# Introduction to the FSO Approach.

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# FSO task force

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This presentation uses material from the FSO task force, and from the Symposium on FSO held at the IFT 2006 annual meeting, but it is not an "official or authorized" representation of the FSO task force.

All the mistakes belong to the author.

The information in this presentation is of basic-exploratory nature.
No warranty is given of its suitability for any particular application.
No reference is made to Baxter's procedures, systems or processes.

$$FSO = H_0 + \sum_i R_i + \sum_j I_j$$

"A statement of the maximum frequency and/or concentration of a microbiological hazard in a food at the time of consumption that provides the appropriate level of protection."

- Traditional regulatory criteria –pasteurize milk for 15s at 72C., etc. – inflexible, not conductive to innovation.
- Performance criteria – SLR 5 for E. coli 0157:H7 in juice, etc. - not directly related to public health and rate of illness.
- Process risk management – from raw ingredients to consumption and possible illness.

# FSO

Maximum frequency and/or concentration of a microbiological hazard in a food at the time of consumption that provides the appropriate level of protection.

A line in the sand.

Level of a specified pathogen for a particular food that will not be exceeded in a serving.

“Companies would decide on specific Performance Objectives(cfu/g), Process Objectives (SLR) for each step and the process criteria (degrees, min) that will meet the FSO”

FSO

“

Links microbial charges to public health objectives

Provide target for process design, validation and verification

Allow greater flexibility in achieving public health objectives

Evaluates entire process.

“

# Bioburden or initial contamination level (Ho),

Hazard analysis questions

Does the food contain ingredients that may present microbiological hazards?

Will the food permit survival or growth of pathogens?

What is the normal microbial load, does it change ? Do changes affect safety of food?

Target pathogen – needs to be destroyed to ensure safety of a food.

Ingredients: origin and preparation means, globalization of food supply.

Examples:

*Clostridium botulinum* spores, *Listeria monocytogenes*, *Staphylococcus aureus*, etc.

Reliable Bioburden data is often insufficient.

*Clostridium botulinum* data are very difficult to generate due to restrictions and cost.

Methodology, sample size, sample origin and control, etc.

Use of literature data, or use data for a similar microorganism or group or microorganisms in the ingredients of food – mesophilic PA's to estimate *C. botulinum* concentration.

“If we have no data:

Worst case – looking at data from all foods estimate the highest realistic value of Ho.

What ingredients are likely to have a high level of contamination?

How do we deal with soil data? Data exists for C. botulinum in soil from different regions of the world and aquatic environments.”

“Data to be used for Ho should be preferably quantitative and include mean and standard deviation values.  Address Frequency distribution: Normal, log normal, empirical, etc.

Ho, the concentration of the target pathogen(s) must be considered to deliver an appropriate FSO.”

Prevention of microbial growth (I).

Commercial sterility: Heat, Aw (<0.8 0.94 for *C. botulinum*), pH (<4.6)

Low acid canned foods are a good environment for growth of *Clostridium botulinum* spores – spores must be destroyed or controlled through pH, Aw or $NO_2$+NaCl

Prevention of growth – formulation, GMP and GHP.

Prevention of recontamination – cooling water, package integrity, recontamination prior to filling in aseptic.

Thermophilic spoilage

"Maintenance of free chlorine levels of 2-5 ppm at pH 5-7.7 gives a reasonably good sanitation control of cooling water"

Package integrity – "probability of botulism from container leakage is about $3.8 \times 10^{-12}$"

“Aseptic – GMP, GHP are critical in this area to maintain Ho

Thermophilic spoilage – PNSU $10^{-3}$ (Pflug)

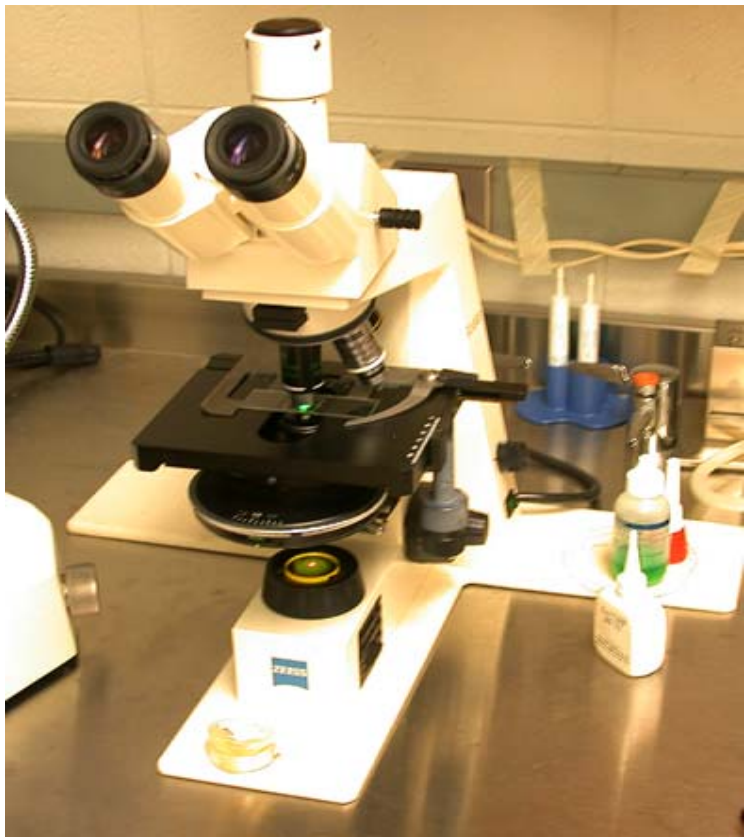
We can’t let down our guard and rely solely on sigma r”

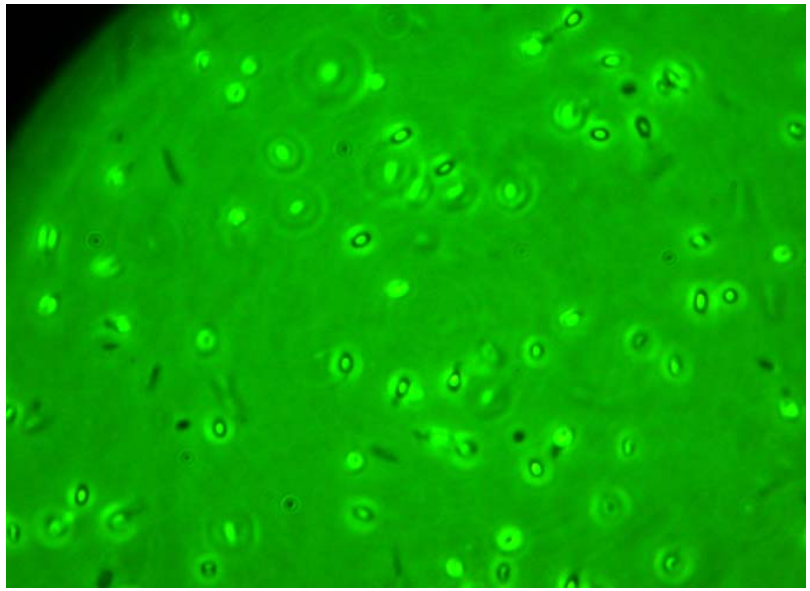
The formula for the food safety objectives (FSO) includes a term that describes the inactivation of microorganisms by one or more processes: $\sum_i R_i$

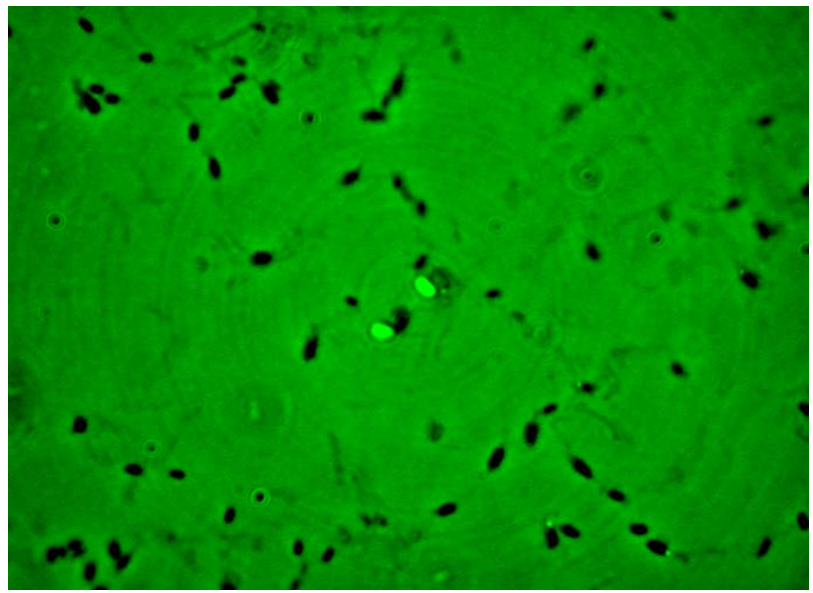
$$FSO = H_0 + \sum_i R_i + \sum_j I_j$$

The safety of the product is most often not affected by the sterilization processes because the bacteria that may induce microbiological alterations are usually more resistant than the bacteria that may cause safety risk. An exception is high pressure sterilization.

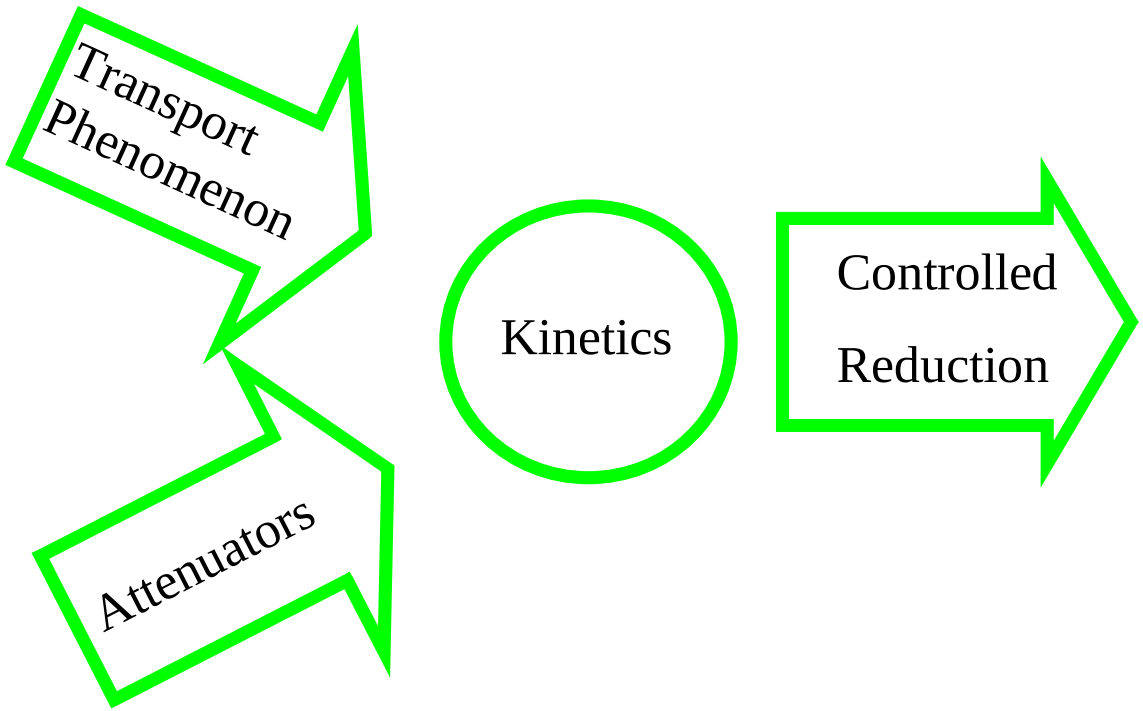
However, the modeling techniques described here can also be applied to describe the inactivation of bacteria and molds that may cause microbiological alterations.

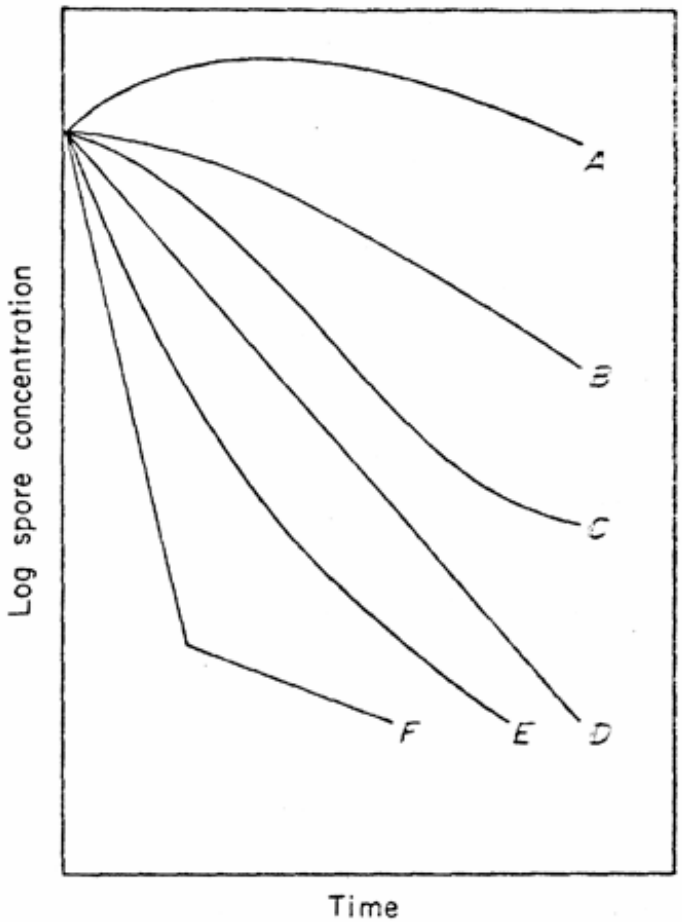






# Control Measures






Cerf, 1977

Fig. 1. Types of survival curves. *A* and *B*, 'shoulder'; *C*, 'sigmoid'; *D*, logarithmic curve; *E*, 'upward concavity'; *F*, 'biphasic curve' with a 'tail'.

