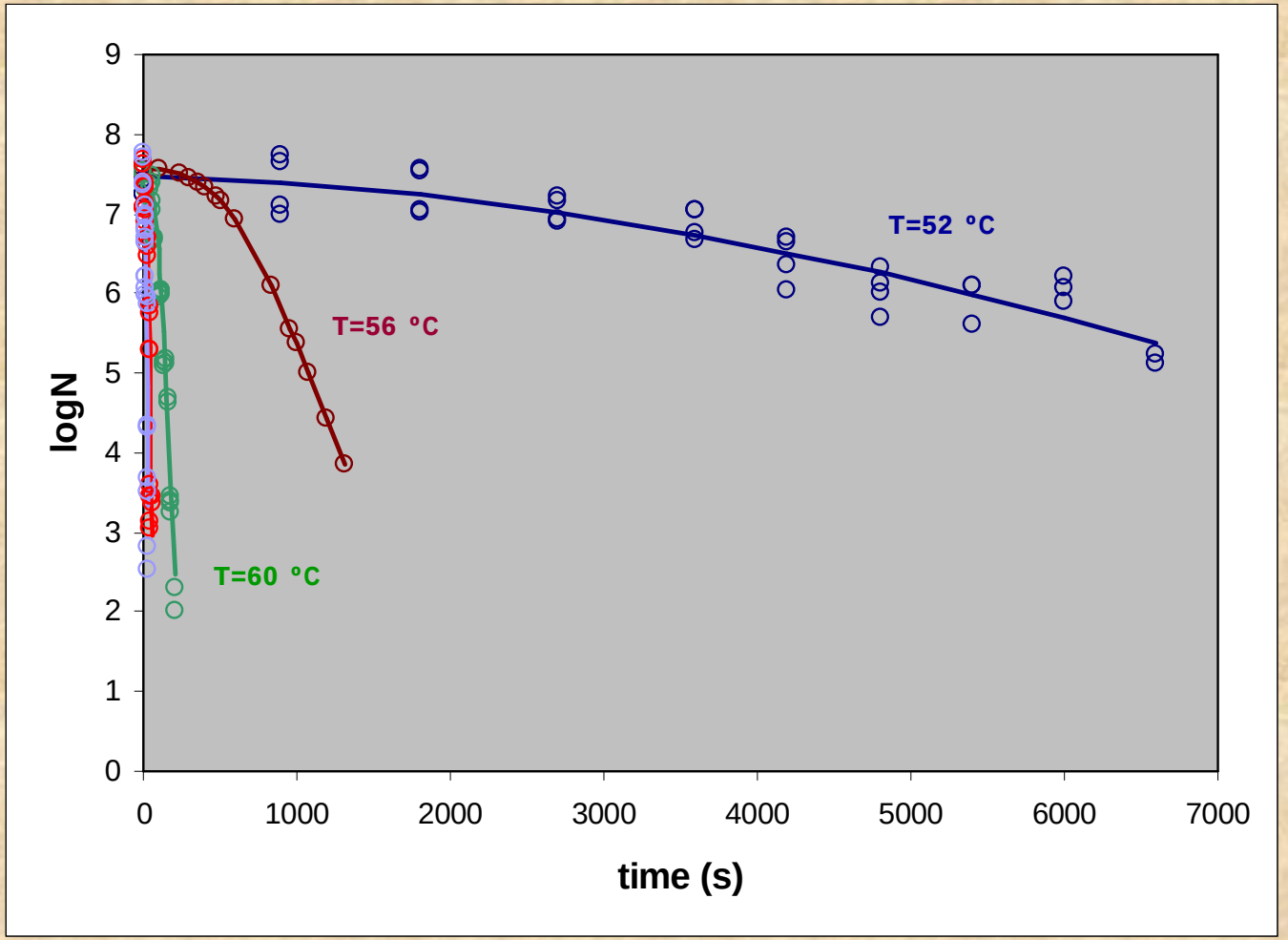
Exemplos

Data of *L.monocytogenes* Scott A at 52,56,60,64,68°C

(24 hours incubation at 5°C in half cream)

Casadei et al. (1998)

Gompertz



Gil (2002)

Statistica 6.0

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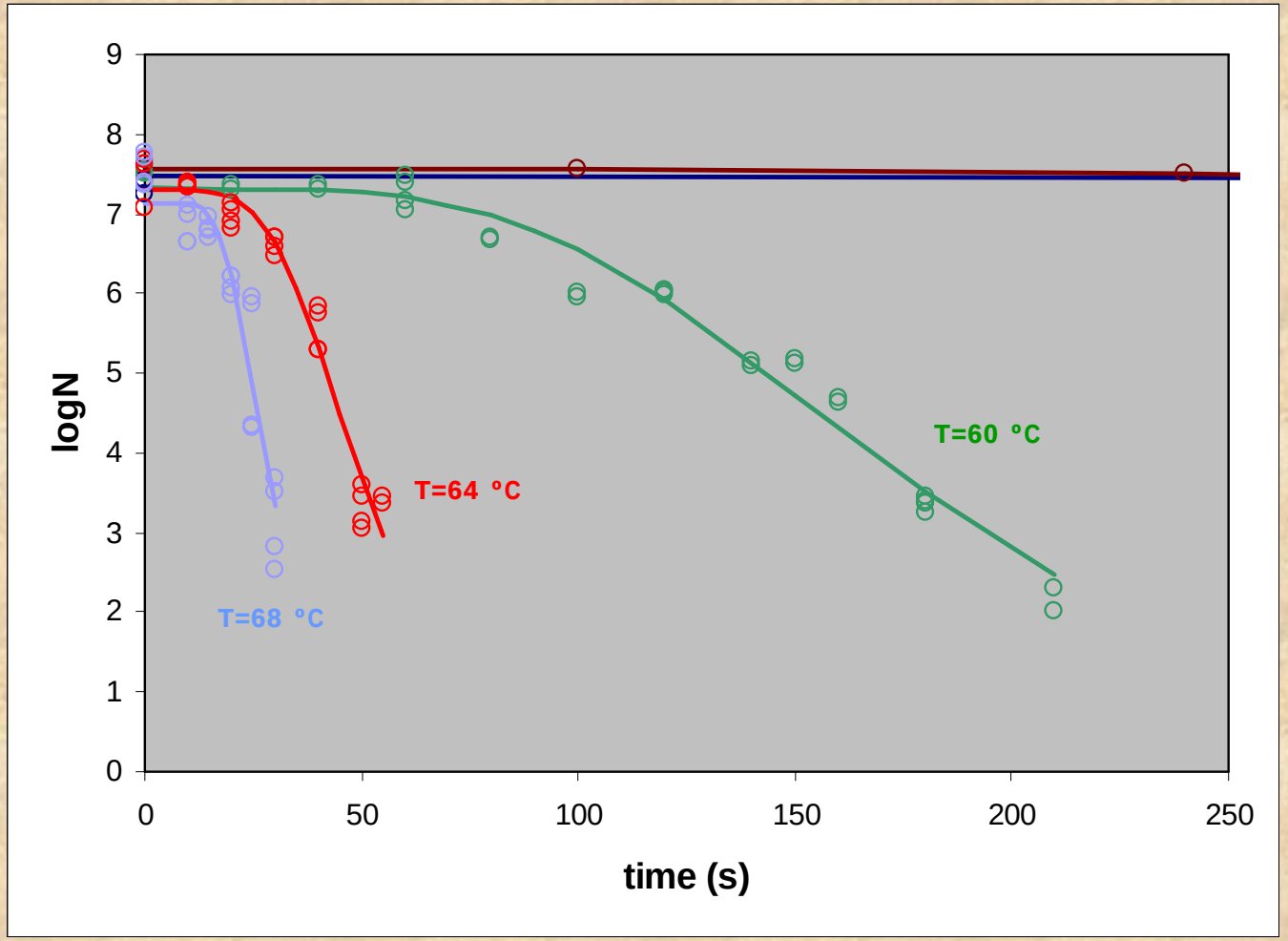

