

**CONCIA 2012**

Congreso Nacional de Calidad e Inocuidad Alimentaria

**OBTENTION AND DATA  
MANAGEMENT IN  
PREDICTIVE  
MICROBIOLOGY MODELS**

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*5<sup>th</sup> September 2012*



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# OUTLINE

- Objectives of food industry
- The challenge
- Predictive microbiology
- How to obtain the data
- Available software
- Validation studies
- Acknowledgement by regulation
- The complexity of dynamic conditions
- Conclusions

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# Objectives of food industry

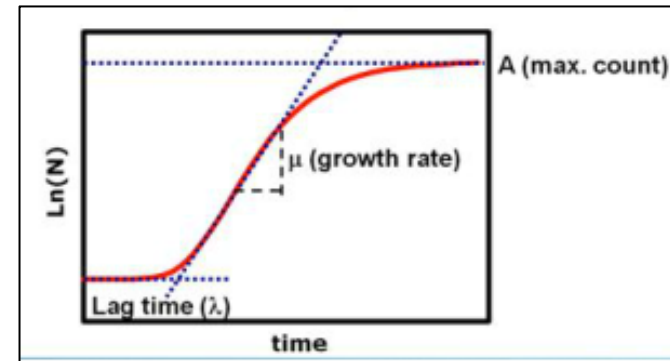
- Prediction of shelf life



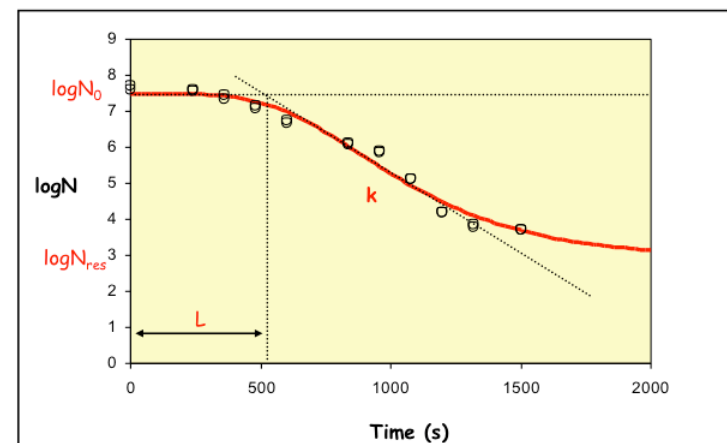
- Preservation of foods



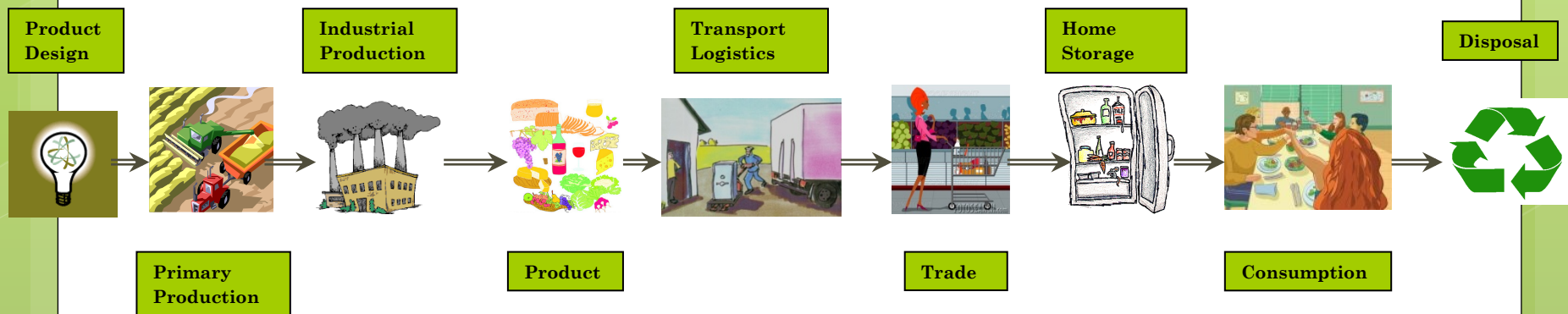
- Control/monitor the growth of microorganisms



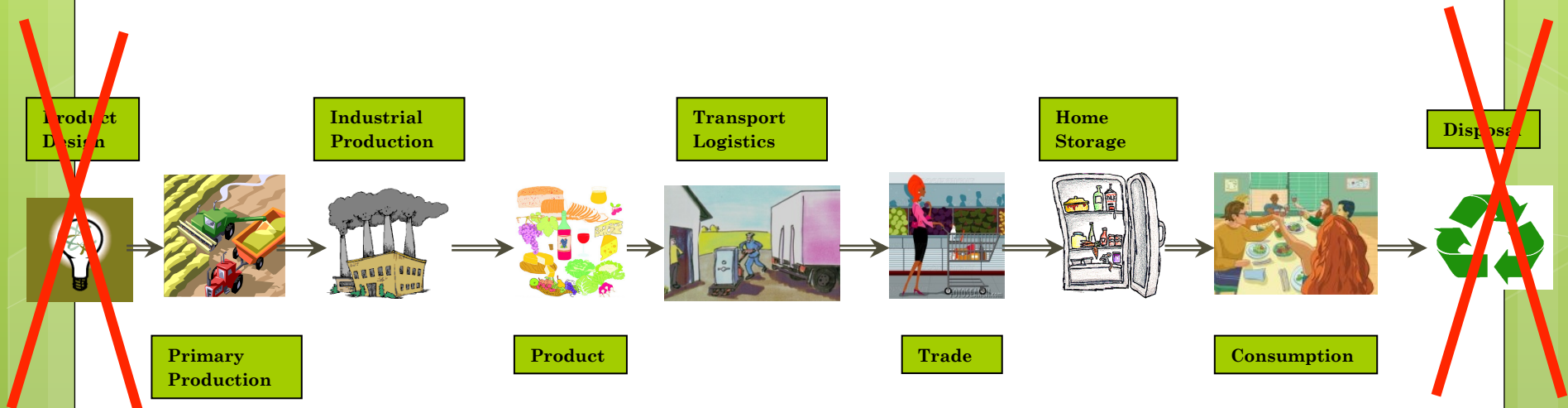
- Predict microbial death



## ○ Distribution chain



## ○ Distribution chain



# OUTLINE

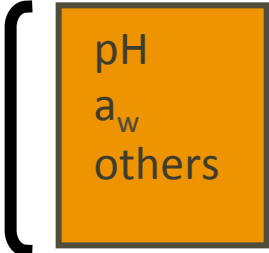
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# The challenge

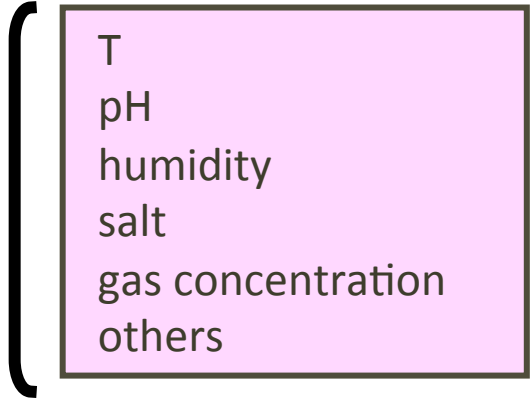
- Microorganisms response depends on:

- **Intrinsic factors**



pH  
 $a_w$   
others

- **Extrinsic factors**



T  
pH  
humidity  
salt  
gas concentration  
others

- **System dynamics**

- **microbial interaction**
- **natural strains diversity**
- **history of initial population**
- **complexity of food structure**
- **interaction food/microorganism**
- **predictions in real and varying environmental conditions**

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# Predictive microbiology

The use of **mathematical models** in the description of **microbial responses** to environmental stressing factors

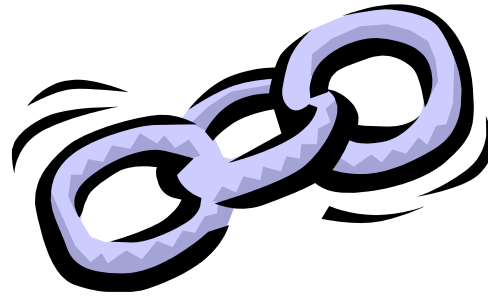
## The idea is not even recent!