The complexity and diversity of industrial sterilization processes precludes the selection of a single approach to describe mathematically the inactivation of microorganisms. The model to select should be the simplest model that describes the inactivation process properly.

# Main types of models for the inactivation transformation:

- Probabilistic/mechanistic.
  - Provide information on the scientific nature of phenomena.
  - Enable design, and what-if trouble shooting.
  - Extrapolation possible.
- Empirical
  - Extremely useful in industrial applications.
  - Work must be re-done if any factor changes beyond the validated range.
  - Interpolation only.

# Mechanistic models

- Single transformation/subpopulation (normally inactivation).
  - Simpler, should be used unless additional transformations/subpopulations force more complex models (Bigelow, Chick, etc).
- Multiple transformations/subpopulations (activation, more/less resistant subpopulations - tailing).
  - More complex – a lot more work.
  - Capable of describing more complex situations in agreement with current scientific knowledge (physics, chemistry, thermodynamics, etc).

# Empirical (curve-fitting) models.

- Linear combination of functions (for instance, polynomials).
  - Enable statistical testing of hypotheses, and calculation of prediction and confidence intervals.
  - Always find a solution (no initial guess needed)
- Nonlinear regression.
  - Good to perform parameter determination for nonlinear mechanistic models.
  - Requires initial guess, may have more than one solution (or none).
  - Statistical tools more scarce, complex and less well understood.

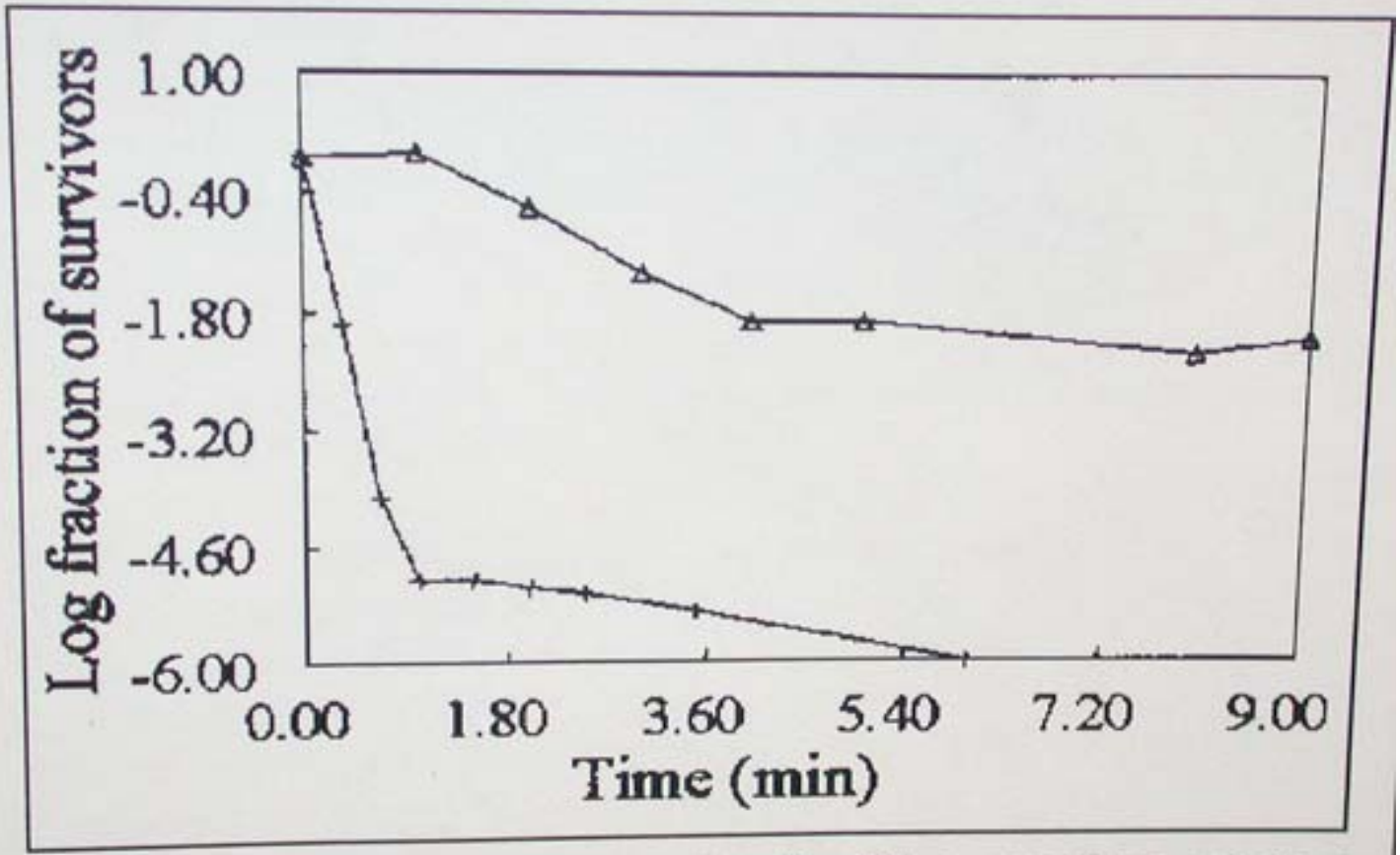



**Figure 3— Survivor curves for *Bacillus pumilus* spores heated at 104 °C. + = original population. △ = spores obtained from tail survivors**

Jolis et al., 2001.

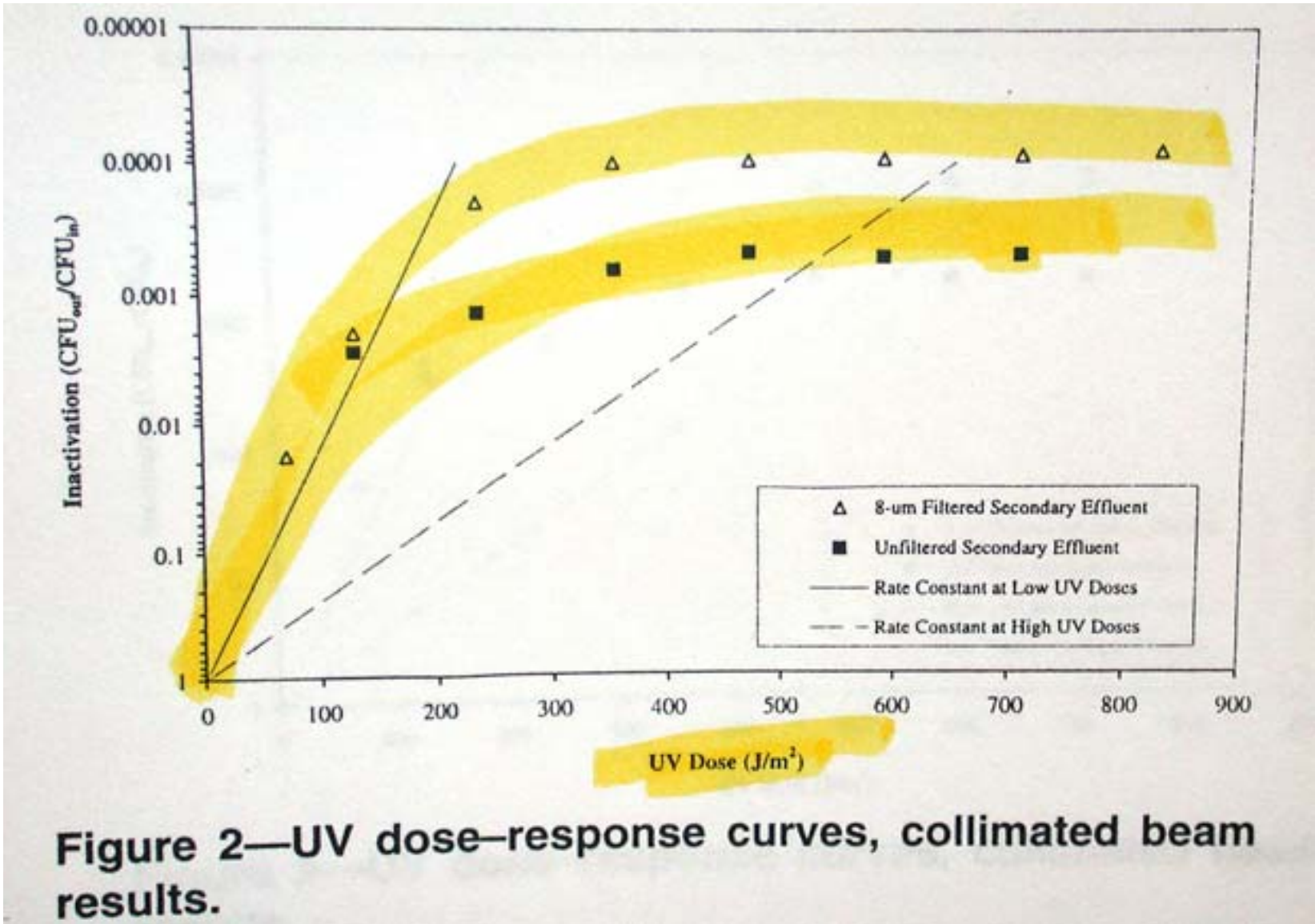


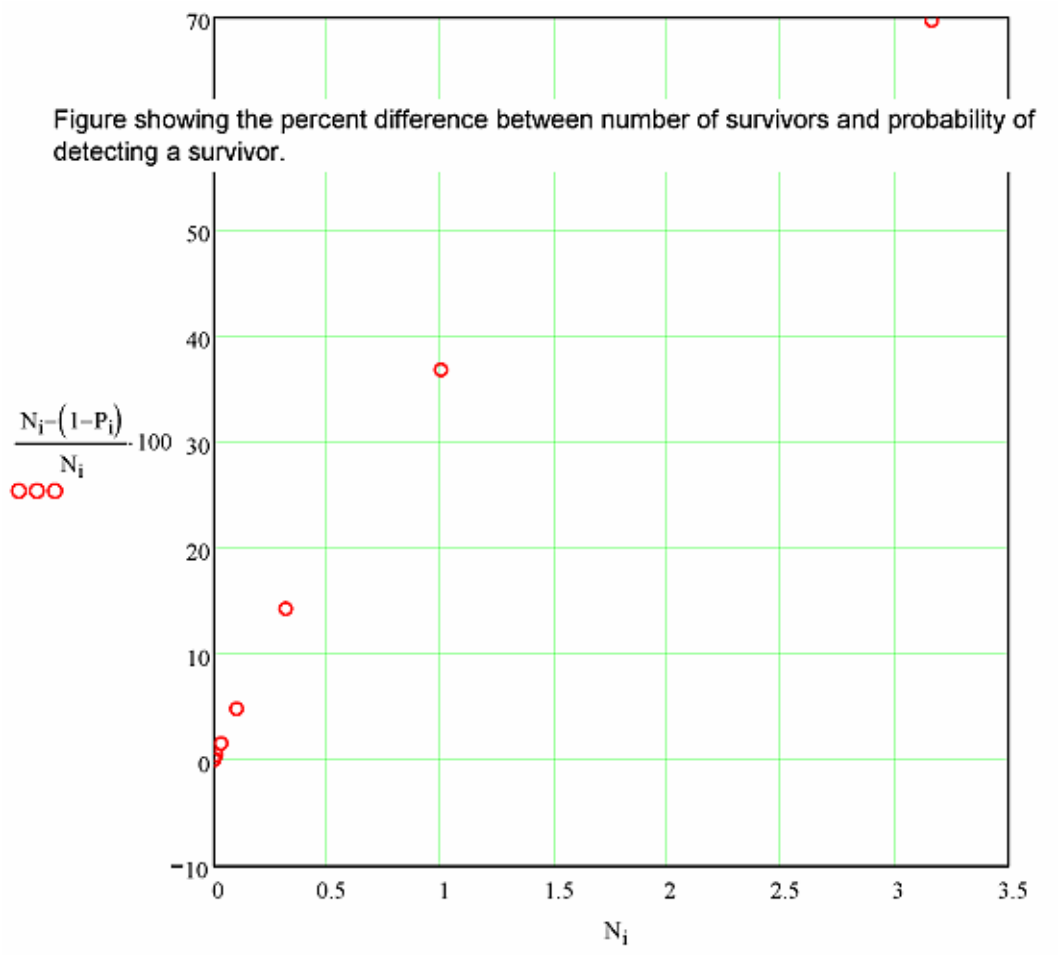

**Figure 2—UV dose–response curves, collimated beam results.**

Modeling microbial inactivation:

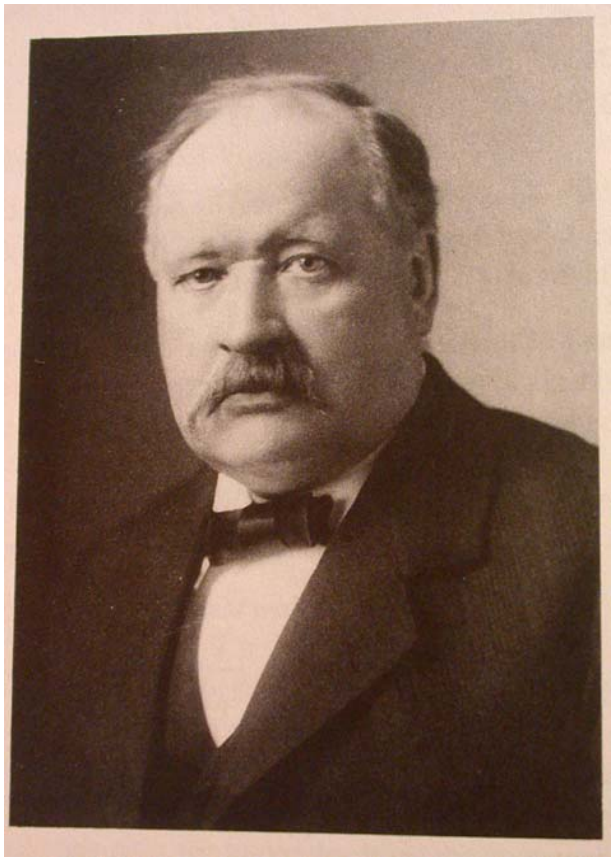
Probabilistic
- Quantal
- Life-span

Mechanistic
- Single transformation and/or population
- Multiple transformations
- Multiple populations with different resistance

Empirical (statistical curve-fitting)
- Linear combination of functions
- Nonlinear regression.




