

Microbial Modeling in Foods

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ABSTRACT: Predictive food microbiology is a field of study that combines elements of microbiology, mathematics, and statistics to develop models that describe and predict the growth or decline of microbes under specified environmental conditions. Models can be thought of as having three levels: primary level models describe changes in microbial numbers with time, secondary level models show how the parameters of the primary model vary with environmental conditions, and the tertiary level combines the first two types of models with user-friendly application software or expert systems that calculate microbial behavior under the specified conditions. Primary models include time-to-growth, Gompertz function, exponential growth rate, and inactivation/survival models. Commonly used secondary models are response surface equations and the square root and Arrhenius relationships. Microbial models are valuable tools in planning Hazard Analysis, Critical Control Point (HACCP) programs and making decisions, as they provide the first estimates of expected changes in microbial populations when exposed to a specific set of conditions. This review describes the models currently being developed for food-borne microorganisms, particularly pathogens, and discusses their uses.

KEY WORDS: models, microbiology, prediction, pathogens.

I. INTRODUCTION

A. Modeling

The objective of predictive food microbiology is to describe mathematically the growth or decline of food-borne microbes under specific environmental conditions. With this ability to describe comes the ability to predict for combinations of conditions where no experimental data exist within the appropriate range of each condition. This environment includes both intrinsic factors (e.g., pH, a_w) and extrinsic factors (e.g., temperature, gaseous atmosphere). A large number of factors undoubtedly affect the microorganism; however, in most foods only a

few exert most of the control over a microorganism's growth or decline. The effect of a factor is assumed to be independent of whether the microbe is in a broth or food (assuming other relevant factors are equivalent).

Microbial modeling began in the 1920s with thermal death time calculations; D and z values were used successfully to ensure that canned foods were free from risk of food poisoning by *Clostridium botulinum*. With the advent of personal computers (PCs), microbial modeling became an area of increasing interest because a model can now be used easily by food technologists and microbiologists; predictions are literally at their fingertips. Before the PC, only relatively simple models were developed; the

SYMBOLS USED: a_w , water activity; a_i , parameters; e , $\exp(1)$; E, enthalpy; F_s , fraction of population in subgroup; k_x , parameters, usually rate parameters; M, microbial population expressed as actual value (nonlogarithm); n, total number of inoculated samples; N_t , microbial population at a specified time (\log_{10} CFU/ml); N_0 , initial microbial population (\log_{10} CFU/ml); N_{max} , maximum population (\log_{10} CFU/ml); N_{turb} , population when first turbid (\log_{10} CFU/ml); N_s , specified population (\log_{10} CFU/ml); P, probability; P_{max} , maximum probability; pH, acidity on pH scale; q, number of samples that failed to grow or show toxin; R, gas constant; s, number of spores inoculated into a sample; t, time; t_l , lag time; t_{turb} , time to turbidity; T, temperature; T_{opt} , optimum temperature; T_{max} , maximum temperature; T_{min} , minimum temperature; x_i , factors of a secondary model (temperature, pH, a_w); Y, calculated value for secondary model; μ , growth rate; τ , time when function has an inflection point.

extensive effort necessary to create a multifactor model was not justified by the limited use it would receive.

Although modeling does not usually reveal unexpected microbial behavior,⁹⁵ it does quantify the effects from the interactions between two or more factors and allows interpolation of combinations of factors not explicitly tested.⁴⁸ Much of the food microbial literature before the advent of predictive modeling defined the limiting conditions for growth when all other factors were near their optimum.⁵⁷ However, in many foods, practical control of pathogens depends on a combination of preservative factors, with none of the factors at levels capable of inhibiting the microorganisms by themselves. Mathematical models are the best way to make predictions in these circumstances.

This article surveys recent advances in the field of microbial modeling of interest to food microbiologists. Primary emphasis is on the development of various models and modeling techniques; less attention is given to describing papers that use existing models. Earlier^{9,69,82} and less extensive^{19,32,33,117} articles have reviewed this rapidly advancing field. The proceedings of the recent International Conference on the Application of Predictive Microbiology to the Food Industry⁴⁰ includes monographs by many of the leaders in modeling. McMeekin et al.¹³⁹ have authored a book that presents the theory and practice of predictive microbiology. This review does not consider the field of fermentations or biotechnology where extensive literature exists.^{18,114,201} Fermentation models usually are concerned with substrate or product concentrations, whereas the models described in this review are usually concerned with cell numbers and preservative factors like temperature, pH, and a_w .

B. Classification of Models

Several schemes have been proposed to categorize microbial models. In this article, the initial differentiation will be into growth models and inactivation/survival models. Within each category, models are described as being at the primary, secondary, or tertiary level.²⁰⁶

1. Primary Level Models

Primary level models describe changes in microbial numbers or other microbial responses with time. The model may quantitate colony-forming units per milliliter (CFU/ml), toxin formation, substrate levels, metabolic products (which are direct measures of the response), or absorbance or impedance (which are indirect measures of the response). A mathematical equation or function describes the change in a response over time with a characteristic set of parameter values. Examples of primary models are the exponential growth rate, Gompertz function, and first-order thermal inactivation. The parameters may, in turn, be reformulated into derived parameters, such as the Gompertz lag time or generation time.

2. Secondary Level Models

Secondary level models describe the responses by parameters of primary models to changes in environmental conditions such as temperature, pH, or a_w . Examples of secondary models are the response surface equation, Arrhenius relationship, and square root model.

3. Tertiary Levels

Tertiary levels are computer software routines that turn the primary and secondary level models into "user-friendly" programs for model users in the forms of applications software and expert systems. These programs may calculate microbial responses to changing conditions, compare the effects of different conditions, or contrast the behavior of several microorganisms.

Primary and secondary level models can be characterized as linear or nonlinear, segregated (defined population of heterogeneous cells) or nonsegregated (averaged cells), or structured (multicomponent) or nonstructured (single component).^{61,63,64} These models may also be described as purely descriptive (empirical) or based on microbiological criteria (mechanistic, kinetic).

The modeling process depends on regression techniques; therefore, the standard criteria for regression analysis must be taken into consider-

ation, including normal distributions and homogeneous variances. Guidelines for developing models include considerations of:

- Accuracy of fit
- Ability to predict untested combinations of factors
- Incorporation of all relevant factors
- Possessing the minimum number of parameters for ease of use (parsimonious)
- Specification of the error term
- Parameters having biological meaning and realistic values
- Reparameterization if it improves statistical properties¹⁴⁰

II. MODELING INCREASING POPULATIONS

Perhaps the simplest situation to model was growth vs. no growth. Bell and Etchells²⁰ observed the concentrations of acetic acid and sugar that prevented the growth of spoilage yeasts in pickles. Equations were determined to calculate the quantities of acid or sugar required to prevent the yeast's growth.

A. Time-to-Growth Models

A model where the time from inoculation to growth/turbidity or toxin formation is the measured parameter can be more informative than the growth/no growth model. This model was used extensively for *C. botulinum*, which originates from spores and produces a potent neurotoxin.^{97,98} The rates of growth and toxin formation were relatively unimportant compared with predicting the time for germination/outgrowth or the initial appearance of toxin. Some workers simply noted the time for turbidity¹³⁵ or toxin^{172,174} and drew block figures indicating positive conditions.

Time-to-growth primary models were incorporated into several types of secondary models. Smith et al.¹⁸⁹ used a second-order response surface equation to predict the effects of a_w , pH, storage temperature, potassium sorbate, carbon dioxide headspace concentration, and inoculation level on the number of days for visible mold

growth on bakery products. The time to detectable *C. botulinum* toxin in fresh fish stored between 4 and 30°C under modified atmosphere packaging for up to 60 d was modeled by a polynomial equation.¹⁰ The time for botulinal toxin formation in vacuum-packaged, cook-in-bag turkey products at an abuse storage temperature of 27°C was fitted to quadratic equations, with sodium lactate and sodium chloride as the independent factors.^{127,128}

The most probable number (MPN) technique was utilized in a time-to-turbidity model when serial dilutions of spores were inoculated into defined media by Lindroth and Genigeorgis.¹²⁰ Using the pattern of turbidities in the dilution series and the MPN tables, the probability of a single spore germinating and growing to cause turbidity was determined at each time period. The probability of growth by a single spore at a particular time was defined as

$$P (\%) = (\text{MPN} \times 100)/s \quad (1)$$

where P was the probability in percent, MPN the most probable number of grown spores, and s the inoculum size.

Cooked potatoes with altered pH and added sodium chloride were inoculated with proteolytic *C. botulinum* and vacuum packaged.⁶⁷ The percent probability of toxin production with storage at 25°C was determined by serial inoculations and MPN tables by Equation 1. Multiple regression equations were calculated to predict the time for toxin formation and the probability of toxin formation by a single spore at a specified condition.