- Thermal lethality
  - D and z values – Bigelow model

$$F_c = \int_0^{t_p} 10^{(T_c - T_{refm})/z_m} dt$$

**predictive  
microbiology**

**microbiology**



**mathematics**

**statistics**

Model → mathematical expression

$$y_i = f(x_{ij}, \theta_k) + \varepsilon_i$$

$i=1,2,\dots,n$  (number of experimental runs/observations)

$j=1,2,\dots,v$

$k=1,2,\dots,p$

Minimize differences

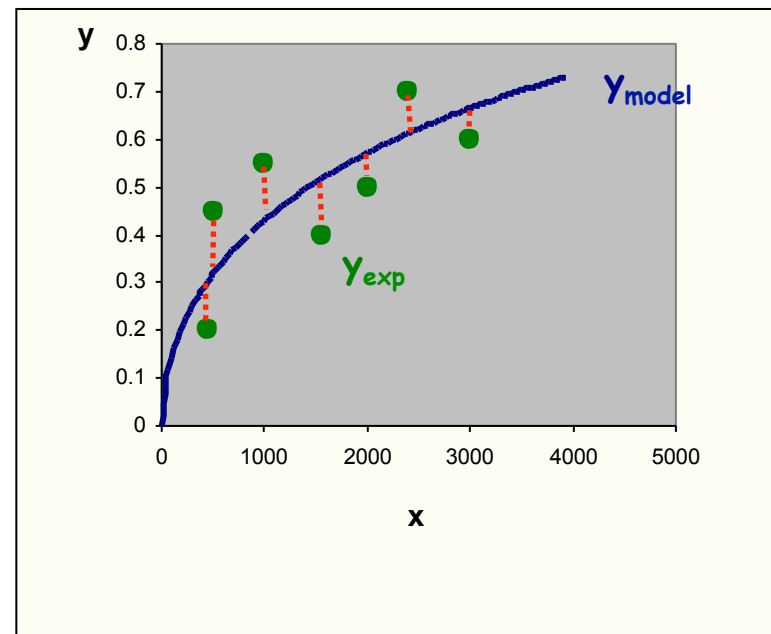
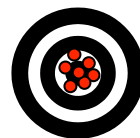


Precise ?



$\theta^*$

Accurate ?

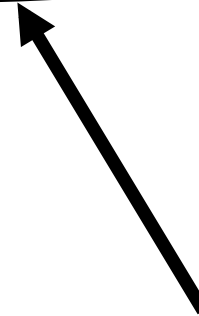


**objective**

**precise and accurate** description of observations

**model adequacy**

**quality of model parameters**

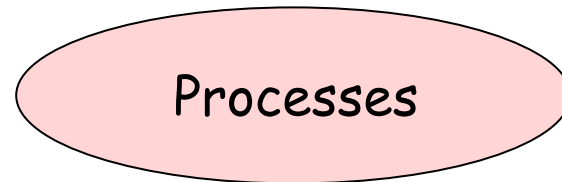




## **advantages**

- **knowledge of the process**
- **process effects on product**
- **control of process variables**

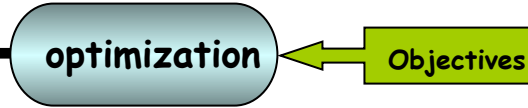
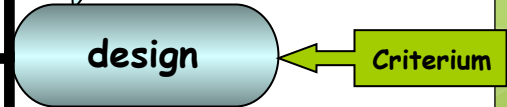
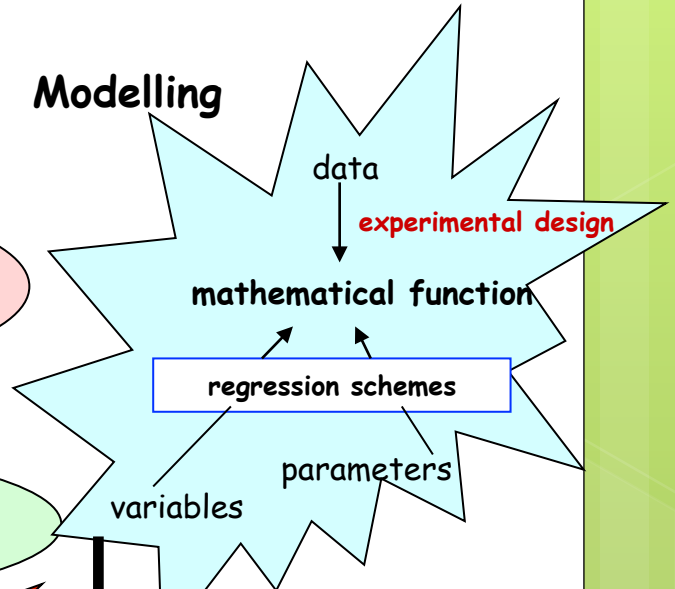
Transport Phenomena  
• heat  
• mass  
• *momentum*  
Reaction kinetics  
Properties