Halvorson &Ziegler's probability expression enabled their development of the MPN method and may be used to understand why when survivorship is small it coincides with the value of the probability of finding a survivor.



Svante Arrhenius
1859-1927
Nobel Prize 1903

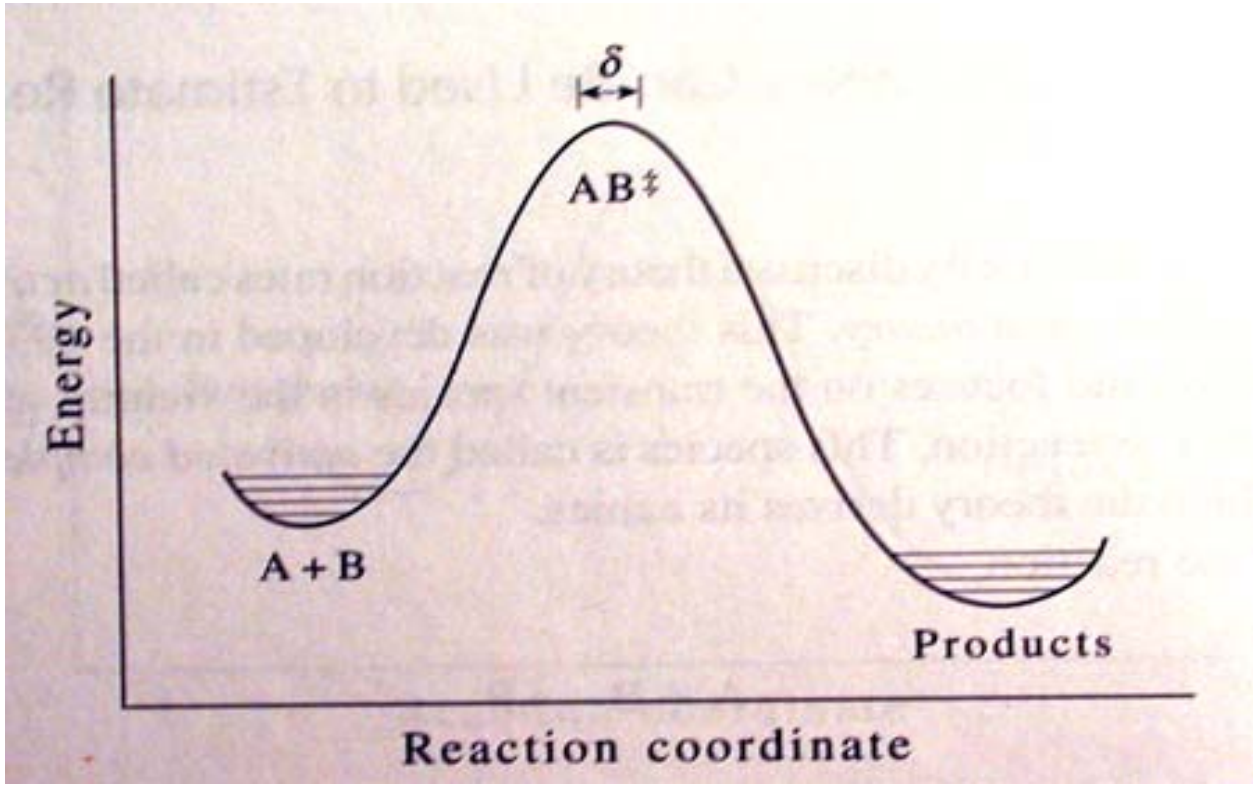
$$\ln k = \ln A - \frac{E_a}{RT}$$

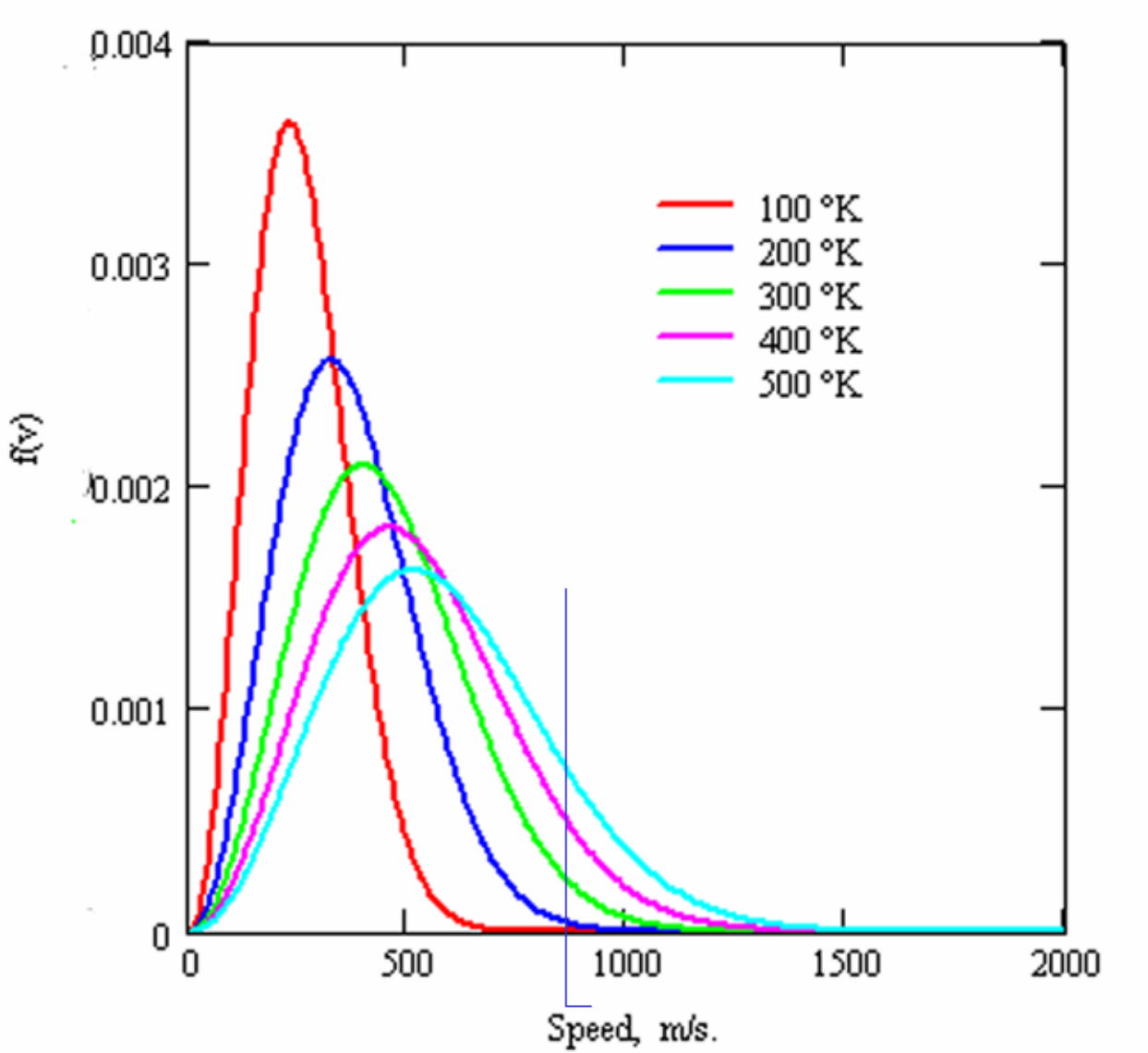
$$k = Ae^{-E_a/RT}$$

$$k(T) = \frac{e^2 k_B T}{hc^\circ} e^{\Delta^\ddagger S^\circ/R} e^{-E_a/RT}$$

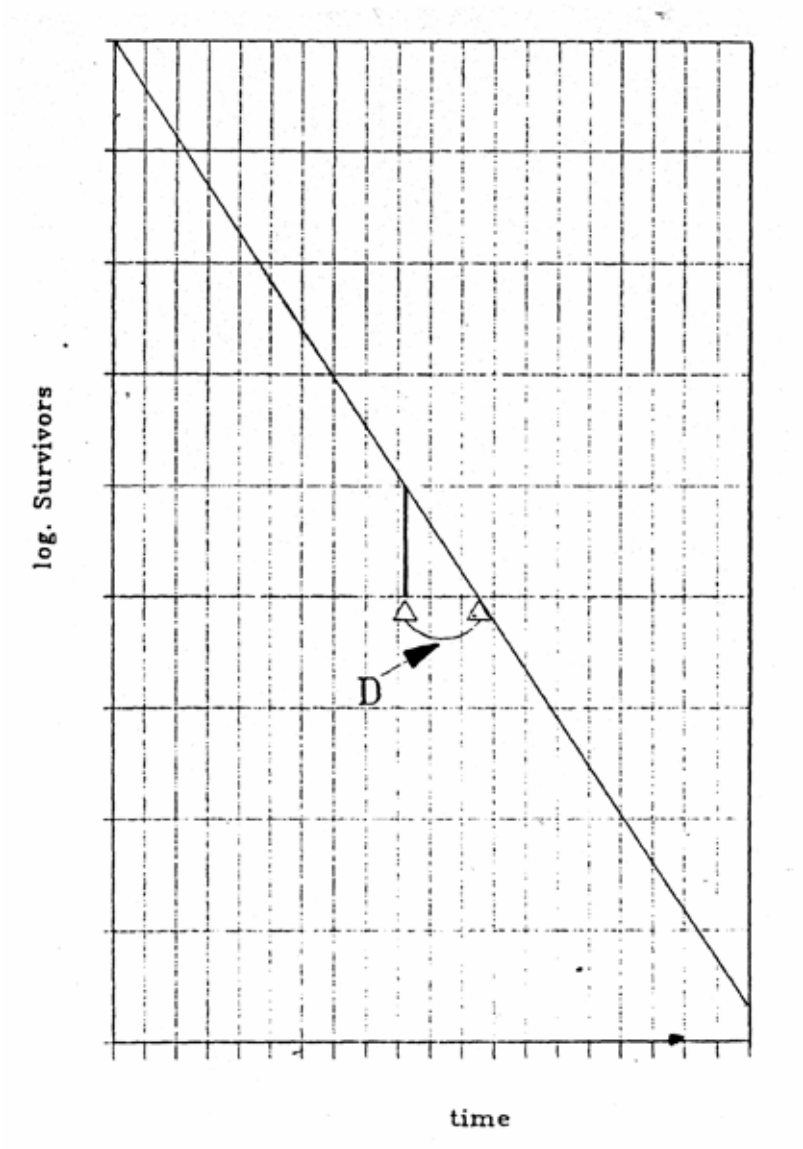
$$A = \frac{e^2 k_B T}{hc^\circ} e^{\Delta^\ddagger S^\circ/R}$$

$$k = aT^m e^{-E'/RT}$$


δ
AB‡
Energy
A + B
Products
Reaction coordinate




Maxwell-Boltzman distribution of the speed of molecules.


log. Survivors
D
time

Experiments with DNA of different chain lengths reveal that the constant $A$ is nearly independent of $Z_{\text{basepair}}$. The agreement of experiment and theory indicates that our very simple model is essentially correct.

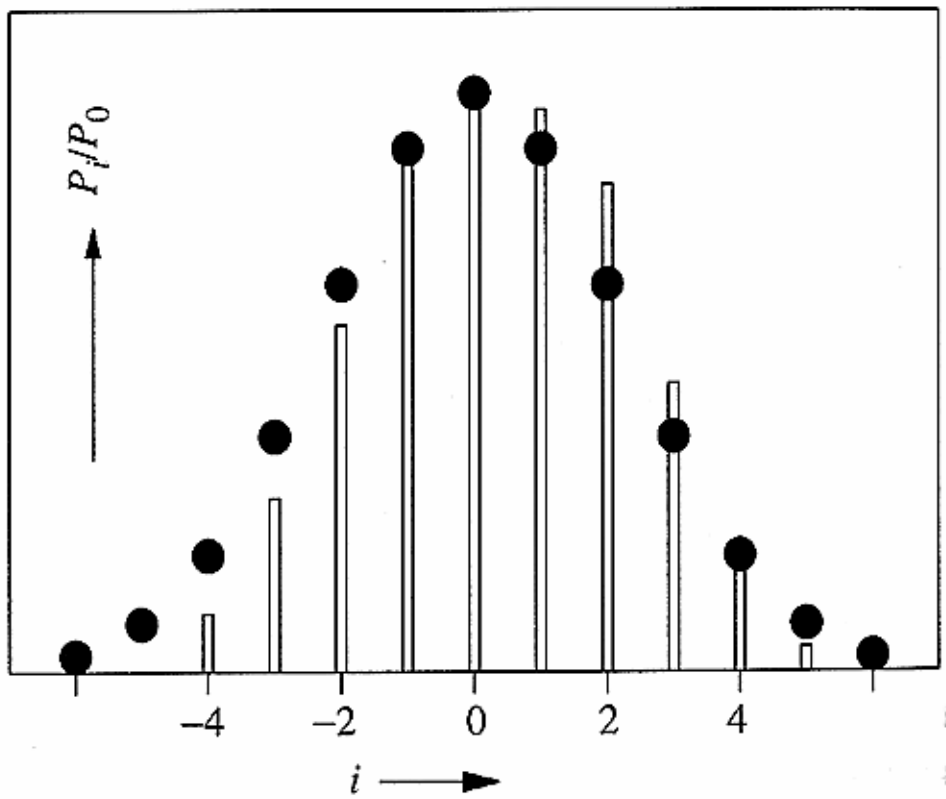



**Figure 12.29** Comparison of the experimental probabilities $P_i$ (bars), to the values calculated from the Boltzmann equation (dots). Experimental data: R.E. Depew, J.C. Wang, Conformational functuation of DNA helix, *Proc. Nat. Acad. Sci.* USA 72, 4275 (1975) J.C. Wang, Helical repeat of DNA in solution, *Proc. Nat. Acad. Sci.* USA 76, 200 (1979)).