Probability of *C. botulinum* growth and toxin formation in broths at different pHs was modeled by the equation⁹¹

$$P = (\ln n/q)/s \quad (2)$$

where n was the total number of inoculated samples, q was the number of samples that failed to show growth or toxin, and s was the number of spores inoculated into each sample. The interactions of modified atmosphere packaging, irradiation, and storage temperature on *C. botulinum* in fresh pork were modeled with Equations 1 and 2 and second-order polynomial equations.⁶⁸

Changes in log P with time were calculated by

$$\log P (\%) = 5[e^Y/(1 + e^Y)] - 3 \quad (3)$$

where Y was a function of temperature, days, and lag period.^{78,120} The change in the logarithm of the probability with temperature was described by a cubic regression equation for Y (secondary model). Separate equations for Y were developed for vacuum, 100% CO₂, and 70% CO₂ modified atmosphere packaging. This approach modeled a variety of situations for *C. botulinum* toxin production in fish^{11,12,74,75,104,107} and cooked turkey.⁷⁸ The effects of redox potential, sodium chloride, temperature, and pH on the probability of growth in broth cultures were explored in a series of papers.¹²³⁻¹²⁶ In these papers, the change in log P with time was fitted to a linear function¹²³

$$\log P = a_1 - k(t_{\max} - t) \quad (4)$$

where a_1 was the maximum value, k was the slope, t_{\max} was the time to reach a maximum value, and t was the incubation time.

Roberts et al.¹⁶⁸⁻¹⁷⁰ described the proportion (p) of meat slurry samples that were toxic after inoculation with *C. botulinum* by

$$Y = (180/\pi) \arcsin (p^{1/2}) \quad (5)$$

where Y was a polynomial equation that included terms for sodium chloride, nitrite, nitrate, isoascorbate, polyphosphate, and heating and storage temperatures. Roberts et al.¹⁷⁰ expanded the meat slurry model by use of a logistic regression function

$$p = 1/(1 + e^{-Y}) \quad (6)$$

where Y was a polynomial with no squared terms. High and low pH pork had separate equations. The polynomial for Y had squared terms (second-order) in Roberts et al.¹⁷¹ and in subsequent papers.^{83,84,173}

The effect of pH, fructose concentration (°Brix), benzoic acid, sorbic acid, and sulfur dioxide on the probability of growth of a spoilage

yeast, *Zygosaccharomyces bailii*, in a model fruit drink after 3 weeks at 23°C was modeled with the logistic probability function and a polynomial equation.⁵²

$$P = 1/(1 + e^{-Y}) \quad (7)$$

$$Y = a_0 + a_1x_1 + a_2x_2 + a_{12}x_1x_2 \dots \quad (8)$$

The probability of finding *Campylobacter* in water as a function of temperature and the presence of indicator fecal coliforms was modeled by a logistic function¹⁸⁴

$$P = e^Y / (1 + e^Y) \quad (9)$$

where P was the probability of the occurrence of the *Campylobacter*, $Y = -4.38 + 1.62 \log(\text{FCB}) + a_{\text{temp}}$. FCB were the counts for fecal coliforms, and the value for a_{temp} varied, depending on which of four temperature ranges from 0 to 12°C the prediction was in. At a measured water temperature, the probability of occurrence of *Campylobacter* had a sigmoidal relationship to the logarithm of the indicator coliform numbers.

The pattern of toxic samples in a set of packages inoculated with clostridial spores and stored under abuse conditions was characterized as an initial period without any toxic samples, a period of increasing numbers of positive samples, and then no additional positive samples.^{105,146} Whiting and Call²⁰⁷ modeled the increasing probability of positive samples of proteolytic strains of *C. botulinum* in broth tubes by a logistic function.

$$P = P_{\max} / (1 + e^{k(\tau - t)}) \quad (10)$$

where P was the probability of a sample being positive (0.0 to 1.0), P_{\max} was the maximum probability of being positive at the end of the modeling period, k was the slope term for the rate of increasing positive samples, τ was the time of the inflection point where P equals half of the P_{\max} , and t was the time. Polynomial regression equations were then calculated for each of the three parameters (P_{\max} , k , and τ) to describe their changes with sodium chloride (0 to 3%), pH (5 to 7) and temperature (15 to 37°C).

B. Growth Models

1. Primary Growth Models

Precomputer modeling was often performed by plotting the logarithm of cell numbers per milliliter with time. The slope of the portion of the growth curve judged to be linear [\log_{10} (CFU/ml)/h] was a first-order relationship

$$N_t = N_0 e^{kt/\ln 2} \quad (11)$$

where N_t was the log population per milliliter at a specified time, N_0 was the log initial population per milliliter, k was the slope, and t was time. The readily calculated version of this model is

$$k = [\log N_2 - \log N_1]/(t_2 - t_1) \quad (12)$$

Baranyi and Roberts¹⁶ pointed out that the growth rate properly refers to the increase in numbers per time (dM/dt) and the commonly referred to growth rate is correctly termed the specific growth rate.

$$\mu = (dM/dt)/M = d(\ln M)/dt = d(\log_{10} M)/2.3 dt \quad (13)$$

This relationship (Equation 12) was fitted by linear regression to describe the growth of *Mycobacterium*,¹¹⁸ growth of *Listeria* in milk¹³³ at 10°C, effects of modified atmosphere packaging on the growth of normal flora and *Listeria monocytogenes* on raw chicken,²¹⁰ and the influence of temperature and pH on the aerobic growth of *L. monocytogenes* on beef lean and fatty tissue.⁹⁰

The lag phase was incorporated into the model by Zamora and Zaritzky²¹⁴ and Bailey and Ollis.⁸

$$M = M_0 \exp(\mu(t - t_l)) \quad (14)$$

where μ was the growth rate during the exponential phase ($dM/dt = \mu M$), and t_l was the lag time.

Additional models have been developed for describing growth data. Pruitt and Kamau¹⁶¹ explained the relationships between exponential, Gompertz, and logistic functions. The Gompertz equation has become the most widely used primary model for describing microbial growth. In

the first use of this function in food microbiology, Gibson et al.⁸⁰ compared the logistic and Gompertz curves for parameterizing the growth of *C. botulinum*, preferring the closer fit by the Gompertz equation. The equation is

$$N_t = N_0 + a_1 \exp(-\exp(-a_2(t - \tau))) \quad (15)$$

where a_1 was the difference in \log_{10} counts between the inoculum and the stationary phase, a_2 was a slope term, and τ was the time at the inflection point.

The parameter values can be determined by fitting the data to the Gompertz function by Gauss-Newton or other iterative estimation procedures that determine the best-fit according to least squares criteria. Bratchell et al.²⁷ examined the number of data points in a growth curve and the resulting errors of the fits. They suggested that more than ten data points per curve were necessary for good fitting. Buchanan et al.⁴³ found that the stationary phase population of *L. monocytogenes* was constant at $10^{9.2}$ for nearly all combinations of sodium chloride, nitrite, pH, and temperatures, allowing the substitution of

$$a_1 = 9.2 - N_0 \quad (16)$$

Secondary models needed to be derived only for parameters a_2 and τ because the model user provides the initial populations (N_0). Gompertz equation parameters can be used to calculate parameters more familiar to microbiologists that are independent of the inoculum size.⁴¹

$$\text{Growth rate } \log_{10} (\text{CFU/ml})/\text{hr} = a_1 a_2 / e \quad (17)$$

$$\text{Lag time (hr)} = \tau - (1/a_2) \quad (18)$$

$$\text{Generation time (hr)} = \ln 2(e)/a_1 a_2 \quad (19)$$

Another way to calculate the lag phase was to determine when the second derivative of the Gompertz function equals zero.³⁵ Zwietering et al.²¹⁷ compared these methods to calculate lag phase and the time a culture was in the exponential growth phase. They found the traditional Gompertz and second derivative to be slightly

different, for lag times the second derivative method was equal to

$$\text{lag time} = \tau - (0.96/a_2) \quad (20)$$

Zwietering et al.²¹⁶ statistically compared different sigmoidal functions for describing the growth of *Lactobacillus plantarum*. After contrasting the logistic, Gompertz, Richards, Schnute, and Stannard functions, they concluded that the Gompertz function was statistically sufficient to describe the growth and was the easiest to use.

The Gompertz function was reparameterized by Zwietering et al.^{215,216} to have the parameters directly represent the growth rate and the lag phase

$$\ln(M_t/M_o) = a_2 \exp\{-\exp[(\mu e/a_2)(t_1 - t) + 1]\} \quad (21)$$

where a_2 was the log difference in cell numbers from inoculum to stationary phase, μ was the maximum specific growth rate, and t_1 was the lag time. These parameters can be converted to the parameters of the previous form of the Gompertz Equation 15 by

$$\text{Gompertz } a_1 = \mu e/a_2 \quad (22)$$

$$\text{Gompertz } a_2 = (\mu e/a_2)t_1 + 1 \quad (23)$$

Equation 21 was used to fit *Aeromonas hydrophila* growth curves.¹⁰² Garthright⁷⁶ further refined the function to have

$$a_2 = N_{\max} - a_4 \quad (24)$$

where N_{\max} was the stationary phase, and the lower asymptote was now defined by

$$a_4 = \log(N_o) - y(o) \quad (25)$$

The incorporation of $y(o)$ into the model gives an unbiased approximation of N_o . Garthright noted that the Gompertz function had several inherent characteristics: the τ time occurred when growth was at $0.37 a_2$, and the lag time ended when the population equaled $a_1 + 0.066 a_2$.

