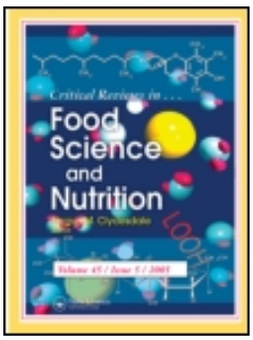

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# Microbial Growth Curves: What the Models Tell Us and What They Cannot

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*Most of the models of microbial growth in food are Empirical algebraic, of which the Gompertz model is the most notable, Rate equations, mostly variants of the Verhulst's logistic model, or Population Dynamics models, which can be deterministic and continuous or stochastic and discrete. The models of the first two kinds only address net growth and hence cannot account for cell mortality that can occur at any phase of the growth. Almost invariably, several alternative models of all three types can describe the same set of experimental growth data. This lack of uniqueness is by itself a reason to question any mechanistic interpretation of growth parameters obtained by curve fitting alone. As argued, all the variants of the Verhulst's model, including the Baranyi-Roberts model, are empirical phenomenological models in a rate equation form. None provides any mechanistic insight or has inherent advantage over the others. In principle, models of all three kinds can predict non-isothermal growth patterns from isothermal data. Thus a modeler should choose the simplest and most convenient model for this purpose. There is no reason to assume that the dependence of the "maximum specific growth rate" on temperature, pH, water activity, or other factors follows the original or modified versions of the Arrhenius model, as the success of Ratkowsky's square root model testifies. Most sigmoid isothermal growth curves require three adjustable parameters for their mathematical description and growth curves showing a peak at least four. Although frequently observed, there is no theoretical reason that these growth parameters should always rise and fall in unison in response to changes in external conditions. Thus quantifying the effect of an environmental factor on microbial growth require that all the growth parameters are addressed, not just the "maximum specific growth rate." Different methods to determine the "lag time" often yield different values, demonstrating that it is a poorly defined growth parameter. The combined effect of several factors, such as temperature and pH or $a_w$, need not be "multiplicative" and therefore ought to be revealed experimentally. This might not be always feasible, but keeping the notion in mind will eliminate theoretical assumptions that are hard to confirm. Modern mathematical software allows to model growing or dying microbial populations where cell division and mortality occur simultaneously and can be used to explain how different growth patterns emerge. But at least in the near future, practical problems, like translating a varying temperature into a corresponding microbial growth curve, will be solved with empirical rate models, which despite not being "mechanistic" are perfectly suitable for this purpose.*

**Keywords** Logistic growth, lag time, growth rate, Verhulst's equation, Gompertz model, kinetics

## *INTRODUCTION*

Microbial growth is the most common cause of food spoilage and when a pathogen is involved, of food poisoning. Consequently, microbial growth patterns and what affects them have been extensively studied. Microbial growth has also been described by a variety of mathematical models. Their properties and how well they fit and predict experimental growth data are discussed in numerous research articles, reviews, and book chapters (e.g., McKellar and Lu, 2004; López et al., 2004; Peleg, 2006; van Boekel, 2009). By and large, the models developed in the context of food quality and safety primarily describe and predict microbial growth in what is approximately a "closed habitat." In a "perfectly closed habitat," all life-sustaining resources are finite and non-renewable while the metabolites

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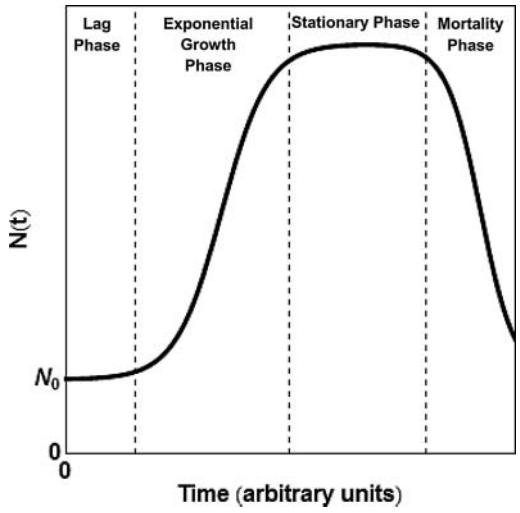


**Figure 1** Schematic view of a typical microbial growth curve in a "closed habitat."

excreted by the living cells, and the cells that have died are not removed. This is in contrast with a continuous fermentation process, for example, where resources are continuously replenished and metabolites or cells are harvested or removed. Typical growth in a closed habitat has four discernible stages as shown schematically in Fig. 1, namely, a "lag phase," "exponential growth phase," a "stationary phase," and a "mortality phase" (McKellar and Lu, 2004). However, with very few exceptions, the models presented and discussed in the food microbiology literatures, only deal with the first three and disregard the last. This makes good sense since most foods become inedible or unsafe to eat long before microbial massive cell mortality begins, and sometimes even before the stationary phase is reached.

An ideal microbial growth curve is a plot of the number of living cells as a function of time. The actual microbial growth curve is a record of the countable cells determined at certain time intervals during the population's evolution. The common expression of the population size is in the form of "Colony Forming Units" (CFU's). Thus, cells that are injured or have failed to form a colony for other reasons are not included in the count. Also, when two or more cells remain attached to each other and hence form a single colony, they are counted as one. These shortcomings are rarely a serious problem because comparisons are almost always made between curves that have been determined in the same manner and in at least some cases after an attempt to separate adhering cells.

In studies of growth kinetics, the data are usually collected at several constant temperatures and other controlled "environmental" or "ambient" conditions such as the pH of the medium, its salt or sugar concentration, water activity, the presence of an antimicrobial, etc. The growth medium can be a model system or an actual food. It is generally assumed that only the principal factors, such as the ones mentioned above, play a significant role in the growth kinetics. Thus factors like nutrients and gases diffusion, changes in solubility, or heat transfer are usually neither monitored nor taken into account, unless they can explain considerable deviations from an expected growth pattern.

Once determined, the experimental relationship between the actual count, $N(t)$, growth ratio, $N(t)/N_0$, or their logarithm, and time is fitted with a mathematical growth model known as the "primary model." The coefficient's dependence on the primary model on the temperature, pH, $a_w$ and/or other factors, is then described mathematically by what is known as "secondary models." These can be reincorporated into the equation of the primary model to produce the "tertiary model," which can then be used to generate growth curves under a variety of static or dynamic conditions. When the tertiary model is an algebraic expression, it can then be used to "predict" growth curves under a variety of fixed temperatures, pH, and/or $a_w$ combinations, for example, using interpolated values of its parameters. When the primary growth model is a differential rate equation, the tertiary model can be used to predict growth patterns under a variety of dynamic conditions by solving the differential equation numerically. [See below]

The most commonly used growth models in food microbiology are of two types: empirical algebraic expressions of which the Gompertz is the most familiar and frequently employed, and growth rate models, almost all variants of the continuous logistic equation, the Verhulst model. Models of these two types will be introduced in the next section and their mathematical properties discussed. A research group at the US Army Natick Soldier RD & E Center (Taub et al., 2003; Doona et al., 2005; Ross et al., 2005) has introduced the third class of models. The starting point of the formulation of these models is not the microbial population size but processes or events at the cellular and/or sub-cellular levels. Most of what follows will focus on the first two kinds and we will only briefly discuss the merits of models of the third kind and explore their potential. The emphasis will be on the issue raised in the title, that is, on whether the amount of information in an experimentally recorded growth curve is sufficient to draw conclusions on what actually happens at the cellular level. We will also address the question of whether any of the various offshoots of the logistic (Verhulst's) equation, notably the Baranyi-Roberts model (Baranyi and Roberts, 1994), is really more "mechanistic" than the purely empirical models as many in the food microbiology community believe.

The evaluation of the models will be primarily based on their mathematical structure and properties, what they imply, and whether these implications are consistent with observed microbial growth patterns in and on foods. Our analysis and evaluation will be solely based on published works and not on new experimental data. We assume that what has been reported in the literature has been properly determined. However, many published growth curves have been recorded in a single set of experiments. Thus in at least some cases, the error bars that accompany the counts or growth ratios only represent the data scatter in the particular experiment and not necessarily the reproducibility of the growth pattern itself. Determination of the

reproducibility of a pattern requires repeating the whole experiment with fresh cultures and media, preferably from different sources, which is frequently judged as cost-prohibitive or too time consuming and hence not done. This statement should not be construed as criticism of the experimentalists who frequently have to struggle with formidable logistic obstacles. Its purpose is only to alert the reader that experimentally determined growth parameters might be influenced not only by the chosen growth model but also by procedural details.

There is much emphasis in the Food Microbiology literature on experimental design, statistical analysis of the data, and fit criteria. Excellent summaries and discussions of these topics can be found in Ratkowsky (2004a), Ross and Dalgaard, 2004, López et al. (2004), and van Boekel (2009). But even under the best experimental design, appropriate experimental procedure, and most sophisticated statistical analysis, the validation of a model never comes from its goodness of fit alone. A true validation of the model should come from its ability to predict correctly experimental results not used in its parameters determination and through confirmation of its parameters by especially devised independent tests. This also applies to any proposed mechanistic interpretation of a growth pattern. A mechanistic interpretation ought to be confirmed by microscopic observations and/or biochemical assays, not by ad hoc theoretical arguments. That experimental results are consistent with the predictions of a model does not establish its uniqueness. Therefore, an agreement between prediction and observation alone only supports the modeling approach. But by itself it does not confirm the validity of the underlying assumptions, especially if alternative models, based on different assumptions can also explain and predict the same results. As a mathematician would say, agreement between prediction and observation is a necessary condition but not sufficient.

In many food publications, fitted curves, or tabulated values calculated with regression parameters, are reported as the "model's prediction." Actually, they are not true predictions in the scientific sense, only fitted curves or values. As to much weight given to fit criteria in the Food Microbiology literature, one should be reminded that regression based on minimizing the mean square error (MSE) might yield different parameters, including the degree of fit itself, if the mean absolute error (MAE) is minimized instead (Peleg, 2006). The difference can be particularly noticeable when there are outliers in the data, which is not uncommon in microbial count records. Consequently, a slightly better fit of a particular model to a specific set of experimental growth results should not be interpreted as evidence of this model's "superiority" over alternative models. Unless a large database consisting of several organisms tested repeatedly is employed, the relative merits of different mathematical models might be better judged by utilitarian criteria and not on the basis of the MSE or $r^2$ alone. Apart from demonstrating predictive ability and internal consistency, which is a must, the usefulness of a model should also be judged by its mathematical simplicity, flexibility, the number of its adjustable parameters and, where appropriate, whether they have intuitive meaning.

The objective of this critical review is neither to compare the performance of published models nor discuss the statistical aspects of their determination. These, as already stated, have already been discussed at great length and depth by others, and the results can be found in numerous research publications, reviews, and book chapters. The main objective here is to critically assess the meaning of mathematical growth models, the interpretation of their parameters, and theoretical implications. For example, although the state, history, and interactions of the microorganism with its habitat indeed determine the course of events at the cellular level, and hence the observed growth pattern, the inverse need not be generally true. For example, one can almost always identify three growth phases in a sigmoid growth pattern, but the observation of a sigmoid growth pattern does not mean that it has been produced by the same chain of events at the cellular level (see below). As previously mentioned, until confirmed by especially designed tests, any mechanistic interpretation of the parameters of a growth model is and will remain merely a conjecture. Unfortunately, reports of such tests have been conspicuously rare in the literature on "Predictive Microbiology" and hence the need to raise the issue.

## *THE CHARACTERISTICS OF SIGMOID GROWTH CURVES*

### *Microbial Growth Parameters*

Much of the literature on the use of microbial growth models in foods deals with three growth parameters, how they are defined or determined, and what affects their magnitude. They are: the "lag time" ($\lambda$), "maximum specific growth rate" ($\mu_{max}$), and "maximum growth level" ($N_{max}$), better referred to as the "asymptotic growth level" ($N_{asymp}$) (see below). As shown in Fig. 1, the three have clear intuitive meaning, and their usefulness has been taken for granted by food microbiologists everywhere. Yet, despite their almost universal acceptance in the field of Food Microbiology, their merits and limitations deserves a fresh look and more critical assessment than has been given to them in many previous reviews.