Physical      Chemical



### Modelling



## **aplication**

- **prediction / simulation**
- **development of efficient processes**

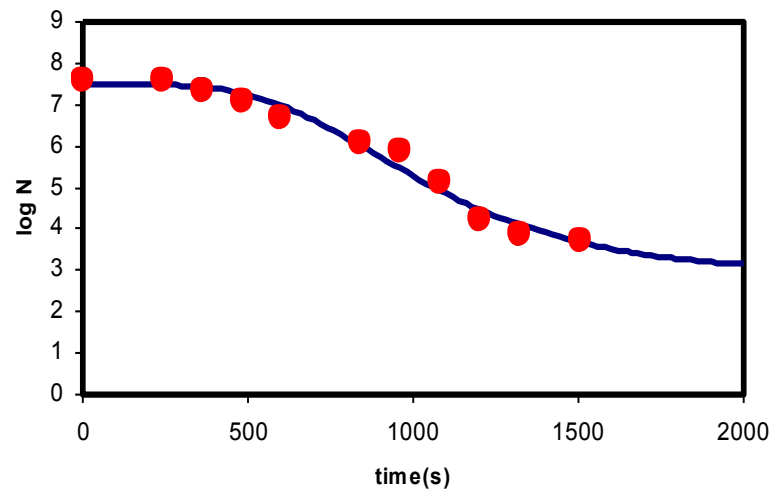


**contribution to **safety****

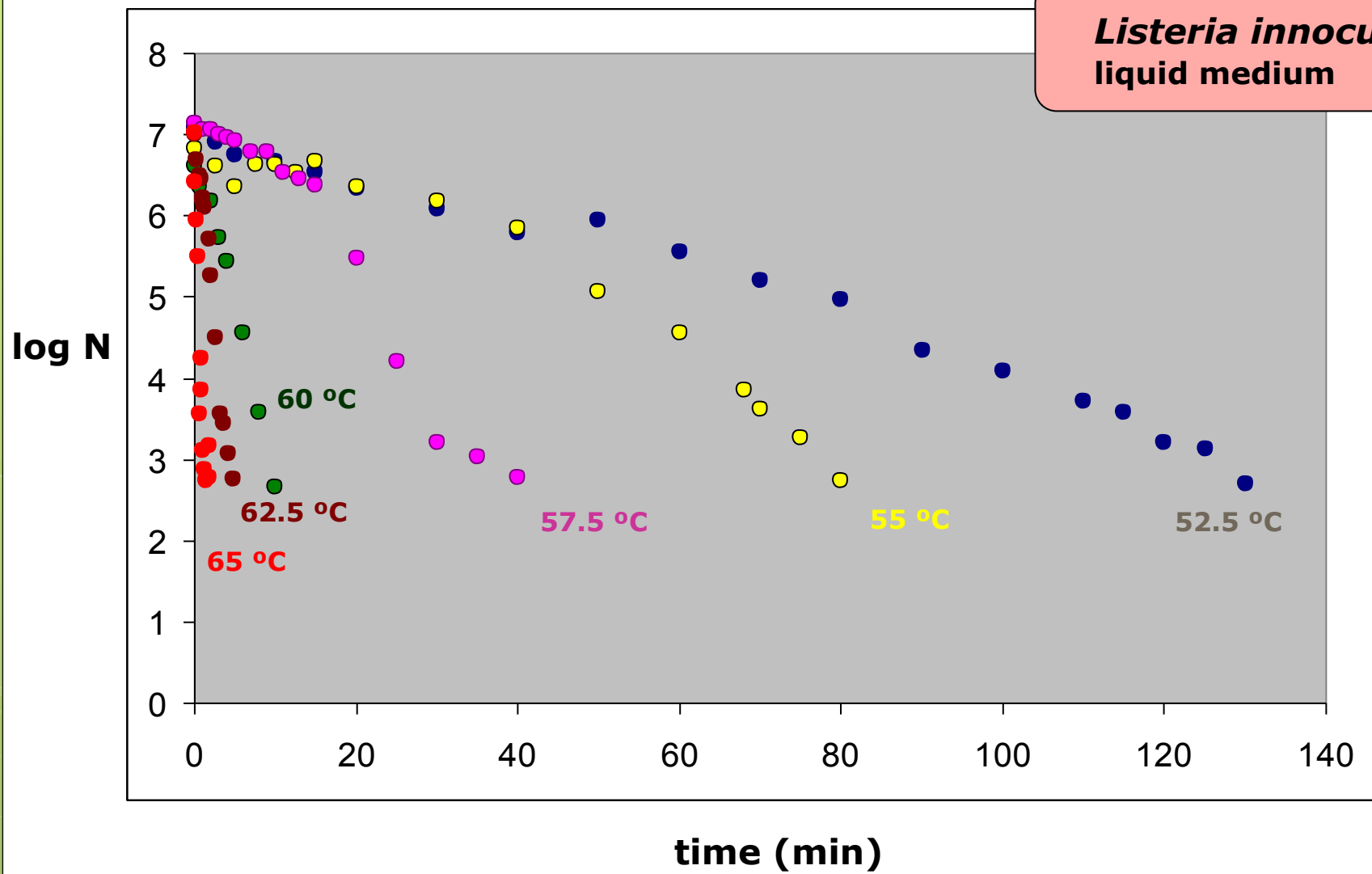
# inactivation



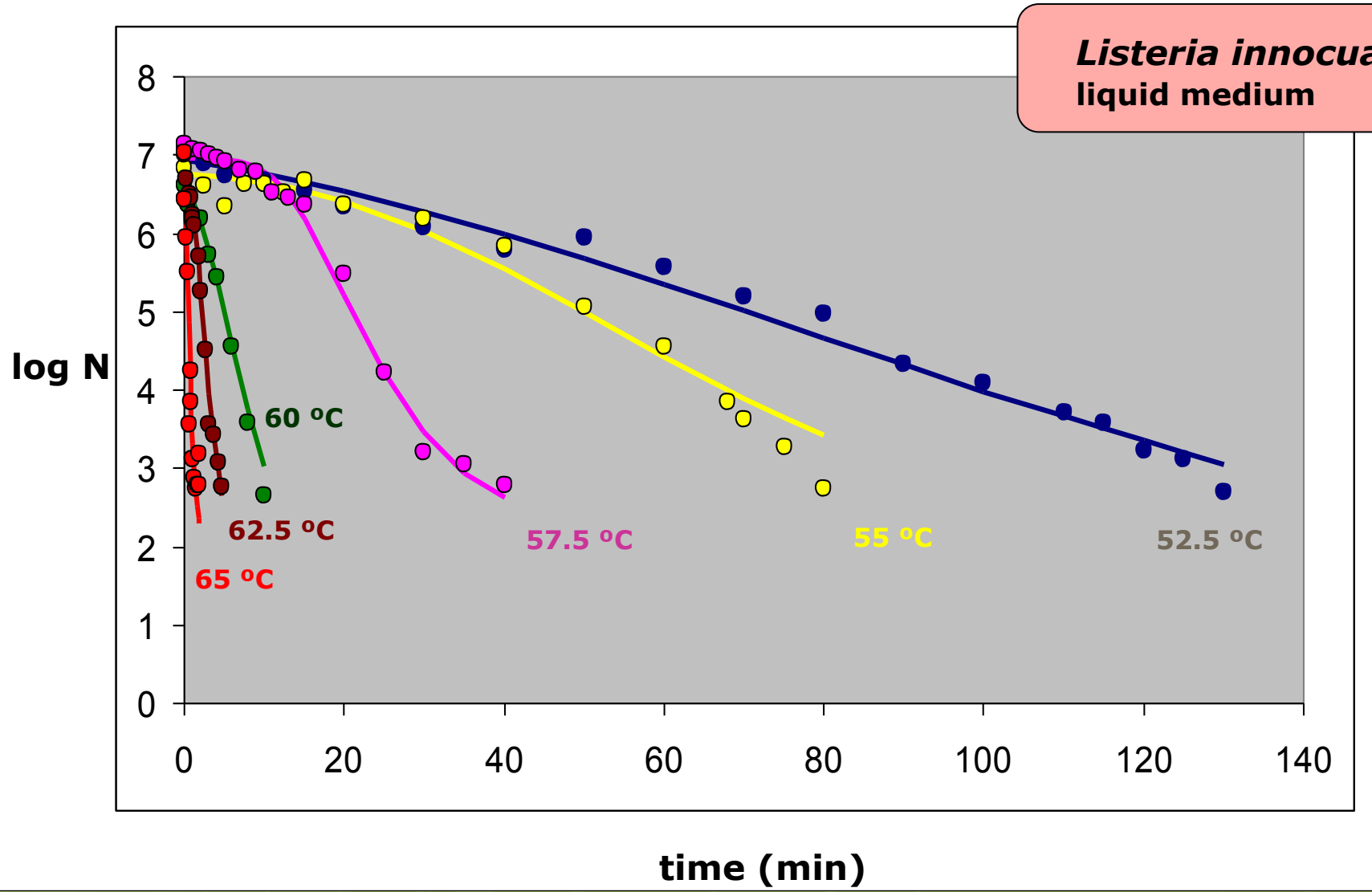
**sigmoidal behaviour**



presence of aggregated microorganisms or sub populations  
more **heat** (or other **stress factor**) **resistant**



Miller (2004)

