

## **Predictive Microbiology and Its Use in HACCP**

Protection of food from microbial contamination is a major concern for the food industry from the safety and quality aspects.

A key issue in controlling foodborne pathogens is understanding the factors influencing their growth. The growth of microorganisms in foods is affected by a large number of variables (depending on the microorganism and environment). Examples include temperature, pH, sodium nitrite ( $\text{NaNO}_2$ ), sodium chloride ( $\text{NaCl}$ ), and presence of gaseous atmosphere.

Understanding the effect of various conditions on the growth of microorganisms is essential in evaluating their survival potential and identifying factors important in controlling their existence and minimizing potential risks.

Predictive microbiology provides objective means for evaluating the effect of processing operations on microbial growth, and shelf life and safety of food products. Predictive microbiology is a possible alternative to traditional or rapid microbiological methods, and it can be complementary to the HACCP concept.

**HACCP AND PREDICTIVE MICROBIOLOGY<sup>1</sup>****HACCP**

1. Identify potential hazards and assess their severity at different stages of processing or operations.
2. Identify the Critical Control Points (CCP) where control measures need to be implemented.
3. Specification of control criteria and methods to ensure a control has been achieved (when necessary).
4. Establish and implement monitoring procedures, and response measures to non-compliance situations.

**Predictive Microbiology**

1. Identify the microorganism(s) of concern.
2. Develop an understanding of the ecology of the microorganism to better identify the source and the likelihood of contamination.
3. Compare information with preset control specifications (i.e., accept/reject criteria).
4. Incorporate the available information into monitoring systems that indicate microbial proliferation.

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<sup>1</sup> McMeekin, T.A., Olley, J.N., Ross, T., and Ratkowsky, D.A. 1993. Predictive Microbiology: Theory and Application. RSP, Taunton, England.

## Growth Curves

In a non-limiting nutrient environment bacteria will reproduce and increase in number. Studying the growth kinetics of microorganisms is usually carried out experimentally using laboratory growth medium (growth curve studies). Growth curves are valuable in explaining some trends observed in processing operations, and assist in assessing methods for improving the overall process effectiveness and risk assessment.

Bacterial growth curves relate the change in the number of microorganisms with time as influenced by a set of intrinsic and extrinsic parameters (or conditions) that dictate the growth, survival, and control of desirable and undesirable microorganisms in food systems.

If the logarithm of the density (count) of bacteria is plotted against time, a characteristic curve such as that shown in Fig. 1 results. This curve represents an environment desirable for bacterial growth, and is characterized by four main phases:

1. The **lag phase** (region 1) that encompasses the lag time in which the cells are adjusting their physiology and biochemistry to exploit the environment in which they find themselves.
2. The **exponential (log) phase** (region 2) where the cells grow in their environment as rapidly as possible and at a relatively constant rate.
3. The **stationary phase** (region 3) where the reproduction rate=death rate. The accumulation of waste metabolites leads to some reduction in the growth rate of the microorganisms.
4. The **death phase** (region 4) where a further increase in the accumulation of toxins increases bacterial lysis, and the rate of cell death exceeds the rate at which the cells divide.

Studying the growth kinetics (parameters characterizing growth/survival curves) and response of bacteria provides information that can be used to predict the microbial safety or shelf life of food products.

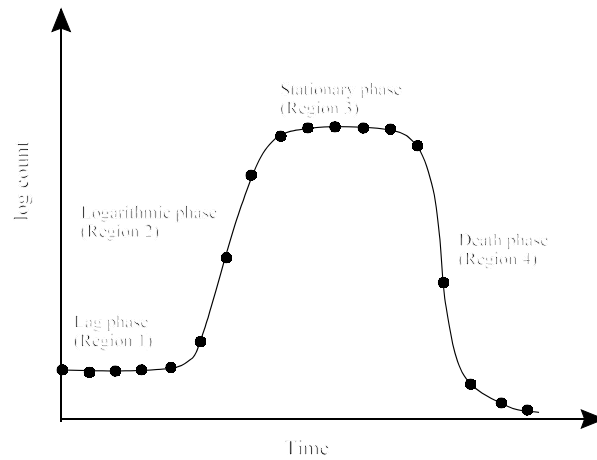


Fig. 1 Schematic of a typical growth curve under constant, initially favorable conditions showing the four phases.