### *The "Lag Time"*

Since the transition from the "no-growth phase" to the "exponential" growth regime is continuous rather than abrupt, the "lag time" ($\lambda$) has no clearly or uniquely defined duration, a point always known to modelers (e.g., Baty and Delignette-Muller, 2004). Traditionally, the "lag time" has been determined in two ways; graphically as the intersection of the tangents to the growth curve at the "lag" and "exponential" phases as shown in Fig. 2, or through non-linear regression by fitting the experimental data with a growth model that has a "lag time" as one of its adjustable parameters. Although what follows might be

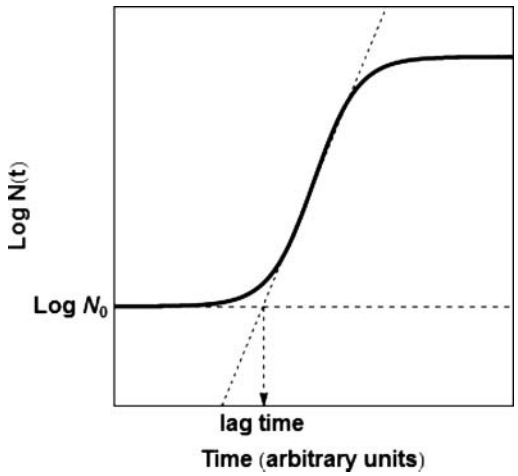


**Figure 2** Schematic view of the traditional graphical "lag time" determination.

considered a "hair splitting argument," both "lag time" determination methods are conceptually problematic. One potential difficulty with the "lag time," determined by either method, will always arise when the growth curve has a steep slope at the inflection point and very moderate climb at the region preceding it as shown in Fig. 3. The problem with the curve fitting method is that different growth models might yield very different values when used to fit the same experimental data (see McKellar and Lu, 2004; Arroyo et al., 2005, for example). These problems should not come as a surprise. They are the inevitable penalty of attempting to assign special significance to a point on a segment of a curve that rises (or falls) monotonically—the inflection point being a notable exception. Although in many cases either method provides a marker of the onset of the accelerated growth regime, the rendered value need not accurately indicate when the acceleration of the growth actually started. Consequently the practice of reporting the "lag time" in three or more digits is conceptually questionable regardless of how the value has been determined. In growth curves showing no "lag time" at all, that is, where the population size rises without any discernible delay, the intersection of the tangent of the curve at its "exponential phase" with the abscissa has no meaning at all (Fig. 3). In such a case, the attempt to save the reality of the lag time by claiming that the lag phase had ended prior to the beginning of the experiment is an example of a circular argument, akin to the classic "begging the question fallacy." One can equally claim that there has been no "lag time." Had the microbial population experienced an adjustment period prior to the observed growth onset, its existence should have been demonstrated experimentally and not by argumentation. Until the results of such experiments are reported and confirmed, the universality of the existence of the lag time and its alleged inherent relation to the "maximum specific growth rate" will remain merely a speculation (see below).

Without a totally novel approach yet to be proposed, the "lag time" issue can be resolved in two ways, both long known, but which require an arbitrary criterion. The first, and quite old, is to define the "lag time" (when it obviously exists) as the time

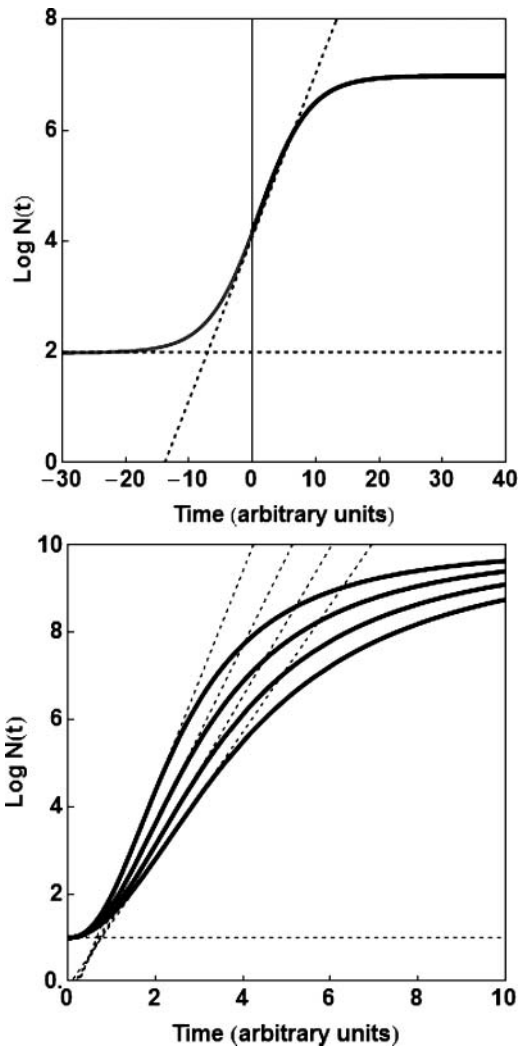


**Figure 3** Potential problems with the traditional definition of the "lag time" when applied to non sigmoid or asymmetric growth curves.

to reach 50 or 100% growth, or a factor of .5 or 1. log units (natural or ten based) increase, etc. When defined in this way, every mathematical model that adequately fits the experimental growth data will yield a similar value. Since the arbitrariness of the definition is admitted at the outset, any confusion concerning the connection of this value to the physiological state of the population and its supposedly universal relation to other growth parameters will be naturally eliminated.

The second and promising approach, now being developed, is to base the "lag time" determination on microscopically monitored cell division frequencies (Elfwing et al., 2004; Brehm-Stecher and Johnson, 2004; Guillier et al., 2006; Li et al., 2006; Niven et al., 2006; Pin and Baranyi, 2006; 2008; Dupont and Augustin, 2009). No doubt, such observations indeed provide direct record of what happens at the cellular level and hence could be considered a direct measure of the lag time duration. However, determining the division times of a cell unambiguously and identifying the distribution of the division times, which marks

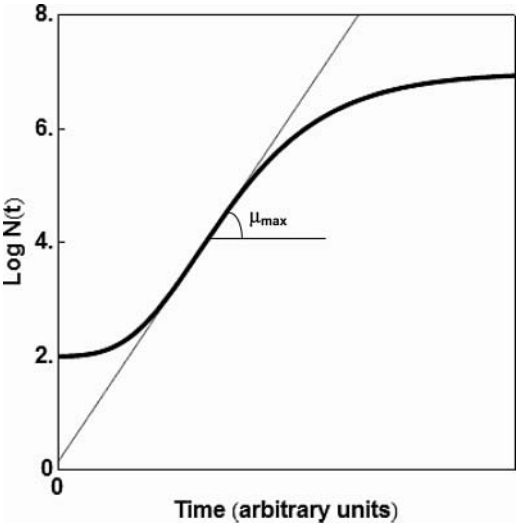


**Figure 4** Schematic view of the traditional "maximum specific rate" definition.

the transition to the exponential growth phase, will still be a challenge.

### *The "Maximum Specific Growth Rate"*

Many, probably the majority of food microbiologists, consider the "maximum specific growth rate" ($\mu_{max}$) as the measure of the growth intensity of an organism and hence of its physiological rigor. As a result, most secondary models (see the following sections) notably the Arrhenius equation, Ratkowsky's square root model, and their various modifications and combinations have been developed exclusively for this parameter and how it is affected by temperature and the properties of the habitat. Mathematically, $\mu_{max}$ is defined as the slope of the (sigmoid) growth curve at its inflection point (Fig. 4). Had all microbial growth curves been symmetric or approximately symmetric and had the same time range and asymptotic growth level ($N_{max}$ in the common parlance), $\mu_{max}$ would indeed be a universal growth parameter. This refers to comparisons of the growth of the same organism at different temperatures or under different conditions, such as different pH or $a_w$ levels, salt, or sugar concentrations, the presence or absence of antimicrobials, etc. The "$\mu_{max}$" issue always arises in comparison to the growth patterns of the same or different organisms if the degree of symmetry of their growth curves, or asymmetry, is not the same and/or if they achieve different growth levels and have different "lags," however defined. In all such cases, reference to $\mu_{max}$ alone might not be of very limited value, if at all, because the difference in the other growth characteristics might be much more significant in practice (See Fig. 5). Here again, an attempt to establish the universality of a link between the "maximum specific growth rate," "$\mu_{max}$" and the "lag time," "λ," which might not even exist, would be difficult to justify (see below).

### *The Initial Number and "Maximum Growth Level"*

The initial number of countable cells is a measurable quantity and so is the number of cells at the "stationary phase" if it has been definitely reached during the experiment. Yet, and not infrequently, these two numbers have been reported in the literature as adjustable regression parameters. A case in point is the Gompertz model (Eq. (1)) where the initial number is "embedded" in the parameter $A$. (According to this model, $A$ should be $\log_e N_0$, where $N_0$ is the initial number). Why not insert the experimentally determined number into the equation directly, which will reduce the number of adjustable parameters in the regression? The statistical rationale for not doing so, apart from the fact that $A$ could slightly deviate from the true value of $\log N_0$, is that like any other point on the growth curve, $N_0$ too might have an experimental error. According to this argument the relative weight of this error would be reduced by considering it together with the scatter of all the other data points that constitute the curve. This is a valid argument, which is upheld in other scientific disciplines. Nevertheless, the value of $N_0$ calculated in this manner might depend on the model chosen for the regression and how well it would have represented the growth pattern had there been no experimental errors. Moreover, if the pattern is described in terms of a growth ratio vs. time relationship, then one would expect that the growth curve will always start at unity if the ratio is defined as $N(t)/N_0$, or zero if it is defined as $\log[N(t)/N_0]$ or $[N(t)\text{-}N_0]/N_0$. Growth curves not starting at these values may look awkward, be difficult to compare visually, and may become a serious problem when the model is converted into a rate model and used to predict dynamic growth (see below).

### *The Asymptotic Growth Level*

Estimation of $N_{asymp}$ ("$N_{max}$") or corresponding growth ratio by regression can be tricky. If a clearly discernible "stationary phase" has not been reached during the experiment, the asymptotic number or ratio calculated in this way would be an extrapolated value. In such a case, the chosen model could have a dramatic effect on the magnitude of the calculated asymptotic level. The uncertainty will only increase when combined with that created by the scatter of the experimental data. Also, the relative weight of experimental errors might differ when alternative growth models are used. Because of all these, the significance of an asymptotic growth level or ratio obtained by extrapolation might not be clear. In systems where the growth ratio rises by several orders of magnitude, the discrepancy between results rendered by alternative models and the "true value" can be quite large. Also, unless a plateau in the experimental growth data has been reached, the regression procedure itself can be unstable and starting with different initial guessed values can result in different growth parameters or failure of the iterations to converge. Here too, different statistical fit criteria might yield different results because of outliers. Moreover, and just as