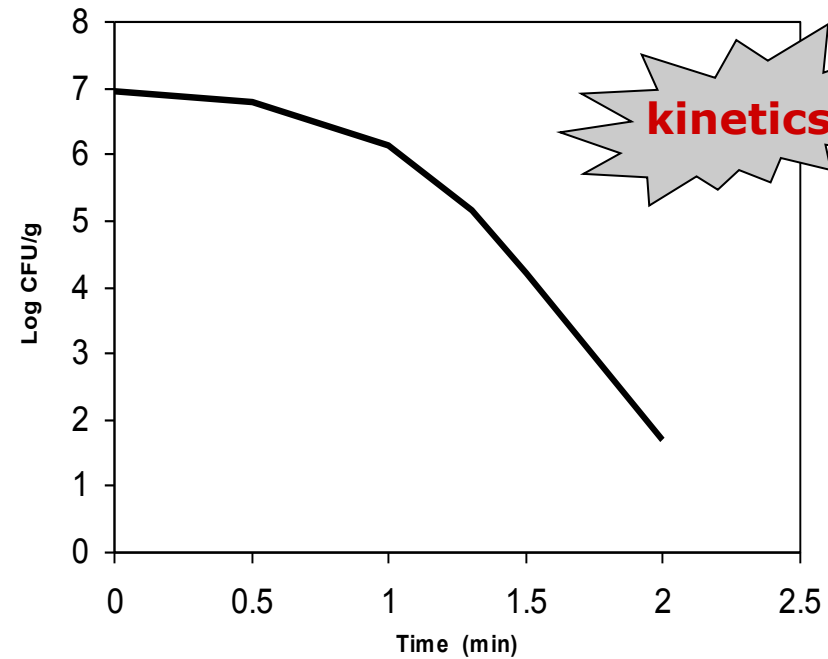


## Types of models

❖ **primary**



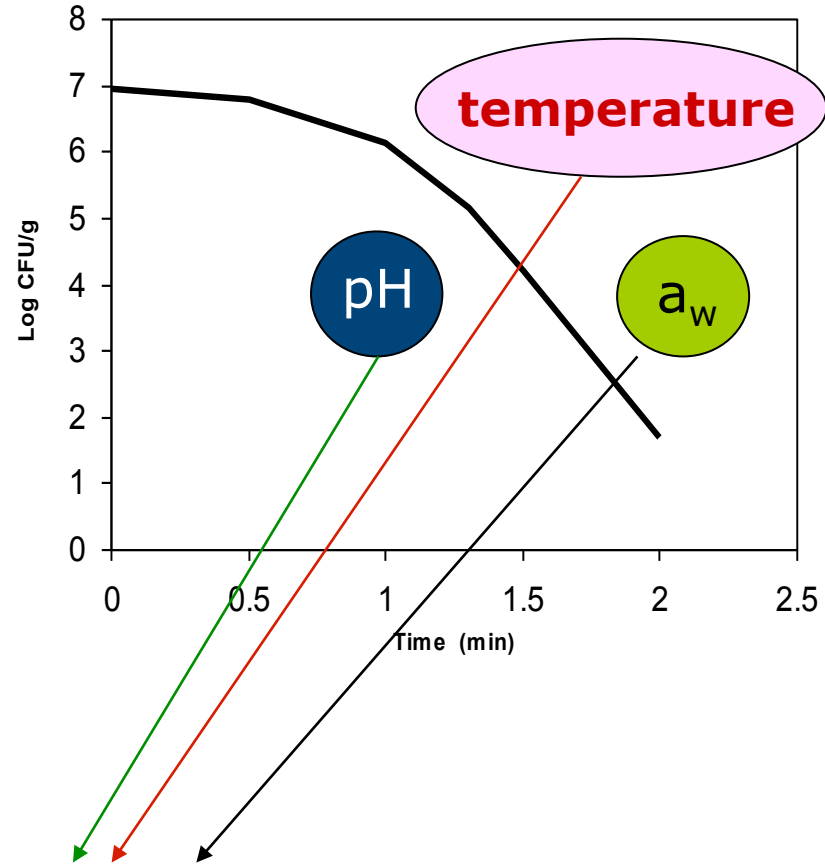
**parameters**



❖ **primary**

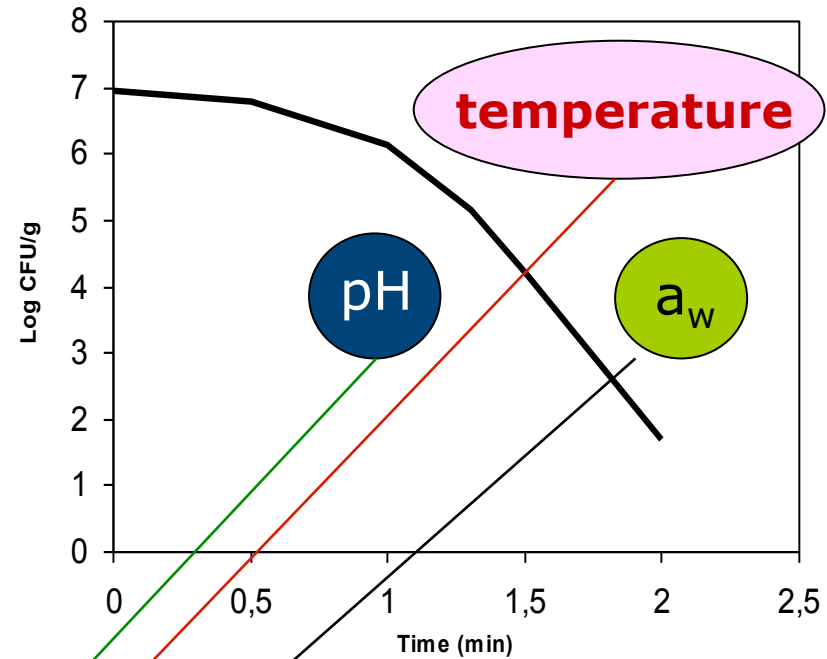
❖ **secondary**

**parameters**





❖ **primary**



❖ **secondary**

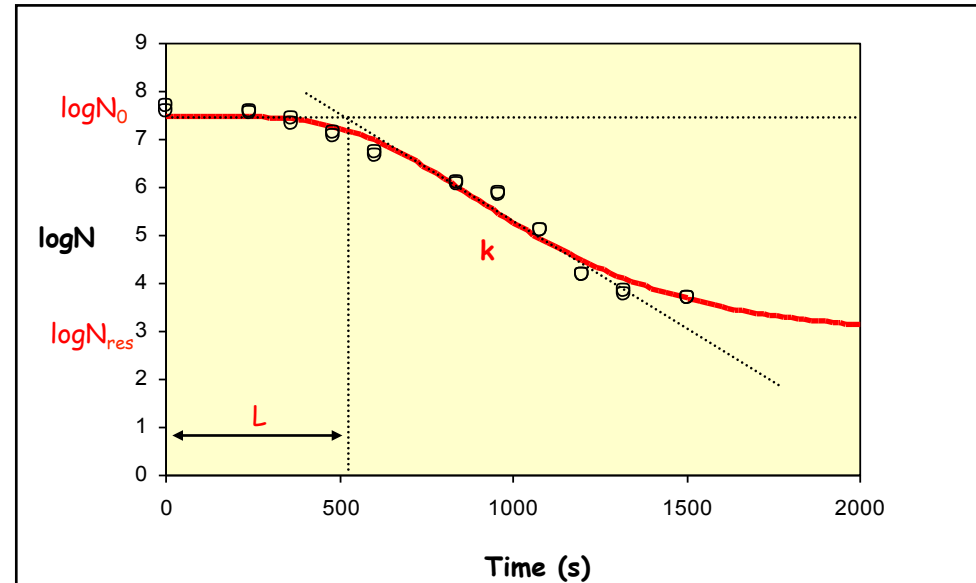
**parameters**

❖ **terciary** - integration of the previous models - **software**

# Inactivation models

## ❖ primary

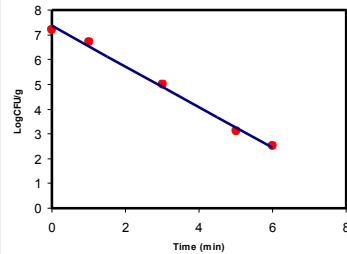
$N_0$  number of initial viable spore cells  
 $N_{res}$  number of residual spore cells  
 $k$  maximum inactivation rate  
 $L$  lag or shoulder



empirical

fundamental

❖ **primary**



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



$$\log N = \log N_0 - \frac{t}{D} \quad \text{First order}$$

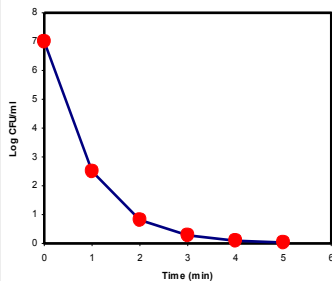
D – decimal reduction time

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

**Cerf (1977)**

**biphasic**

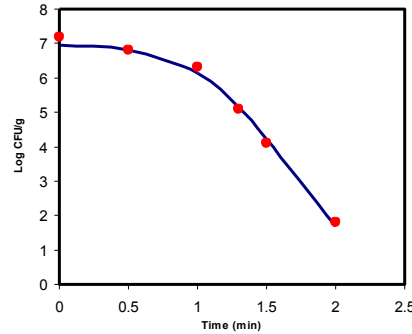
$F_1$  – fraction of inactivated microorganisms  
 $k_1$  e  $k_2$  – kinetic constants



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

**Kamau et al. (1990)**

❖ primary



Whiting &  
Buchanan  
(1992)

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

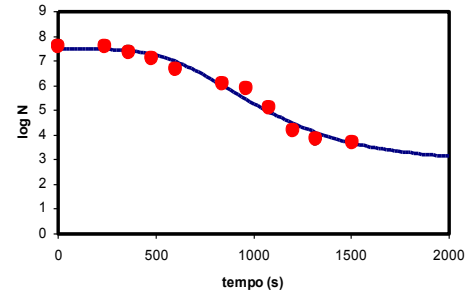
L – lag or shoulder

Cole et al.  
(1993)

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

distribution of heat  
sensitivity of  
microbial populations

❖ primary



Baranyi et al.  
(1993)

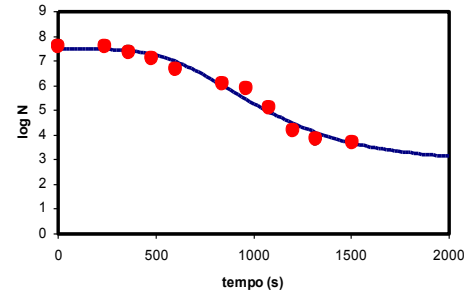
$$\frac{dN}{dt} = -k \alpha(t) \beta(t) N$$

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

‘tail’ function

‘lag’ function

❖ primary



Baranyi et al.  
(1993)

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

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

'tail' function

'lag' function

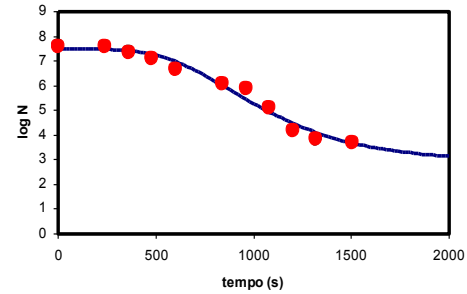
Geeraerd et al.  
(2000)

$$\begin{aligned} \frac{dN}{dt} &= -k_{\max} k_Q(Q) N \\ \frac{dQ}{dt} &= -k_{\max} Q \end{aligned}$$

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

Q - variable related to the physiological state of the cells

❖ primary



## Gompertz

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



*Listeria monocytogenes*

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

reparameterized for inactivation based in Zwietering (1990)

## Logistic

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

c – constant

❖ **secondary**

Arrhenius

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

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

Davey / Arrhenius modified

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

“Square-root type models”

Ratkowsky *et al.* (1982)

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

McMeekin *et al.* (1987)

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

Adams *et al.* (1991)

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

McMeekin *et al.* (1992)

$$\sqrt{k} = b(T - T_{\text{min}}) \sqrt{(a_w - a_{w\text{min}})} \sqrt{(\text{pH} - \text{pH}_{\text{min}})}$$



❖ **tertiary**

**softwares**

**Microbial growth**

**Shelf life prediction**

**Microbial inactivation**

## Difficulties in food processes modelling:

- Dynamic processes
- Complexity and heterogeneity of products
- Structural and physicochemical changes

## Gompertz

dynamic situation of temperature

$$\downarrow \frac{d(\log N)}{d(\text{time})}$$

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

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

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

Linear

dynamic situation of temperature

$$\downarrow \frac{d(\log N)}{d(\text{time})}$$

$$\frac{N}{N_0} = 10^{\left( \frac{-1}{D_{T_{\text{ref}}}} \int_0^{PT} 10^{\frac{T-T_{\text{ref}}}{z}} dt \right)}$$

$$D = D_{\text{ref}} 10^{-\frac{1}{z}(T-T_{\text{ref}})}$$

different temperature histories

approach by Vieira et al. (2002)