# Model of the inactivation transformation.

$$N(t) = \frac{N_0}{10^{\left[\frac{1}{D_{250}}\int_0^t 10^{\frac{T(t)-250}{z}} dt\right]}}$$

$$\frac{dN}{dt} = -kN$$

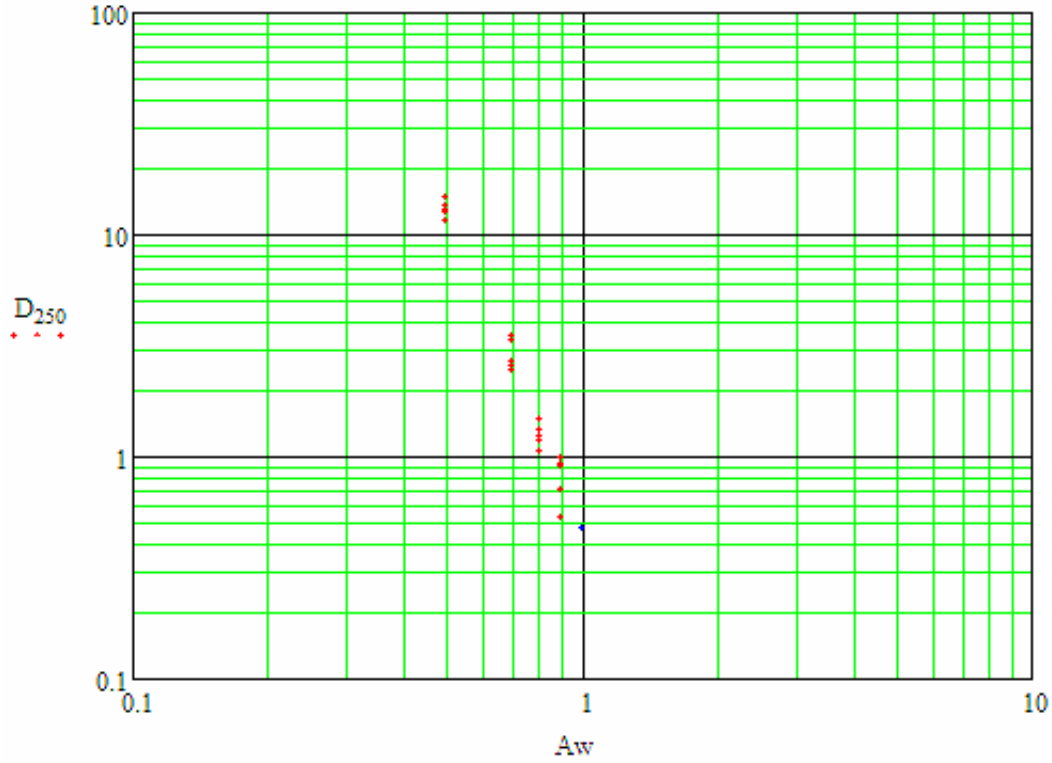
$$D = \ln(10)/k$$

$$Fo = \int_0^{time} 10^{\frac{T(t)-250}{18}} dt$$

$$\ln(D) = f(T, P, C, Aw, pH, dose, \ldots)$$

$$SLR = \log\left(\frac{N_0}{N}\right) = \int_0^{time} \frac{dt}{D(T(t), C(t), P(t), pH, \ldots)}$$

$$\sigma^2(\ln(D)) \approx \frac{\partial f}{\partial T}\sigma^2 T + \frac{\partial f}{\partial P}\sigma^2 P + \frac{\partial f}{\partial C}\sigma^2 C + \frac{\partial f}{\partial A_w}\sigma^2 A_w + \frac{\partial f}{\partial pH}\sigma^2 pH + \ldots$$




$X := Aw - 1$

$\overrightarrow{slope(X, \log(D_{250}))} = -3.12$

$10^{\overrightarrow{intercept(X, \log(D_{250}))}} = 0.337$

$\left(\overrightarrow{corr(X, \log(D_{250}))}\right)^2 = 0.973$

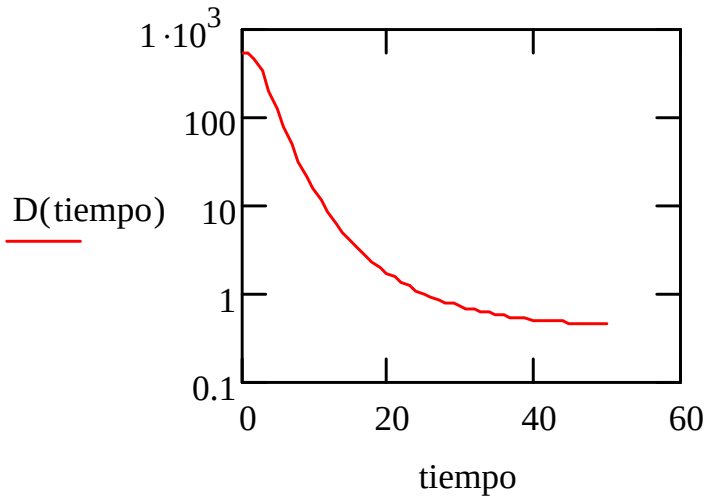
The decimal antilogarithm of the ordinate to the origin corresponds to the $D_{250}$ value when Aw = 1.0.

# Empirical description of the relationship between D250 and Aw.

Values of model parameters to calculate the decimal reduction time as function of Aw and T.

$D_0 := 0.4$ $\quad m := -3.12$ $\quad z := 11$

$$D(x) := D_0 \cdot 10^{m \cdot (Aw(x)-1)+\left(\frac{T_0 - T(x)}{z}\right)}$$




D(40) = 0.501

$$SLR(x) := \int_0^x \frac{1}{D(y)}\, dy$$

This function calculates the SLR. It uses the concentration and temperature regimes together with the kinetic parameters D0, m, and z .

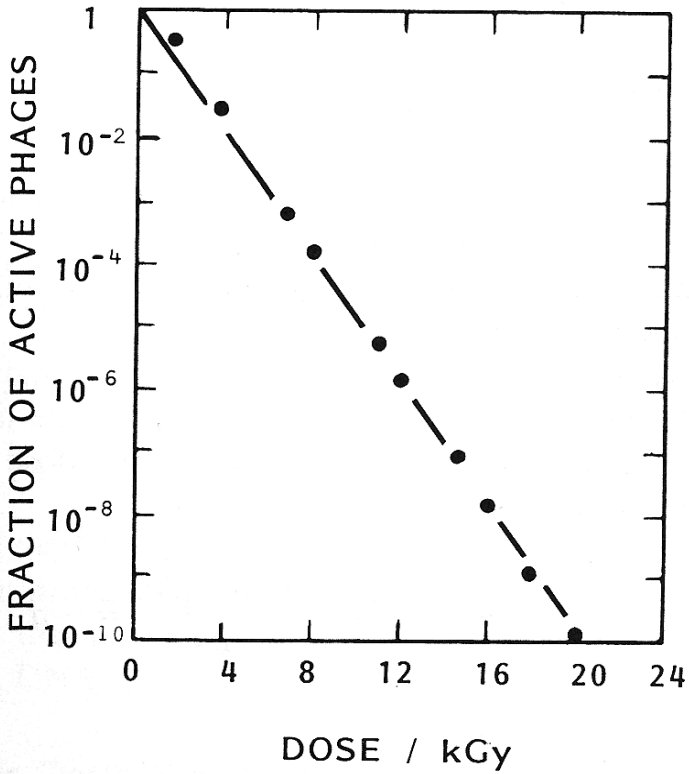
$$\frac{dN}{dt} = -kC^n N$$ Chick, 1908.

$$\frac{dN}{dt} = -kN$$ Chick, 1910 (C = 1).

Where

*N* is the number of survivors.
*t* is time.
*k* is the rate constant
*C* is the concentration of the lethal agent.
*n* is an exponent.

From Kellerer (1987).



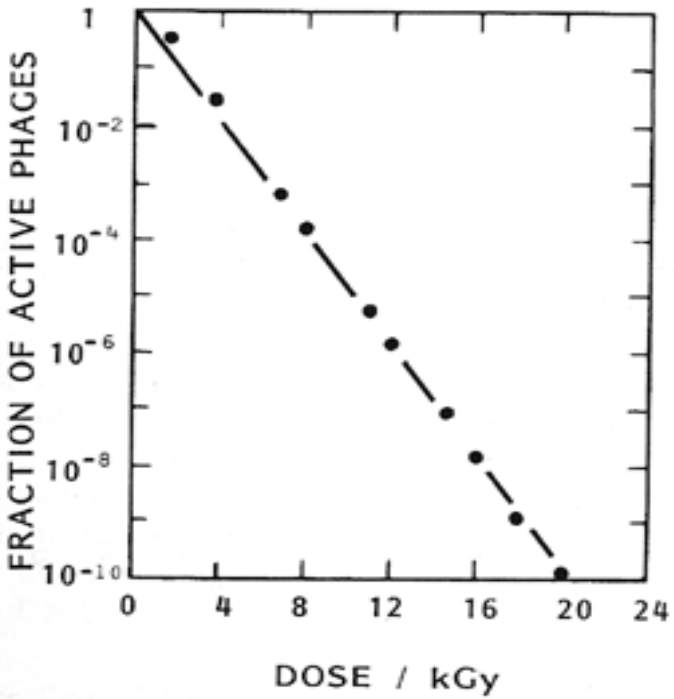

Exponential dose dependence for the inactivation of T1 phages by Co $\gamma$ rays

$$\frac{dN(D)}{dD} = -a \times N(D)$$

$$\frac{d\ \ln\left(N\left(D\right)\right)}{dD} = -a$$

$$S(D) = \frac{N(D)}{N_0} = \exp(-a \times D)$$

From Kellerer (1987).




Exponential dose dependence for the inactivation of T1 phages by Co γ rays

$a = 0.0011\ Gy^{-1}$

mean number of hits in the target region = a x D

E = mean energy transfer for primary ionization.

E = 80 eV

1 eV =1.602x10-19J,
1Gy = 1J/kg.

mean energy per target region = a D E = D m

mass (m) = E a = $1.4 \times 10^{-17}$ kg.

The actual mass of DNA double-strand molecule of the T1 phage is $5 \times 10^{-17}$ g.

$$\frac{dN}{dt} = -kC^n N$$

$$F \equiv \frac{1}{kC^n} \ln\left(\frac{N_0}{N}\right)$$

$$\log\left(\frac{k_2}{k_1}\right) = \frac{T_2 - T_1}{z}$$

Basic equations that describe the inactivation rate, lethality and the effect of temperature on the rate constant.

Rodriguez, et al., 2001.

$$N(t) = \frac{N(t=0)}{e^{\left[ K_{T_R} \int_0^t C^n(t) * 10^{\frac{T(t)-T_R}{z}} dt \right]}}$$

Formulae that enable us to estimate the survivors and to compare transient sterilization cycles.

$$F_{T_R, C_R, z} = \frac{1}{C^n{}_R} \int_0^t C^n(t) 10^{\frac{T(t)-T_R}{z}} dt$$

Rodriguez, et al., 2001.