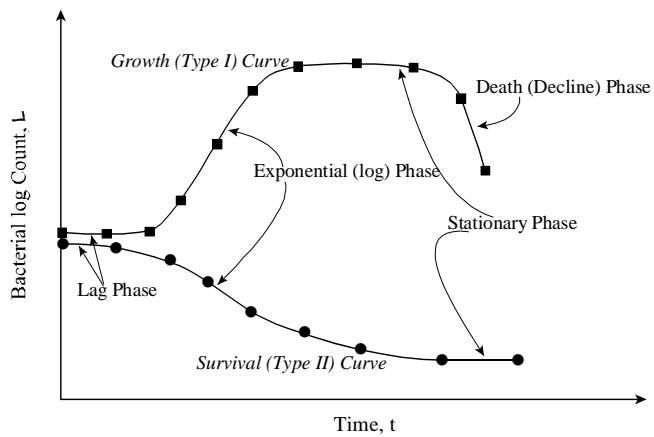


Fig.2 Schematic of growth curve (Type I) and survival curve (Type II) showing the various phases.

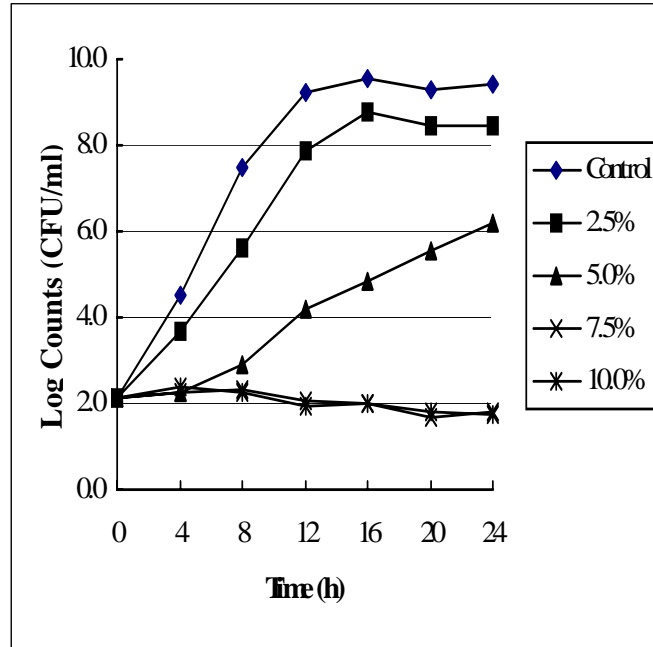


Fig.3 Growth of *Escherichia coli* O157:H7 over time at different concentrations of NaCl.

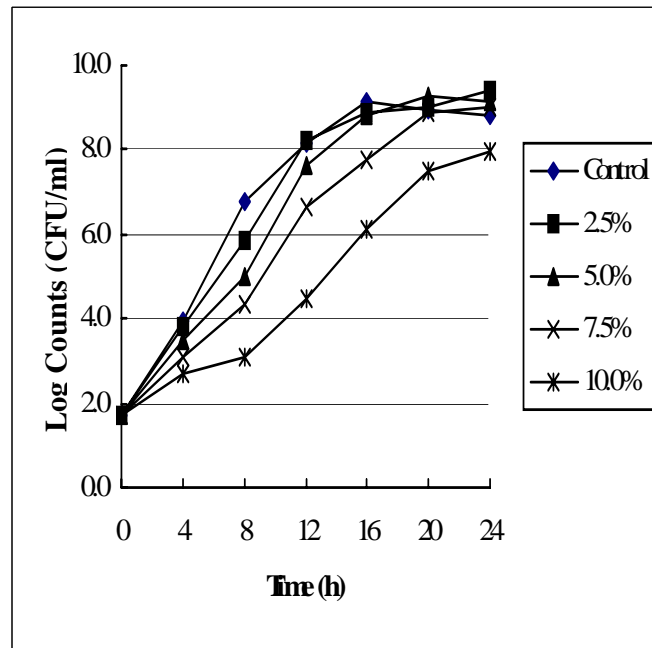


Fig.4 Growth of *Staphylococcus aureus* over time at different concentrations of NaCl.

## **Predictive Models**

The use of simple kinetic parameters descriptive of the curves is one effective means in illustrating and quantifying the differences between the curves and can enable simple comparisons between the various scenarios studied.