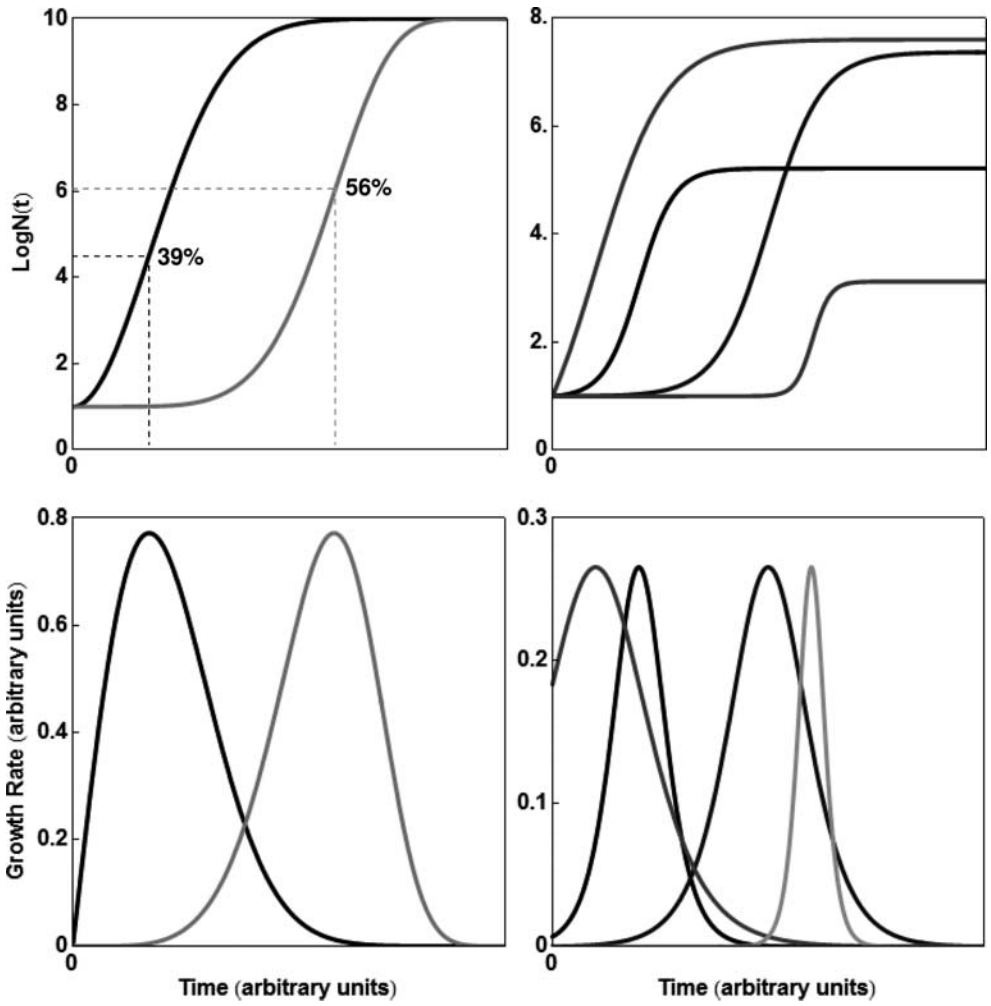


**Figure 5** Demonstration of the weakness of the "maximum specific growth rate" concept when applied to asymmetric and other growth curves. Notice that the two growth curves shown on the left and the four on the right here the same "maximum specific growth rate."

important, since the experiment might be of limited duration for logistic or other reasons, the actually reached microbial level in the stored food could be significantly higher than predicted by the model through extrapolation, and it might not even reach a plateau if mortality sets in.

### *Comparison of Sigmoid Growth Patterns*

A symmetric sigmoid curve has three characteristics: its center is along the time axis (the location of the inflection point), its "steepness" is in the intensive growth region, or the "span of this "phase," and the overall growth level as illustrated in Fig. 6. When a growth curve is grossly asymmetric, its characterization would require additional parameters to account for its shape. Sigmoid growth curves encountered in food microbiology are rarely perfectly symmetric but they are rarely grossly asymmetric either. Therefore, their shape can be usually specified in terms of these three characteristics, except that the inflection point location replaces the geometric center, and the slope at this point ("$\mu_{max}$") becomes the steepness measure of the curves. The asymptotic cell number, the net growth and/or growth ratio, linear or logarithmic, remain measures of the overall growth level. External factors might affect these three characteristics in a manner that can only be revealed experimentally. For example, in many cases, elevated storage temperature, or raised pH level, is expected to shorten the time to reach the inflection point, increase the growth rate at this point and also the overall amount of growth. But there is no reason that the higher temperature or pH will always have these three characteristics changing in such a manner that monitoring one will be sufficient to predict the effect on the other two. The same can be said about the effect of antimicrobials. It is not inconceivable that the major effect of some is the delay in the growth onset, with little or no influence on the growth rate and its asymptotic level. Or alternatively, that their main effect will be on both the growth level and time to reach the maximum growth rate but not on the growth rate itself. Moreover, there may also exist a possibility that exposure to a mild antimicrobial or antimicrobial mixtures may increase the growth level of an organism and/or its maximum growth rate,

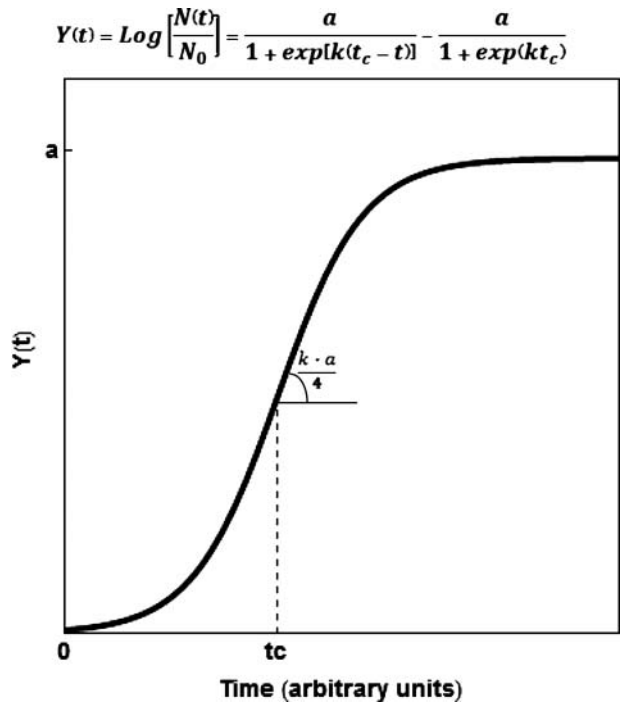


**Figure 6** A "typical" shifted logistic growth curve and its three characteristics.

possibly as a result of adaptation and/or selection during the induced delay. At least theoretically, it is also not impossible that a chemical growth promoter or a suppressor at different levels of intensity will not only affect these three main growth characteristics quantitatively but will also alter the overall shape of the growth curve. This may be manifested not only in the degree of symmetry or asymmetry of the curve but also in whether the onset of the mortality of the cells is advanced or delayed. All the above suggests that studies of the effect of external conditions and presence of antimicrobials on the growth of a microorganism should not focus on the "lag time" or "maximum specific growth rate" only, but on the growth pattern in its entirety.

## *PHENOMENOLOGICAL AND EMPIRICAL PRIMARY MODELS*

An empirical or phenomenological model is a mathematical expression whose purpose is to describe and quantify experimentally observed patterns. Such a model does not explain why a particular pattern has emerged and it is not meant to. Not being derived from any basic principles or mechanistic considerations, the only criteria of a phenomenological model's utility are its mathematical convenience and goodness of fit. Like other models, however, a phenomenological model ought to conform to the parsimony principle, also known as Ockham's razor, according to which the number of adjustable parameters should be held to the minimum. In other words, when two models describe the same pattern adequately, preference should be given to a three-parameter growth model over one having four or more. For example, although a polynomial model of high order can almost always adequately describe the sigmoid shape of microbial growth curves, it has rarely if ever been used for this purpose. This is despite the mathematical simplicity of polynomial models and the relative ease of their coefficients calculation by (linear) regression. A main reason is the frequently encountered difficulty to assign intuitive meaning to the coefficients of a polynomial model, especially with an alternating sign.

### *The Gompertz Model*

Among the empirical growth models, the most prominent and widely used is the Gompertz model, which can be written in the form (McKellar and Lu, 2004):

$$\log_e N\ (t) = A + C \exp\{\exp[-B\,(t - M)]\} \qquad (1)$$

where $N(t)$ is the momentary number of cells in the population, $A$ represents the logarithm of the initial number, that is, $A \approx \log_e N_0$, $C$ is the asymptotic logarithmic growth ratio, that is, $N(t)/N_0$ when $t \to \infty$, $B$ is the relative growth rate, and $M$ the time that corresponds to the maximum growth rate, that is, it marks the inflection point location of the sigmoid growth curve. Theoretically, even for growth curves having a long "lag time," A can only be approximately equal to $\log_e N_0$ because the second term of the right side of the equation is never exactly zero. Although this characteristic of the Gompertz model rarely has practical consequences when applied to isothermal growth curves, it can be a serious problem when specifying the boundary condition if converted to a rate model for predicting dynamic growth.

The Gompertz model is an extremely flexible mathematical model and hence its widespread use in many disciplines. It was introduced in 1825 to describe the mortality of humans. Its formulation was based on the empirical observation that (with the notable exception of early infancy) the mortality rate is very low among young people, rises as they mature and age, and then declines again when they become old. In many growing populations, the same pattern is observed in reverse, that is, the growth rate is initially very low, and then, after a period of intensive increase in the population size, it diminishes and approaches a plateau. Notice that although the growth version of the Gompertz model was originally based on changes in the growth rate rather than in the actual number, it provides no explanation of what causes the exponential rise of the population, what triggers its initiation, and why it slows down when reaching a certain size. Thus, unlike the Verhulst model and its various offshoots, which will be discussed in the next section, the Gompertz model has always been classified as purely phenomenological. By adjusting its parameters, the Gompertz model can describe growth curves having a long or short "lag time," or none at all. In food microbiology, it has been primarily used to fit isothermal growth curves or recorded under static environmental conditions. Like other phenomenological models, the Gompertz model as expressed in Eq. (1) can be converted into a rate model once the dependence of its coefficients on temperature or other factors

has been determined experimentally and expressed algebraically (see below).

### *The Logistic Function*

Isothermal sigmoid microbial growth curves of the kind frequently encountered in food and related systems can be described by a logistic model (Zwietering et al., 1990; Ross and Dalgaard, 2004; López et al., 2004), which can be written in several forms, such as:

$$N\ (t) = \frac{N_{asymp} N_0 \exp(kt)}{N_{asymp} - N_0[\exp(kt) - 1]} \tag{2}$$

or

$$N\ (t) = N_0 + \frac{N_{asymp} - N_0}{1 + \exp[k(t_c - t)]} \tag{3}$$

where $N_0$ is the experimentally determined initial number, $N_{asymp}$ the number approached at the "stationary phase," $k$ a "rate constant," and $t_c$ a marker of the inflection point. The magnitude of the three growth parameters $N_{asymp}$, $k$ *and* $t_c$ are likely to depend on the growing conditions, mainly temperature and the medium's composition in manners that have already been discussed. According to Eq. (2), $N(0) = N_0$ as it should be. This is not the case with Eq. (3), which like the empirical version of the Gompertz model (Eq. (1)), implies that $N(0)$ is not exactly equal to $N_0$. This issue, to which we will return, may create a problem in establishing a boundary condition if the model is converted into a rate equation and used to predict dynamic growth.

Equation (2) is the isothermal solution of the original Verhulst model (see Eq. (8) below), that is, when its coefficients are not functions of time. Being a solution of a rate equation, it can be automatically converted back into the dynamic model version and applied to growth under varying temperature conditions. As will be shown later, the main drawback of the Verhulst model, in both versions (Eqs. (2) and (3)), is its inability to account for growth patterns that exhibit a "long logarithmic lag." We will use this term to describe growth curves that start with a long flat region when plotted on semi logarithmic coordinates, that is, as log$N(t)$ vs. *time* instead of $N(t)$ vs. *time* relationships (Peleg et al., 2007). Examples of such plots are given in Fig. 7.