The logistic function is similar in shape to the Gompertz, only symmetrical.¹⁷⁶

$$M_t = a_5 + [a_6/(1 + \exp(\tau - t/g))] \quad (26)$$

where M_t was the population at time t measured in optical density units, a_5 was the value of the lower asymptote ($\approx M_o$), a_6 was the maximum population, τ was the time at the inflection point, and g was the generation time. Another version of the logistic function was developed by Lambrecht et al., who measured ¹⁴CO₂ release.¹¹⁸ The logistic function was found to fit plate count data less closely than the Gompertz function by Gibson et al.⁸⁰ for *C. botulinum*.

Another approach to determine the growth rate was to observe the time to turbidity of tubes containing varying concentrations of a microorganism.⁵⁶ The slope of the time to turbidity vs. log of the inoculum size was linear with the following relationship

$$t_{\text{turb}} = t_1 + (N_{\text{turb}} - N_o)/k \quad (27)$$

where t_{turb} was the time to turbidity, t_1 was the lag time, N_{turb} was the population when turbidity was observed, N_o was inoculum ($N_o < N_{\text{turb}}$), and k was the growth rate constant from the slope of the line. The value for k was determined from the times for two inocula to become turbid.

$$k = (N_{o1} - N_{o2})/(t_{\text{turb}2} - t_{\text{turb}1}) \quad (28)$$

The values for t_1 and N_{turb} were not needed. In this approach, it was assumed that lag time was not affected by the inoculum size.

Several authors constructed models built on assumptions about microbial growth. These models also addressed several criticisms of the Gompertz function, principally: (1) the actual exponential growth is linear (constant specific growth rate), not a continuous curve with an inflection point at the maximum growth rate, and (2) the curve fitting routine frequently calculates a negative lag time.

Baranyi et al.^{15,17} constructed a model beginning with a basic growth model

$$dM/dt = \mu_{(N)} M \quad (29)$$

where dM/dt was the change in population with time, M was the cell population, and $\mu_{(N)}$ was the specific growth rate. The specific growth rate was

greatest at time zero and declined to zero at M_{\max} (stationary phase). In Baranyi et al.,^{15,17} modification of the lag phase reflected the time necessary for a cell to adjust to the new environment; the greater the change from old to new environment, the longer the lag phase. This transition was modeled by

$$dM/dt = \alpha_{(t)} \mu_{(N)} M \quad (30)$$

where $\alpha_{(t)}$ was termed the adjustment function whose value depended on the environmental change. Its initial value was small for large changes and it increased with time to eventually reach a value of 1.0. Therefore, the term $\mu_{(N)}$ became the potential specific growth rate and $\alpha_{(t)}\mu_{(N)}$ the actual specific growth rate. Generally, the smaller the initial value of α , the longer the lag phase and the slower initial growth rate. The adjustment function was expanded based on concepts from Michaelis-Menten kinetics to have a lag time parameter (λ) and a curvature parameter set to 4. This model described a lag and exponential growth phase.

To add the stationary phase, these concepts were incorporated into a logistic curve.

$$N_t = N_{\max} - \ln[1 + (\exp(N_{\max} - N_0) - 1) \exp(-\mu_{\max} A(t))] \quad (31)$$

where N_t was logarithm of population, N_0 was the logarithm of initial population, N_{\max} was the logarithm of the maximum population, μ_{\max} was the maximum specific growth rate, and $A(t)$ was the definite integral of the adjustment function.

This four-parameter model fitted growth data better than the Gompertz function as judged by goodness-of-fit and standard errors of the estimates. The value for the lag time parameter (λ) was close to the time that the second derivative of the Gompertz function was at a maximum. This model did not give negative lag periods and had an essentially linear exponential growth phase. The fits of a set of *Listeria* growth data by this function estimated the growth rate to be about 10% slower than those determined using the Gompertz function. This discrepancy was attributed to the Gompertz slope being too steep at the inflection point relative to the entire exponential phase slope. The concept that the duration of the

lag phase was partially dependent on the previous environment was very important in this model. This model was used to determine that the doubling times for type B *C. botulinum* ranged from 42.3 h to 22 min at 3.9 to 35°C, respectively.⁹²

Whiting and Cygnarowicz-Provost²⁰⁸ constructed a growth and decline model by assuming that spore germination, lag phase, or recovery from injury was a first-order process or that the apparent rate was controlled by a single first-order step. This had a k_1 parameter describing the rate

$$M_B = M_A \exp(k_1 t) \quad (32)$$

where M_A and M_B were the populations for the initial and activated cells. The exponential growth rate was

$$M_C = M_B 2^{(t/g)} \quad (33)$$

where M_C was the population of actively growing cells and g was designated as the generation time. The generation time was shortest at the beginning of growth and increased with accumulating toxic metabolites or depleting substrates. The increase was proportional to the sum of the populations at each time period from t_0 to t .

$$g = a_2 + a_3 \sum (\text{population} \times \text{time}) \quad (34)$$

where a_2 was the basic generation time and a_3 was the sum-growth parameter. The sum of the population over time did not significantly affect the generation time (g) until the stationary phase was approached, ca. 1 log cycle below the maximum. This resulted in a linear exponential growth phase when the log of the population was plotted for this model in contrast to the Gompertz function, which was continuously curving. At the maximum population, g reached a large value. The decline after the maximum population was assumed to be first-order.

$$M_D = M_C \exp(k_4 t) \quad (35)$$

where M_D was the number of dead cells and k_4 was the death rate parameter. This model was shown to describe the germination, growth, and decline of *C. botulinum* and growth of *L.*

monocytogenes. The growth rates were found to average 16% less than those determined with the Gompertz function. A second version of this model had the first-order decline apply to both the inoculum (M_A) and growing cells (M_C). In this form, the model described a series of environmental conditions, such as increasing NaCl levels, where the microorganism changes from growth to decline. Jason¹⁰⁶ proposed a model where the growth of each bacterium was accompanied by the production of a constant amount of toxic end-products. The growth rate declined in proportion to the ratio of the accumulation of these substances to the mass of nutrients, assuming the nutrient substrates were not limiting.

Another model where population was a balance between growth and death was proposed by Jones and Walker.¹⁰⁹ The population was

$$M_t = M_0 2^{(Y_{1t} - Y_{2t})} \quad (36)$$

with

$$Y_{1t} = a_1 [1 - (1 + (t/a_2) + (t/a_3)^2 + (t/a_4)^3/6) \exp(-t/a_5)] \quad (37)$$

representing a growth function and

$$Y_{2t} = \exp((t-a_6)/a_5) - \exp(-(t-a_1)/a_5) - \exp(-a_1/a_5) + \exp(a_1/a_5) \quad (38)$$

representing a death function, where a_1 to a_5 were parameters, M_t was the population, M_0 was the initial population, and t was time. This model accurately fitted the growth and decline of *Yersinia enterocolitica* in broths of varying pH and sodium chloride concentrations stored at different temperatures. The mean square errors of this model were lower than those of the Gompertz function, Equation 15.

A descriptive model for the influence of environmental conditions on mold growth and aflatoxin production assumed that the rate of growth was proportional to the net growth rate plus current concentration of mold.¹⁵⁸ The growth rate was the optimal rate multiplied by a series of factors for temperature, a_w , pH, and mold mass. An Arrhenius-like function was postulated for temperature, a linear function for a_w , a parabolic model for pH, and a Monod model for

colony size. Toxin formation was related to the production of new cell mass, and toxin degradation was proportional to the dead cell mass (degradative enzymes released during mycelial lysis).

2. Secondary Growth Models

Secondary models determine the parameter values of the primary models for different environments. The three approaches most frequently used with growth models were the response surface equation (multiple polynomial), Arrhenius relationship, and square root model (Bélehrádek). The *response surface* model is a regression equation that is fitted using standard regression techniques and may contain linear, quadratic, cubic, or reciprocal terms and includes interaction or cross product terms. Frequently, the logarithm of a parameter gives better fits (smaller error sums of squares). The full form of the equation may be presented, or backward partial regression techniques may simplify the equation to the statistically significant terms. This equation is entirely descriptive of the particular data set used to calculate the equation and does not imply any theoretical or mechanistic relationship.

An example of a simple linear relationship was reported by Spencer and Baines¹⁹⁰ for the spoilage of fresh fish. They found that for the temperature range of -1 to 25°C , the rate of spoilage was described by the equation

$$k = k_0(1 + aT) \quad (39)$$

where k was the rate of spoilage at a temperature T , k_0 was the spoilage rate at 0°C , and a was the linear constant.

Second-order response surface or multiple regression equations were used frequently for secondary modeling when many factors influenced the primary model. Roberts et al.¹⁷⁰ modeled the Y parameter of the logistic model

$$P = 1/(1 + e^{-Y}) \quad (40)$$

by a polynomial equation with no squared terms. A second-order equation for Y was used by Rob-

erts et al.¹⁷¹ and Robinson et al.¹⁷⁵ Polynomial equations for the Gompertz a_2 and τ parameters were calculated for the effects of pH, sodium chloride, and storage temperature on the growth of *Salmonella*.⁸¹ Bratchell et al.²⁸ compared graphical models of the Gompertz parameters by simple linear regression, contour, and three-dimensional surface plots. Hudson¹⁰² fitted quadratic and cubic equations; the latter models had larger R^2 values, but were not necessarily considered to be the "best" without verification with additional data.