**Cupuaçu nectar**

## Case studies:

1

### Square-root

$$\sqrt{k_{\max}} = \sqrt{c} (T - d)$$

c, d constants

### Arrhenius

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

$k_{\text{ref}}$  reaction rate at  $T_{\text{ref}}$   
 $E_a$  activation energy

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

3

4

### Arrhenius

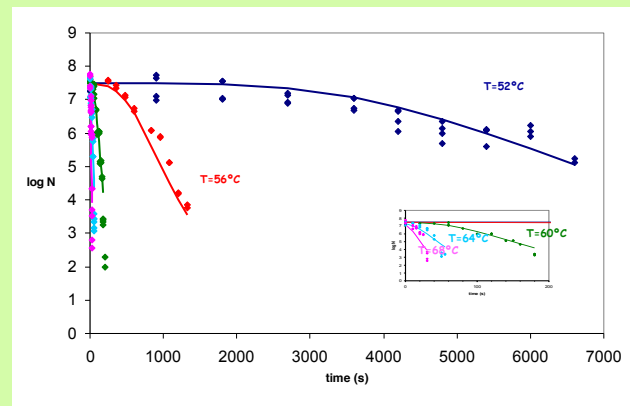
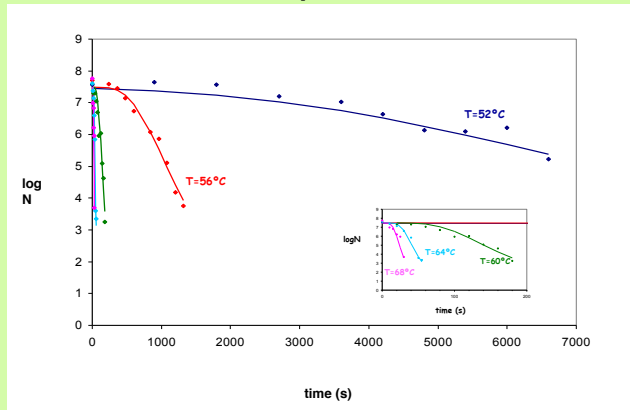
$$L = a \exp\left(b \left(\frac{1}{T} - \frac{1}{T_{\text{ref}}}\right)\right)$$

a, b constants

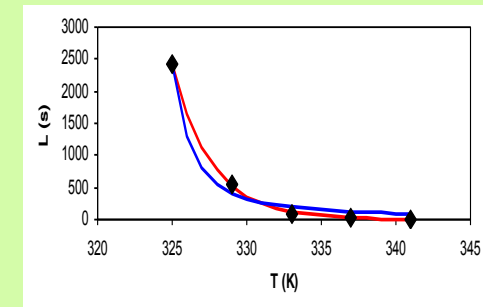
### Williams-Landel-Ferry

$$L = 10^{\left(\frac{a(T - T_{\text{min}})}{b + (T - T_{\text{min}})}\right)}$$

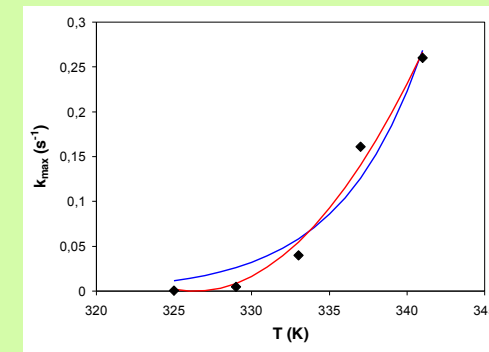
## Gompertz



Two-step



$$L = f(T)$$



$$k_{\max} = f(T)$$

One-step

Equations 1 and 4 selected

*L. Monocytogenes* in half-cream at 52, 56, 60, 64 e 68°C (Casadei *et al.* 1998)



Tank with water  
+  
*L. innocua*



15 minutes

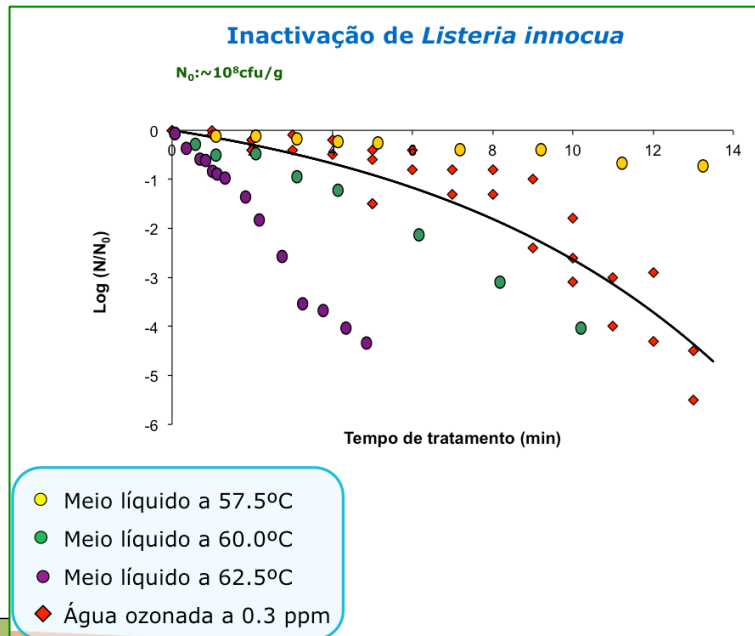


Inumeration



**Weibull  
model**

Ozonation



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# How to obtain the data

- Experiments in broth at various conditions (pH, T,  $a_w$ , [growth inhibitors], etc.)
- Inoculation studies in foods under various conditions
- Derive model parameters

## Sampling:

- Heuristic sampling
- Experimental design

Minimize variance of:

- *predicted response*
- *parameter estimates*

## Data analysis:

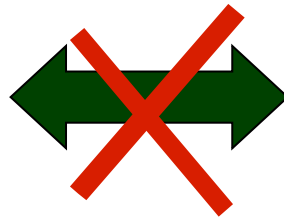
- Regression schemes

$$SSR = \sum_{i=1}^n e_i^2 = \sum_{i=1}^n [y_i - f(x_{ij}, \theta_k)]^2$$

Least-squares  
method

- Analysis of residuals

**Mathematical  
complexity**



**Adequate description**

**quality**

**model**



**parameters**

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# Available software



DTU Aqua  
National Institute of Aquatic Resources

Technical University of Denmark



## Seafood Spoilage and Safety Predictor (SSSP) ver. 3.1

Copyright © 2009 National Institute of Aquatic Resources (DTU Aqua), Technical University of Denmark (DTU)

### Table of content

[Introduction to SSSP](#)

[Installing SSSP](#)

[Using SSSP](#)

- [Relative rate of spoilage \(RRS\) models and general information about SSSP](#)
- [Microbial spoilage models \(MSM\)](#)
- [Options and zoom functions available in SSSP to modify graphs](#)

#### Relative rate of spoilage (RRS) models




- [Introduction](#)
- [Fresh seafood from temperate waters](#)
- [Fresh seafood from tropical waters](#)
- [Cold-smoked salmon](#)
- [Cooked and brined shrimps](#)
- [RRS models with user-defined temperature characteristics](#)
- [Comparison of observed and predicted RRS data](#)

#### Microbial spoilage (MS) models

- [Introduction](#)
- [Photobacterium phosphoreum](#)
- [Shewanella putrefaciens](#)
- [MS models with user-defined parameter values](#)

→ predicts shelf-life as well as growth of spoilage and pathogenic bacteria in seafood

→ evaluates the effect of constant or fluctuating temperature storage conditions (Dalgaard et al. 2002, 2003, 2008)

USDA United States Department of Agriculture Food Safety and Inspection Service  United States Department of Agriculture Agricultural Research Service   EASTERN REGIONAL RESEARCH CENTER

### Predictive Microbiology Information Portal

**PMIP Home** You are here: PMIP Home

- Getting Started
- Overview of Predictive Microbiology
- PMIP Tutorial
- Resource Locator
- Pathogen Modeling Program
- ComBase


**Welcome to the Predictive Microbiology Information Portal**

The USDA Food Safety & Inspection Service (FSIS) and the USDA Agricultural Research Service (ARS) have joined together to produce the [Predictive Microbiology Information Portal \(PMIP\)](#). This portal is geared to assist food companies (large and small) in the use of predictive models, the appropriate application of models, and proper model interpretation. Our vision is that the PMIP will be the highway to the most comprehensive websites that brings together large and small food companies in contact with the information needed to aid in the production of the safest foods.

The PMIP links users to numerous and diverse resources associated with models (PMP), databases (ComBase), regulatory requirements, and food safety principles.

We hope you find the PMIP to be a useful resource!

To start, you can click on the icon below or the navigational links to the left.



[FSIS.USDA.gov](http://FSIS.USDA.gov) | [ARS.USDA.gov](http://ARS.USDA.gov)

- more than 40 models for different bacterial pathogens
- the software allows growth or inactivation of pathogens to be predicted for different combinations of constant temperature, pH, NaCl/ $a_w$  and, in some cases, other conditions such as organic acid type and concentration, atmosphere, or nitrate

**ComBase** ComBase Browser Predictive Models

Home About ComBase Resources Events News Downloads Picture Gallery Help & Support

### ComBase - a Combined dataBase for predictive microbiology

**Welcome to the new home page of ComBase**, a freely available website, the no.1 web-based resource for quantitative food microbiology.