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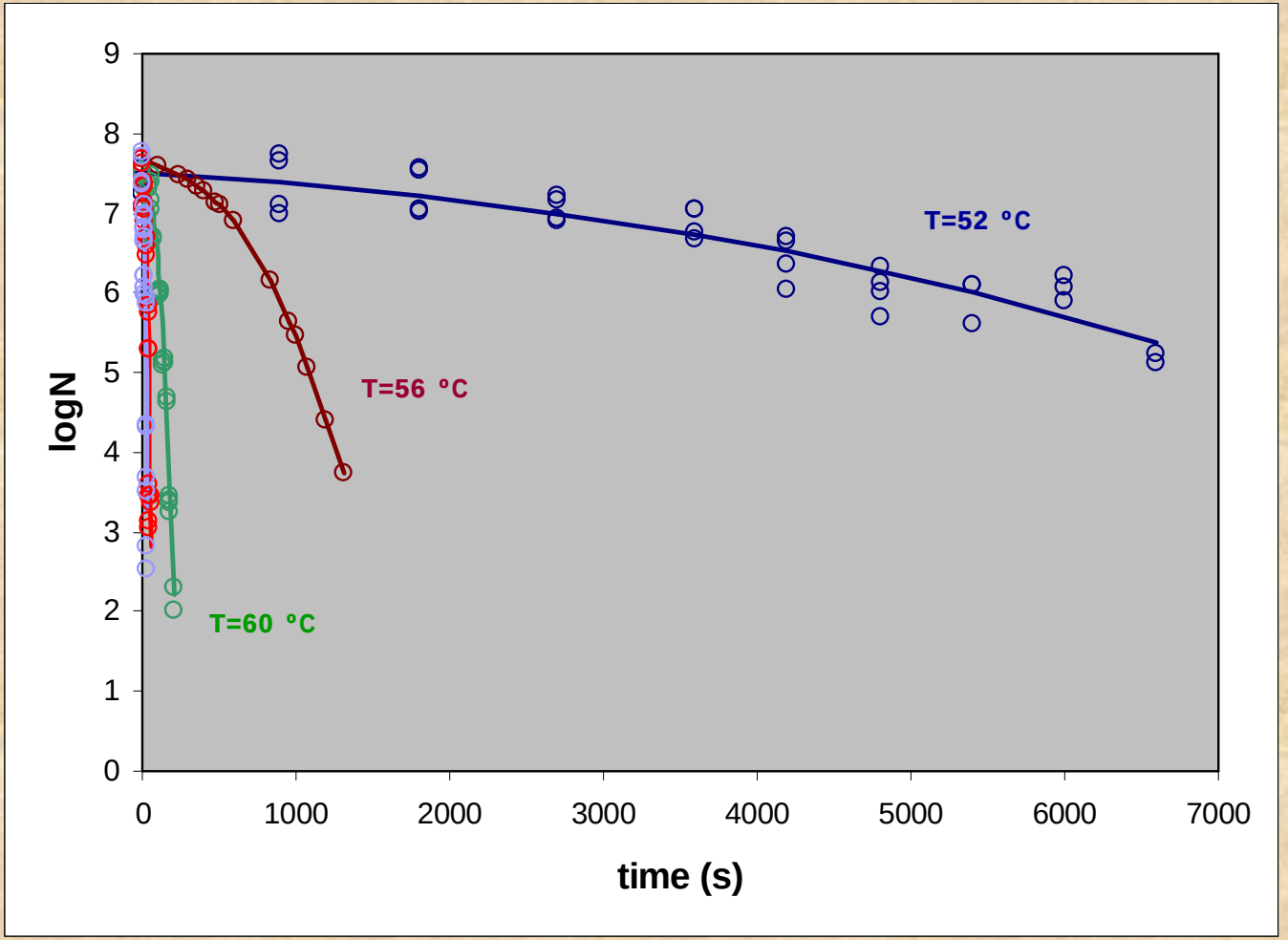

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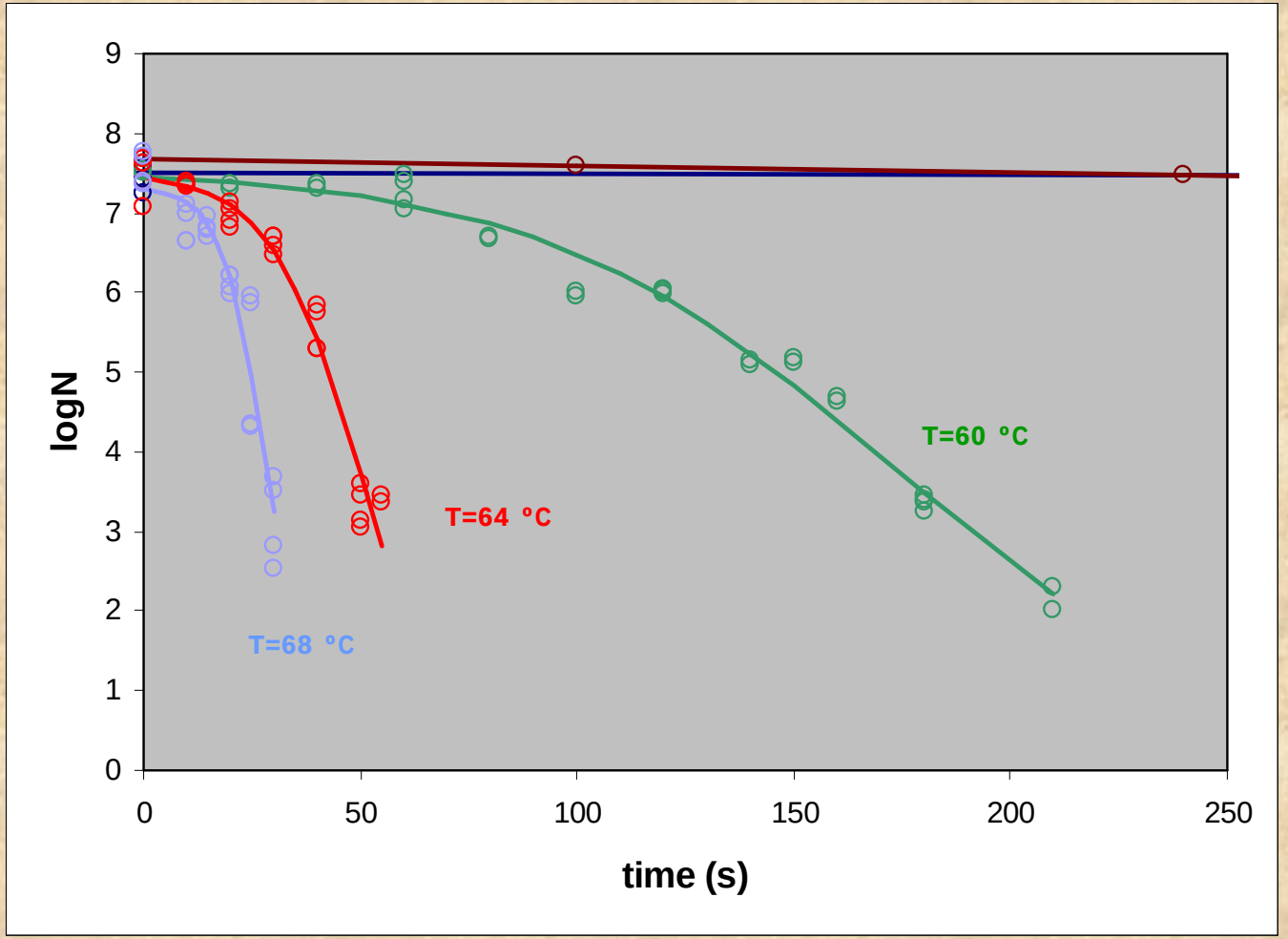