### *The Shifted Logistic Function*

A way to describe a curve having a short or long lag phase, be it linear or "logarithmic," is to use the simpler and purely

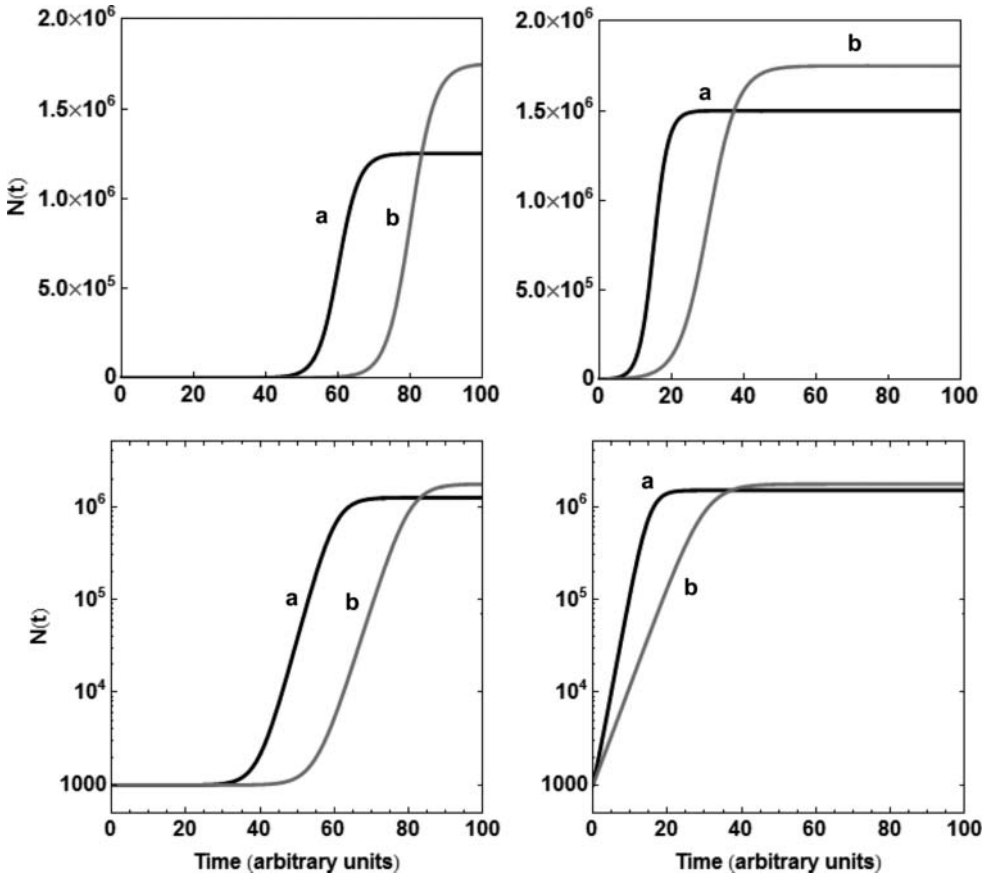


**Figure 7** Schematic view of growth curves with a short and "long logarithmic lag", right and left, respectively, drawn on linear and semi-logarithmic coordinates.

empirical version of the logistic model (Eq. (3)), and apply it to the growth ratio rather than to the actual momentary count (Corradini and Peleg, 2005; Peleg, 2006). The formulation of the model in terms of a ratio facilitates comparison of growth curves starting with a different initial number of cells. It also makes the model more suitable for conversion into a dynamic rate model. The growth ratio, $Y(t)$, can be defined as:

$$Y(t) = \frac{N(t) - N_0}{N_0} \tag{4}$$

or

$$Y(t) = \log\left[\frac{N(t)}{N_0}\right] \tag{5}$$

depending on whether the population size rises by a few folds only (Eq. (4)) or several orders of magnitude (Eq. (5)), in which case the logarithm base can be either $e$ or 10.

Here again, $N(t)$ and $N_0$ are the momentary and initial counts respectively. Regardless of whether the ratio is linear or logarithmic, it requires that the $Y(t)$ vs. *time* relationship start at the origin, that is, at $t = 0$, $Y(0) = 0$ by definition. To accommodate this requirement, one can use the simplest form of the logistic function to which a "shift factor" or "correction term" is added to the right side of the equation. Adding the term eliminates the problem and guarantees that at time zero, the ratio will always be exactly zero and hence could be used as a boundary condition in the rate version of the model. The result is the "shifted logistic model" written in the form:

$$Y(t) = a\left\{\frac{1}{1+\exp\left[k\left(t_c - t\right)\right]} - \frac{1}{1+\exp\left[k\,t_c\right]}\right\} \tag{6}$$

where $Y(t)$ is the linear or logarithmic growth ratio and $k$ and $t_c$, as before, are the temperature or conditions dependent model's coefficients (Corradini and Peleg, 2005; Peleg, 2006). According to Eq. (6), when $t \to \infty$, $Y(t) \to a\{1\text{-}1/[1+\exp(kt_c)]\}$. But since almost always $1/[1 + \exp(kt_c)] \ll 1$, one can treat $a$ as the asymptote of the growth curve, $Y_{asymp}$, for practical purposes. [The interested reader can generate and view growth curves generated by the shifted logistic model with a freely downloadable interactive program posted in the Internet as a contribution to the Wolfram Demonstrations Program.] Open: http://demonstrations.wolfram.com/RatioBasedModifiedLogisticIsothermalMicrobialGrowth/. This Wolfram Demonstration generates growth curves, which can be viewed on linear or semi-logarithmic coordinates according to the user's choice. The user can also enter and change the initial number, $N_0$, the parameters of the model, $a$, $k$, and $t_c$, and the coordinates scales of the plot, by moving sliders on the screen with the mouse. The CDF Player, the software that runs the Demonstration, can be downloaded free of charge following the instructions on the screen. Once the CDF Player has been downloaded, it can be used to open, view, and manipulate any of the thousands other Demonstrations of the Project. [To see only an animated version of the Demonstration, just click on the little arrow.

Although to the best of our knowledge it has never been tried, the Gompertz model (Eq. (1)) can also be "corrected," in a similar way, in order to force it to comply with the condition that at $t = 0$, $N(0) = N_0$. The "shift" term in that case would be: $-C\exp[-\exp(BM)]$.

### *The Three Phase Linearized Growth Model*

It has long been known that the three phases of many growth curves can be approximated by three straight lines when plotted on semi-logarithmic coordinates; a flat segment representing the "lag phase," an inclined segment representing the "exponential growth phase" and a second flat segment representing the "stationary phase" (Ingraham et al., 1983; Buchanan et al., 1997). Mathematically this translates into:

$$\begin{aligned} &\text{If } t \le t_1 && \log N\ (t) = \log N_0 \\ &\text{If } t_1 < t < t_2 && \log N\ (t) = \log N_0 + k\,(t - t_1) \\ &\text{If } t \ge t_2 && \log N\ (t) = \log N_0 + k\,(t_2 - t_1) \end{aligned} \tag{7}$$

where again $N(t)$ is the count at time $t$, $t_1$ the "lag time," $t_2$ a marker of the end of the "exponential growth phase" and beginning of the "stationary phase," and $k$ the slope of the straight line representing the "exponential growth phase," that is, the exponential growth rate. Like the Gompertz and logistic models (Eqs. (1), (3), and (6)), this linearized model too has three adjustable temperature dependent parameters, namely, $t_1$, $t_2$, and $k$, and they all have very clear intuitive meaning. Also, once the temperature dependence of these parameters has been determined experimentally, the model can be used to generate non-isothermal growth curves in a manner similar to that of biphasic survival curves (Corradini et al., 2007). Nevertheless, because of its two "If" statements, the linearized model might be judged less convenient for describing and predicting growth under dynamic conditions where the transition from one phase to another is unlikely to be as sharp as in some isothermal curves. Moreover, the three phase linearized model (Eq. (7)) requires that in isothermal growth, the exponential phase initiation must always mirror its termination. As many asymmetric growth curves testify, this need not be always the case and there is no reason that it should be (Peleg, 2006).

### *"Power Type" Growth*

A totally empirical model whose parameters are almost completely devoid of intuitive meaning was used by Corradini and Peleg (2005) to predict dynamic growth. For isothermal growth curves it has the form:

$$Y(t) = \frac{a^m t^m}{b^m + t^m} \tag{8}$$

where *a, b,* and *m* are temperature or other conditions dependent parameters.

This model has excellent fit to sigmoid growth curves with a relatively short "lag time" and it satisfies the condition that at $t = 0$, $Y(t) = 0$. According to Eq. (8), the asymptotic level of the linear or logarithmic growth ratio is $a^m$. The location of the inflection point of the growth curve can be calculated numerically and the corresponding growth rate at this point is determined by a complicated relationship between *b* and *m*. This is in contrast with the logistic models where it is primarily determined by *k*. Like the Gompertz and logistic models, Eq. (8) has no marker of a "lag time." The sole purpose of its introduction was to demonstrate that, in principle, any model that adequately fits isothermal growth data can be converted into a rate model that could correctly predict dynamic growth patterns. In other words, for a growth model to be predictive it neither needs to be unique nor mechanistic, an issue to which we will return in more detail.

The list of primary models in this section is by no means exhaustive. One should not be surprised if new models or new versions of existing ones will continue to be proposed. The three-parameter "Stretched exponential model" is a yet untried empirical growth model that might serve as an example. It can be written in the form $Y(t) = a[1\text{-}\exp(\text{-}bt^n)]$ where *a* is the asymptotic growth ratio, $Y_{asymp}$, and *b* and *n* are parameters that control the time to reach the inflection point of the growth curve. It satisfies the condition of $Y(0) = 0$ and can describe growth curves showing either short or long ("logarithmic") lag time. According to this model the inflection point time $t_c = (n\text{-}1)/bn$ and the maximum growth rate $\mu_{max} = abt_c^{n\text{-}1}\exp(\text{-}bt_c^n)$. It can also be easily transformed into a dynamic rate equation. Its major disadvantage is that its parameters have no intuitive meaning.

The fit of the Gompertz, logistic, shifted logistic, and "power type" model (Eqs. (1), (3), (6), and (8)) to the same sets of

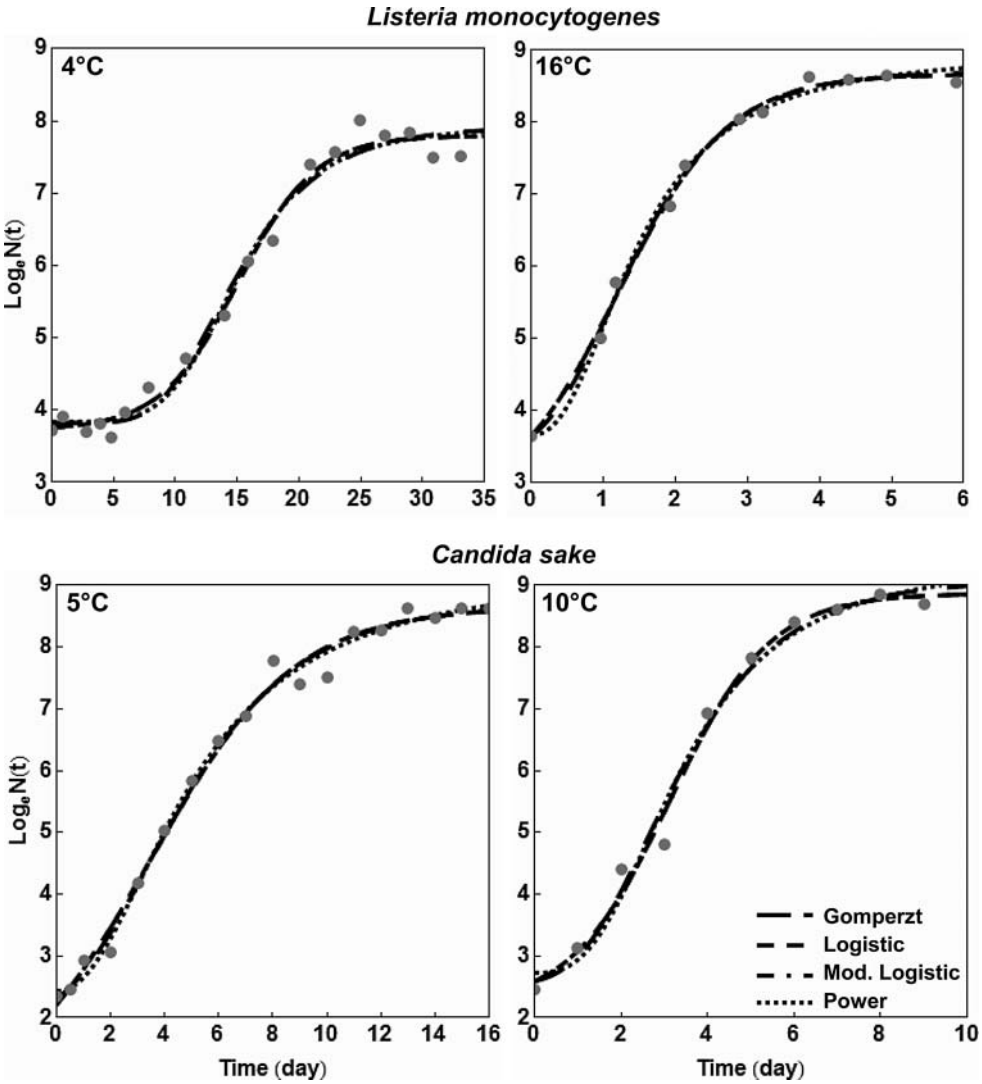


**Figure 8** The fit of the Gompertz, logistic, shifted logistic and "power growth" models to experimental isothermal growth data of *Listeria monocytogenes* and *Candida sake*. Notice that despite their different mathematical structure and not having any mechanistic interpretation, the four models can have excellent fit and be used interchangeably. The original data are from Xanthiakos et al. (2006) and Tyrer et al. (2005), respectively.

published growth data is demonstrated in Fig. 8. The figure illustrates that despite their very different mathematical structures, the four models could be used interchangeably, at least for the shown data sets. A slightly smaller mean square error (MSE) or higher regression coefficient ($r^2$) in this case would be more likely due to a random deviation of one or more data points rather than to the inherent superiority of any particular model over the others. As will be shown below, the same can be said about rate models, which too can adequately fit the experimental data shown in the figure.