Buchanan et al.⁴³ began a series of papers by the U.S. Department of Agriculture that utilized the Gompertz function to model the growth of food-borne pathogens. Polynomial equations to describe the effects on the a_2 and τ parameters from aerobic-anaerobic atmosphere, pH, sodium chloride level, sodium nitrite, and storage temperature on *L. monocytogenes* were calculated. These were followed by equations for $\ln(a_2)$ and $\ln(\tau)$ with cubic terms.⁴¹ Palumbo et al.¹⁵² performed quadratic and cubic fits to the natural log and square root of both a_2 and τ values from *A. hydrophila* data. From the results, it was concluded that the best fit was the natural log-quadratic equations because these did not calculate negative lag times, negative generation times, or occasional poor predictions as did the other equations, even though the equation did not give the highest R^2 values. The natural log-quadratic model was also used to model the growth of *L. monocytogenes* at refrigeration temperatures;³⁶ *A. hydrophila* under aerobic conditions,¹⁵¹ anaerobic conditions,¹⁵² and with organic acids:¹⁵⁰ *Shigella flexneri*;^{212,213} *Bacillus cereus*;²¹ *Staphylococcus aureus*;⁴² *Escherichia coli* O157:H7;^{34,37} and *Salmonella*.¹⁹⁶

The Arrhenius relationship, the logarithm of the rate vs. the reciprocal of the temperature (K), has wide use in physical chemistry. When applied to microbiology, the basic model assumes that the growth rate is governed by a single rate-limiting enzymatic reaction. Specific growth rates were measured by turbidity increases at various temperatures for 12 bacterial strains and plotted as Arrhenius profiles.¹⁴² A simple smooth curve with a single slope at suboptimal temperatures was observed for some bacteria and a curve with two distinct slopes at subopti-

mal temperatures was observed with others. The latter relationship generally occurred with bacteria having $T_{opt} > 37^\circ\text{C}$. However, Reichardt and Morita¹⁶⁷ reported that Arrhenius profiles for psychrophilic and psychrotrophic bacteria also had profiles with two distinct slopes at sub-optimal temperatures.

Schoolfield et al.¹⁸¹ reformulated earlier equations into one suited for biological temperature-dependent rate models. The model features inactivation at high temperatures and a two-slope or broken activation curve below the optimum temperature. Stannard et al.¹⁹¹ cited other efforts to model microbial growth with the Arrhenius equations; various authors had reported linear or two-phase relationships. Zwietering et al.²¹⁵ tested the Schoolfield and Hinshelwood versions with μ determined by the modified Gompertz function and found that these equations satisfactorily described the data. The Schoolfield model was used successfully in ultra-high temperature (UHT) milk with lowered a_w by addition of D-glucose for *S. aureus* and *S. typhimurium*.²⁹ This model was then expanded to include pH.³⁰ The lag phase was estimated, and both the lag and generation times were described effectively by modified nonlinear Arrhenius equations.

A natural log transformation of the Schoolfield model without the enthalpy term for the low temperature inactivation of the enzyme (four parameters) had lower sum of squares error than the standard six-parameter model.⁷ The former equation's variance was not significantly higher than the natural log, transformed, six-parameter model. The transformed four-parameter model was considered superior to the original Schoolfield model because it was more parsimonious and had smaller confidence limits (larger parameter t-values).

An additive Arrhenius model was described by Davey⁶⁰

$$\ln k = -E/RT + a_1(\text{pH})^2 + a_2(\text{pH}) + a_3 \quad (41)$$

where E was the enthalpy; R was the gas constant; T was the temperature in K; and a_1 , a_2 , and a_3 were model parameters. A linear Arrhenius model for the effect of temperature and a_w was determined in foods by Davey^{59,64} to satisfactorily predict growth rates.

$$\ln(k) = a_0 + a_1/T + a_2/T^2 + a_3a_w + a_4a_w^2 \quad (42)$$

where k was the growth rate and a_n were model parameters. The lag phase duration was modeled by a similar function⁶²

$$\ln(1/\text{lag time}) = a_0 + a_1/T + a_2T^2 + a_3a_w \quad (43)$$

The lag and generation times from the Gompertz equation for *Salmonella* on the surface of bovine tissues were modeled as exponential-decay functions of temperature.⁶⁶

$$\text{lag time (generation time)} = a_0 + a_1 \exp(a_2 T) \quad (44)$$

The development of the **Bélehrádek** or **square root** model was reviewed recently.^{140,179} This model was based on the linear relationship between the square root of the growth rate and temperature. An important feature was the concept of a biological zero, the value for temperature when the growth rate was extrapolated to zero. This model was first used by Ratkowsky et al.¹⁶⁵ to model the temperature effect on growth rate of 14 cultures from 5 species and 29 data sets from the literature. The simplest version of the model for temperatures below the optimum growth rate was

$$\sqrt{k} = a(T - T_0) \quad (45)$$

where k was the growth rate or other rate term, such as the reciprocal of the lag time, T_0 was the temperature when the line was extrapolated to $k = 0$, and a was the slope. The T_0 was termed the notional temperature and did not necessarily mean the lowest temperature where growth was observed or would occur.¹⁶³ This model was extended to the full temperature range by adding two parameters, a_2 and T_{\max} .¹⁶⁴

$$\sqrt{k} = a_1(T - T_{\min})\{1 - \exp[a_2(T - T_{\max})]\} \quad (46)$$

where a_1 was the slope for the increasing rate, T_{\min} was the extrapolated temperature at $k = 0$ for the increasing rate, a_2 was the slope for the decreasing rate, and T_{\max} was the extrapolated temperature at $k = 0$ for the decreasing rate. This model was fitted to data from 30 strains of microorgan-

isms (12 species) and the authors reported that it was easy to fit and produced unbiased and normally distributed errors. Zwietering et al.²¹⁵ concluded that the proper form of the square root model should be

$$k = (a_1(T - T_{\min}))\{1 - \exp[a_2(T - T_{\max})]\}^2 \quad (47)$$

to have homogeneous variances. These authors modified the equation further by squaring the below-optimal temperature parameters

$$k = [a_1(T - T_{\min})]^2\{1 - \exp[a_2(T - T_{\max})]\} \quad (48)$$

Alber and Schaffner^{6,7} maintained that the natural logarithm of growth data

$$\ln k = \ln [(a_1(T - T_{\min}))^2 (1 - \exp(a_2(T - T_{\max})))] \quad (49)$$

corrected for heterogeneity of variance more effectively than the square root transformation of Equation 48,

$$\sqrt{k} = a_1(T - T_{\min})\{1 - \exp[a_2(T - T_{\max})]\}^{1/2} \quad (50)$$

Alber and Schaffner⁶ also showed that the square root model was more accurate than the Schoolfield model when both were transformed by taking the natural logarithm.

Gill and Phillips⁸⁸ tested Equation 46 using growth of *E. coli* in nine media. In six of the media, the fits of the data at the lower or upper temperature ranges to the model were not good; however, the authors asserted that the model was suitable for describing the temperature dependence of the growth rate in a particular media. Lobry et al.¹²² compared Equation 46 with the cardinal temperature model

$$k = k_{\text{opt}}(1 - \{(T - T_{\text{opt}})^2 / [(t - t_{\text{opt}})^2 + T(T_{\max} + T_{\min} - T) - T_{\max} \times T_{\min}])\}) \quad (51)$$

where k_{opt} was the optimum rate, T_{opt} was the temperature of the optimum rate, and T was in K. Equation 51 had a smaller residual sum of squares than Equation 46.

Heitzer et al.⁹⁹ compared three models for describing the effect of temperature on growth.

The first was called the master reaction model and was based on Arrhenius and Schoolfield ideas, wherein the single limiting reaction was affected by both high- and low-temperature inactivation. The second model was a damage/repair model based on the Arrhenius relationship with the addition that activity was affected by the rate of damage and the rate of repair. The last model was the square root model with T_{\max} and T_{\min} (Equation 46). Growth of *Klebsiella pneumoniae*, *E. coli*, and *Bacillus* sp. at 13 temperatures was compared by residual errors of least-square fits. They concluded the damage/repair model was over parameterized, whereas the master reaction model was good, although a two-step fit was often necessary. The square root model (Equation 46) was considered good "even though devoid of any conceptual basis."

A series of straight lines from growth rates of *S. xylosus* at various temperatures in media with different a_w from added sodium chloride all extrapolated to a common T_{\min} .¹³⁷ When the square root model was extended to include a_w , the equation was

$$\sqrt{k} = 0.0205 (a_w - 0.838)^{1/2} (T - 275.9) \quad (52)$$

The values 0.838 and 275.9 represented the extrapolated minimum a_w and T_m (K), respectively. A feature of this model was an absence of cross product terms, thus implying that the factors acted independently of each other. This equation also modeled growth by *S. xylosus* without an interaction between temperature and a_w .⁴⁷

The incorporation of pH into the square root model was made by Adams et al.⁴ by multiplying $(\text{pH} - \text{pH}_{\min})$ by the temperature terms.

$$\sqrt{k} = a(T - T_{\min})(\text{pH} - \text{pH}_{\min})^{1/2} \quad (53)$$

This model gave good fits for two pathogenic and one environmental serotype of *Y. enterocolitica*. The inhibitory pH depended on the acidulant with pH_{\min} of 4.8, 4.7, 4.4, and 4.0 for acetic acid, lactic acid, citric acid, and sulfuric acid, respectively.