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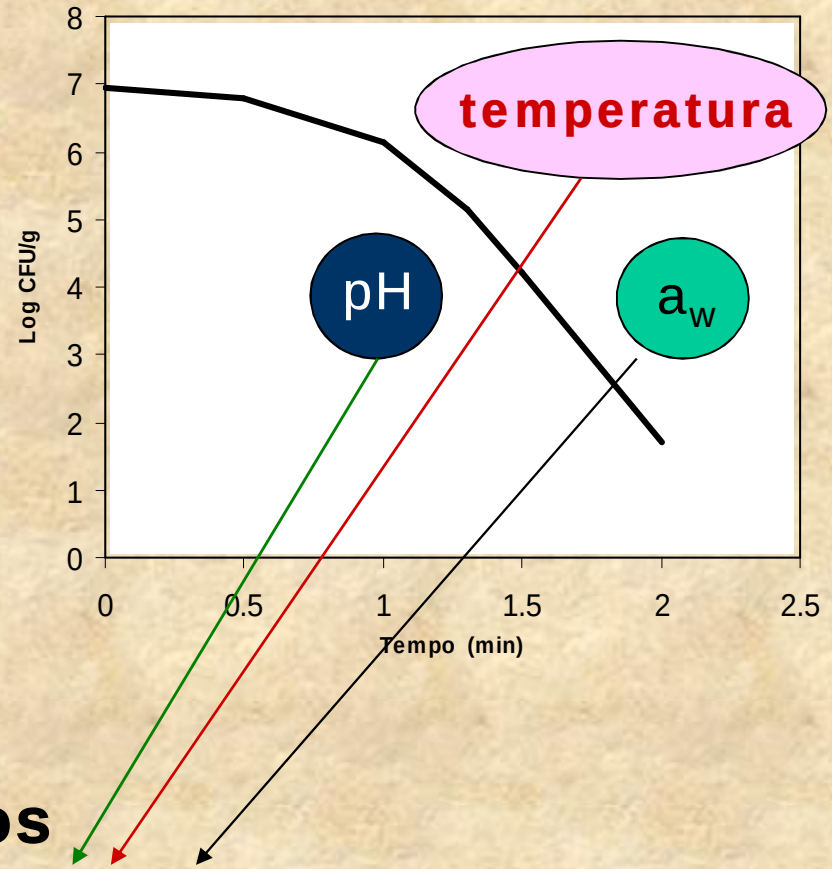
Statistica 6.0

Modelos matemáticos

- **primários**



- **secundários**

  parâmetros

Modelos matemáticos

❖ secundários

Arrhenius

$$k = k_0 \exp\left(-\frac{E_a}{RT}\right) \rightarrow \ln k = \ln k_0 - \frac{E_a}{RT}$$

$$k = k_{ref} \exp\left(-\frac{E_a}{R}\left(\frac{1}{T} - \frac{1}{T_{ref}}\right)\right)$$

Davey / Arrhenius modificado

$$\ln k = C_0 + \frac{C_1}{T} + \frac{C_2}{T^2} + C_3 a_W + C_4 a_W^{\ 2}$$

"Square-root type models"

Ratkowsky *et al.* (1982)

$$\sqrt{k} = b(T - T_{min})$$

McMeekin *et al.* (1987)

$$\sqrt{k} = b(T - T_{min})\sqrt{(a_w - a_{w_{min}})}$$

Adams *et al.* (1991)

$$\sqrt{k} = b(T - T_{min})\sqrt{(pH - pH_{min})}$$

McMeekin et al. (1992)

$$\sqrt{k} = b(T - T_{min})\sqrt{(a_w - a_{w_{min}})}\sqrt{(pH - pH_{min})}$$

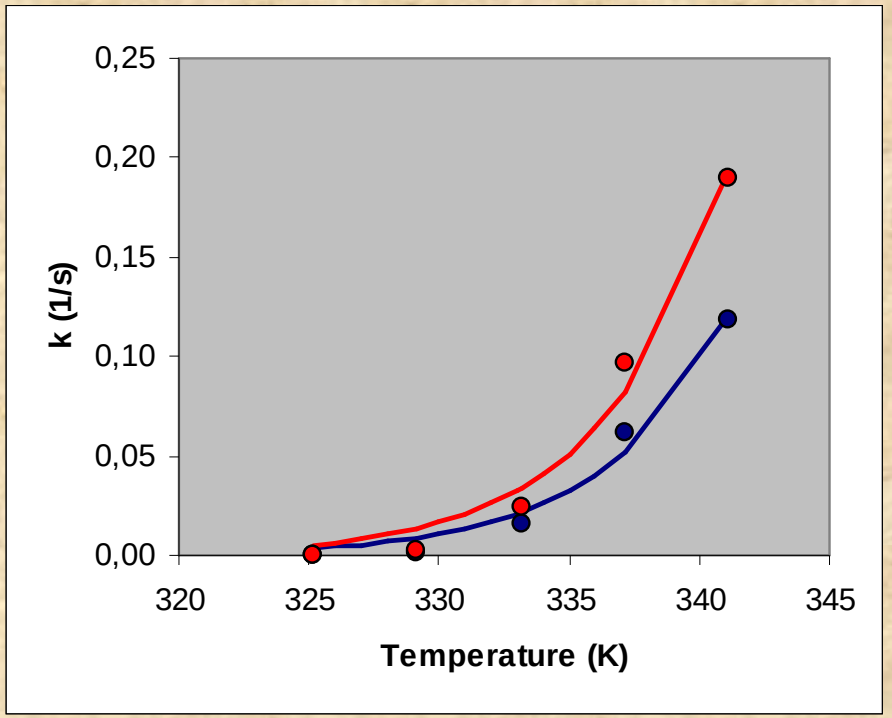
min – valor mínimo para crescimento

Exemplos

Data of *L.monocytogenes* Scott A

(24 hours incubation at 5°C in half cream)

Arrhenius



k = f(T)

Gompertz $k=\mathbf{0.0216}\exp(-\mathbf{203.3}/R*(1/T-1/333.15))$ | DEa=28.85 kJ/mol | $DK_{ref}=4.58\times10^{-3}\ s^{-1}$

Logística $k=\mathbf{0.0337}\exp(-\mathbf{206.6}/R*(1/T-1/333.15))$ | DEa=27.56 kJ/mol | $DK_{ref}=7.31\times10^{-3}\ s^{-1}$

Gil (2002)

Statistica 6.0

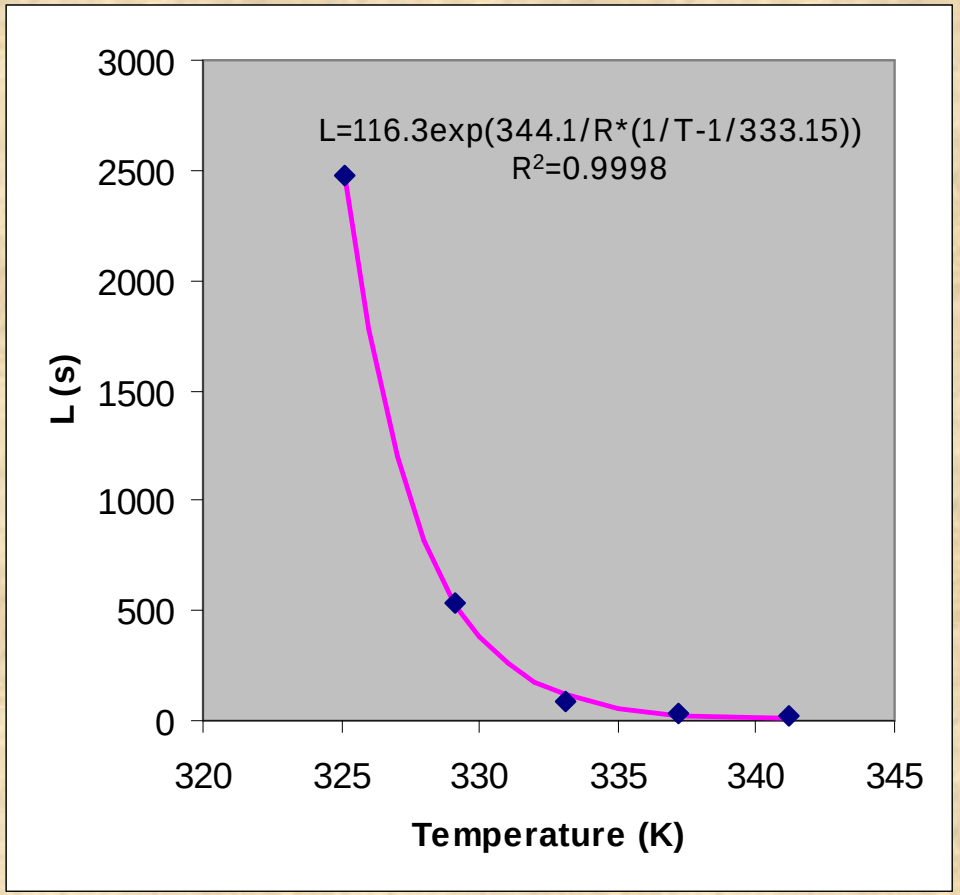
Exemplos

Data of *L.monocytogenes* Scott A

(24 hours incubation at 5°C in half cream)