### *Phenomenological Growth and Mortality Models*

Obviously, as already mentioned, a crowded habitat depleted of its material resources and polluted by discarded metabolites cannot support a large population indefinitely. Thus at some point the population will have to decline. Although rarely an important issue in food spoilage or health risk, as also already mentioned, the mortality phase can become consequential in long refrigerated or frozen storage, for example, especially of foods containing "probiotic organisms" in which we are interested. Discussions of growth models that account for mortality are rather scarce in the food literature. In principle, every phenomenological model can be modified to produce a mortality phase by multiplying its original growth term by a decay factor, which can be logistic, exponential, stretched exponential or any algebraic term that starts at one and drops to zero. A recent example is the model developed for complex chemical reactions and biological processes governed by competing mechanisms (Peleg et al., 2009). When adapted to isothermal microbial growth and mortality it will have the form:

$$N\ (t) = N_0 \exp\left(\frac{t}{t_{c1}}\right)^{m1} \cdot \exp\left[-\left(\frac{t}{t_{c2}}\right)^{m2}\right] \quad m_2 > m_1 \quad (9)$$

where $N(t)$ is the momentary number at time $t$, $N_0$ the initial number, $t_{c1}$ the characteristic time of the exponential growth had it been unimpeded, $t_{c2}$ the characteristic time of the mortality stage, and $m_1$ and $m_2$ are coefficients that account for the steepness of the curve at its growth and mortality phases, respectively.

Examples of curves generated with this model are given in Fig. 9. Notice that as in every other growth (or inactivation) model, the parameters $t_{c1}$, $t_{c2}$, $m_1$, and $m_2$ depend on the temperature of the medium, pH, $a_w$, etc. The advantage of this model, apart from its mathematical flexibility, is that $t_{c1}$ and $t_{c2}$ have an intuitive meaning. For example, if $t_{c2}$ is much longer than $t_{c1}$ and the experiment duration, no peak growth will be observed, only monotonic growth with a rate determined by $m_1$. When $t_{c2} \to \infty$ and $m_1 = 1$, the model represents linear growth. When $m_1 > 1$, the the initial part of the growth curve is concave upward and when $m_1 < 1$ downward. When $t_{c2} < t_{c1}$, cell mortality could become predominant well before

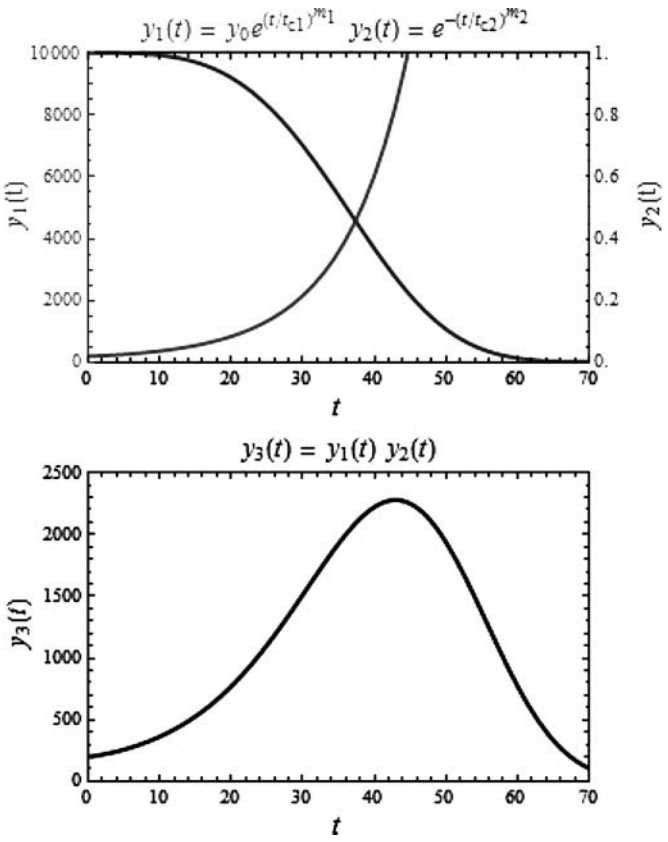


**Figure 9** Simulated growth-mortality curves generated with Eq. (9) as a model and plotted as a Wolfram Demonstrations. Notice that the model's four parameters, $t_{c1}$, $t_{c2}$, $m_1$ and $m_2$ are all temperature, pH, $a_w$ and other ambient factors dependent.

the "stationary phase" is even reached (see figure) resulting in a clearly peaked growth curve. According to Eq. (9), pure inactivation is just a special case of its equation where $t_{c1} \to \infty$. For more on the model, and how it can be converted into a dynamic model, see Peleg et al. (2009). A free interactive program to simulate isothermal growth/mortality curves, using Eq. (9) as a model, is also available as a Wolfram Demonstration at http://demonstrations.wolfram.com/IncipientGrowthProcesses WithCompetingMechanisms/.

The model can be written in terms of the growth ratio, $Y(t) = N(t)/N_0$, vs. *time* relationship by dividing both sides of the equations by $N_0$, in which case $Y(0) = 1$ by definition. However, for isothermal growth curves where the growth ratio is defined as $Y(t) = [N(t)\text{-}N_0]/N_0$ or $\log[N(t)/N_0]$, the first term in the right side of the equation of the model would have to be modified in order to comply with the requirement of $Y(0) = 0$. The result would be a model similar to Eq. (21) below, except that it would be formulated in terms of time instead of temperature. More about this issue, its ramifications, and how it has been resolved can be found in Peleg et al. (2009). A free interactive program to simulate peaked curves starting at $Y(0) = 0$ can be downloaded at http://demonstrations.wolfram.com/ DeNovoGrowthProcessesWithCompetingMechanisms/. [As in the previously mentioned Wolfram Demonstrations, here too,

an animated version of the plots can be seen by clicking on the little arrow.]

Certain peaked isothermal growth curves, especially showing a short "lag time" and mild drop in the cells number at the mortality phase, can be modeled with a modified version of the empirical sigmoid "power law" model (Eq. (8)) in which $t^m$ in the denominator is replaced by $t^n$ where $n > m$. The resulting model is:

$$Y(t) = \frac{a^m t^m}{b^m + t^n} \quad n > m \tag{10}$$

where again $Y(t)$ is the logarithmic or linear net growth ratio $\log[N(t)/N_0]$ or $[N(t)\text{-}N_0]/N_0$ respectively, and $n$ like $a$, $b$, and $m$ is a parameter whose magnitude depends on the temperature, pH, $a_w$, etc. (Peleg, 2006).

Equation (9), like Eq. (10), has only four adjustable parameters, namely *a, b, m,* and *n,* and is therefore equally parsimonious. However, its parameters do not have the same intuitive meaning as that of $t_{c1}$, $t_{c2}$, $m_1$ and $m_2$ in Eq. (9), and therefore it will be less likely to be widely used in modeling microbial growth and mortality. Equation (10) is also unsuitable for the description of peaked growth curves that have a "long logarithmic lag." Still, it is possible that despite being totally devoid of any "mechanistic interpretation," Equations (9) and (10), and probably other empirical four parameter models, could be used to fit experimental isothermal growth and mortality data as demonstrated in Fig. 10.

## *RATE GROWTH MODELS*

### *The Verhulst (Logistic) Model and its Offshoots*

The Verhulst logistic model is an excellent example of a conceptual population dynamics model of growth in a closed habitat. First published in 1845, it can be written in the form:

$$\frac{dN(t)}{dt} = rN(t)\left[1 - \frac{N(t)}{N_{asymp}}\right] \tag{11}$$

where again $N(t)$ is the momentary population size, the number of countable cells in our case, $N_{asymp}$ the asymptotic number, which corresponds to the population size at the "stationary phase," and $r$ a rate constant, which depends on temperature, pH, $a_w$, etc. What Eq. (11) says is that a momentary ("instant") growth rate of a population is proportional to its momentary size $N(t)$ – the more cells there are the more can divide—and the momentary fraction of the still available resources in the habitat represented by $1\text{-}N(t)/N_{asymp}$. In other words, $N_{asymp}$ represents the carrying capacity of the habitat, expressed as the number of cells that it can support. As stated earlier, the common use of $N_{max}$ in the food microbiology literature instead of $N_{asymp}$ can be misleading because according to this model and its offshoots, the population never actually peaks. Equation (11) is known as the "continuous logistic equation." It should not be confused with "discrete logistic model," which has the form

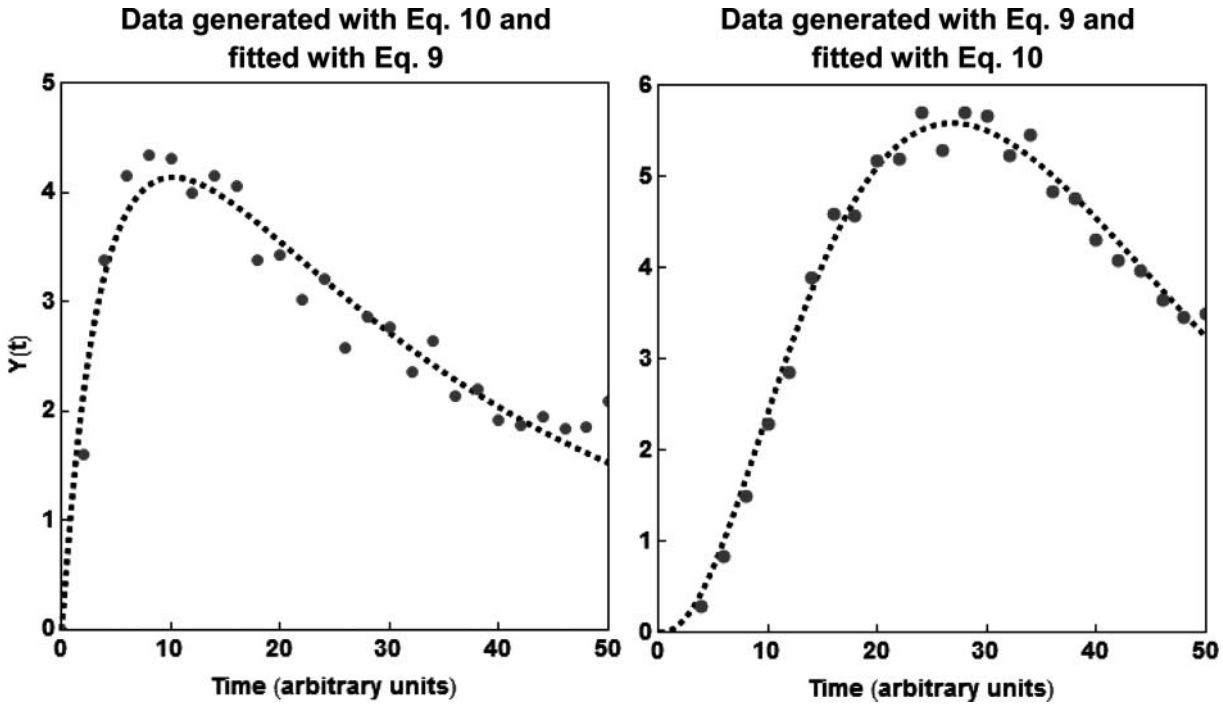


**Figure 10** Simulated growth-mortality curves fitted with Eqs. 9 and 10 as models. They demonstrate that the two models, despite their mathematical dissimilarity, could be used interchangeably for describing the same data sets, and hence that the model need not be unique to have a good fit.