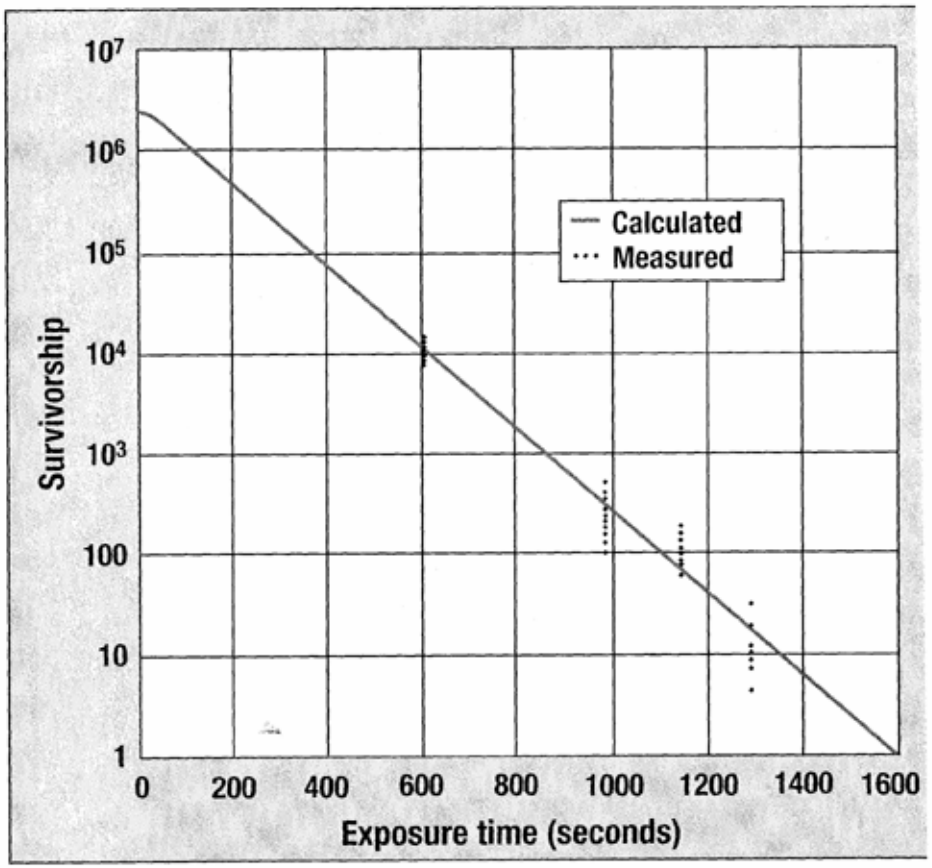



*Figure 6. Comparison of measured and calculated values for the lethal effect of EtO on* **B. subtilis** *var.* **niger** *using a resistometer. The concentration was 600 mg/L and the temperature 54°C.*

Rodriguez, et al., 2001.

$$\frac{dN}{dt} = -kN$$

Equations for the inactivation rate and the dependency of the rate constant with respect to pressure and temperature.

$$d\ln k = \left(\frac{\partial \ln k}{\partial T}\right)_T dT + \left(\frac{\partial \ln k}{\partial P}\right)_P dP$$

$$\left(\frac{\partial \ln k}{\partial T}\right)_P = \frac{Ea}{RT^2}$$

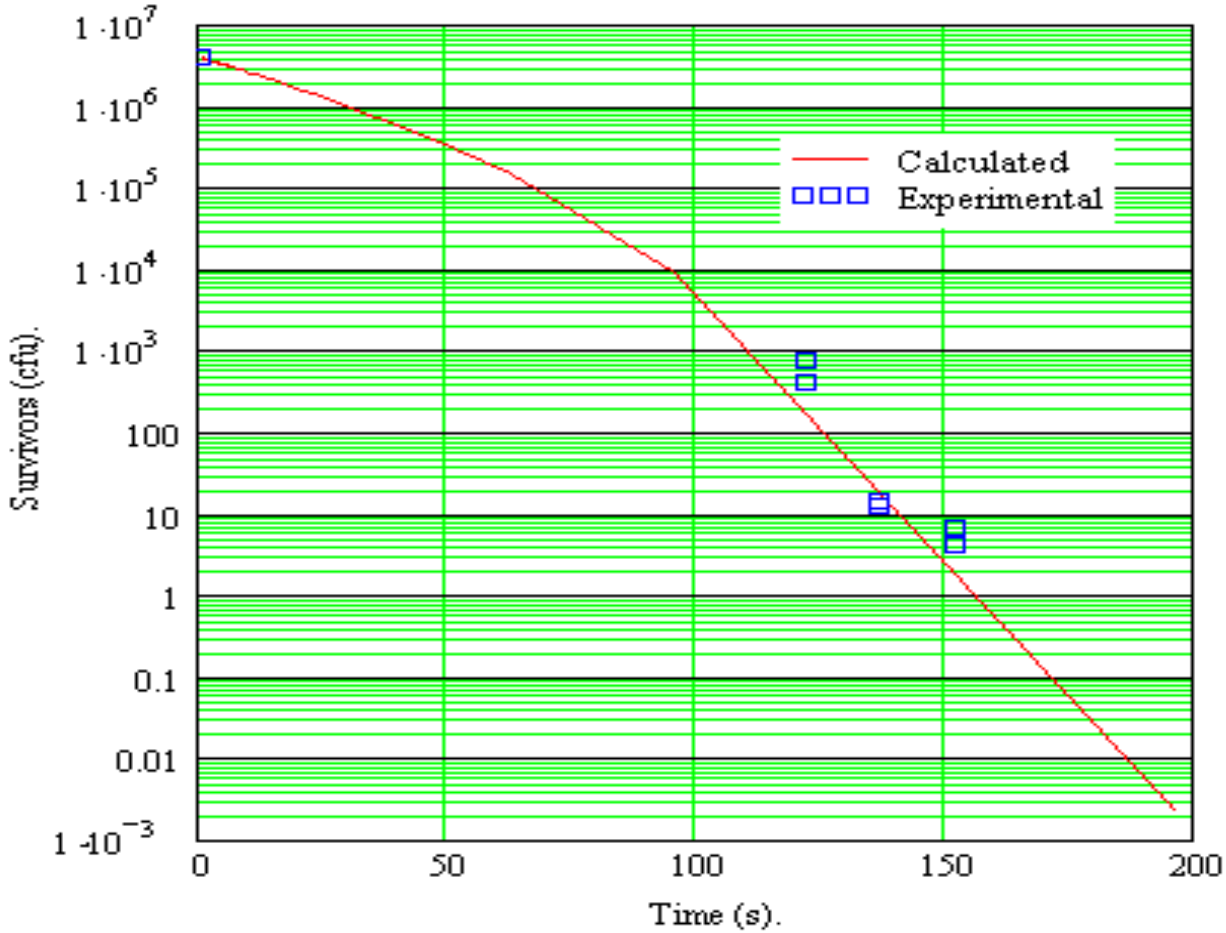
$$\left(\frac{\partial \ln k}{\partial P}\right)_T = -\frac{\Delta V^*}{RT}$$

$$k = k_o \exp\left\{-\left[\frac{\Delta V^*}{RT_0}\left(P - P_0\right) + \frac{Ea}{R}\left(\frac{1}{T} - \frac{1}{T_0}\right)\right]\right\}$$

# Formulae that enable us to estimate the survivors and to compare transient UHP sterilization cycles (Rodriguez, 2002).

$$N(t) = N_o \exp\left\langle -k_o \int_0^{processTime} \exp\left\{ -\left[ \frac{\Delta V^*}{RT_0}\left(P(t) - P_0\right) + \frac{Ea}{R}\left( \frac{1}{T(t)} - \frac{1}{T_0} \right) \right] \right\} dt \right\rangle$$

$$F_0 = \int_0^{processTime} \exp\left\{ -\left[ \frac{\Delta V^*}{RT_0}\left(P(t) - P_0\right) + \frac{Ea}{R}\left( \frac{1}{T(t)} - \frac{1}{T_0} \right) \right] \right\} dt \ldots\ldots\ldots (11)$$




*B. stearothemophilus*,
transient ~700mPa, ~110°C.

# Kinetic Models and Response Equations for multiple transformations/subpopulations:

Moist-heat (system analysis),
Gas (chemical),
and High-Pressure Sterilization.

System analysis enables us to handle combinations of the transformations.

It has been shown to apply to biological systems.

See Smerage, 1979 for additional information.

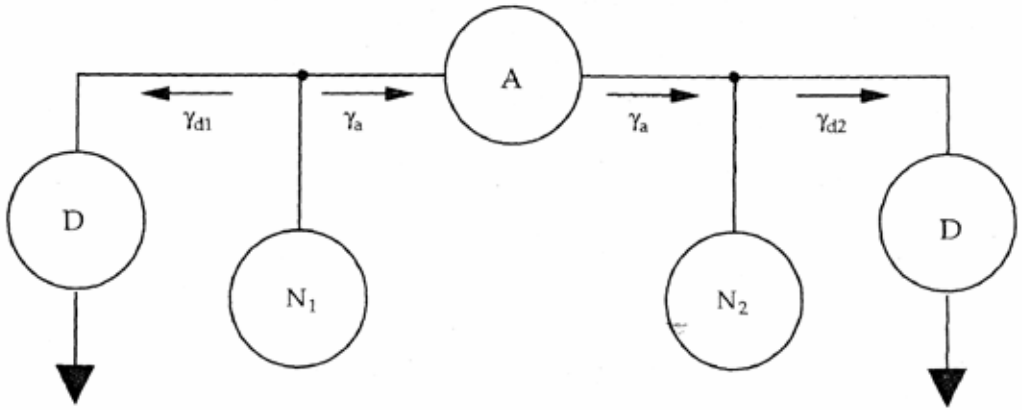



**Figure 1–Dormant and activated spores, $N_1$ and $N_2$, respectively, affected by activation, A, and inactivation, D. The triangle is the sink for dead spores.**

D denotes inactivation transformation
$\gamma_a$ = rate of activation of dormant spores
$\gamma_{d1}$ = rate of inactivation of dormant spores
$\gamma_{d2}$ = rate of inactivation of activated spores
The triangles represent the sink for dead spores.

Rodriguez &Smerage, 1996.

Busta and Ordal, 1964

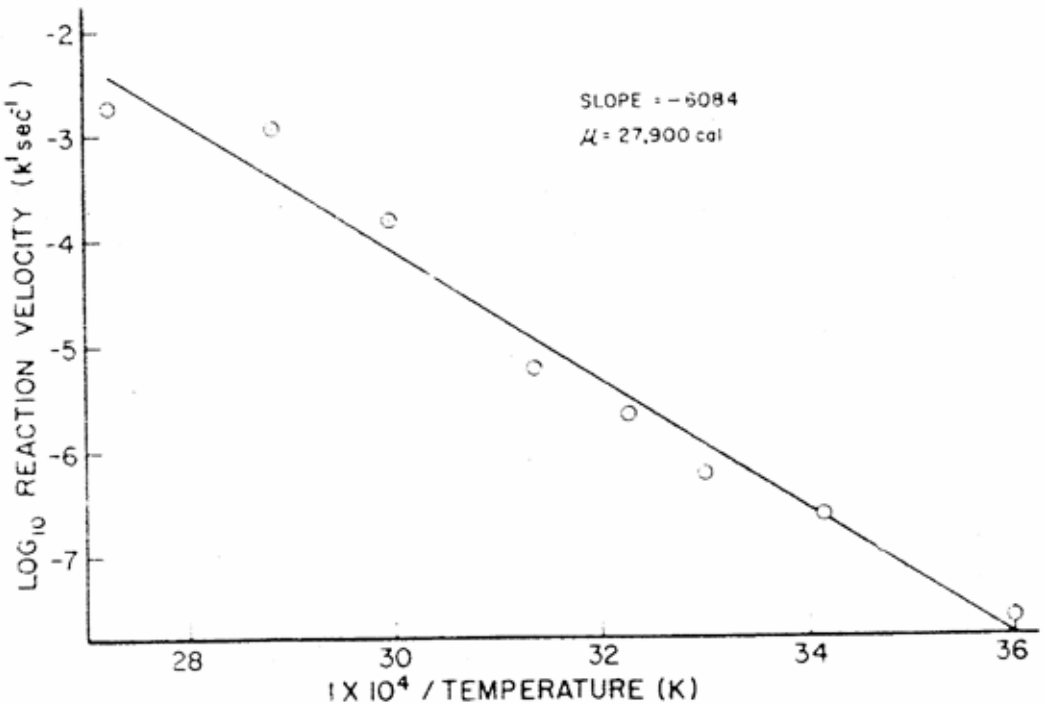



Fig. 6. Effect of temperature on the rate of heat activation. An Arrhenius plot of the reaction velocity vs. the reciprocal of absolute temperature.

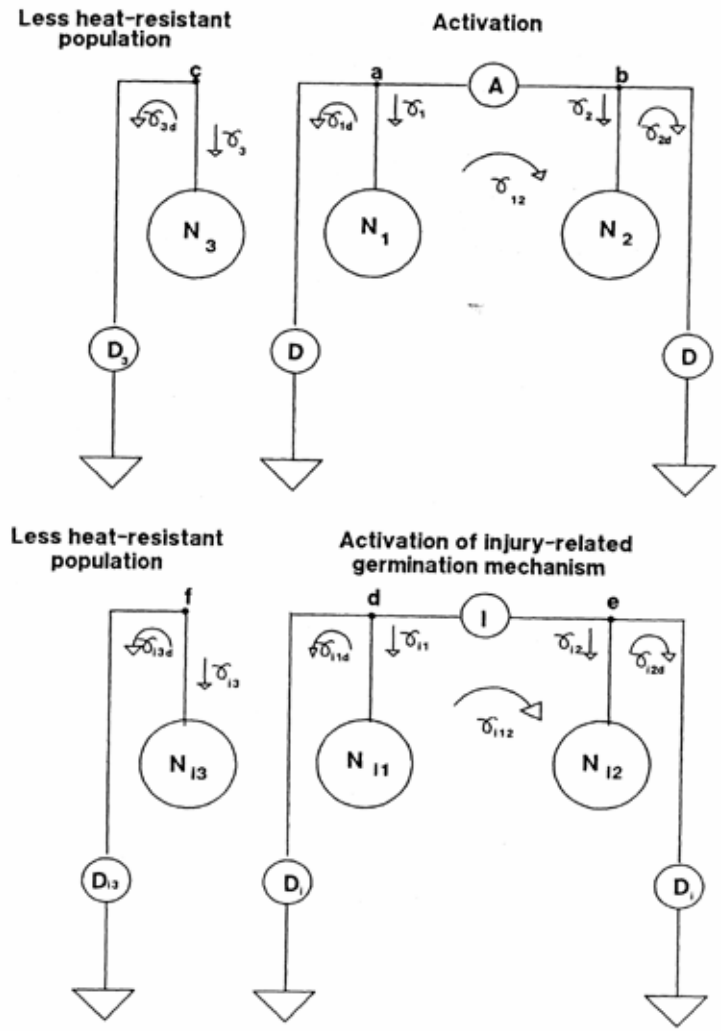



Fig. 1—System diagram for a population of bacterial spores at lethal temperature.