McMeekin et al.¹⁴⁰ suggested the next expansion of the model could include a_w , pH, and temperature.

$$\sqrt{k} = f [(a_w - a_{w\min})(\text{pH} - \text{pH}_{\min})]^{1/2} (T - T_{\min}) \quad (54)$$

This model was acceptably fitted to growth rates of *L. monocytogenes* in tryptone soya broth determined with the Gompertz model.²⁰⁹ Comparison to published growth rates in foods showed a good but safe estimate by this model.

The secondary model for growth rates determined by the time-to-turbidity primary model (Equation 27) was⁵⁶

$$k = a(\text{pH} - \text{pH}_{\min})(\text{pH}_{\max} - \text{pH})(T - T_{\min})^2 \quad (55)$$

with an adjustment to $T_{\min} = T_o + a_2(\text{pH} - \text{pH}_o)^2$ because the pH fit was poor at highest pH values. T_o was the lowest value of T_{\min} and pH_o was the pH at which T_{\min} has its lowest value. To adjust for variance increases with square of time, non-linear regression using variance weighted regression with $1/t^2$ was used to calculate the parameters.

The fermentation of meats by *Pediococci* was modeled using the square root of the time to attain a selected pH (5.3 or 5.0).¹⁶² The different T_{\min} values were considered to reflect the relative abilities of the different cultures to perform at the lower temperatures. The effect of an antioxidant (TBHQ) and the inoculation level of *Pediococci acidilactici* was also modeled. Modeling the latter allowed prediction of the inoculation levels needed to reach a desired pH within a specified fermentation period.

The square root model (Equation 45) was used to model the effect of temperature on the rate of bacterial growth in raw mutton^{187,188} and on the Gompertz growth parameters in minced beef.¹²⁹ The inverse of the generation time (generations per hour) and the inverse of the lag time (h^{-1}) were used for the Gompertz rate terms. Data for the growth of *L. monocytogenes*, *B. cereus*, and *Y. enterocolitica* on rice and noodle products showed promising fits to the square root model.²⁵

Stannard et al.¹⁹¹ defined the rate parameter as the time to achieve a specified increase in bacterial numbers, thereby taking the lag phase and growth rate into consideration simultaneously. Similarly, the growth rates of *Halobacterium* were calculated by the reciprocal of the time to reach an optical density (turbidity) of 0.3 and modeled

by the square root Equation 45 with temperature at different sodium chloride concentrations (as a_w).⁴⁶ The lag time of spoilage microorganisms in milk^{45,93} was modeled by the square root of the reciprocal of the lag time. The temperature effect on the shelf life of pasteurized milk was fitted to the square root model by setting the growth rate to the reciprocal of the time for the count to reach log CFU/ml 7.5.⁹⁴

A comparison of the linear equation,¹⁹⁰ Arrhenius equation,¹⁴⁸ and the square root model¹⁶⁵ for predicting the shelf life of poultry and meat products by Pooni and Mead¹⁵⁹ found that the square root model was the most appropriate for predicting the effect of temperature. They noted discrepancies between the square root model and actual growth, particularly at the higher abuse temperatures. This was attributed to different flora predominating at the higher temperatures. The Arrhenius and Bélehrádek (square root) models were also compared by Phillips and Griffiths,¹⁵⁷ McMeekin et al.,¹³⁸ and Ratkowsky et al.;¹⁶⁶ all authors favored the square root model.

Grau and Vanderlinde⁹⁰ compared several versions of Arrhenius and square root models for their ability to fit growth of *L. monocytogenes* on lean and fatty beef tissue. All models were poor predictors of lag times and growth rates on fatty tissue. For growth on lean tissue, the linear Arrhenius Equation 46 accounted for over 99% of the variance.

$$\ln(\text{gen/h}) = a_0 + a_1/T + a_2/T^2 + a_3/\text{pH} + a_4/\text{pH}^2 \quad (56)$$

where a_n were parameters and T was the temperature (K). A modified square root model

$$\sqrt{\text{Gen/h}} = a_0 + a_1 T K + a_2 (1/(\text{pH} - \text{pH}_\lambda)) + a_3 (T/(\text{pH} - \text{pH}_\lambda)) \quad (57)$$

where a_n were parameters and pH_λ was found to be 4.8 accounted for over 98% of the variance.

However, Little et al.¹²¹ found major deviations between the square root model and experimental values at the suboptimal temperature range of *Y. enterocolitica*. The quadratic response surface was able to describe the growth rate (time for 10^2 increase) in both laboratory media and UHT

milk with a lower mean square error than the square root model. The influence of four acids on the time for growth also had a lower mean square error for the response surface model than the square root model at nearly all combinations of pH and temperature.

Adair et al.³ found that the Arrhenius (Schoolfield) model predicted the growth of six species better than the square root model. Others disputed this conclusion, claiming the square root model fitted the data well, was close to linear, and had good estimates of the parameters, had interpretable parameters, was appropriate to the stochastic properties of growth rates, and was easy to use.^{61,179}

Other substrate-based models were used by Rochet and Flandrois,¹⁷⁶ Petrova and Stepanova,¹⁵⁶ and Korte et al.¹¹⁵ These models, based on the exhaustion of a limiting nutrient, were based on the Monod equation.¹⁹ They have received little use in food microbiology because the limiting substrates and their quantities were usually unknown. An interesting variation associated the growth rate of *E. coli* to subinhibitory levels of antibiotics.⁵⁴

Models of binary populations on biofilms were based on cell mass and biochemical products specific to one of the species.¹⁸³ Tokatli and Özilgen¹⁹⁸ used the model of Luedeking-Piret to model exotoxin production. Toxin production rate was proportional to the rate of growth plus the production related to absolute population numbers.

$$d\text{Toxin}/dt = a_1(dM/dt) + a_2M \quad (58)$$

where Toxin was the toxin concentration, M was the cell population, and a_1 and a_2 were parameters. They merged growth and stationary phases by incorporating into Equation 58

$$dM/dt = \mu M(1 - M/M_{\max}) \quad (59)$$

where μ was the growth rate and M_{\max} was the stationary phase population. Papageorgakopoulou and Maier¹⁵³ modeled substrate utilization and cell growth (increase in biomass) in terms of two potentially rate-limiting enzyme systems for substrate inhibition and enzyme repression. Other substrate models were described by Tan and Gill¹⁹⁵

and Straight and Ramkrishna.¹⁹³ A model for the growth rate of lactic acid fermentations was based on undissociated lactic acid being the primary inhibitory agent during the fermentation and Monod-type relationships to substrates.²¹¹ The model calculated the concentration of undissociated acid and determined the specific rates and parameters for growth and lactic acid production at various pHs. By postulating noncompetitive inhibition by lactic acid, the model generated close fitting curves for both growth and acid production. Another model, based on a biochemical process,¹³² had *E. coli* cell growth controlled by the protein-synthesizing system through negative feedback on the ribosomal RNA. The rates responded to the precursor metabolite concentrations in the medium rather than the flux of ATP.

III. AUTOMATED DATA COLLECTION

Collecting sufficient data to fit primary models required numerous samples and enumerations over the entire time period of growth or inactivation.²⁷ Secondary models with several factors usually needed parameter values from several hundred primary curves. Conventional plating techniques were executed faster and with fewer dilutions and plates using spiral plating and automatic plate counting (Spiral Systems, Inc., Baltimore, MD). Replacing sampling, diluting, and plating with automated techniques potentially provides more data points for primary fits and allows inclusion of more factors and combinations in the secondary model with less manual effort and cost.¹⁷³ Fully automated techniques for measuring microbial growth have been based on turbidity (Bioscreen, LabSystems Oy, Helsinki, Finland) or conductance (Malthus Microbiology Systems, Westlake, OH).

A. Turbidity Methods

Turbidity, less properly termed optical density or absorbance, has a long history of use in microbiology. Many studies and models measured the growth rate in absorbance units per hour for bacteria and mold.⁵³ The development of equip-

ment to automatically read 96-well ELISA plates allowed recording of a sufficient number of readings to plot a smooth growth curve regardless of how rapidly or at what time of day or night growth occurred. The number of wells permitted numerous combinations of environmental parameters (except atmosphere and temperature, which must be varied by separate runs) and sufficient replication. The technique had two limitations: (1) high counts (ca. 10^6 CFU/ml) were necessary before turbidity was detected, and (2) the relationship between turbidity and microbial count was neither linear nor log-linear.

Damoglou and Buick⁵⁸ transformed turbidity to CFU/ml with a predetermined polynomial regression equation and then fitted the Gompertz function. The estimates of the Gompertz parameters came from the portion of the curve after the τ time (midpoint or inflection point) and before the stationary phase. McClure et al.¹³⁴ developed a procedure to fit a logistic function and a calibration equation for cell population to optical density. A quadratic response surface was then fitted to the curve parameters for the factors of temperature, sodium chloride, and hydrogen ion concentration. With high-quality original data, this approach gave reasonable values for the growth rate. When the original data had to be extrapolated to extend to the lag period, the accuracy decreased. The other problem with this method was that marginal growth conditions may not allow sufficient growth to cause turbidity, leading to an erroneous conclusion of no growth. These limitations made this a suspect technique for the initial modeling of an organism; however, once the general nature of the organism's growth characteristics were known, it could be used to increase the accuracy of selected levels or to develop specific food applications. The time to turbidity model (Equation 27)⁵⁶ also was suitable for automated reading.