Growth models (predictive models) developed to fit experimental growth curves usually include a number of useful growth kinetic parameters:

- Lag phase duration (LPD): The amount of time needed by the microorganisms to adjust to the growth environment.
- Exponential growth rate (EGR): The speed by which the population doubles within the exponential phase.
- Generation time (GT): The time taken for the population within the exponential growth phase to double, also called doubling time. GT can be calculated from the maximum slope within the exponential phase ( $GT = \log_{10}2/\text{slope} = 0.301/\text{slope}$ ).
- Maximum population density (MPD): The highest level in microbial count pertaining to saturation phase.

## **The Modeling Process**

The modeling process encompasses four main points:

1. Planning
2. Collection and analysis of data
3. Mathematical description of data (model development)
4. Validation and maintenance of model

**Predictive Models**

Example on predictive models is the mathematical **modified** Gompertz equation

$$\text{Log}N = A + D e^{-e^{-B(t-M)}}$$

where N is the number of microorganisms, A, B, D, and M are empirical constants, and t is time.

A = value of the lower asymptote { i.e.,  $\text{Log} N_{(-\infty)}$  }

D = difference in value between the lower and upper asymptote { i.e.,  $\text{Log} N_{(\infty)} - \text{Log} N_{(-\infty)}$  }

M = time at which exponential growth rate is maximal

B = a constant related to the slope of the curve at point M

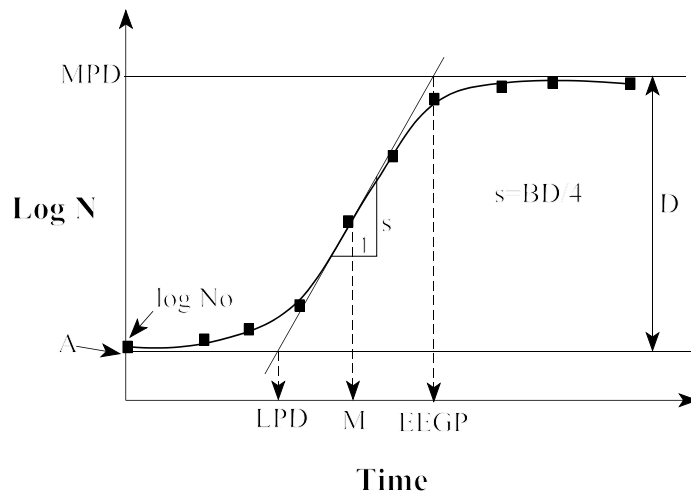
### Kinetic Parameters

$$LPD = M - \frac{1}{B} [1 - e^{1 - e^{BM}}]$$

$$EGR = \frac{BD}{e}$$

$$GT = \frac{0.301 e}{BD}$$

$$MPD = D + A$$



Several examples on the use of predictive microbiological models can be found in the PowerPoint supplemental material. The examples were generated using the Pathogen Modeling Program (PMP) developed at the USDA-ARS eastern regional research center. Additional information can be found at <http://www.arserrc.gov/mfs/PATHOGEN.HTM>

Models for heat inactivation of *Clostridium botulinum* spores were developed in the 1920s, and later for vegetative cells of other bacteria. Inactivation of viruses and other foodborne pathogens generally follows the same pattern, but only bacteria and some fungi can multiply in food.

Heat inactivation of bacterial spores and most vegetative cells is exponential, meaning that a straight line is obtained when the logarithms of survivors are plotted against heating time.

*Clostridium botulinum* (proteolytic) spores heated at 250°F (121.1°C) in medium with near-neutral pH:

Heating time (sec)	Survivors	Log Survivors
0	10 million	7
15	1 million	6
30	100,000	5
45	10,000	4
60	1,000	3
75	100	2
90	10	1
105	1	0
120		

The time for 90% destruction — the D-value or Decimal Reduction Time (DRT) — at this temperature is 15 seconds.