Rodriguez et al., 1988.

# Mathematical model:

$$\frac{dN_1}{dt} = -(k_d + k_a)N_1 \ldots\ldots (41)$$

$$\frac{dN_2}{dt} = k_a N_1 - k_d N_2 \ldots\ldots (42)$$

$$\frac{dN_1}{dt} = -k_{d_3} N_3 \ldots\ldots\ldots (43)$$

$$N_1(0) = N_{10} \ldots\ldots (44)$$

$$N_2(0) = N_{20} \ldots\ldots (45)$$

$$N_3(0) = N_{30} \ldots\ldots (46)$$

Rodriguez, 1988

Where:

$N_1$ is the subpopulation of dormant spores.

$N_2$ is the subpopulation of activated spores.

$N_3$ represents a subpopulation that has different resistance to moist-heat sterilization.

$k_a$ is the activation rate constant.

$k_d$ is the inactivation rate constant.

$k_{d3}$ is the inactivation rate constant for the subpopulation with different resistance.

# Response Equations:

$$N_1(t) = N_{10} \exp\left[-(k_d + k_a)t\right] \ldots\ldots\ldots (47)$$

$$N_2(t) = (N_{10} + N_{20}) \exp\left[-k_d t\right] - N_{10} \exp\left[-(k_d + k_a)t\right]$$

$$N_3(t) = N_{30} \exp\left[-k_{d_3} t\right] \ldots\ldots\ldots (49)$$

$N_{10}$ is the initial value of the number of dormant spores.

$N_{20}$ is the initial value of the number activated spores.

$N_{30}$ is the initial value of the population with different resistance.

Rodriguez et al., 1988

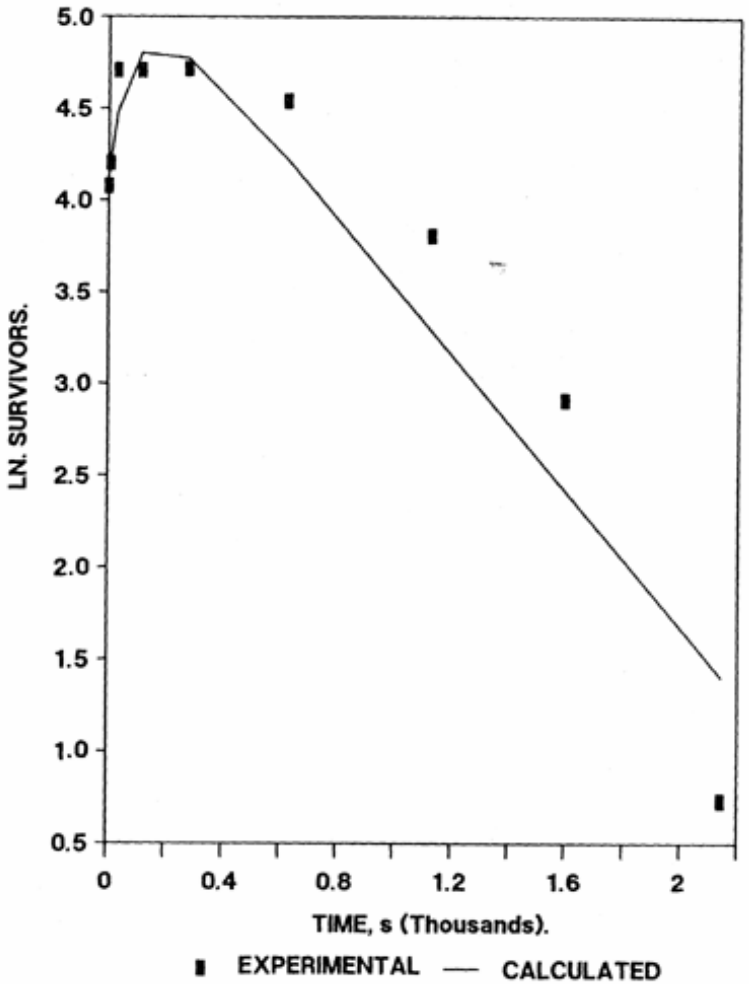



**Fig. 4—Comparison between calculated and experimental survivor values for *B. subtilis* spores treated at 93°C and incubated at 45°C (standard incubation condition).**

Rodriguez et al., 1988.

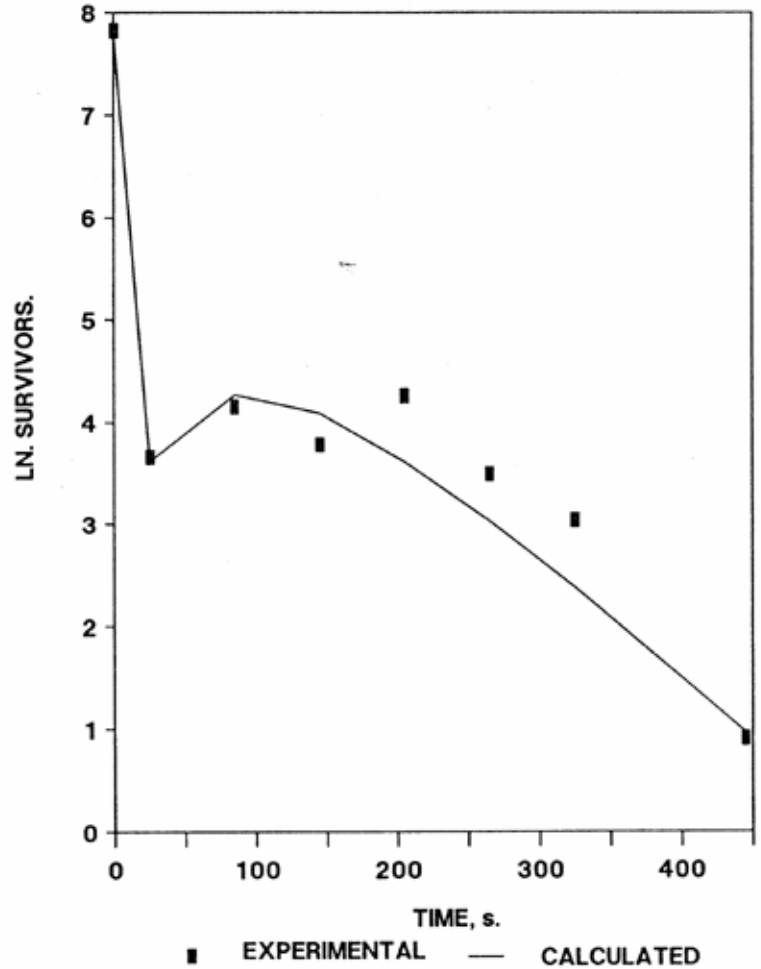



**Fig. 6—Comparison between calculated and experimental surviv values for *Cl. botulinum* spores treated at 120°C (data provided by Ababuch).**

Rodriguez et al., 1988

“ It is evident that ungerminated spores are resistant to hydrostatic pressures of well over 8000 atm, and yet much lower pressures than these, of the order of a few hundred atm, can initiate spore germination”

G.W. Gould, 1972

# The corresponding mathematical model for high pressure is:

$$\frac{dN1}{dt} = -(kdD + kA)\, N1 \quad \ldots\ldots \quad 1$$

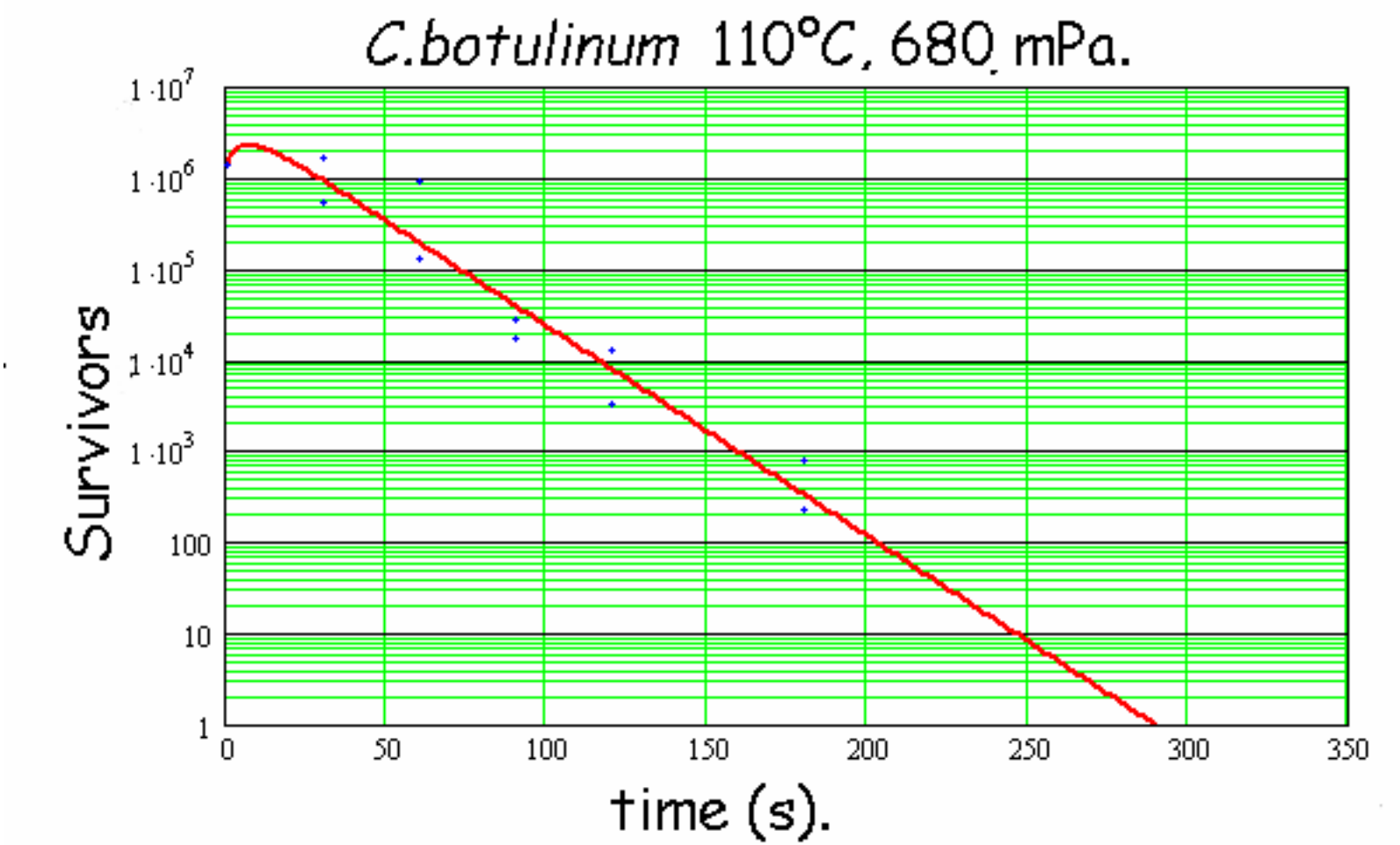
$$\frac{dN2}{dt} = kA\, N1 - kdA\, N2 \quad \ldots\ldots \quad 2$$

(Rodriguez, A.,High Pressure Sterilization of Pharmaceutical Products- Presented at the 2005 annual Meeting of the Parenteral Drug Association.)

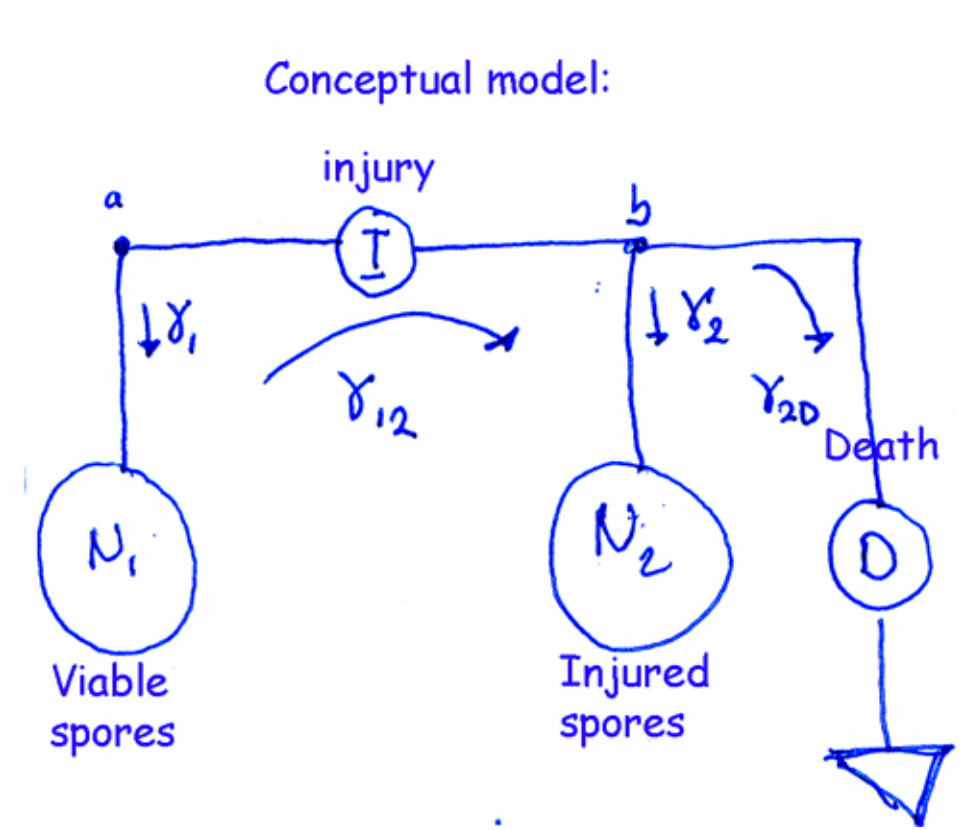
The isothermal/isobaric response equations are:

$$N1[t] \to e^{-(kA+kdD)\,t}\, N1O$$

$$N2[t] \to \frac{e^{-kdA\,t}\,(kA\,((-1 + e^{-(kA-kdA+kdD)\,t})\,N1O - N2O) + (kdA - kdD)\,N2O)}{-kA + kdA - kdD}$$


C.botulinum 110°C, 680 mPa.
Survivors
1·10^7
1·10^6
1·10^5
1·10^4
1·10^3
100
10
1
0
50
100
150
200
250
300
350
time (s).