Growth of microorganisms in gradient agars was measured by turbidity.^{135,136,155} Two-dimensional gels were made with increasing pH in one direction and increasing temperature in the other. A third factor, the sodium chloride level, was incorporated by running a series of gels. By mapping turbidity changes with time over the gel, multidimensional models were developed.

B. Conductivity Methods

Conductivity techniques are based on the metabolism of a microbial population causing changes in a growth medium, which, in turn, results in conductivity changes.¹⁴⁹ This technique has been used in two ways. For the first, there was an inverse relationship between the cell population in a sample at a given time and the time for their metabolism to produce detectable amounts of conductants in a specified analytical medium. Samples must have been taken throughout the growth curve, inoculated into the analytical medium, and the time for the conductivity change noted. This means of determining cell populations had little advantage over traditional plate counting procedures. The second approach was to follow the magnitude of the conductivity changes in the medium treatment being modeled with incubation time.^{26,149} These conductivity changes from the production or utilization of charged compounds were related to metabolic activity of the microorganisms, although not necessarily directly to growth. The magnitude of the response depended upon the specific microorganism, composition of the medium, and environmental factors. Because the inoculum must grow to ca. 10^8 CFU/ml before detectable conductivity changes resulted, many of the considerations and limitations of the turbidity technique also applied.

The modeler converted the measured electrical units to cell populations and extrapolated the growth rate and lag time from the late exponential phase of growth. This assumed that conductivity changes in all of the media were the same for a given microbial growth, which may not be accurate. Because of these limitations, this conductivity technique would not be recommended for the initial model development. It would be an excellent technique for modeling food products as part of the quality assurance or HACCP program. Plate counts initially would be correlated with conductivity measurements for a specific food, then subsequent analyses and modeling could use conductivity methods.

A deterministic model for bacterial growth¹⁰⁶ was partially based on conductance measurement data. The influence of temperature, pH, and lactate levels on the growth rates of *Y. enterocolitica*

O:3 was modeled in broth using the Gompertz function and polynomial regression.²⁶ Growth rates in pork samples with adjusted pH and added lactate were close to those predicted by the broth model.

IV. HEAT INACTIVATION MODELS

Thermal process times for inactivation of clostridial spores in low acid foods used the first-order inactivation model, that is, a constant proportion of spores were inactivated in each successive time period. The D value was the time for one log decrease in viable spores at a given temperature, menstruum, etc. The z value was the rate of change in the log of the D value with temperature. An excellent anthology of the pioneering papers in the development of this model was edited by Goldblith et al.⁸⁹

This classic model is still used frequently for thermal inactivation. Mackey et al.¹³⁰ found the D values for *L. monocytogenes* in fresh and cured beef and chicken. Hutton et al.¹⁰³ determined the decrease in D values of PA3679 and *C. botulinum* 213B with decreasing pH and increasing sodium chloride concentrations. The rate of thermal inactivation in egg yolk with sodium chloride or saccharose¹⁴³ and the destruction of *E. coli* by microwave heating⁷³ followed a first-order inactivation with time and an Arrhenius relationship between the rate and reciprocal of temperature (K). Regression equations for temperature and each of three medium parameters (a_w , osmotic pressure, and water-binding energy) on the D value of *S. aureus* showed increased survival with increasing sodium chloride or potassium chloride.¹⁹⁹

However, deviations from the linear declines in the log numbers with time were frequently observed,^{22,100} even in the earlier literature.^{44,144} Deviations were of two general forms, a shoulder or lag period before any death occurred and a tailing from an apparently resistant subpopulation. The shoulder was attributed to a requirement for more than one damaging event or the need to activate the spores to make them more susceptible to thermal destruction. Two concepts were proffered for tailing.⁴⁴ The first was a vitalistic mechanism wherein resistance was possessed in

varying degrees by the different cells that constitute a suspension of apparently identical cells. The relative heat resistance was permanent for an individual cell, and a collection of cells presumably formed a normal distribution of cells. The second concept, termed mechanistic, assumed that resistance was the result of cellular processes. At different times of its life cycle or in specific environments, an individual cell has different degrees of thermal resistance. At the time of heating, a small population of cells was in a resistant state. Tailing may also be a treatment artifact, a result of a heterogeneous population, nonuniform treatment, clumping, or enumeration errors.⁴⁴

Rodriguez et al.,^{177,178} Teixeira and Rodriguez,¹⁹⁴ and Smerage and Teixeira¹⁸⁶ advanced a population dynamics theory for thermal inactivation of spores. The cause for various patterns of initial decrease or increase in spore populations was a combination of rapid inactivation of the less heat-resistant population, a period of activation of remaining spores to a more heat-sensitive state, and final inactivation. Activations and inactivations were first-order processes. UHT sterilization was modeled by dormant spores being either inactivated or activated by heat.¹⁸⁰ The activated spores were then subject to inactivation. The temperature dependence of each of the three parameters followed the Arrhenius equation, and the model successfully predicted spore inactivation during variable heating regimes.

Abrahm et al.¹ also hypothesized that the initial shoulder before the linear decline resulted from the necessity for dormant spores to be activated before being destroyed by heat. Both activation and inactivation were first-order processes and the first step was the limiting process.

The inactivation of *L. monocytogenes* in a submerged coil heating apparatus was determined at 45 combinations of temperature (56, 60, and 62°C), hydrogen ion concentration (0.1 to 57.5 μM; pH 7.0 to 4.24), and added sodium chloride (0, 3, or 9%).⁵⁰ The survivor data did not fit the traditional log-linear relationship. A logistic function of log number of survivors vs. log time described the results.

$$N_t = a_1 + [(a_2 - a_1)/(1 + \exp((4k(\tau - t)/(a_2 - a_1)))] \quad (60)$$

where N_t was the log number of survivors, a_1 was the upper asymptote ($\approx N_0$), a_2 was the lower asymptote, τ was the time of the maximum slope, k was the maximum slope, and t was the \log_{10} time. For these 45 combinations, the values for a_1 , a_2 , and k were not significantly different. A polynomial regression equation for τ was determined with terms for temperature, salt, and hydrogen ion concentration.

The thermal death time (TDT) method for determining process times (D and z values) was compared with an Arrhenius approach, with the former calculating that 16% less time was necessary for processing than the latter.¹³¹ The TDT method had a reaction rate parameter that was a function of temperature, whereas the Arrhenius method had a reaction rate parameter that was a function of the inverse of the temperature. The two turned out to be proportional to each other over a narrow temperature range. The authors concluded that the TDT method was not as good a model as the Arrhenius method; however, most workers continue to prefer the TDT method.

V. INACTIVATION/SURVIVAL MODELS

It would be desirable in chilled foods and shelf or semi-shelf stable foods to have any pathogens present in the foods decline in numbers during storage. For many foods, inactivation of microorganisms results from a combination of inhibitory factors, none sufficient by themselves to cause death.¹⁴⁵ Declines in numbers of environmental microorganisms were observed to follow first-order kinetics.^{79,96,112} Whether the process was termed inactivation or survival was frequently arbitrary. Inactivation implied a faster rate of killing by an active agent, whereas survival implied a slower and more passive rate of decline. However, modeling was the same for both.

A. Linear Model

Survival curves frequently showed lag or shoulder periods where the cell numbers remained constant. This period was followed by a decline approximating a first-order process. In some sur-

vival curves, a tailing was observed from a resistant or long-lived subpopulation. A simple regression equation was determined to relate the lag time to the pH of the broth.¹⁵⁴ Buchanan et al.^{38,39} modeled the shoulder and first decline by two discontinuous equations. At times less than the lag time, the population equaled the inoculum.

$$N_t = N_o \quad t < t_l \quad (61)$$

where t_l was the lag time. After that time, the decline was

$$N_t = N_o + a(t - t_l) \quad t > t_l \quad (62)$$

where a was the slope and equal to $-1/D$. This pair of equations was run under the ABACUS curve fitting program (ERRC, ARS, USDA, Philadelphia, PA)⁴³ using a Gauss-Newton iteration routine.

B. Logistic Model

A logistic model was proposed by Kamau et al.¹¹¹ for enhanced thermal destruction of *L. monocytogenes* and *S. aureus* by the lactoperoxidase system. For data exhibiting one slope

$$\log (M_t/M_o) = 2/[1 + e^{(kt)}] \quad (63)$$

A second model described survival data exhibiting two slopes

$$\log (M_t/M_o) = \log \left\{ \frac{2F_1}{1 + \exp(k_1 t)} + \frac{2(1-F_1)}{1 + \exp(k_2 t)} \right\} \quad (64)$$

where F_1 was the fraction of population in the major group, k_1 was the inactivation rate parameter for the major population, and k_2 was the inactivation rate parameter for the subpopulation. A final expression modeled survival data having a shoulder followed by a decline

$$\log (M_t/M_o) = \log [1 + \exp(-kt_{1/2})] - \log [1 + \exp\{k(t - t_{1/2})\}] \quad (65)$$

where $t_{1/2}$ was the time for $M = (M_o/2)$, a measure of the lag time.