Gompertz

atraso = f(T)



DEa=7.485 kJ/mol

DL_{ref}=7.595 s^{-1}

Gil (2002)

Statistica 6.0

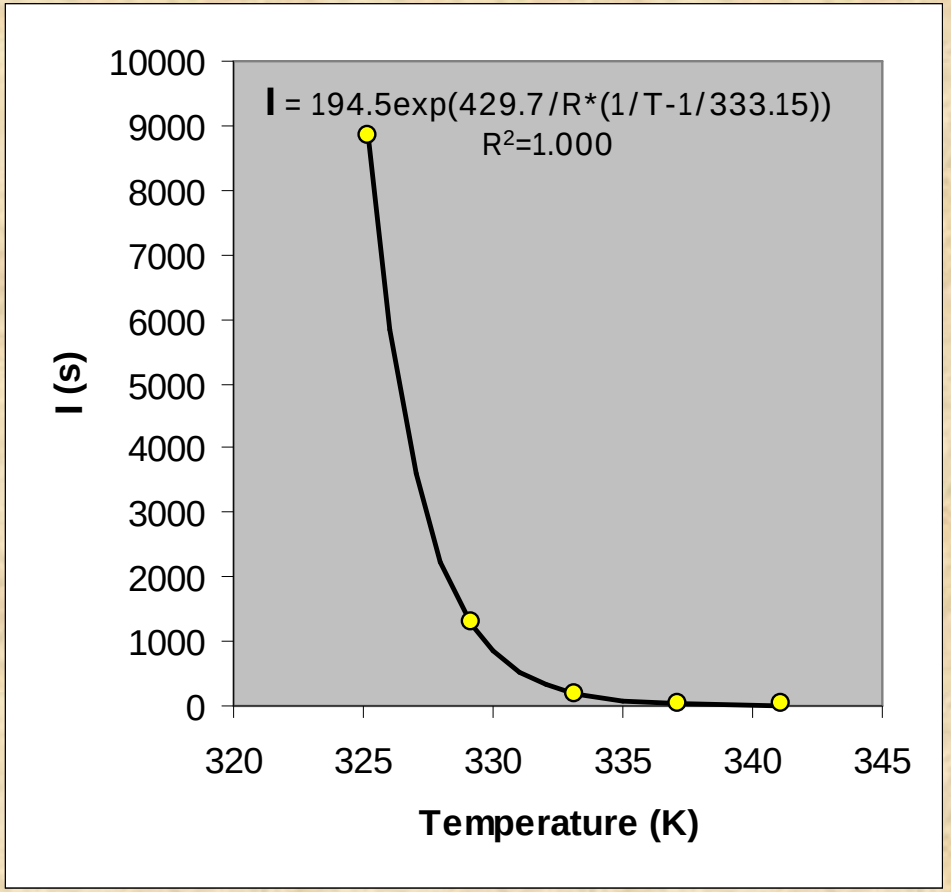
Exemplos

Data of *L.monocytogenes* Scott A

(24 hours incubation at 5°C in half cream)

Logística

atraso = f(T)



DEa=3.591 kJ/mol

Dl_{ref}=6.154 s^{-1}

Gil (2002)

Statistica 6.0

Modelos matemáticos

Condições variáveis de

temperatura

pH

a_w

maior complexidade !!

$$\frac{d(\log N)}{d(\text{tempo})}$$

Modelos matemáticos

Gompertz

situação dinâmica de temperatura

$$\frac{d(\log N)}{d(\text{tempo})}$$

$$\log N = \log N_0 - \int_0^t \left[k \exp(1) \exp\left(\frac{k \exp(1)}{\log\left(\frac{N_0}{N_{res}}\right)} (L - t') + 1 \right) \exp\left(- \exp\left(\frac{k \exp(1)}{\log\left(\frac{N_0}{N_{res}}\right)} (L - t') + 1 \right) \right) \right] dt'$$

$$k = k_{ref} \exp\left(- \frac{E_a}{R} \left(\frac{1}{T} - \frac{1}{T_{ref}} \right) \right)$$

$$L = a \exp\left(b \left(\frac{1}{T} - \frac{1}{T_{ref}} \right) \right)$$

Modelos de inactivação

- **terciários**

softwares

crescimento de microrganismos √

previsão de tempo de prateleira

inactivação de microrganis

lacunas

Limitações

- interacções entre microrganismos
- diversidade natural das estirpes
- estrutura complexa dos alimentos
- alimento / microrganismo
- modelagem da fase 'atraso'
- modelagem da fase 'cauda'
- previsões reais com condições ambientais variáveis

Envolvimento da equipa num projecto internacional ...

2001-2004

BUGDEATH

www.frperc.bris.ac.uk/bugdeath.htm

microbiologia predictiva

pasteurização de alimentos à superfície

fronteira importante de contaminação

heiro Editar Ver Favoritos Ferramentas Ajuda

roceder Avançar Parar Actualizar Home page Procurar Favoritos Histórico Correio Imprimir Editar Debate

ereço http://www.frperc.bris.ac.uk/bugdeath.htm Ir para Hiperligaçõ

"BUGDEATH" (QLRT-2001-01415)

Micrograph of a Salmonella bacteria

Predicting the reduction in microbes on the surface of foods during pasteurisation.

- Tell me more about the Bugdeath project.
- What are the objectives of the Bugdeath project?
- Who are the project partners?
- Meetings
- Industrial Advisory Group (IAG)
- Partner area of web site (specifically for project partners - password needed to enter)

Bugdeath

BUGDEATH is a research project funded by the European Commission to produce accurate predictive models of the reductions in microbial numbers that can be achieved on the surface of foods during surface pasteurisation processes.

Food poisoning is increasing throughout the European Union (EU). Over 60% of outbreaks are associated with meat, fresh fruit and salad vegetables. Most of the contamination by pathogenic and spoilage organisms is present on the surface of foods at the time of harvesting or is transferred to the surfaces during slaughter and processing. Accurate microbial death models would be of considerable help to the food industry in the development of surface pasteurisation systems for meat, fruit and vegetables. This will in turn lead to safer foods with improved quality and shelf life.

Personnel involved in Bugdeath from left to right

Internet

Bugdeath

Parceiros

1. Food Refrigeration and Process Engineering Research Center (**FRPERC**)
   University of Bristol

2. Escola Superior de Biotecnologia (**ESB**)
   Universidade Católica Portuguesa

3. Department of Chemical Engineering
   Katholieke Universiteit Leuven (**KUL**)

4. Teagask, The National Food Center (**NFC**)

5. Campden & Chorleywood Food Research Association (**CCFRA**)

6. Faculty of Applied Science
   University of West England (**UWE**)

7. Laboratoire de Ginie des Procidis Alimentares (**ENITIA**)
   Ecole Nationale d'Inginieurs des Techniques des Industries Agricoles et Alimentaires

8. Institute National de la Recherche Agronomique (**INRA**)

Bugdeath

Objectivos

- estudos de inactivação à superfície de alimentos sólidos
- desenvolvimento de modelos de inactivação precisos e exactos
- desenvolvimento de equipamento para pasteurização à superfície
  'rig apparatus'
- desenvolvimento de software para previsão