# Inhibition of spore outgrowth for injured C. botulinum spores in cured meats.

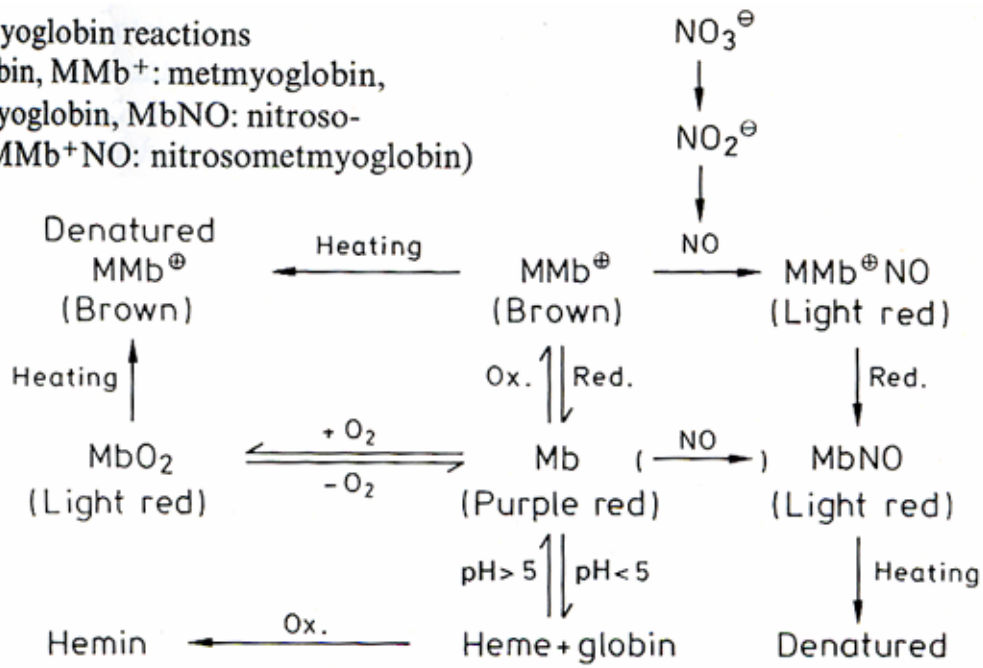



Stores:

Viable spores: $N_1$: $\frac{dN_1}{dt} = \gamma_1$

injured spores: $N_2$: $\frac{dN_2}{dt} = \gamma_2$

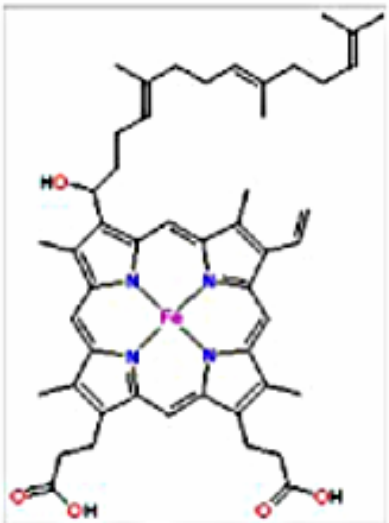
**Fig. 12.14.** Myoglobin reactions (Mb: myoglobin, $MMb^+$: metmyoglobin, $MbO_2$: oxymyoglobin, MbNO: nitroso-myoglobin, $MMb^+NO$: nitrosometmyoglobin)




Belitz, H.D., and W. Grosch. 1987 Food Chemistry. Springer-Verlag Berlin:425.

Haem $d_1$

- Cytochrome $cd_1$ nitrite reductase ($d$



Haem $o$

- Cytochrome $o$ oxidase

Transformations:

Injury:

$$\gamma_{12} = K_1 N_1$$

Inactivation:

$$\gamma_{2d} = K_d N_2$$

a) $\frac{dN_1}{dt} = -K_I N_1$

b) $\frac{dN_2}{dt} = K_D N_2 - K_I N_1$

say $N_1(t=0) = N_0$

$N_2(t=0) = 0$

No is the initial number of viable spores. The initial number of injured spores is zero.

Response equations for the isothermal case (K's are constants):

$$N_1(t) = e^{-KI \cdot \tau}$$

$$N_2(t) = -\frac{e^{-KI \cdot \tau}\left(-1 + e^{(KD \cdot \tau + KI \cdot \tau)}\right) \cdot KI \cdot N\emptyset}{KD + KI}$$

Summation of flows at node a:

$$-\gamma_1 - \gamma_{12} = 0 \quad \text{or} \quad \gamma_1 = -\gamma_{12}$$

Summation of flows at node b:

$$\gamma_{12} - \gamma_2 - \gamma_{2D} = 0 \quad \text{or} \quad \gamma_2 = \gamma_{2D} - \gamma_{12}$$

Review of the literature shows that injured spores cannot grow when $NO_2$ and NaCl are present in the growth medium. Spores that have not been injured (not exposed to moist-heat sublethal treatment) are not inhibited by the salts. Adding salts to heating menstrum does not change the rate of injury/inactivation significantly.

A mathematical model was developed based on these concepts, and the response equation corresponding to an isothermal process found solving the system of differential equations using the initial conditions that the initial number of injured spores was zero, and the initial number of viable spores was found in the 0 min exposure ($N_{0)}$.

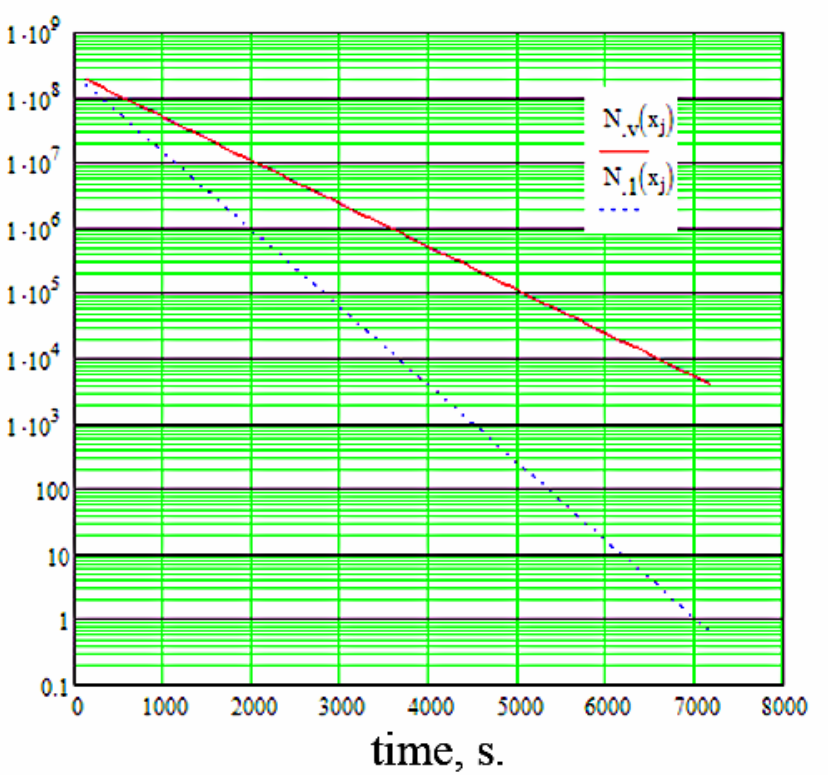



Figure showing the calculated values for injured spores + salts and viable spores.

# ISO Types of Sterilization Cycles

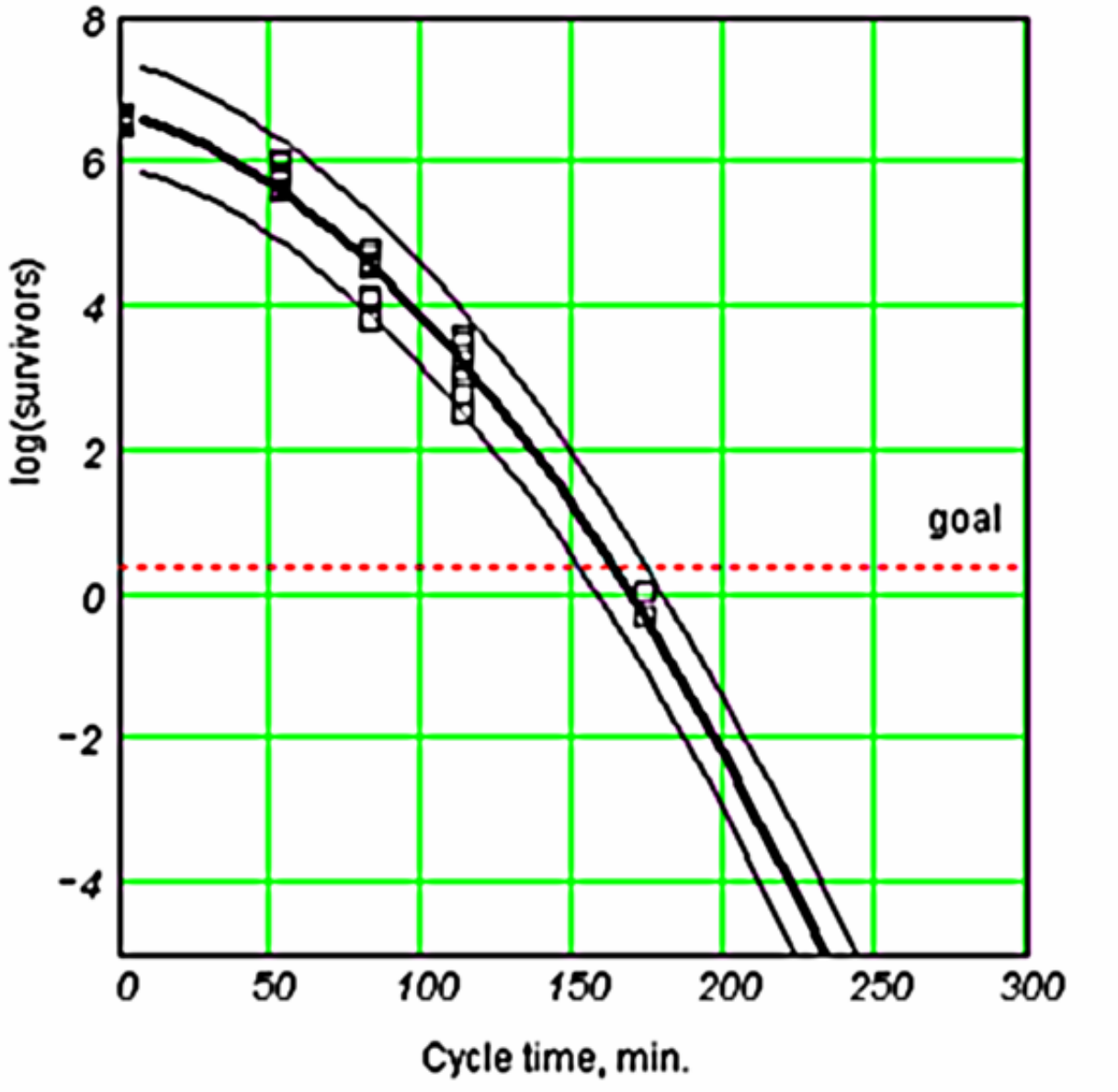
- Bioburden –Adjust exposure to lethal agent to the resistance of the bioburden.
  - Work needs to be done again if measured resistance of the bioburden increases.
  - Example: radiation sterilization of medical devices.
- Overkill – Find exposure needed to get zero positives and multiply it by two.
  - Safer/easier leads to more expensive/harsher cycles.
- Combined bioburden – biologic indicator (surrogate).
  - Relationship between the measured resistance of the bioburden and the indicator organism needs to be determines.
  - Leads to intermediate cycles with respect to the other two methods.
  - Provides a margin of adjustment if measured resistance of the bioburden increases.