The classic D value for the first log decline was

$$D = 2.9/k \quad (66)$$

Whiting²⁰⁴ expanded this model to include a shoulder and two slopes

$$\log(M/M_o) = \log[F_1(1 + \exp(-k_1 t_l))/(1 + \exp(k_1(t - t_l)))] + \log[(1 - F_1)(1 + \exp(-k_2 t_l))/(1 + \exp(k_2(t - t_l)))] \quad (67)$$

where t_l was the lag period, F_1 the fraction of cells in the major population, and k_1 and k_2 the respective rate parameters ($D = 2.3/k$). When the subpopulation did not exist, the fraction of cells in the subpopulation was set to an insignificantly low value. If the shoulder was not present, t_l was set to 0.0 and the model became a nearly straight line. Whiting²⁰⁴ compared the linear (Equations 61 and 62) to the logistic (Equation 67) model and found the linear model calculated slightly larger D values or slower rates of inactivation. This was explained by the curving nature of the logistic model over estimating the slope when fitted by least-squares procedures.

This model (Equation 67) was used to simulate survival in uncooked meat products.²⁰⁴ BHI broths with added lactic acid, sodium chloride, and sodium nitrite were inoculated (10^8 CFU/ml) with *L. monocytogenes* or *S. aureus* and stored at different temperatures. The broths were sampled until the counts declined to undetectable levels. The logistic model (Equation 67) was fitted to several hundred survival curves and the resulting parameter values were used to calculate the time for 10^4 -fold inactivation, which were then described by second-order regression equations.

An interaction between pH and the concentration of lactic or acetic acid on the inactivation times of *L. monocytogenes* was observed.³⁸ For both acids, the logarithm of the time for 10^4 -fold inactivation was inversely related to the square root of the undissociated acid concentration. The equation for lactic acid was

$$\text{time } 10^4\text{-fold inactivation (h)} = \exp(7.348 - 0.1773[\text{HA}]^{1/2}) \quad (68)$$

The undissociated acid concentration was calculated from the pH and total acid concentration by the Henderson-Hasselbach equation.

Miller¹⁴¹ used the shoulder and two-slope model for decreases in the population of *L. monocytogenes* in broths with various concentrations of sodium chloride, glycerol, or propylene glycol. With sodium chloride, declines occurred at a_w at and below 0.91. As the a_w decreased, the shoulder shortened and the D values became smaller. With propylene glycol, declines occurred at $a_w = 0.93$ and resistant subpopulations were observed for a_w of 0.87 and lower.

VI. MODELING CHANGING CONDITIONS

Most modeling was conducted under presumed constant conditions to determine the respective parameter values. However, conditions of temperature, pH, or atmosphere composition seldom remained constant during the storage of actual chilled foods.¹¹⁷ Gill⁸⁵ estimated the growth of *E. coli* during the cooling of liver and organ meats by calculating the growth in a specific period of time using the rate of growth for the average temperature during that period. Total growth was obtained by summation of the time periods. This approach was applied to *E. coli* growth during cooling of beef carcasses.⁸⁷ The growth and inactivation rates (first-order) of *C. perfringens* were determined at various temperatures used in meat cooking and found to follow an Arrhenius relationship.¹⁹⁷ The model accurately predicted growth during constant and increasing temperature conditions. This approach was similar to the traditional thermal process calculations that summed the lethality at the temperature of each heating period until the desired amount of killing was achieved.

Gill and Jones⁸⁶ compared predictions of *E. coli* growth during the cooling of beef offals with bacterial counts. Some predictions were good; however, highly variable cooling rates in some of the offal-containing cartons led to large errors in the predictions.

Blankenship et al.²⁴ developed a model for the growth from spores of *C. perfringens* in chili during a 5- to 15-h cooling process after cooking. It combined a logistic function for the lag time

$$1/t_1 = a_2[1 - \exp(-a_0(t - a_1))] \quad (69)$$

(where t_1 was the lag phase time and a_0 , a_1 , and a_2 were adjustable parameters) with an exponential function for growth

$$N_t = N_0 e^{kt} \quad (70)$$

The values for k came from a square root model (Equation 46) with parameters for T_{\min} , T_{\max} , a_1 , and a_2 . The number of germinating spores and growth were calculated each hour using the respective chili temperature. The growth of *Salmonella* during the cooling of bovine tissues was predicted by interpolation of the lag time and generation times that were calculated from isothermal determinations of Gompertz parameters and an exponential-decay function for temperature.⁶⁶

Powers et al.¹⁶⁰ found that *Aerobacter aerogenes* and *S. aureus* grew faster under square wave or sinusoidally cycling temperatures from 40 to 80°C that when incubated at a constant 60°C. They proposed a method to integrate reaction rate with temperature. Fu et al.⁷² combined a sinusoidally fluctuating temperature with Arrhenius or square root models. Abrupt temperature transitions in the growth of *Pseudomonas fragi* were effectively modeled, although an effect of the temperature change was still present.

A dynamic model combining the Gompertz function, the square root equation, and an inactivation term when the microorganism was placed into a lethal environment described the microbial population as a function of time and temperature.²⁰⁰ The model dealt with both varying temperatures and temperatures above the maximum growth rate temperature.

Exponentially growing *E. coli* cultures subjected to abrupt changes in temperature had an initial growth rate that was intermediate to the rates normal for the initial or final temperatures.¹⁴⁷ Shaw¹⁸² demonstrated that an adjustment time occurred depending on the extent of the changes that yeasts make when changing environments. The greater the change, the longer the adjustment period. They concluded that growth at low temperatures altered or damaged the cell in a way that reduced the growth rate. Preincubating *L. monocytogenes* at temperatures of 28°C or higher

increased the lag times when subsequently incubated aerobically at 5°C or preincubated at 13°C when subsequently incubated anaerobically.³⁶ The exponential growth rates at 5°C were not affected by the preincubation temperatures.

For most current growth and survival models, the inocula were usually overnight cultures in optimal media at favorable temperatures. It is probable that the lag phase of microorganisms after transfer to a 15°C abuse environment would not be the same as that of microorganisms naturally present in a food stored at 5°C and then abused at 15°C. Baranyi et al.¹⁷ included the effect of the state of the inoculum culture on the observed lag period in his growth model, but did not explore the magnitude of the effect with quantitative data. In nonmodeling papers, culture age and preincubation temperature and pH were shown to affect microbial survival,^{55,70,71,116,185} including that in cheese.¹¹⁹ Starved *Vibrio* and *E. coli* had increased resistance to lethal temperatures.¹¹⁰ Kolter¹¹³ discussed some of the physiology of stationary, nongrowing, or extremely slow-growing bacteria, including survival genes, protein synthesis, and mutations. Additional research is needed to model both one-time changes in temperature and various cycling regimes more accurately. Similar comments could be made regarding changes in other environmental factors, such as pH or atmosphere.

VII. TERTIARY LEVEL

Growth models for six food pathogens based on the primary Gompertz function and secondary-response surface equations were combined into a DOS-based PC program (Pathogen Modeling Program).³¹ The program has a series of menu screens asking for input on the desired microorganism, aerobic or anaerobic atmosphere, initial bacterial population, pH, sodium chloride level, temperature, and nitrite concentration. The program then asks whether growth parameter values, an estimated time for a specified growth, or a graph showing growth is desired and calculates the requested prediction.

The Micromodel program in the U.K. has predictive equations for growth, survival, and death of pathogens.^{23,49,65,108} Growth models for

L. monocytogenes, *Y. enterocolitica*, *B. cereus*, *Campylobacter jejuni*, psychrotrophic *C. botulinum*, *A. hydrophila*, *Salmonella*, and *S. aureus* include the factors of temperature, pH, and a_w . Validation of these equations using several foods from each of six major groups (meat, fish, vegetables, dairy products, bakery products, and eggs) is underway. This modeling program will use the results of predictive microbiology research in the context of an expert system.

Expert systems are computer programs that emulate the reasoning and decision making of human experts. They consist of a set of rules and descriptive information. To use an expert system, the user starts with a query, the system then applies the rules to ask further questions, and through dialog with the user retrieves or calculates the desired information. Adair and Briggs² described the development of a microbiological system for chilled ready-to-eat foods that consists of four sections: a database of information on the manufacture of these foods, a database of information on microorganisms, a meal-design knowledge base, and a predictive modeling spreadsheet system. Other software provided an interactive interface with the user, an expert system shell that contained the rules and information and conducted the logical processes, and a graphics presentation program that displayed the predicted growth. Expert systems can be a rapid means to retrieve appropriate information and also ensure consistent decision making. The authors also pointed out the process of developing an expert system of formalized knowledge and identified inadequacies in the current information.

Zwietering et al.²¹⁸ depicted a system wherein the pH, a_w , temperature, and oxygen availability affected the growth kinetics of the microorganisms expected to predominate in different foods. A series of rules determined the probable microorganisms. The growth rate at a specific condition was calculated as a proportion of the optimal growth rate. The proportional rates for temperature and pH were determined by the square root model (Equation 47) and a_w by a linear relationship.¹³⁷ The overall rate was the product of the proportional rates for each factor.