tarefa da ESB

Bugdeath

alimentos estudados

batata

frango

bife

microrganismos

Listeria monocytogenes

E. coli

Salmonella

Campylobacter

rig apparatus

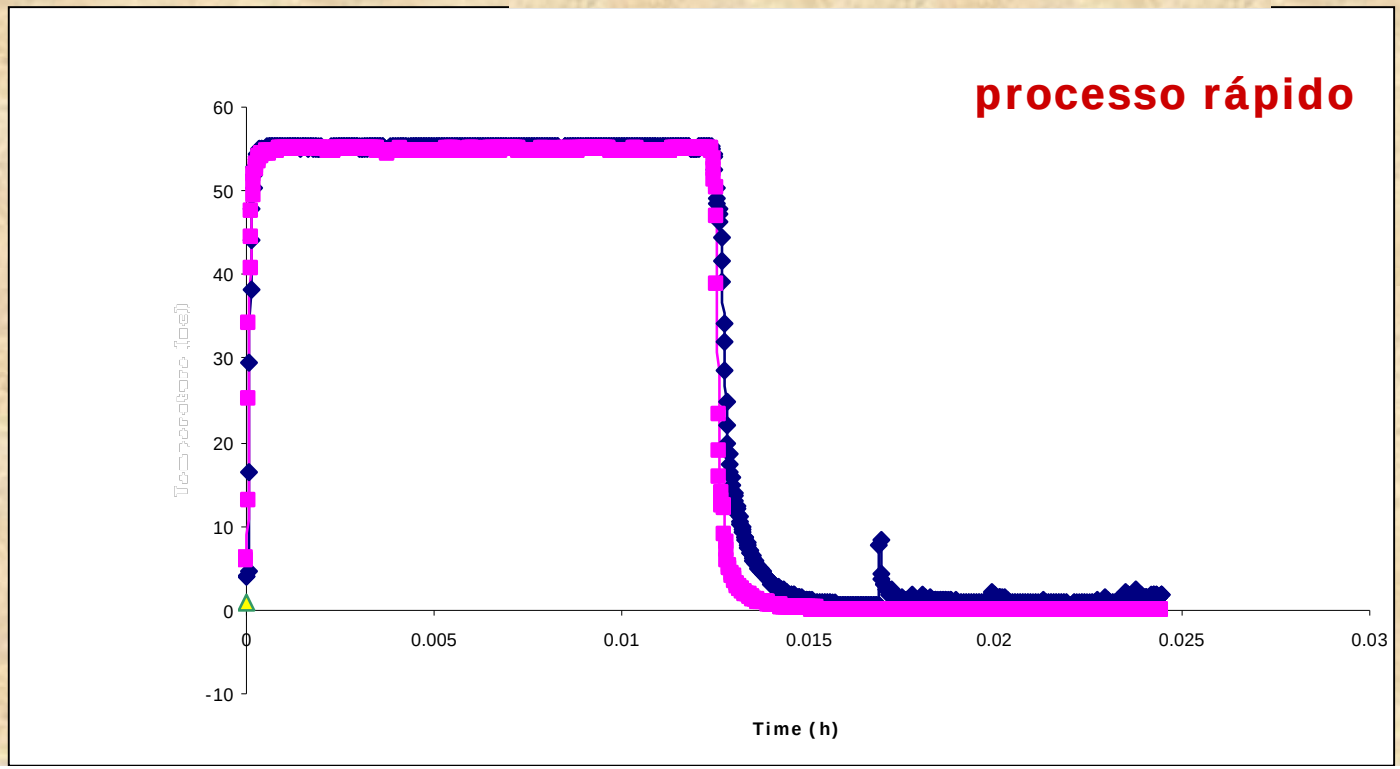
Bugdeath

processo térmico

- vapor
- ar seco

história de temperatura

rig apparatus



podem ser testadas diferentes histórias de temperatura

Escola Superior de Biotecnologia, Universidade Católica Portuguesa

Team

Pedro Pereira
Maria Manuel Gil
Teresa Brandão
Cristina Silva

Sessão de disseminação
Chipping Campden, Agosto 2004

Development of a user-friendly combined heat, mass transfer and microbial death model

Objectives

- Create a software application

User-friendly tool

Simulation of inactivation kinetics of microorganisms on the surface of foods during pasteurisation treatments

'Bugdeath' - funded by the European Commission under the EC Framework 5;
Quality of Life and Management of Living Resources Programme

Introduction

- Need to obtain reliable data on relationship between microbial death and the surface temperature of real foods

Lead to the design, construction and commission of equipment

Software application to simulate results obtained in the rig apparatus

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Modelling

Heat transfer phenomena

Global model

Integrates

Prediction/simulation purposes

Microbial inactivation under constant and time-varying temperature conditions

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Modelling

two modelling approaches

- Accurate modelling of heat transfer
  - Description of the phenomena induced to the food surface by a thermal process

- Modelling microbial inactivation behaviour under such temperature conditions

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Heat transfer model

estimation of surface temperature

- One dimensional heat transfer model
- Combination of different phenomena
  - Conduction
  - Convection
  - Evaporation/condensation of water or steam
  - Radiation (not considered)

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Heat transfer model

- Simplified model

  Conduction/convection - Fourier

  Geometry – plane sheet

  No mass transfer phenomena considered

- Limits

  Air velocity ranged from 15 m/s to 25 m/s

  Extrapolation locked outside of conditions that can be verified in the test rig

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Heat transfer model

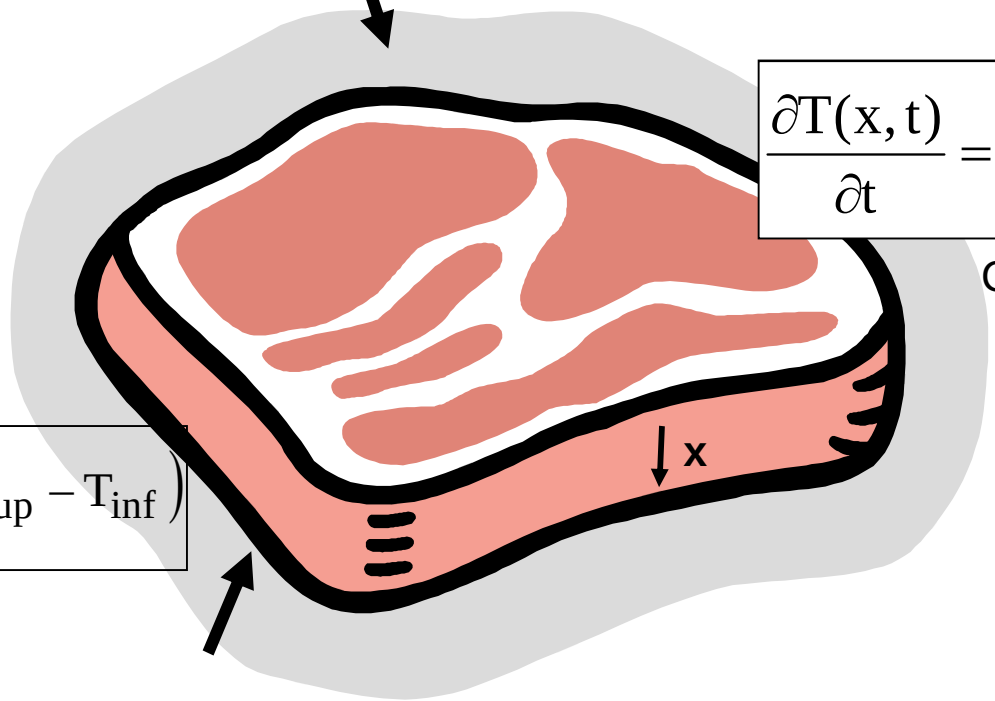
$$\lambda\left(\frac{\partial T}{\partial x}\right)_{top} = h_{eff}\left(T_{air\ or\ steam} - T_{sur}\right)$$

3

1

$$\frac{\partial T(x,t)}{\partial t} = \alpha\,\frac{\partial^2 T(x,t)}{\partial x^2}$$

Crank-Nicholson method



$$\lambda\left(\frac{\partial T}{\partial x}\right)_{bot} = h_{inf}\left(T_{sup} - T_{inf}\right)$$

2

t – time
T – temperature
h – heat transfer coefficient
α – thermal diffusivity
λ – Thermal conductivity

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Boundary conditions - bottom

Exchanges between the bottom of the product and the support can be neglected – product thickness < 0.5 cm