**It includes:**

- A systematically formatted [database](#) of quantified microbial responses to the food environment with more than 50,000 records
- The [ComBase Predictive Models](#) - based on ComBase data to predict the growth or inactivation of microorganisms in food.

**It can be used for:**

- Predicting and improving the microbiological safety and quality of foods*
- Designing, producing and storing foods economically*
- Assessing microbiological risk in foods.*

#### Database Browser

The *ComBase Database* consists of thousands of microbial growth and survival curves that have been collated in research establishments and from publications

Access the [ComBase Browser](#)

#### Predictive Models

The *ComBase Predictive Models* are a collection of software tools based on ComBase data to predict the growth or inactivation of microorganisms

Access the [ComBase Predictive Models](#)

#### Upcoming Events

[FoodMicro 2012](#)

The [25th Food Microbiology conference of ICFMH](#) (International Committee of Food Microbiology and Hygiene of the International Union of Microbiology Societies) will take place in Istanbul, from 3-6 September, 2012.

[ICPMF 2013](#)

The [8th International Conference on Predictive Modelling in Food](#) will take place in Paris from 16-19 September, 2013

Search on the web site

- includes more than 40.000 curves/data on growth, survival or inactivation of microorganism in foods.
- data has been obtained from the literature or provided by supporting institutions
- the modelling toolbox within ComBase includes the Combase Predictor (previously Growth Predictor and Food MicroMoodel).



**SYM'PREVIUS**  
Accélérer l'innovation, garantir la qualité

Le système Sym'Previus    Pourquoi utiliser Sym'Previus    Comment utiliser Sym'Previus    La microbiologie prévisionnelle

Accueil ■ Accueil

Contact ■ **SYM'PREVIUS, UN SYSTÈME OPÉRATIONNEL**

Rechercher ■

Partenaires ■ Ensemble d'outils d'aide à l'expertise en sécurité des aliments, Sym'Previus est conçu pour les professionnels de l'alimentation pour :

Bibliographie ■

Accès logiciels ■

Plan du site ■

- Renforcer les plans HACCP,
- Développer de nouveaux produits,
- Mieux comprendre et quantifier le comportement microbien,
- Déterminer les durées de vie et produire des aliments plus sûrs.

Sym'Previus est destiné à des responsables qualité ou des responsables recherche et développement. Son utilisation permet de réduire les durées de mise en oeuvre, et le nombre d'épreuves expérimentales. Il apporte des arguments basés sur les modèles de microbiologie prévisionnelle les plus récents. Des formations à son utilisation peuvent être proposées par les centres experts Sym'Previus.

Sym'Previus est un outil en évolution permanente, avec un enrichissement de la base de données et des outils de simulation au travers de la participation à des programmes de recherche nationaux et européens.

C'est un outil fiable répondant aux exigences des règlements européens (EC) n° 2073/2005 et n°1441/2007 concernant les critères microbiologiques applicables aux denrées alimentaires.

Recommandations pour l'utilisation de la microbiologie prévisionnelle appliquée aux aliments

→ French decision support system that includes (i) a database with growth and inactivation responses of microorganisms in foods and (ii) predictive models for growth and inactivation of pathogenic bacteria and some spoilage microorganisms

**purac**  
pure by nature

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**Food** Natural solutions for safe, healthy and delicious food

## Calculators

› Listeria Control Model  
› Beverage Fortification Calculator

**Live webinar:**  
Predictive micro modeling in today's food industry  
Reduce R&D cost & increase speed-to-market  
Click on the calculator icon to view the webinar

**Welcome to the Purac calculator portal**

In this portal you will find a wide variety of fast, easy-to-use software tools specifically designed by Purac to save you valuable R&D time. All software on this page is tailor-made and is the culmination of 80 years of Purac ingredient expertise and food processing experience.

Try now, R&D efficiency is just a mouse click away.

**Quick links**

- Tell a friend
- Add to favorites
- Request a sample
- Keep me updated

**Beverage fortification calculator**  
Start Beverage Fortification Calculator  
Calculate calcium fortification impact on:

**Listeria control model**  
Start Listeria Control Model  
Predict Listeria outgrowth with various Purac

→ predicts the effect of organic acids, temperature, pH and moisture on growth of *Listeria monocytogenes* in products

## Shelf Stability Predictor

Developed by the Center for Meat Process Validation at the University of Wisconsin - Madison

### About

Our **Shelf Stability Predictor** provides a set of models for predicting the growth of *Listeria monocytogenes* (LM) and *Staphylococcus aureus* (SA) on Ready-To-Eat meat products as a function of pH and water activity. Use these tools to help you decide if your product is **shelf stable**.



### A shelf stable product:

- Will not support the growth of *L. monocytogenes* (LM) and,
- Will not support the growth of *Staphylococcus aureus* (Staph) and,
- Is vacuum-packaged, packed under modified atmosphere (MAP), or packed with an oxygen scavenger.

### Shelf Stability Predictor

#### Instructions



1. Enter the **pH** and **water activity** of your product in the spaces indicated.<sup>1</sup>
2. Select **Calculate**.

The predictor will indicate the probability of *L. monocytogenes* and *S. aureus* growth on this product, on a scale of 0 = growth very unlikely to 1 = growth very likely.

A value of 0.20 or lower is a clear indicator that *L. monocytogenes* and *S. aureus* will not grow, while a value of 0.80 or higher indicates that *L. monocytogenes* and *S. aureus* are likely to grow.<sup>2</sup>

predict the growth of *Listeria monocytogenes* and *Staphylococcus aureus* on Ready-To-Eat meat products as a function of pH and water activity

# OUTLINE

- Objectives of food industry
- The challenge
- Predictive microbiology
- How to obtain the data
- Available software
- **Validation studies**
- Acknowledgement by regulation
- The complexity of dynamic conditions
- Conclusions

# Validation studies

- Before market introduction, **validation** has to be carried out for **new or altered products**
  - After the concept/prototype
    - acceptance tests → refinement of models → final formulation → validations in challenge test
- have to be performed

- This is the main difference between free and paid software
- A complete challenge test takes approximately 3 months + evaluation of process variations + identification of acceptable limits for formulation limits → establish a theoretical shelf life
- Industry saves costs and time when using reliable predictive micro modelling

- Industry has to perform validation studies, for final verification
- The analytical values for pH, water activity, moisture, etc are crucial
- However, predictive microbiology does not replace hygiene measure or Good Manufacturing Practices
  - models can not be the only hurdle to pathogens

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# Acknowledgement by regulation

- United States:
  - U.S. 9CFR Regulations
  - 2008 USDA Supplementary Guidance
- European Union:
  - 2005 & 2010 EU regulation

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# The complexity of dynamic conditions

Laboratory research



Industrial scale



Laboratory research

Industrial scale

- Studies are often carried out at constant temperatures

- Time-varying temperature conditions are common

transfer of results is compromised

isothermal

non-isothermal

- The greatest modeller's effort has been given to data obtained under constant (or static) environmental conditions
- From a realistic point of view this is somehow restrictive, since the majority of thermal processes occur under time-varying environmental conditions, and kinetic parameters obtained under such circumstances may differ from the ones estimated at static conditions, which compromises safety control