# Empirical Models of Microbial Inactivation

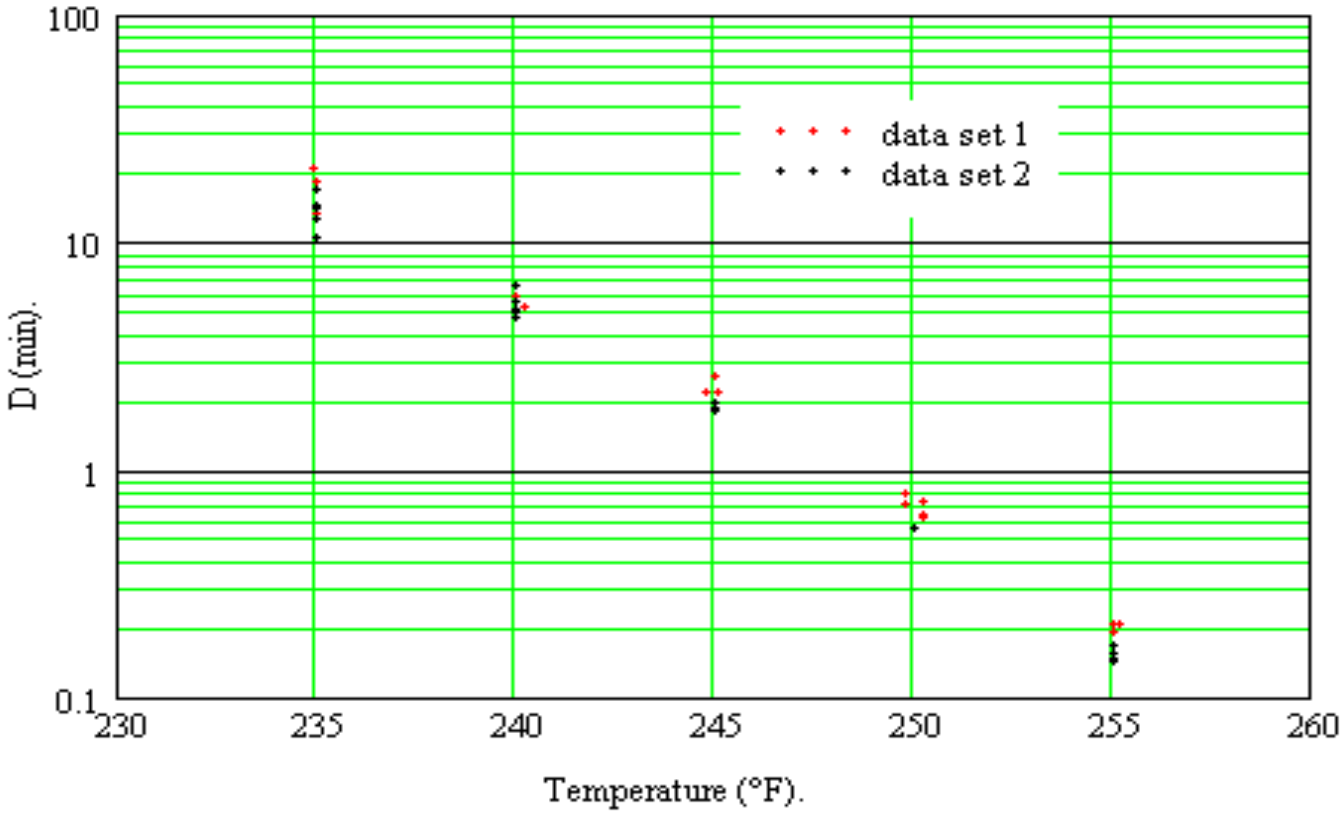
The coefficients ($b_i$) of a linear combination of functions follow the F distribution.

$$y(x) = b_0 + b_1\Phi_1(x) + b_2\Phi_2(x) + \ldots + b_n\Phi_n(x)$$

Polynomials are particularly useful, but the functions $\Phi_i$ may be very diverse including exponentials, trigonometric functions etc.




Curve-fitting applied to the survivorship of USP BI's under the effect of a lethal agent.




Decimal reduction time (D) at different temperatures for *B. subtilis* spores in two different solutions.

Using a linear LSR model we can test if the curves are significantly different.

$$F_{star} := \frac{\frac{SSE_R - SSE_F}{DFR - DFF}}{\frac{SSE_F}{DFF}}$$

$\alpha \equiv 0.001$

$F_{star} = 11.115$

$F_{critical}(\alpha) := qF[(1 - \alpha), (DFR - DFF), DFF]$

$F_{critical}(\alpha) = 8.579$

Conclusion := if$\left[\left(F_{star} \le F_{critical}(\alpha)\right), \text{"Accept C1 "}, \text{"Accept C2"}\right]$

At the selected confidence level $\alpha$,there are two alternative conclusions when we test if two regression lines are the same: $C_1$ implies that the values of the coefficients of the regression lines are equal and $C_2$, that the values of some or all the coefficients of the regression lines are not equal.

Conclusion = "Accept C2"

$$percent(k) := \left(1 - \frac{1}{k^2}\right) \cdot 100$$

Lower limit of the population included as a function of the number of standard deviations from the mean from the Bienayme-Chevishev rule.

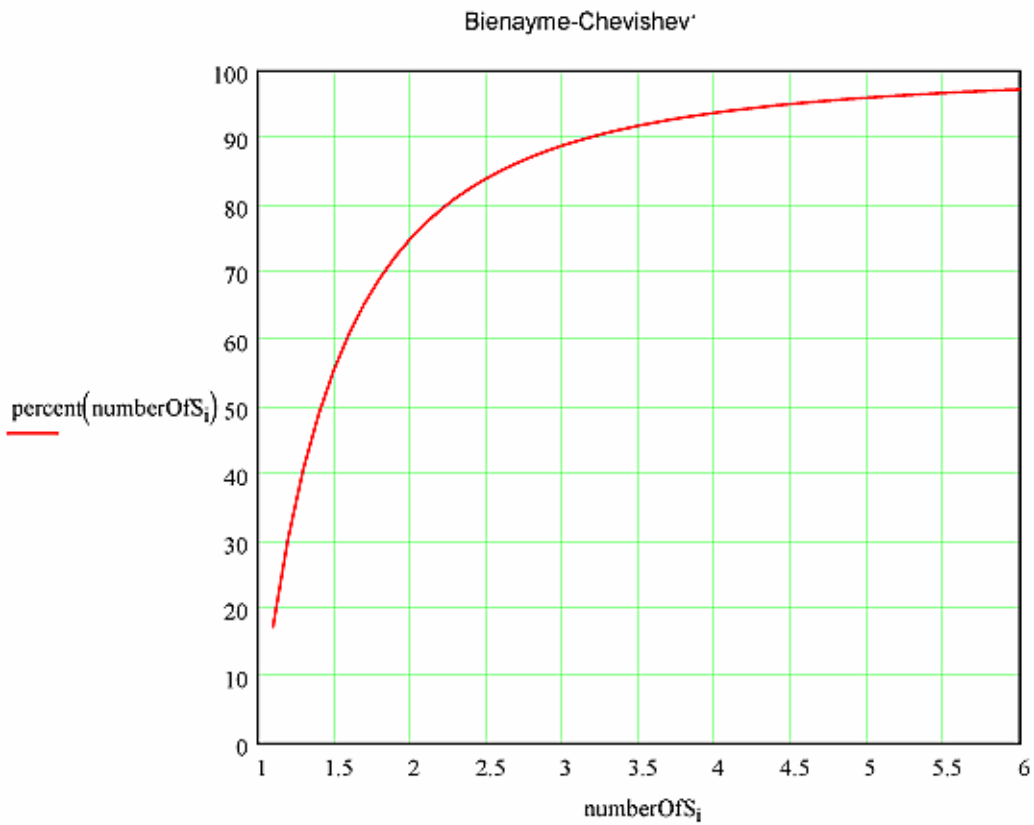



Figure showing the minimum percentage of the population included in an interval of + or - a number of standard deviations from the mean regardless of the distribution of frequencies.

The Bienayme-Chevishev rule provides the minimum percentage of the population that will be included within a certain number of standard deviations from the mean.

TABLE 2.—REACTION KINETICS OF CHLORINE DISINFECTION IN ALGAL-BACTERIAL SYSTEM

| Residual chlorine, in milligrams per liter (1) | $n$-Order rate constant, $K_n$ (2) | $m$-Order and $n$-order rate constant, $m$ (3) | Intercept constant in $m$-order and $n$-order equation, $\log(-Kh'/m)$ (4) |
|---|---|---|---|
| 0.25 | -2.20 | -0.243 | 0.999 |
| 0.50 | -2.61 | -0.300 | 1.034 |
| 1.0 | -3.41 | -0.597 | 1.360 |
| 1.5 | -3.36 | -0.773 | 1.495 |
| 2.0 | -2.73 | -0.793 | 1.413 |

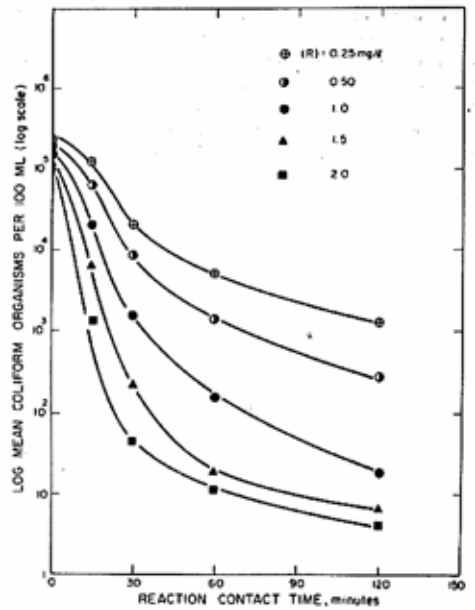



FIG. 6.—FIRST-ORDER REACTION KINETICS OF CHLORINE TREATMENT OF COLIFORM ORGANISMS

## MATHEMATICAL MODEL

On the basis of information derived from the literature review, assuming chlorine as the disinfecting agent, time and concentration are the major controllable variables and therefore are used in the development of the mathematical model. The hypothesis derived by the writer is that the combined effects of time-concentration relationships of the effect of chlorine bacteria

may be expressed by the generalized differential equation:

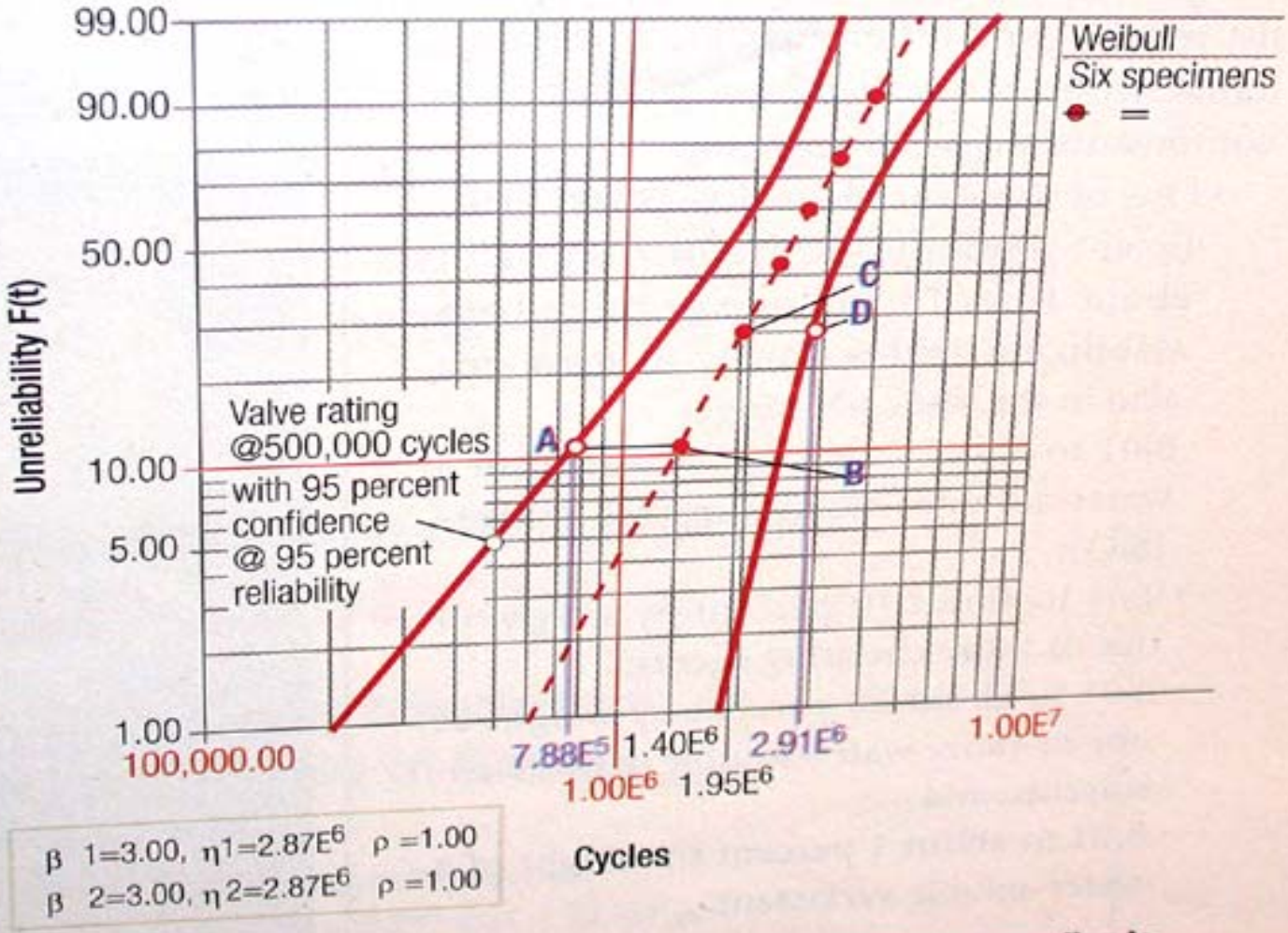
$$\frac{dN}{dt} = -kNt^m C^n \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (4)$$

Under conditions such that $m = 0$ and $n = 0$, Eq. 4 is a first-order reaction in which $dN/dt = -kN$.

Hom, L. presented the use of time to a fractional (< 1) exponent.

(J. of the Sanitary Engineering Division, American Soc. Of Civil Engineers. February, 1972:183-194)

## Figure 2: Weibull plot showing the concept of zero failure case (Weibayes analysis)




**A Weibull plot can be used to demonstrate Weibayes analysis. According to the plot, the first of the six valves would fail on average at 1,400,000 cycles.**

Modeling microbial inactivation:

Probabilistic
- Quantal
- Life-span

Mechanistic
- Single transformation and/or population
- Multiple transformations
- Multiple populations with different resistance

Empirical (statistical curve-fitting)
- Linear combination of functions
- Nonlinear regression.

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