Experimentally observed in the rig

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Heat transfer model

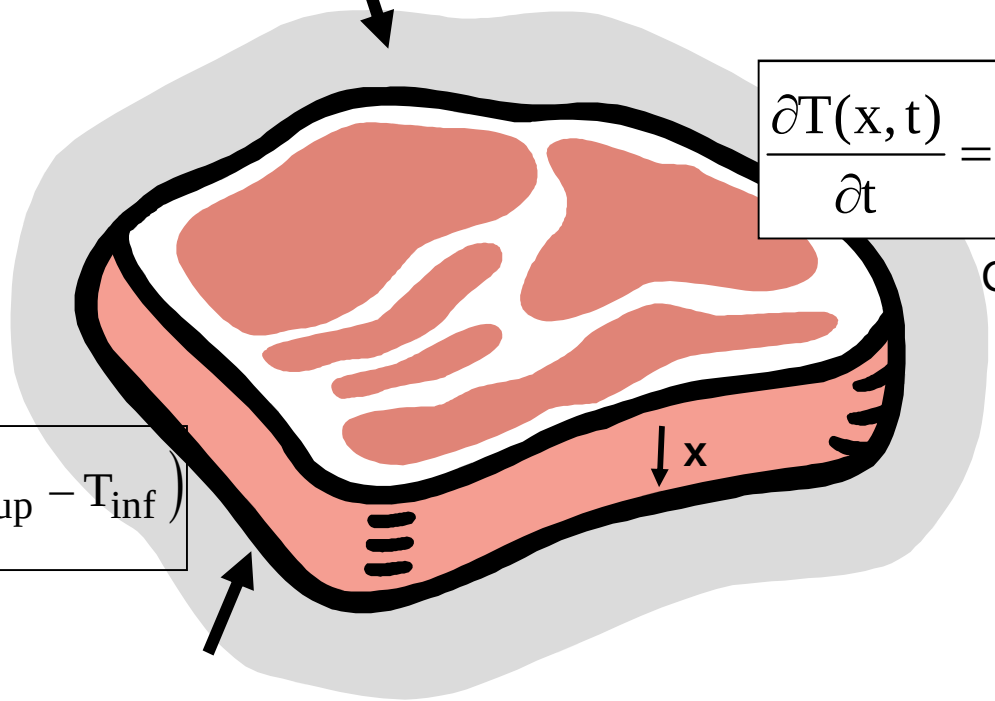
$$\lambda\left(\frac{\partial T}{\partial x}\right)_{top} = h_{eff}\left(T_{air\ or\ steam} - T_{sur}\right)$$

3

1

$$\frac{\partial T(x,t)}{\partial t} = \alpha\,\frac{\partial^2 T(x,t)}{\partial x^2}$$

Crank-Nicholson method



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Effective coefficient – dry conditions

$$h_{eff} = \underbrace{h\,\frac{T_{air} - T_{sur}}{T_{max} - T_{sur}}}_{\text{convection}} + \underbrace{K_m \Delta H\,\frac{P_T - a_{w_{sur}} P_{T_{sur}}}{T_{max} - T_{sur}}}_{\text{evaporation}}$$

k_m – mass transfer coefficient
ΔH – latent heat of water evaporation

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Effective coefficient – wet conditions (steam)

$\cancel{h_{eff}}$

Due to complexity of mathematical expressions to be incorporated in the software

$$T_{sur} = T_{steam} - 3^{o}C$$

Experimentally observed in the rig

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Microbial inactivation model

- To predict microbial content at the surface of foods

- It has the advantage of dealing with time-varying temperature conditions

- Typical of pasteurisation treatments

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Microbial inactivation model

$$\frac{dN}{dt} = -k_{\max} k_Q(Q) N$$

$$\frac{dQ}{dt} = -k_{\max} Q$$

N – microbial cell density
Q – variable related to the physiological state of the cells
k_{max} – inactivation rate constant

$$\log \frac{N}{N_0} = \log(\exp(-k_{\max} t) \frac{1 + Q(0)}{1 + Q(0) \exp(-k_{\max} t)}$$

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Microbial inactivation model

$$k_{\max}(T, a_w) = \frac{\ln 10}{D_{ref}} \exp\left(\frac{\ln 10}{z}(T - T_{ref})\right) \exp\left(\frac{\ln 10}{z_{a_w}}(a_w - 1)\right) + c1$$

T = Tsur → Calculated on the basis of all considerations of heat transport

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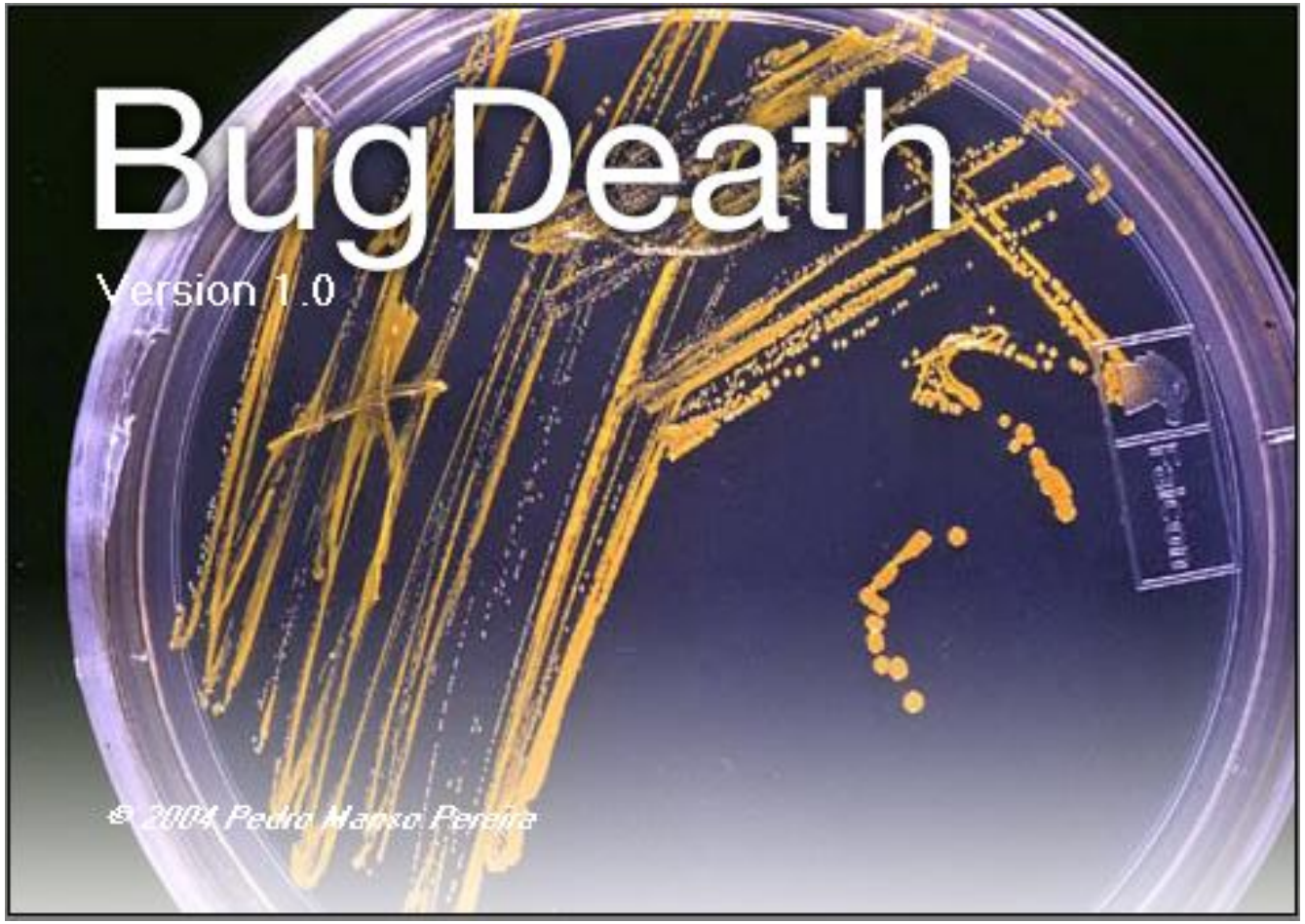
Software Program