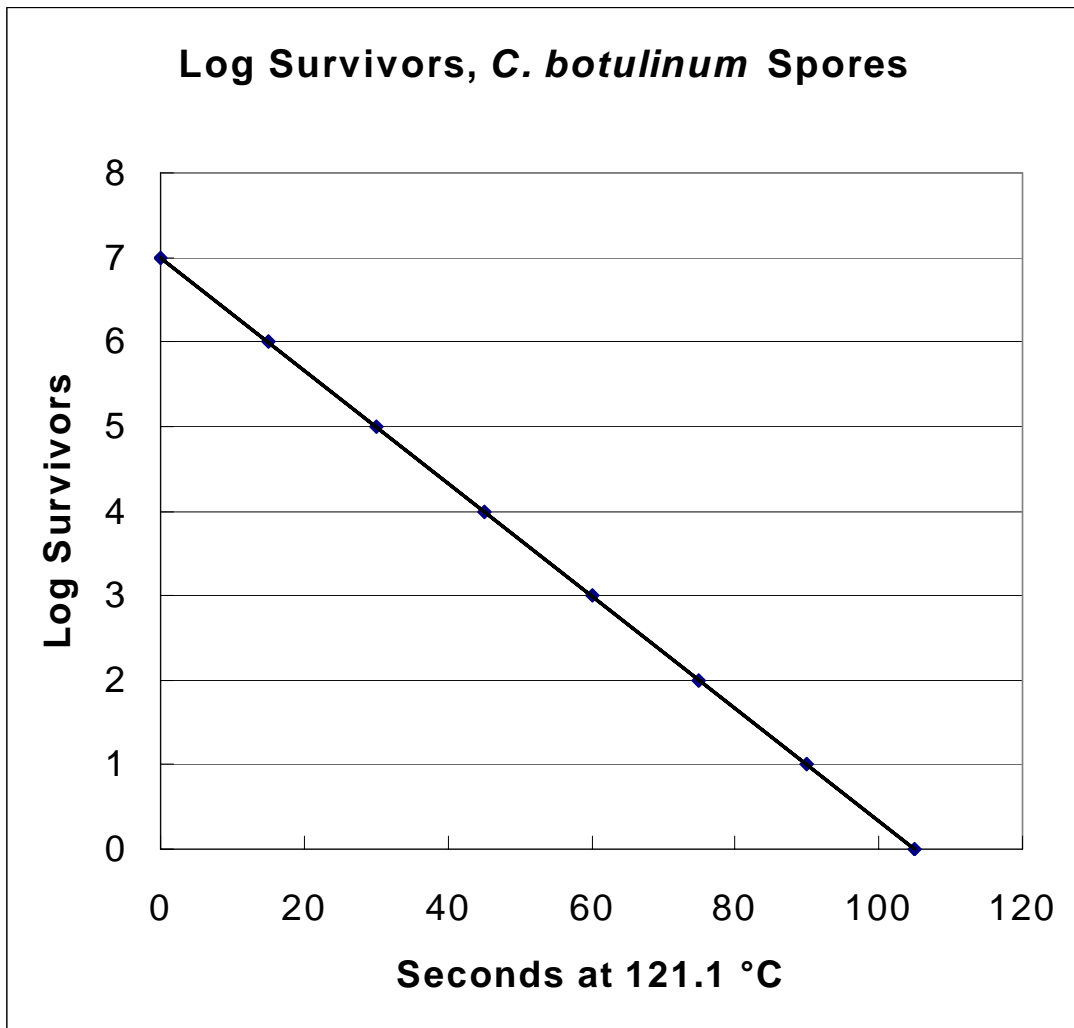


Fig.5 Heat inactivation of *Clostridium botulinum* spores.

Relationship between DRT and heating temperature, for proteolytic *C. botulinum* spores:

Temperature, °C	DRT
121.1	15 sec
111.1	150 sec
101.1	25 min
91.1	4.17 hr

Percent microbial removal as function of  $\log_{10}$  count reductions.

<b><math>\log_{10}</math> reduction (<math>\delta</math>)</b>	<b>Percent reduction in count (<math>\rho</math>)</b>
0.5	68.377
1.0	90.000
1.5	96.838
2.0	99.000
3.0	99.900
4.0	99.990
5.0	99.999

### **Applications of Microbiological Modeling**

1. Hygienic efficiency of meat processing operations, cooling, transport, meat carton thawing
2. Shelf-life studies for meat, poultry and dairy products
3. Validity of regulations, check rationale for mandatory codes of practice
4. Microbial fermentation, finding optimum conditions for growth of desirable microbes (e.g., starter cultures)
5. Conditions for enrichment of target microorganisms in cultures
6. Process optimization and control
7. Product formulation
8. Education

### **References:**

Hajmeer M.N., and Cliver D.O. 2002. Microbiology of Food Preservation and Sanitation. *In* Foodborne Diseases. 2<sup>nd</sup> Ed. Cliver, D.O. and Riemann, H. (Eds.) Academic Press, New York, NY. Chapter 22. pp. 330-352.

Please refer to examples generated using the Pathogen Modeling Program.