$x_{n+1} = r\ x_n\ (1\text{-}x_n)$ where $x_n$ and $x_{n+1}$ being the value of the variable $x$ after the $n^{th}$ and $(n + 1)^{th}$ step, respectively, and $0 < x_n < 1$. This discrete logistic model got wide publicity as an example of how complicated fluctuation patterns and chaos can emerge in a "simple" nonlinear process. [For more on the discrete logistic model and its potential use in food microbiology, see Peleg, 1997.]

### *The Generalized Verhulst Model*

There is no reason to assume that the momentary growth rate of a microbial, or any organismic population, should be exactly proportional to the momentary number of its members, $N(t)$, and the carrying capacity of the habitat, $1\text{-}N(t)/N_{asymp}$. In terms of the Verhulst's logistic model, this can be expressed by adding powers to the two terms that represent them in the right side of the equation. The result would be the generalized version of the logistic model (e.g.,Tsoularis and Wallace, 2002):

$$\frac{dN(t)}{dt} = rN(t)^{\alpha}\left(1 - \frac{N(t)}{N_{asymp}}\right)^{\beta} \tag{12}$$

where $\alpha$ and $\beta$, like $r$, are coefficients that depend on temperature and other ambient factors.

Examples of growth curves generated with model are shown in Fig. 11. They were produced by a program posted in the Wolfram Demonstration Project, which can be downloaded free of charge from the Internet at http://demonstrations.wolfram.com/GeneralizedLogisticVerhulstIsothermalMicrobialGrowth/. The Demonstration allows the user to enter and adjust the values of $r$, $\alpha$, and $\beta$, as well as the coordinates scale of the plot with sliders on the screen.

According to the generalized version of the logistic model, $\alpha > 1$ means that the population initial growth rate exceeds that implied by the original model, while $\alpha < 1$ that it does not exercise its full growth potential. $\beta < 1$ implies that the population is less sensitive to the diminishing resources than the original model indicates, while $\beta > 1$ that it is more sensitive. [For more on the mathematical properties of the original and generalized Verhulst models and their potential implications in the food microbiology, see Peleg et al., 2007.] Both the original and generalized versions of the logistic model entail that at the beginning of the growth, when $N(t) \ll N_{asymp}$, $dN(t)/dt \approx r\ N(t)^{\alpha}$. This entails that the growth ratio rises continuously from the start. Thus, when plotted on semi logarithmic coordinates, that is, as $\log[N(t)]$ or $\log[N(t)/N_0]$ vs. *time* relationship, the growth curve will be climb from the start (see figure.) It will have noticeable upward concavity when $\alpha > 1$, and downward concavity when $\alpha < 1$. When $\alpha = 1$ the beginning of the curve will look linear. Either way, the Verhulst model is unsuitable to describe growth curves characterized by a "long logarithmic lag."

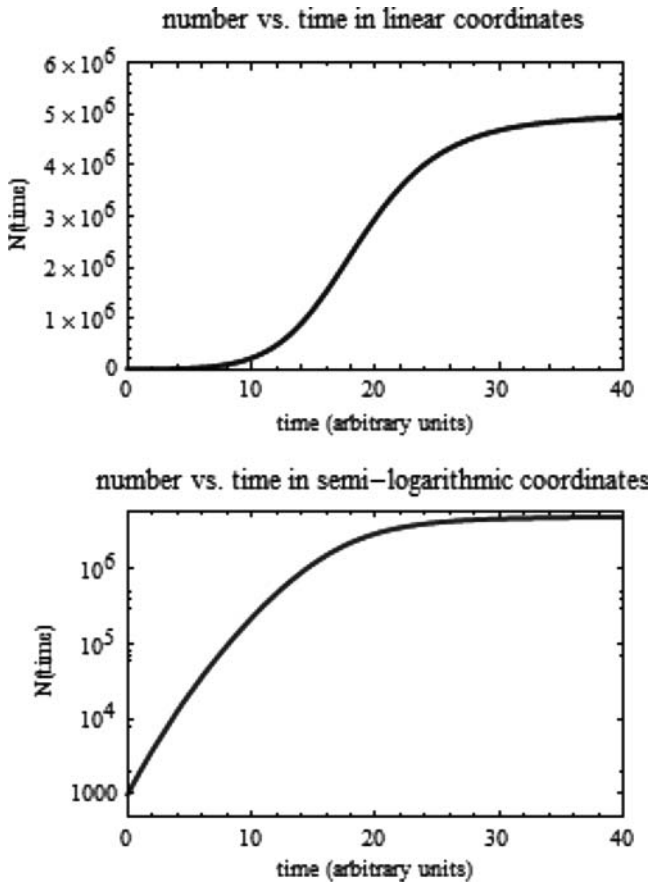


**Figure 11** Simulated growth curves generated with the generalized Verhulst model (Eq. (12)) and plotted as Wolfram Demonstrations on linear and semi logarithmic coordinates. Notice that the model is incapable to describe growth curve with a "long logarithmic lag."

### *Logistic Growth with a Long Lag Time*

A "lag time" of arbitrary duration can be imposed on the logistic equation in different ways. Perhaps the simplest is through the introduction of the "If" statement into the algebraic growth model (e.g., Augustin et al., 2000; Oscar, 2005).

The discontinuity can be eliminated by replacing the "If" statement by a logistic decay term with a range of 0–1, for example, as shown in Fig. 12, that is,

$$\frac{dN(t)}{dt} = \frac{r}{1 + \exp[a(t_{lag} - t)]}N(t)\left(1 - \frac{N(t)}{N_{asymp}}\right) \tag{13}$$

where $a$ is an arbitrary large number. [When $a \to \infty$, the first term of the equation's right side will be indistinguishable from a "true" step function.]

The same can be done using the Generalized Verhulst model (Eq. (12)), that is:

$$\frac{dN(t)}{dt} = \frac{r}{1 + \exp[a(t_{lag} - t)]}N(t)^{\alpha}\left(1 - \frac{N(t)}{N_{asymp}}\right)^{\beta} \tag{14}$$

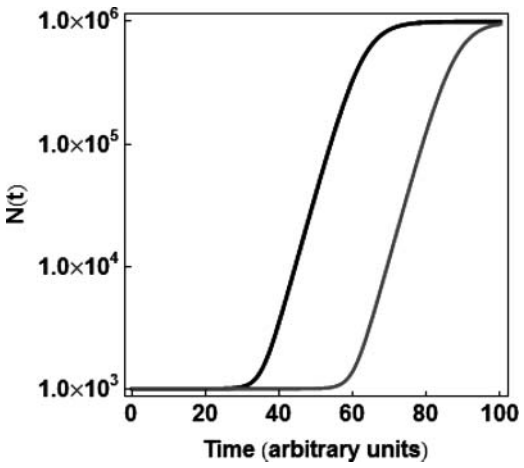


**Figure 12** Simulated logistic growth curve into which the "lag time" has been introduced in the differential (rate) equation by the super imposition of a logistic term (Eq. (13)).

The same can be accomplished by a variety of other expressions, for example, by replacing $r$ with $r\{1\text{-}\exp[-(t/t_{lag})^m]\}$ where again $r$, $t_{lag}$, and $m$ are temperature dependent parameters. Such mathematical manipulations of the equation of the logistic model come at a cost of additional adjustable parameters; two in the case of Eqs. (13) or (14). Here too, the fit of the modified model by itself does not establish the physical reality of the "lag time" and its calculated value should be only viewed a marker of the "lag phase" duration. The same pertains to other modifications of the logistic model based on alternative mathematical terms that achieve the same goal.

### *The Baranyi-Roberts Model*

A logistic rate model popular among food microbiologists is the Baranyi-Roberts model (Baranyi and Roberts, 1994). It is frequently presented in the form (e.g., McKellar and Lu, 2004):

$$\frac{dN(t)}{dt} = \frac{q(t)}{1+q(t)}\mu_{\max}N(t)\left[1-\left(\frac{N(t)}{N_{\max}}\right)^m\right] \tag{15}$$

where according to its authors

$$\frac{dq(t)}{dt} = \mu_{\max}q(t) \tag{16}$$

Equation (16) is the same as saying $q(t) = q_0\exp(\mu_{max}\ t)$ and the rationale for writing it as a rate equation inside a rate equation is unclear. According to this model, the term $q(t)/[1+q(t)]$ is associated with the "lag time," $\lambda$, through the introduced parameter $h_0 = \lambda\mu_{max}$, which appears in the solution of the rate equation. The claim is that $h_0$ accounts for the cells" "initial physiological state" (Baranyi and Roberts, 1994; Baranyi et al., 1995; Baranyi and Pin, 1999.)

Apart from its peculiar mathematical structure, the Baranyi-Roberts model (Eqs. (15) and (16)) has several conceptual problems. The most serious of these is the implication that there is a universal relationship between the "lag time," $\lambda$, and "maximum specific growth rate," $\mu_{max}$. Under favorable conditions to the organism, one would expect that its growth not only be more vigorous (manifested in a high $\mu_{max}$), but also that it start earlier (manifested in a shorter $\lambda$). Therefore, it is not surprising that Brown (2007) who compiled and plotted published "lag times" ($\lambda$'s) and "maximum specific growth rates" ($\mu_{max}$'s) values, found an inverse relationship between them as the $h_0$ concept implies. Upon scrutiny, however, one sees that the $\lambda$ vs. $\mu_{max}$ plot also shows that the larger $\lambda$ values, which correspond to the same $\mu_{max}$, could be several folds bigger than the smaller ones. In other words, although the expected trend is clearly evident, one cannot predict the specific value of one from that of the other with an acceptable degree of accuracy. The "lag time," as has already been pointed out, is a poorly defined entity and the maximum "specific growth ratio" has questionable meaning in asymmetric growth curves. Thus any attempt to estimate the former from the latter, or vice versa, should be considered with caution. But even if the "lag time" and "specific growth ratio" were flawless growth characteristics, there would still be no reason to assume that they must be bound by a universal mathematical relationship as implied by the Baranyi-Roberts model. It is by far more reasonable to assume that varying the growth conditions of an organism will affect the shape of its growth curve in a manner that is not predetermined by a universal mathematical rule, but by the specific characteristics of the system. These include, but are not limited to, the organism type, the population's history, the medium, temperature, pH, oxygen tension, and the presence of different acids, salt and/or antimicrobial agents, etc. While it is true that the observed growth pattern is affected by all these factors and their interactions, the shape of the growth curve does not contain enough information to quantify the physiological effects of the changes in a singular way (Peleg, 2006).

A related issue is that the "work to be done" can be deduced and calculated by extrapolation of the log-linear part of a growth curve (the "exponential phase") to the log$N(t)$ axis. This idea too should be viewed with skepticism for a variety of reasons. The most obvious is the question concerning dimensions and units. The notion that the intercept of a tangent to a growth curve with the log$N(t)$ axis is associated with "work" or "energy" needs supporting evidence. Even if there were a connection between the intercept and slope, which is yet to be demonstrated, why the extrapolation line must be linear and not curved would still need explanation. Another weakness of the Baranyi-Roberts model, and other published modifications of Verhulst's logistic equation, has to do with the term $[N(t)/N_{max}]^m$. While the term $1\text{-}N(t)/N_{asymp}$, or $[1\text{-}N(t)/N_{asymp}]^\beta$, in the original and generalized versions of the logistic models has intuitive meaning, what $1\text{-}[N(t)/N_{max}]^m$ stands for is not at all clear.