Voyer and McKellar²⁰² described an expert system under development by Agriculture

Canada that used input from (1) a flowchart of the production system, (2) the factors affecting survival and growth of microbial pathogens, and (3) the ranges for each factor. The factors were grouped into five types: contamination, formulation, time/temperature, package permeability, and assembly. The ranges of variation for each factor's parameters and the probabilities for different levels of each factor occurring were determined. The system then calculated the accumulated growth/decline for each sequential step in the process.

VIII. VALIDATION OF MODELS

General models for growth or inactivation are typically derived in broth media with controlled factors such as pH, salt, and temperature. After collecting an appropriate number of curves and calculating the primary and secondary models, it is important to test the accuracy of the model with new data and new combinations of factors.^{65,179} This provides an estimate of the goodness of fit and shows where additional data are needed. An overparameterized or excessively complex model usually fits a specific data set better than a simpler one. However, it may not be better than a parsimonious model when tested against new data. The second stage of validation is to compare the predictions to actual microbial behavior in foods. This demonstrates the limitation of the model and, when a particular food is not accurately described, suggests what additional factors (or a consideration of microenvironments) need to be included in the model to increase its applicability. Models cannot be used with confidence to make predictions in foods until this validation is done. Errors in the estimate of growth should tend toward a faster rate than the rates observed in foods to make a conservative or "fail-safe" prediction.

IX. LIMITATIONS OF MODELS

A. Statistical Limitations

An important statistic missing from most current secondary models and tertiary level programs

is an estimate of the variation around the calculated value. With marginal conditions for growth, the variation between replicates becomes large.¹⁶⁶ Transformations were used to homogenize the variances for fitting the models. The logarithm of values for time parameters were frequently closer to being normally distributed than untransformed values.

Fits of both primary and secondary models gave an F value and estimates of the error, either R^2 , mean square error, or residual sum of squares. However, the quality of a model remained a subjective evaluation. Reparameterizing the model made variances more uniform and normally distributed or made the parameters more interpretable; however, the process was controversial.^{13,14,76,77,166,203,215} Ratkowsky et al.¹⁶⁶ demonstrated the consequences of mathematical transformations of the square root model and the effect on the mean square errors. The relationship between the variance of \sqrt{k} , now homogeneous over the temperature range, and the variance in the lag time was given by

$$\text{Var}(t_l) = 4t_l^3 \text{Var}(\sqrt{k}) \quad (71)$$

where t_l was the lag time and k the growth rate.

Several reparameterizations of the square root model having T_{\min} and T_{\max} were tested by Alber and Schaffner^{6,7} with *Yersinia* growth rate data. The model was transformed to the natural logarithmic form because the range of variances of the growth rate increases as the magnitude of the growth rate increases. The version with the minimum sum of squared error was

$$k = \{ \ln[(a_1(T - T_{\min}))^2 (1 - \exp(a_2(T - T_{\max})))] \}^2 \quad (72)$$

This model (Equation 72) fitted data near the maximum growth rate more closely than the other versions of the model after Ratkowsky et al.¹⁶⁴

$$\ln(k) = 2 \ln[a_1(T - T_{\min}) \{ 1 - \exp(a_2(T - T_{\max})) \}] \quad (73)$$

or after Zwietering et al.²¹⁵

$$\ln(k) = \ln([a_1(T - T_{\min})]^2 \{ 1 - \exp[a_2(T - T_{\max})] \}) \quad (74)$$

Alber and Shaffner⁶ weighted the regression calculations and found this compensated for uneven variances as well as the transformations did. Weighted regression analysis factors each parameter value by the reciprocal of its variance to calculate a regression equation with the smallest weighted sum of squares.¹⁹² The use of this technique for determining secondary models needs further consideration by modelers.

Cole et al.^{50,51} claimed that bacterial kinetic responses were more linear with hydrogen ion concentration than pH values (pH being a transformation). The \log_{10} CFU/ml is also a transformation of the actual bacterial population.

B. Biological Limitations

It is important that the model developer clearly specify directly or through the model what the limits of the model are, that is, what microorganisms, what factors, the ranges of each factor, and what combinations of factors give valid answers. The presence of additional inhibitory factors in a food that were not present in the model invalidate the model or require cautious interpretation of the predictions. Currently, growth models do not usually include factors such as anion effects from the acidulant used, phosphates, sorbates and bacteriocins, and humectants other than sodium chloride. No broth models include competition from other microorganisms. Some models developed with foods include the "normal" spoilage flora, but how this flora may change in species and number with plant or season and the effect on the modeled microorganism are largely unknown.

Because pathogens grow in most foods, the important question then is whether the pathogens will grow to a significant population before the spoilage flora cause the food to be rejected by the consumer.¹⁰¹ There is a need for systematic modeling of representative classes of spoilage microorganisms so that tertiary software can then plot comparative growth curves for both pathogenic and spoilage organisms. For some pathogens with very low infective or toxic doses, such as *Listeria*, *Yersinia*, and *C. botulinum*, the criteria may be growth-no growth and the spoilage flora has little signifi-

cance unless they alter the environment by lowering the pH or produce a bacteriocin.

X. APPLICATIONS OF MICROBIAL MODELS

It must be stressed that models are valuable tools for making predictions and planning HACCP programs.^{65,205} Particularly at present, as models are evolving from the basic research laboratory to use by industry and regulatory agencies, models should be considered as initial estimators of microbial behavior and guides for evaluating potential problems. Models do not completely replace microbial testing nor the judgment of a trained and experienced microbiologist. Models can provide very useful information for making decisions in the following situations.

A. Estimate Risk

Time-to-growth and survival models can estimate whether there is likely to be a risk in a particular food after a specified time-temperature storage. Growth models can aid in setting a pull date governed by growth of a pathogenic or spoilage microorganism.

B. Identify Critical Control Points

Identification of critical steps in the process by the model assists in developing an HACCP program. A critical control point can exist where the model indicates that a certain level of a factor permits or suppresses microbial growth. Quantitative estimates of microbial behavior at different levels of the factors can suggest the allowable ranges for that factor.

C. Evaluate Reformulations

The consequences of reformulations on growth or inactivation can be estimated. Models show which factor has the major influence and can identify alternative formulations with similar or enhanced resistance to growth.

D. Evaluate Out-of-Process Product

The consequences of out-of-process events, such as lack of intended salt or inadequate refrigeration, can be immediately determined. Decisions to rework, rapidly utilize, or scrap a product can be made without waiting for testing.

E. Education

By generating graphs or estimates of the time to a specified microbial population, models can be educational tools, particularly for nontechnical people. The model can dramatically demonstrate the importance of maintaining proper refrigeration temperatures or the benefits of high-quality raw materials with lower initial populations.

Using models of microbial response potentially saves resources, time, and money by reducing much of the laboratory work. This permits the laboratory to utilize its resources in other areas. The model will quickly give the ranges of concern for a factor and thereby guide the design of challenge tests, storage trials, and other conventional techniques to assess the probability of pathogen growth. Examining the model's predictions increases the understanding of what governs microbial growth or decline in a particular food and thereby gives the processor greater confidence in his process and product. This knowledge enables the manufacturer to create a more sophisticated and effective HACCP program.

XI. RESEARCH NEEDS

Many avenues for additional research have been alluded to in this review: a brief listing includes: modeling of spoilage microflora, determination of error or confidence intervals in model predictions, incorporation of additional environmental factors into models, identification of effects of food structure, effect of the physiological state and culture history of the inoculated cells, integrating growth and inactivation models, and need for automatic techniques. The sizes of databases needed for multifactor models, the complexities of expert systems, and the requirement

to validate a model make cooperation between researchers imperative.

Most of the current primary and many secondary models are descriptive, therefore, models based upon physical-chemical, physiological, or biochemical considerations would be an advance. This in turn may lead to a renaissance in basic metabolic research. Understanding the mechanisms of heat resistance, acid and salt tolerance, lactate and phosphate inhibition, injury and repair, spore germination, and subpopulation origins, for example, would have benefits for food and other areas of microbiology.

Ultimately, what is desired is a risk assessment: what are the chances of becoming ill from a food-borne pathogen after consuming a specific food? This requires quantitative evaluation of three areas.⁵ The first is to identify and enumerate all possible sources of contamination. Both the frequency of occurrence and the numbers of pathogens are needed. The second is to understand the physiology, biochemistry, and behavior of the pathogens; how fast will they grow or produce toxins under specified conditions? Finally, the characterization of the human response to the pathogen, that is, what is the infectious dose? These areas must be integrated into a publicly trusted, cost-benefit analysis to determine the steps that minimize risk. Current efforts in microbial modeling are making rapid strides in fulfilling the second area.

XII. CONCLUSION

The progress in microbial modeling has been impressive, and models are becoming a standard research tool and a valuable aid in evaluating and designing food processes. However, it is not yet possible to rely solely on models to determine the safety of foods and process systems. Laboratory testing is still necessary to unequivocally determine the propensity for pathogen growth or survival in the food product.

DISCLAIMER

Reference to a firm or brand name does not constitute endorsement by the U.S. Department

of Agriculture over others of a similar nature not mentioned.

This review includes work published or known to the author through February 1993.

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