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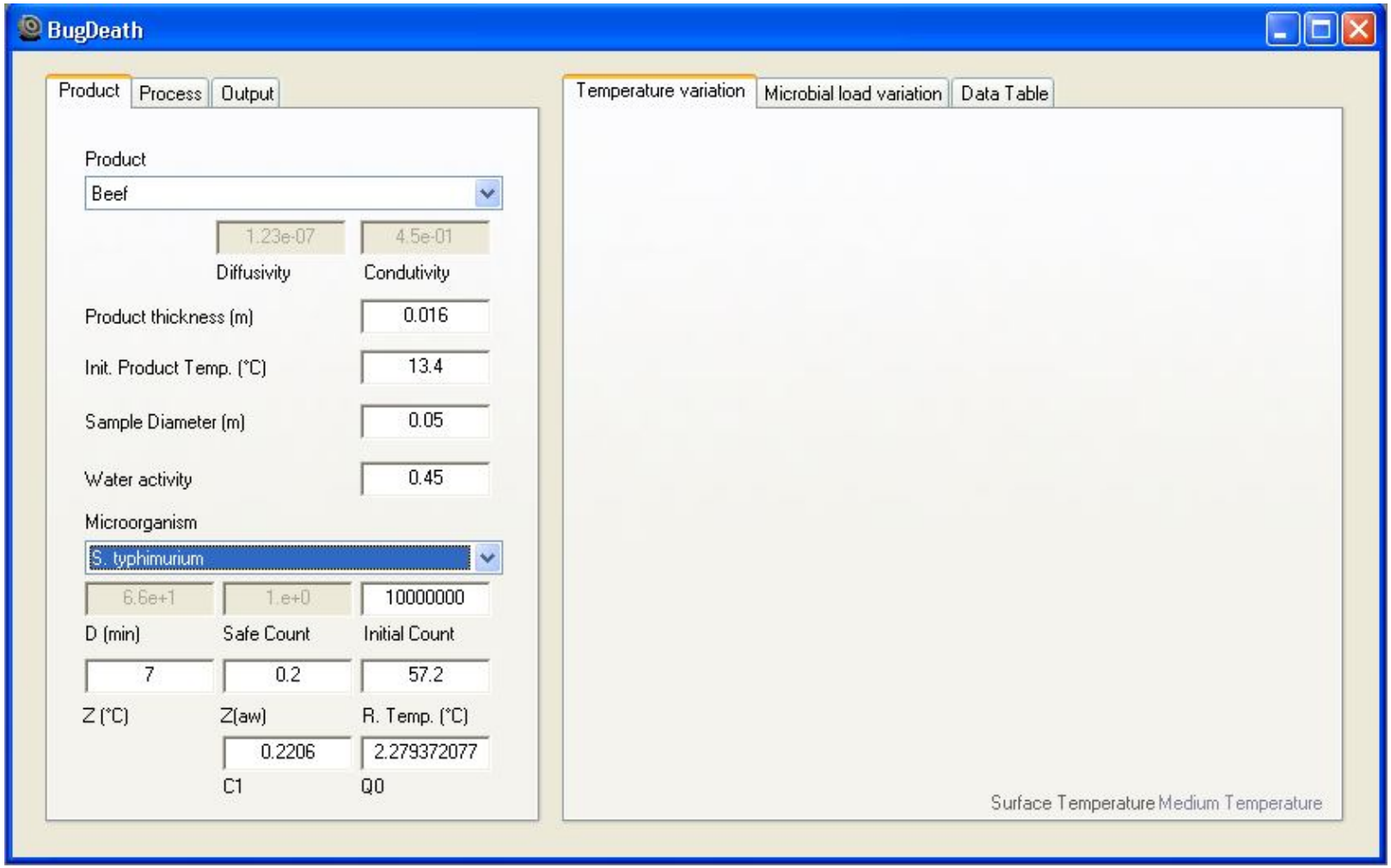
Software Program

- The programme was developed using Real Basic® 5.2 application

- Food/microorganism selection is allowed
  (database of thermal properties and kinetic parameters)

- On the basis of the selection of a heating regime of the medium, the programme allows prediction of the food surface temperature and simulates the microbial load content along the whole process time

First screen – product / microorganism



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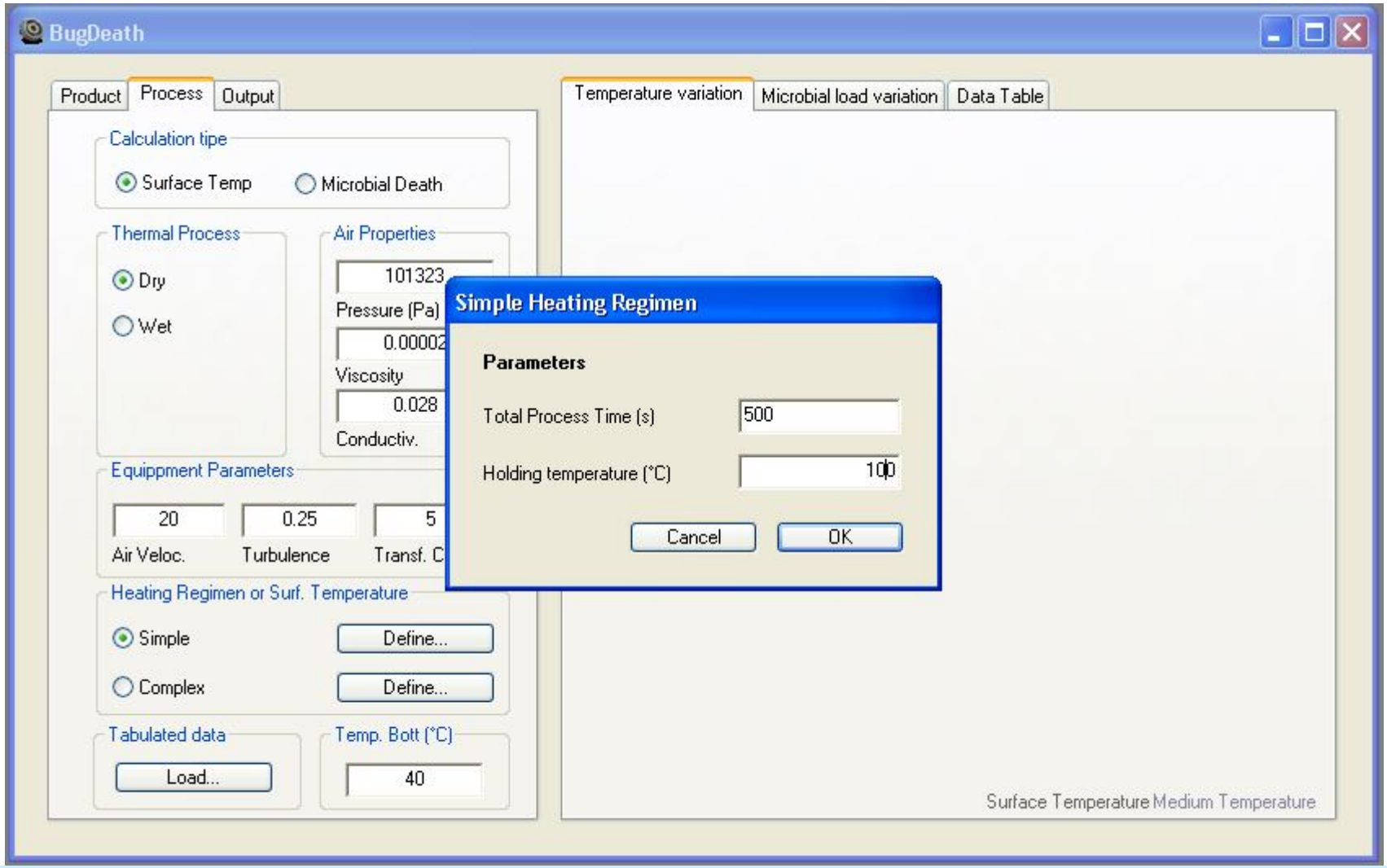
Second screen – process