The Baranyi-Roberts model predicts that the extensions of all the isothermal (semi-logarithmic) tangents of the growth curve

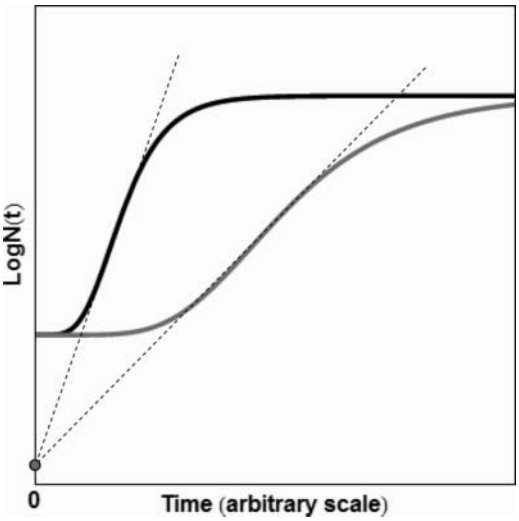


**Figure 13** Schematic view of the concept that the "work to be done" in isothermal growth is manifested in the distance of the intersection point of the tangents to the growth curve from its origin and should be the same at all pertinent temperatures - see Baranyi and Pin (2004).

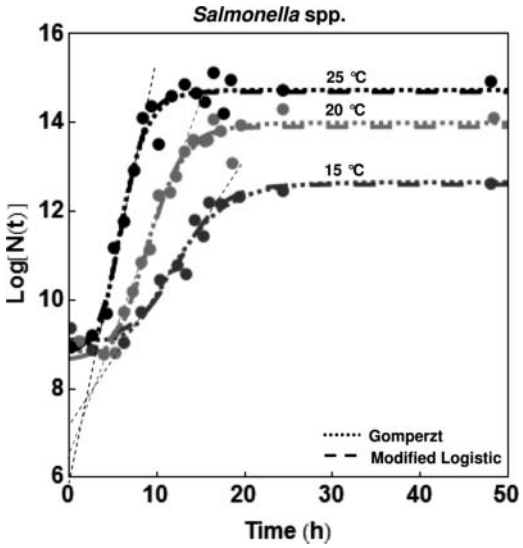


**Figure 14** An example of a failed prediction of the "work to be done" concept. The shown experimental growth curves were fitted with the Gompertz and shifted logistic models, Eqs. (1) and (6), respectively. The experimental isothermal growth data were reported by Koseki and Isobe (2005). Notice that the tangents do not meet at a single point along the logN(t) axis as the concept requires, and there is no reason that they should.

at the inflection point must meet at one point (e.g., Baranyi and Pin, 2004) as shown schematically in Fig. 13. This is not only contradicted by several reported growth data in the literature (Juneja et al., 2009; Huang, 2008; Cornu et al., 2006; Koseki and Isobe, 2005), but can be proven false by constructing realistic growth curves with the Gompertz or shifted logistic function as a model that clearly violate this "rule" (see Fig. 14.) According to the Baranyi-Roberts model, as well as the original and generalized versions of the Verhulst model as already mentioned, the initial growth rate must always be bigger than zero. This implies that every growth curve must be rising from the start, which makes these models ill-suited for growth patterns exhibiting long "logarithmic lag times," where the rate remains practically zero for a considerable time as shown in Fig. 15 (Peleg et al., 2007).

### *The Fujikawa, Kai, and Morozumi's Model*

Another logistic growth model proposed for food has the form (Fujikawa et al., 2004; Fujikawa and Morozumi, 2005):

$$\frac{dN(t)}{dt} = rN(t)\left[1 - \frac{N(t)}{N_{\max}}\right]\left[1 - \frac{N_{\min}}{N(t)}\right]^n \tag{17}$$

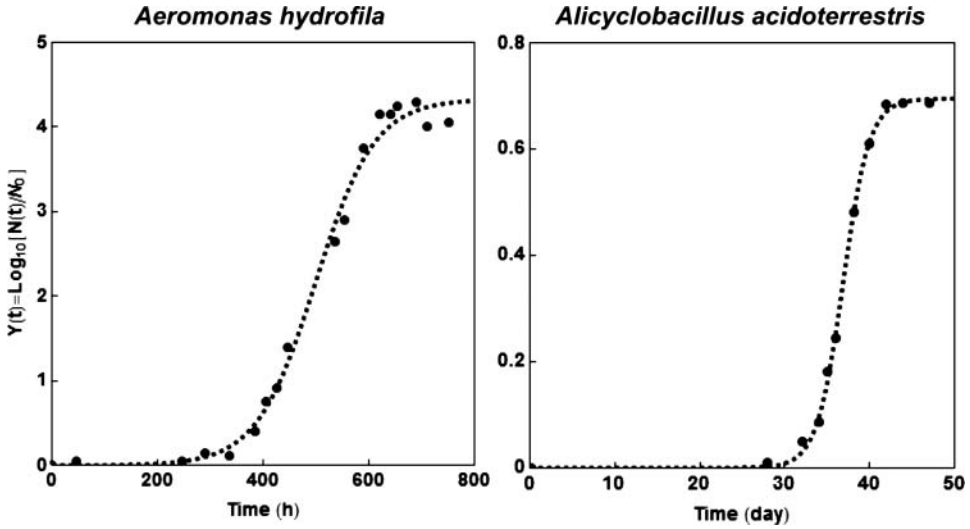


**Figure 15** Examples of sigmoid experimental growth curves with a "long logarithmic lag" fitted with the shifted logistic model (Eq. (6)). The original data are from Blackburn (2000) and Luera Peña and Rodriguez de Massaguer (2006). After Peleg et al. (2007).

where $N_{max}$ is $N_{asymp}$, and $N_{min}$ is $N_0$, in the original Verhulst model, $r$ a temperature and other conditions dependent rate parameter, and $n$ another temperature and conditions dependent coefficient of a magnitude on the order of 1–9.

Equation (17) is basically Verhulst's model to which the term $[1\text{-}N_{min}/N(t)]^n$ has been added. The authors state that the sole purpose of this added term has been to improve the model's fit to their experimental data, especially in the transition region between the "adjustment regime" and "exponential growth phase." Equation (17), like all the logistic model variants already discussed, does not offer any "mechanistic insight," and its authors, correctly, do not claim that it does or should. Still, the model successfully predicted dynamic growth patterns from isothermal data, a demonstration that there is more than one way to modify the original logistic rate model in order to improve its fit and make it predictive. Since Fujikawa, Kai, and Morozumi's model is not mathematically identical to the Baranyi and Roberts model, its success also demonstrates that a correction term added to the original Verhulst equation, unless proven otherwise, cannot have universal mechanistic interpretation or a direct link to the physiology and biophysics of cell growth and division.

## *SECONDARY MODELS*

An excellent comprehensive review of the formulation and application of secondary models for microbial growth in foods can be found in a book chapter written by Ross and Dalgaard (2004). These authors describe, survey the applications, and critically assess the whole gamut of secondary models, including the polynomial. The main emphasis of the chapter is on how the "specific maximum growth rate," $\mu_{max}$, and to a lesser extent the "lag time,"$\lambda$, are affected by temperature, $a_w$, pH, and other factors. In the discussion, Ross and Dalgaard highlight the differences between models that do and do not allow for optimal growth conditions. Notable among the former is the expanded square root model based on Belerádek's and known as the "Ratkowsky model" (Ratkowsky et al., 1982) and its extensions (Gibson et al.,1994; Ross, 1993; Sautour et al., 2001). Among the latter, the most extensively used have been the original and modified versions of the Arrhenius model, which implies that the growth rate always increases monotonically with temperature, or vice versa, and analogously, with factors like pH and $a_w$.

### *The Arrhenius Model*

That the Arrhenius equation is still being used as a secondary growth model in quantitative microbiology today is most surprising. This model is clearly inappropriate for microbial growth because of its inability to account for optimal growth temperature (McMeekin et al., 1993) and other conditions, such as pH. This shortcoming could perhaps be overlooked when the model is only used in the restricted temperature range where the growth rate indeed monotonically rises with temperature or other factors. However, as Ross and Dalgaard (2004) correctly point out, the assumption that microbial growth is regulated by temperature independent "energy of activation" has yet to be justified. But let us go further. The original Arrhenius equation is particular suitable for gases such as oxygen, nitrogen, and hydrogen. The molecules of such gases, unless acquiring enough kinetic energy to overcome the electrons repulsion, will not react and therefore can remain unchanged for years and eons. This is clearly not even remotely the situation in microbial growth and the medium or food that supports it. Thus, the inclusion of the Universal Gas Constant, R, in the model's equation and expressing the energy of activation in a unit such as "Joule per mole," which makes perfect sense for describing the temperature effect on the rate of chemical reactions between gases or other reactants in a solution, makes no sense at all in the description of microbial growth kinetics. [The same can be said about the application of the Arrhenius model in microbial inactivation kinetics (Peleg, 2006), but this should not concern us here.] To drive home the point, consider the following. Since the mass of a typical bacterial cell is on the order of $10^{-12}$ gram, a "mole of bacteria" ($\sim 6 \times 10^{23}$ cells) would have a mass of approximately $6 \times 10^{11}$ g or 600,000 metric tons—hardly a convenient unit for calibration or comparison. The frequently offered explanation that the "mole" in the Arrhenius equation refers to a "limiting enzymatic reaction" is very hard to accept. This putative reaction has yet to be identified and its energy of activation determined independently, by calorimetry for example, for verification. Moreover, in a microbial growth process, one usually monitors the cells' number or number per unit volume or mass as a function of time and not the molar concentration of any chemical reactant. Therefore, the relationship between the "growth rate constant" (which varies with time) and this hypothetical limiting reaction's rate constant is not at all clear. As if all this is not sufficient to dismiss the Arrhenius model applications to microbial growth and mortality, consider the following. The temperature range of 0–40°C, say, is very large as far as microbial growth is concerned, at least in the context of food. Therefore, the rationale of compressing it to the meager $0.0037\text{–}0.0032^{\circ}K^{-1}$ range by using the model is yet to be explained (Peleg, 2006). The same can also be said about the rationale for the logarithmic conversion of the characteristic "growth rate constant," however defined. Unless this "rate constant" indeed varies by several orders of magnitude in the pertinent temperature range, there is simply no reason for this transformation.

### *Mechanistic Modifications of the Arrhenius Model*

We have borrowed this title from a section in Ross and Daalgard's chapter (2004), which deals with published attempts to incorporate thermodynamic considerations into the kinetics of enzymatic activity and enzymes inactivation when described by the Arrhenius model. In light of the already mentioned inherent problems with the Arrhenius model application to microbial

growth and inactivation kinetics, the merit of any such endeavor is unclear. Consider the Ross model (1999) cited in Ross and Dalgaard (2004). This secondary model was made to account for the peak of the growth rate at the optimal temperature and has the form:

$$rate = \frac{CT \exp\left(\dfrac{\Delta H^{+}}{RT}\right)}{1 + \exp(-n(\Delta H^{*} - T\Delta S^{*} + \Delta C_p[(T - T_H{}^{*}) - T \ln(T/T_S{}^{*})])/RT)} \quad (18)$$

where – quoted verbatim - "C is a parameter whose value must be estimated, $\Delta H^{+}$ the activation enthalpy of the reaction catalyzed by the enzyme controlling the overall reaction rate, D$C_p$ the difference in heat capacity (per mole amino acid residue) between the native (catalytically active) and denatured state of the enzyme, $T_H{}^{*}$ the temperature (°K) at which the D$C_p$ contribution to the enthalpy is 0, $T_S{}^{*}$ the temperature (°K) at which the D$C_p$ contribution to the enthalpy is 0, D$H^{*}$ the value of enthalpy at $T_S{}^{*}$ per mole amino acid residue, $T$ the temperature (°K), $R$ the Universal gas constant (8.314 J/K mol), and $n$ is the number of amino acid residues in the enzyme."