# Case study



## Isothermal conditions

52.5 °C

55.0 °C

57.5 °C

60.0 °C

62.5 °C

65.0 °C

*L. innocua* NCTC 10528

*L. innocua* 2030c

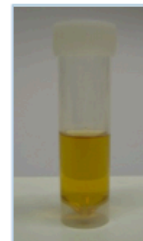
culture media

TSBYE



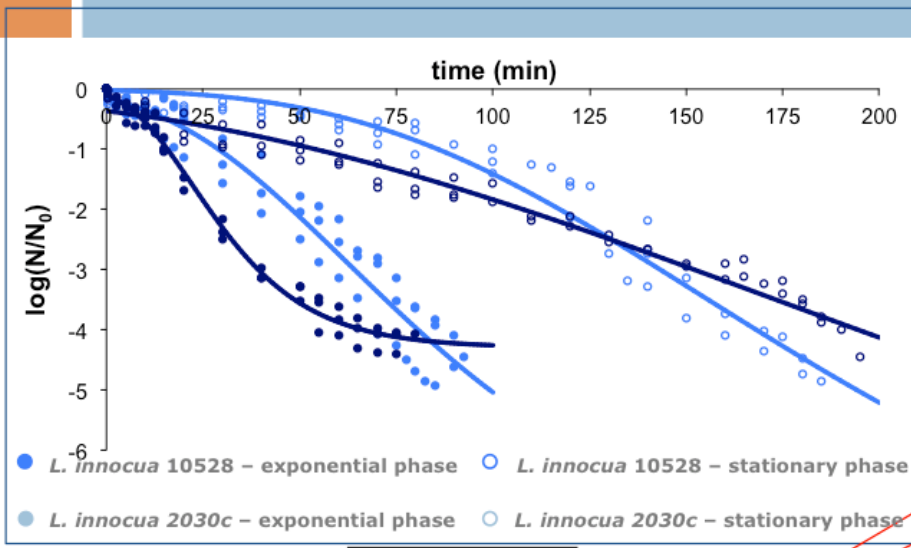
30 °C/9h

30 °C/20h



Exponential phase

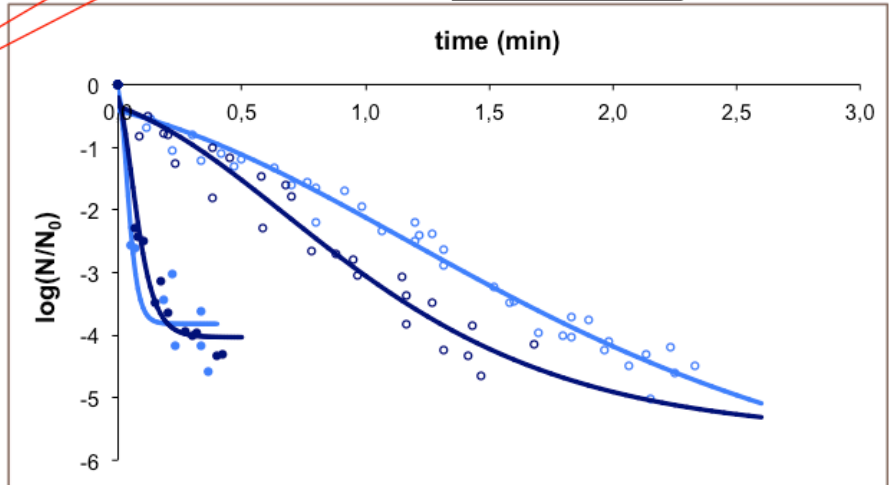
Stationary phase



52.5 °C

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

65.0 °C



Significant temperature effects

N<sub>0</sub> ≈ 10<sup>7</sup> cfu/mL

## Significant temperature effects

### Shoulder parameter

$$L = c(T - d)^2$$

c and d are model parameters

### Maximum inactivation rate

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

$k_{\text{ref}}$  is inactivation rate at temperature  $T_{\text{ref}}$

$E_a$  is the inactivation energy

$R$  is the gas constant

$$\log\left(\frac{N}{N_0}\right) = \log\left(\frac{N_{\text{res}}}{N_0}\right) \exp\left(-\exp\left(\frac{-k_{\max} e^{(L-t)+1}}{\log\left(\frac{N_{\text{res}}}{N_0}\right)}\right)\right)$$



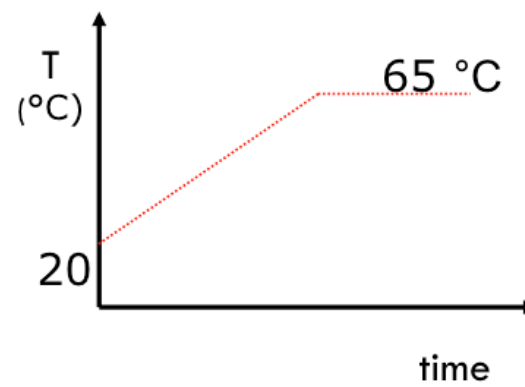


## Non-isothermal conditions

(P1) 1.5 °C / min

(P2) 1.8 °C / min

(P3) 2.6 °C / min



culture media  
TSBYE

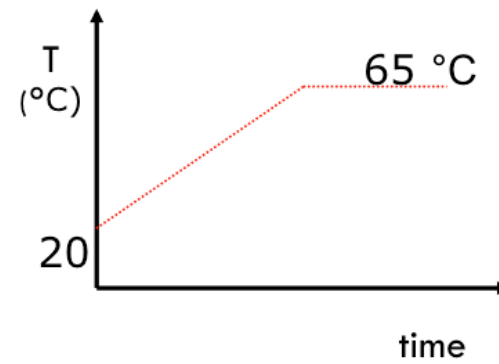


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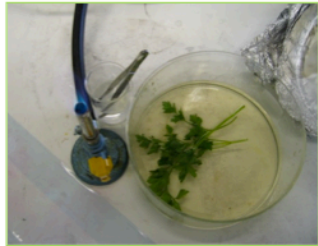


parsley



*Petroselinum crispum*

**parsley** artificially inoculated



TSBYE bacterial suspension  
~ 10<sup>7</sup> cfu/mL of *L. innocua*



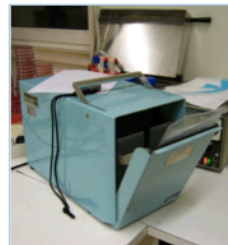
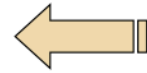
samples vacuum sealed



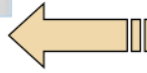
thermal treatment



sampling



stomaker



Palcam agar



30 °C / 5 days

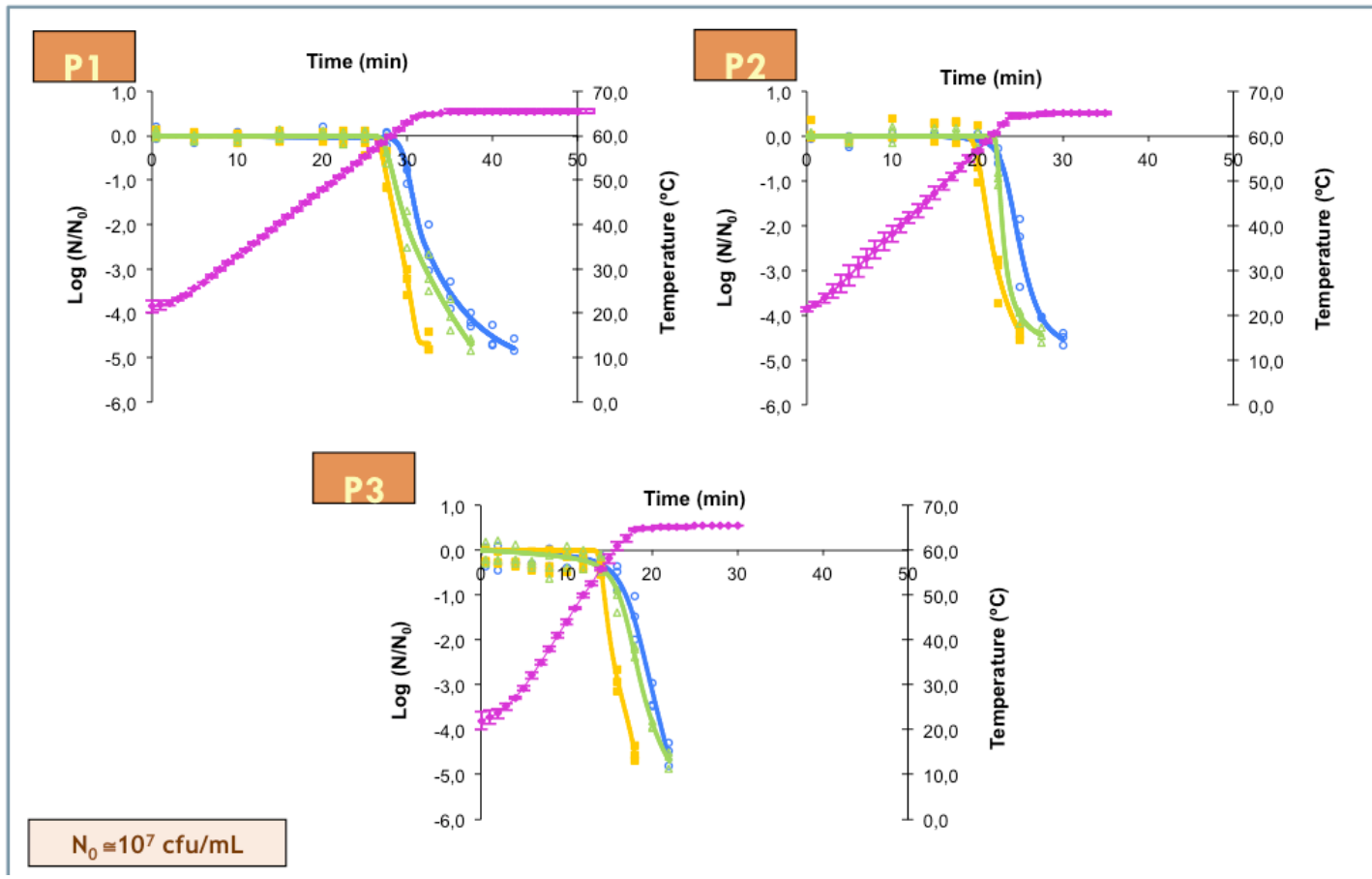
## Gompertz model encompassing the time-temperature effect

$$\log\left(\frac{N}{N_0}\right)_{\text{non-isothermal}} = \int_0^t \frac{d\left(\log\left(\frac{N}{N_0}\right)_{\text{isothermal}}\right)}{dt} dt' \quad T=f(t)$$

$$k_{\max} = k_{\text{ref}} \exp\left(-\frac{E_a}{R} \left(\frac{1}{T} - \frac{1}{T_{\text{ref}}}\right)\right) \quad \Downarrow \quad L = c(T-d)^2$$