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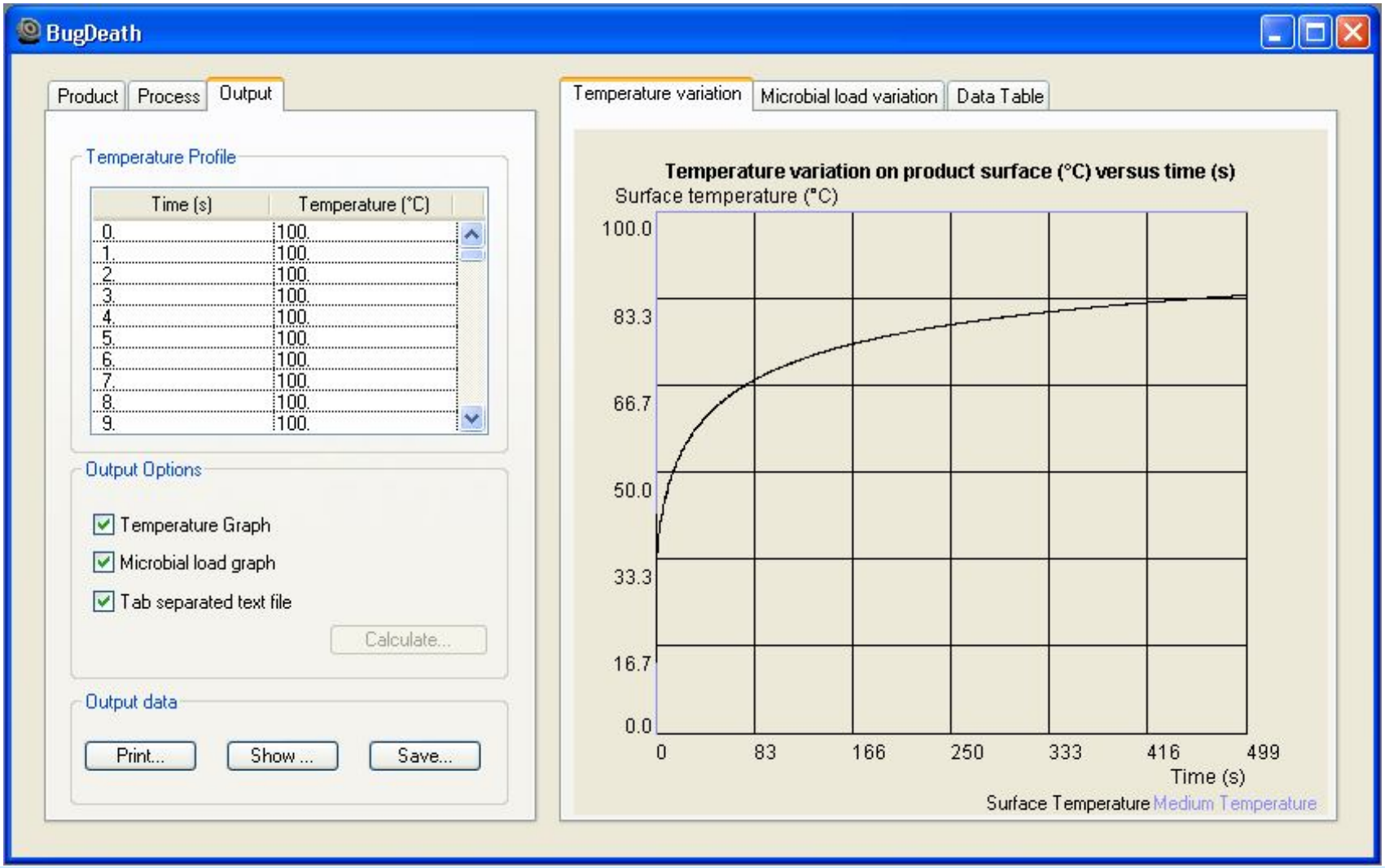
Output – graphic / temperature



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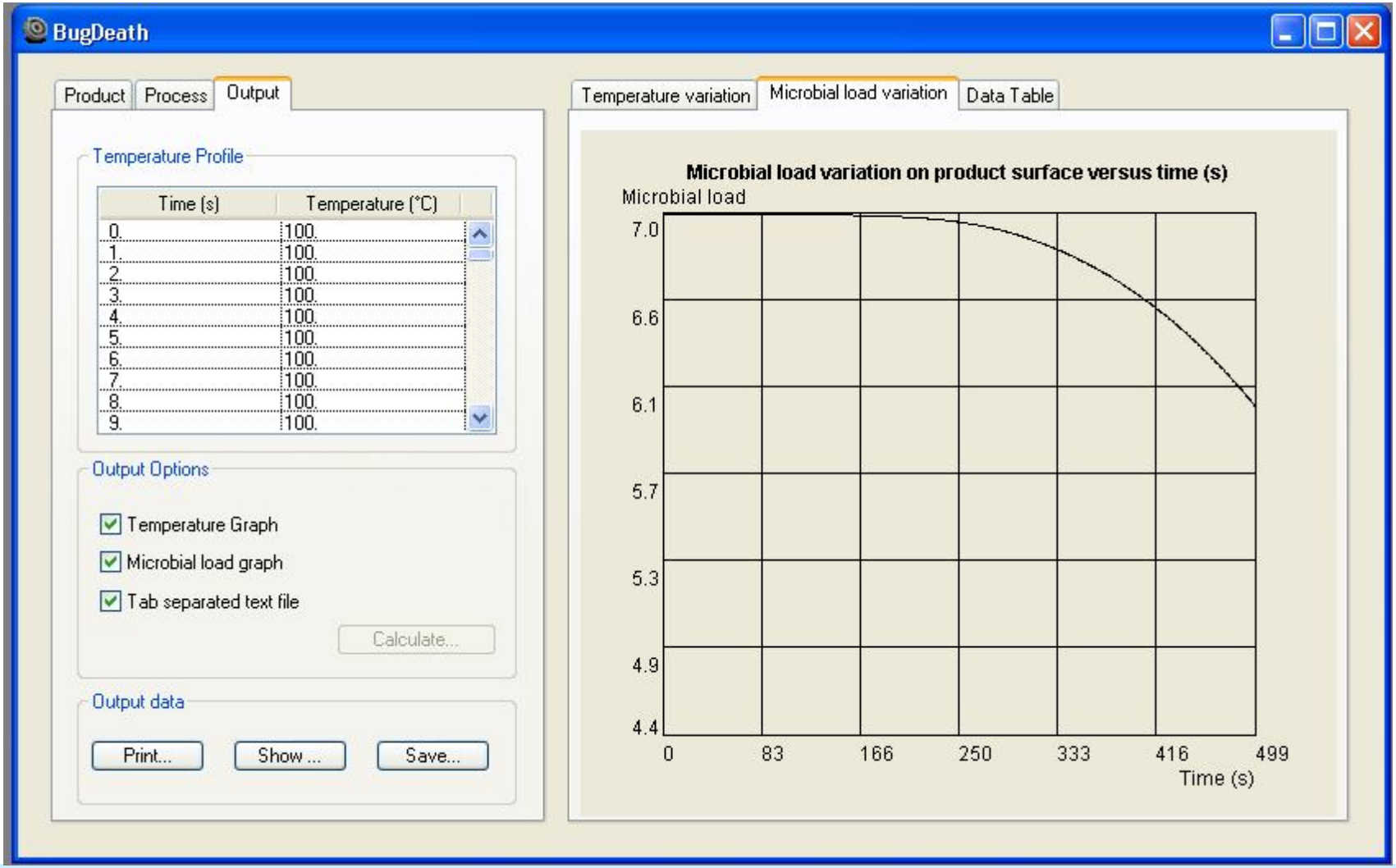
Output – graphic / microbial load



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Output – data table

BugDeath

Product | Process | Output

Temperature Profile

Time (s)	Temperature (°C)
0.	100.
1.	100.
2.	100.
3.	100.
4.	100.
5.	100.
6.	100.
7.	100.
8.	100.
9.	100.

Output Options

- [x] Temperature Graph
- [x] Microbial load graph
- [x] Tab separated text file

Calculate...

Output data

Print... | Show ... | Save...

Temperature variation | Microbial load variation | Data Table

Time (s)	Medium Temp. (°C)	Surface Temp. (°C)	Microbial Load
0	0	14.02522	7
0.6081229	100	39.29868	6.991117
1	100	31.74754	6.991117
1.608123	100	35.68345	6.991117
2	100	36.00534	6.991117
2.608123	100	36.9512	6.991117
3	100	38.14414	6.991117
3.608123	100	38.62585	6.991117
4	100	39.78456	6.991117
4.608123	100	40.16022	6.991117
5	100	41.19636	6.991117
5.608123	100	41.524	6.991117
6	100	42.4504	6.991117
6.608123	100	42.74452	6.991117
7	100	43.58093	6.991117
7.608123	100	43.84836	6.991117
8	100	44.61078	6.991117
8.608123	100	44.85613	6.991117
9	100	45.55682	6.991117
9.608123	100	45.78353	6.991117
10	100	46.43196	6.991117
10.60812	100	46.64271	6.991116
11	100	47.2463	6.991116
11.60812	100	47.44323	6.991116
12	100	48.0079	6.991116
12.60812	100	48.19275	6.991116
13	100	48.72329	6.991115
13.60812	100	48.89746	6.991115
14	100	49.39783	6.991115

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Conclusions / outputs

- Software application simulates the results obtained in the rig apparatus
- Valuable for developing appropriate and safety thermal processes
- Marketed and commercially available
- Educational purposes (simulation of real food processes)

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Workshop

Predictive Microbiology

Aplicações e Novas Fronteiras

Obrigada

Teresa Brandão

Cristina Silva

UNIVERSIDADE CATÓLICA PORTUGUESA
Escola Superior de Biotecnologia

4 de Março 2005