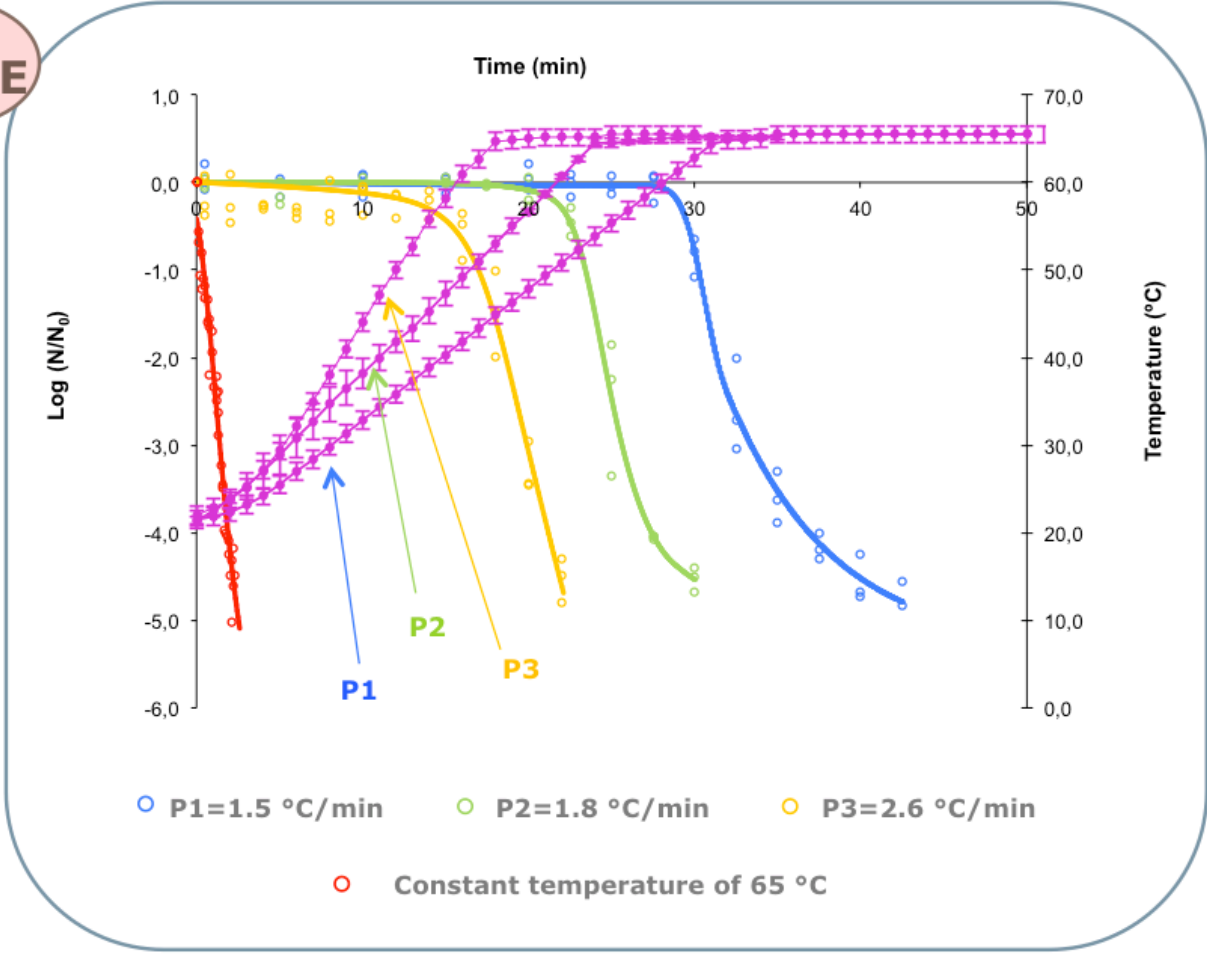
$$\log\left(\frac{N}{N_0}\right)_{\text{non-isothermal}} = \int_0^t \left[ -k_{\max}(t') e \exp\left(-\frac{k_{\max}(t') e}{\log\left(\frac{N_{\text{res}}}{N_0}\right)} (L(t') - t') + 1\right) \exp\left(-\exp\left(-\frac{k_{\max}(t') e}{\log\left(\frac{N_{\text{res}}}{N_0}\right)} (L(t') - t') + 1\right)\right) \right] dt'$$

culture media  
TSBYE



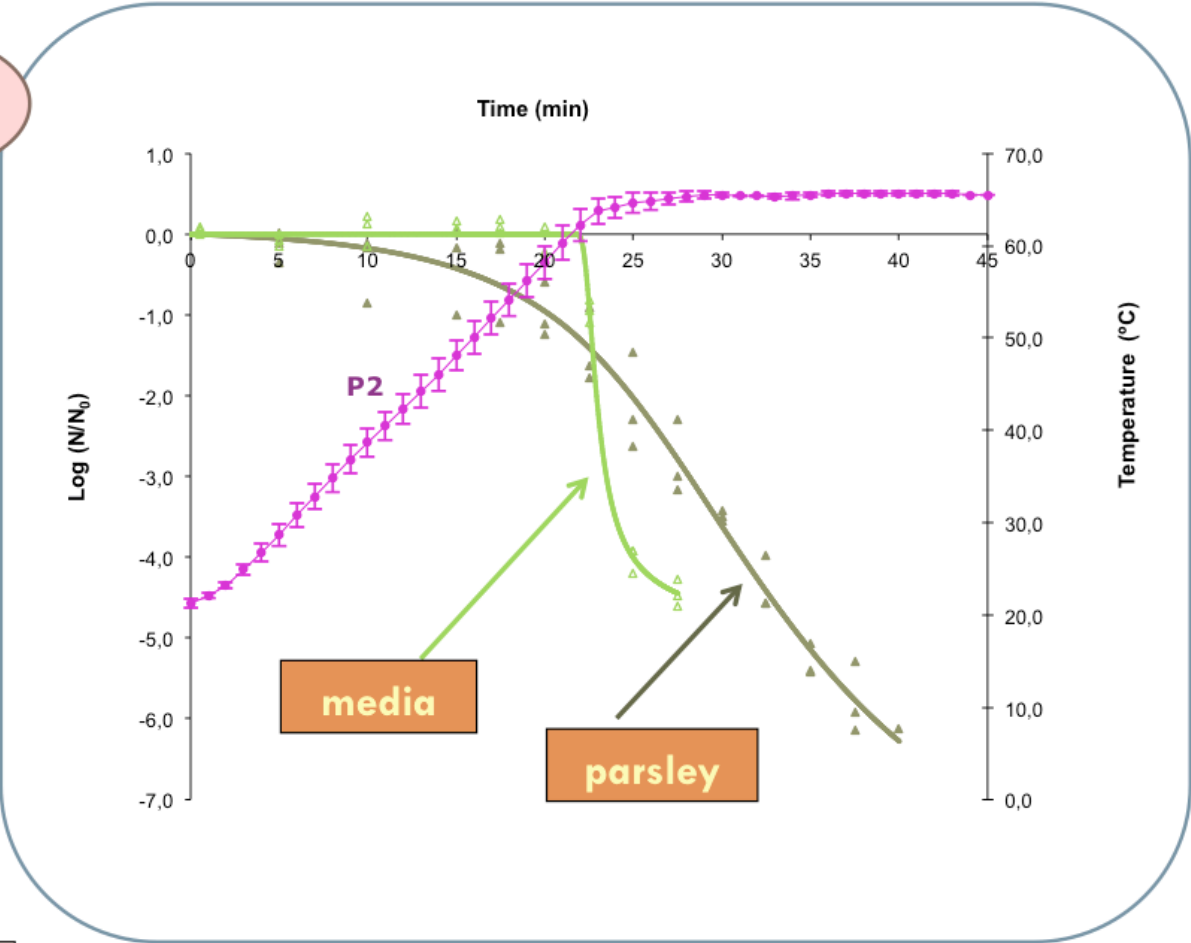
**TSAYE**

culture media  
TSBYE





**Palcam  
Agar**



$N_0 \cong 10^7$  cfu/mL

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# Conclusions

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- Great progresses in the past 20 years
- There are many models available, each with its benefits and limitations
- Yet much work has still to be developed, particularly kinetic studies under dynamic conditions

- Validation studies have to be carried out for new or altered products, due to the complexity of the systems
- Predictive microbiology is a powerful tool, but does not replace hygiene measures or Good Manufacturing Practices



Thank you!

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Elisabeth Alexandre