Although reflecting a valiant attempt to connect the growth pattern to the biochemistry of dividing cells, identification of the "controlling enzyme" and assuring that it is the same and only one over the entire temperature range might be a difficult experimental task. So will be the independent experimental determination of all the relevant thermal and thermodynamic constants that the model's equation contains. Consequently, it is doubtful that a proper database for the model validation will be created and published any time soon. The thermal and thermodynamic constants could probably be assumed, perhaps on the basis of reports or observations in the laboratory model or other biological systems. Doing it, however, would carry its own risks because the continuously changing chemical and physical environment within and outside the growing or dividing cell might well become an issue. This is because it will be very difficult to obtain the needed complementary quantitative information, on the physiology, biochemistry, and biophysics of cells growth and division and on how exactly a microbial population interacts chemically and physically with the growth medium or food.

### *The Square Root and Ratkowsky Model*

The original square root, the Belerádek or Ratkowsky model, is frequently written in the form (Ratkowsky et al., 1982):

$$\sqrt{\mu_{\max}} = b\,(T - T_{\min}) \quad (19)$$

where $b$ is a proportionality constant and $T_{min}$ a marker of the lowest temperature where growth can still be observed. It was later expanded to account for optimal growth temperature (Ratkowsky et al., 1983) at which growth rate peaks, that is:

$$\sqrt{\mu_{\max}} = b\,(T - T_{\min})\,\{1 - \exp\,[c\,(T - T_{\max})]\} \quad (20)$$

where $c$ is a constant and $T_{max}$ marks the end of the temperature range where growth is still possible, that is,, before any further rise of temperature will result in heat inactivation. The model was later further expanded in two ways—by adding terms that identify the optimal temperature and improves the fit of the model (Sautour et al., 2001), and by adding terms that account for the role of the pH, $a_w$, etc., within a single equation—see the "gamma hypothesis" section.

The Ratkowsky model from its inception was only intended to describe experimental results and it has been very successful at that. Making no claims concerning mechanistic interpretation of its parameters, it serves as an example of how a simple algebraic model can be very useful. It also demonstrates that at least for certain practical applications, such an empirical model is preferable to one ostensibly derived from fundamental principles based on mechanistic assumptions that cannot be verified. Yet, and despite being empirical, the Ratkowsky model does raise an issue of theoretical interest. It is unlikely that the frequently observed linear relationship between the square root of the specific maximum growth rate and temperature, over a substantial temperature range, is merely a coincidence. It would therefore be a challenge to researchers to provide a mechanistic explanation of this peculiar "scaling," based on statistical or physiological considerations, for example.

### *Interpretation of the Growth Rate vs. Temperature Relationship*

Raising the temperature almost invariably accelerates physiological processes and therefore it is not unreasonable to expect that increases the rate of cell growth and division, regardless of how the "rate" is defined. However, at the same time, the temperature rise can initiate and eventually accelerate processes that suppress the growth and division of cells. As long as the growth promoting mechanisms dominate, the growth rate will rise with temperature. But once the inhibitory mechanisms set in and then take over, the growth rate will decline and then drop to zero. If the temperature continues to rise, it can reach a lethal level, in which case growth will not only cease but be turned into what we call "thermal inactivation." [The scenario might be more complicated as a result of adaptation triggered by the cells exposure to "sub-lethal" temperatures, but this should not concern us here.]

Making the statement about the existence of competing processes does not require that they be identified or the specific knowledge of their underlying mechanisms and their kinetics. In most cases, we cannot even be sure that these processes are the same at different temperatures. As a matter of fact, it will be prudent to assume that they need not be, but this is immaterial

to the construction of the secondary model that we will present. Once we deal with growth promoting and inhibitory processes in general, we can ignore their details and follow the methodology similar to that used by Ratkowsky et al. (1982) to create their secondary model for the growth rate. An alternative to specifying the upper and lower limits of the growth temperature range (Eq. (20)) is superimposition of a "decay factor" that accounts for growth suppression or even mortality, on a pure "growth term" that accounts for the temperature growth promoting effects. One such possibility is a phenomenological model similar to that developed for "peaking" complex chemical, biological and biochemical processes (Peleg et al., 2009). An example, adjusted to temperature (above zero) as the independent variable is:

$$k(T) = \frac{k_{asymp}\left(\frac{T}{T_{c1}}\right)^{m1} \cdot \exp\left[-\left(\frac{T}{T_{c2}}\right)^{m2}\right]}{b^{m1} + \left(\frac{T}{T_{c1}}\right)^{m1}} \qquad m_2 > m_1 \tag{21}$$

where $k_{asymp}$ is the upper limit on $k(T)$ imposed by physical and physiological considerations, $T_{c1}$ a characteristic temperature of the growth promoting processes, $T_{c2}$ of the inhibitory processes and $m_1$, and $m_2$, constants characteristic of the organism and the habitat in which it grows, that is,, the composition of the medium and pH, etc.

Notice that this model (Eq. (21)) has *6* parameters and is hence less parsimonious than Ratkowsky et al.'s model (Eq. (20)). On the other hand, the model expressed by Eq. (21) neither requires nor implies that the transition between growth and no growth at both ends of the temperature range must be abrupt, which is consistent with observation. Examples of simulated $k(T)$ vs. T plots with Eq. (21) as a model are given in Fig. 16. Their appearance is almost identical to the curves shown by Ross and Dalgaard (2004), Ratkowsky (2004b), Gibson et al. (1994), Sautour et al. (2001) and others, when plotted either as $k(T)$ *vs. T* or $\sqrt{k(T)}$ *vs. T* relationships. Notice that in contrast with an Arrhenius based model, the "growth term" in Eq. 21, $(T/T_{c1})^{m1}/[b^{m1} + (T/T_{c1})^{m1}]$, is a sigmoid function. The "decay term," $\exp[-(T/T_{c2})^{m2}]$, in the equation is a stretched exponential, a flexible function that can describe a delayed fall whose onset is primarily determined by $T_{c2}$ and steepness by $m_2$. A freely downloadable interactive Wolfram Demonstration that generates peaked curves with Eq. (21) as a model (albeit in the form of $Y(t)$ vs. $t$ relationships) can be found at http://demonstrations.wolfram.com/IncipientGrowthProcesses WithCompetingMechanisms/.

Being a purely phenomenological model, Eq. (21) does not explain anything. None of its coefficients has a one to one relationship with a specific enzyme, the biophysical process inside the cells, or their interaction with the growth medium. However, $T_{c1}$ and $T_{c2}$ are markers of the temperature scale of the growth promoting and suppressing processes. Thus, if $T_{c2} \gg T_{c1}$ and

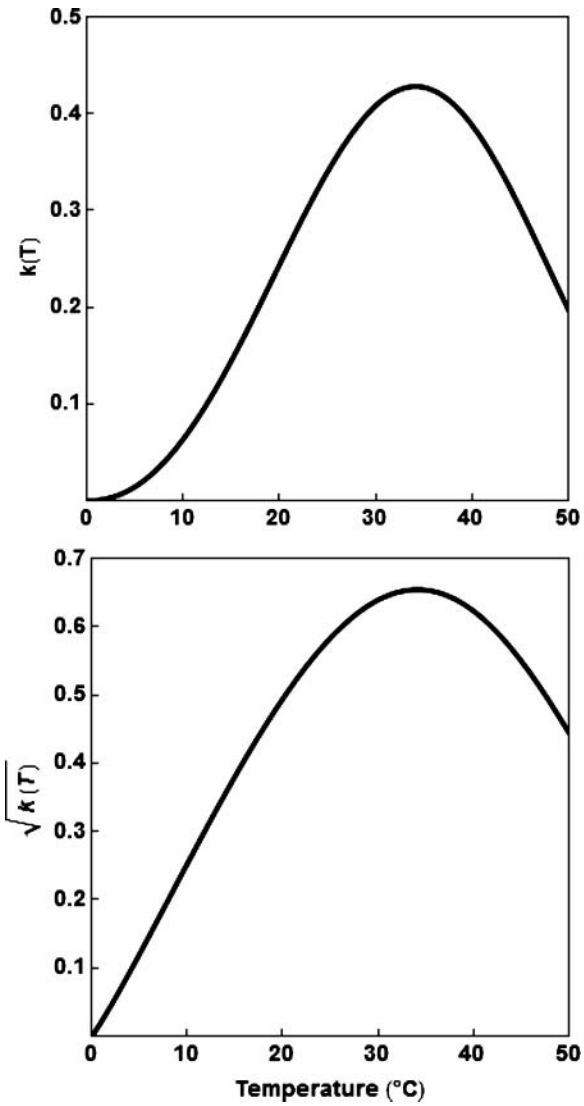


**Figure 16** Simulated growth rate vs. temperature relationship generated with Eq. (21) as a model and plotted as $k(T)$ vs. $T$ and $\sqrt{k(T)}$ vs. $T$.

falls outside the experimental temperature range, no peak rate will be observed in the experimental temperature range. However, if $T_{c2}$ falls within the experiment temperature range, or just beyond it, a peak rate will be observed. The height and shape of the peak will then depend on the other model parameters. In principle, Eq. (21) can be used to quantify and compare the temperature effect on the growth rate measure of the microbial populations, however defined, or any other growth parameter that reaches a maximum value, such as the asymptotic size of the population, $Y_{asymp}$. Notice that as the characteristic growth rate drops to zero, the time to reach it, $t_c$ for example, tends to infinity, in which case no separate modeling will be needed.

By adding a third term to Eq. (21), having its own characteristic temperature, $T_{c3}$, the model is converted into a

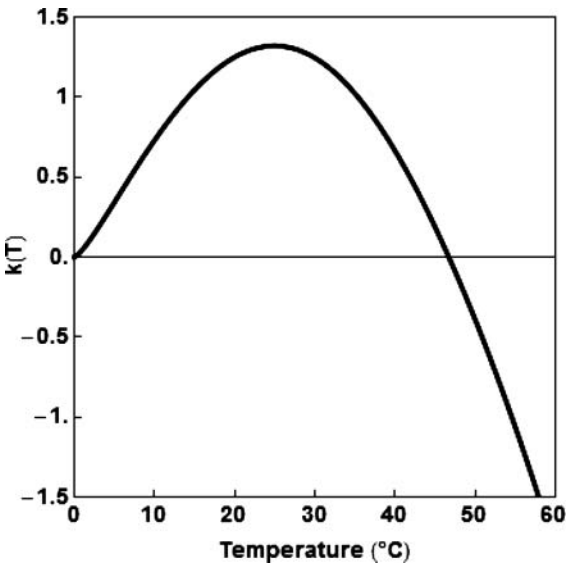


**Figure 17** Simulated growth rate vs. Temperature relationships with a transition between growth and inactivation generated with Eq. (22) as a model.

growth/inactivation model, e.g.:

$$k(T) = \frac{k_{asymp}\left(\frac{T}{T_{c1}}\right)^{m1} \cdot \exp\left[-\left(\frac{T}{T_{c2}}\right)^{m2}\right]}{b^{m1} + \left(\frac{T}{T_{c1}}\right)^{m1}} - \left(\frac{T}{T_{c3}}\right)^{m3}$$

$$T_{c3} > T_{c2} > T_{c1} \tag{22}$$

where $T_{c3}$ is the inactivation temperature range marker and $m_3$ a constant, representing (together with $T_{c3}$) the organism's heat sensitivity or resistance. An example of a simulated $k(T)$ vs. $T$ relationship generated with Eq. (22) as a model is given in Fig. 17. Note that a positive $k(T)$ indicates growth and negative inactivation. This kind of a model can be used to describe continuous transitions between growth and inactivation or vice versa, to complement the current "growth – no growth" models (e.g., Ross and Dalgaard, 2004). A growth/inactivation model of the kind expressed in Eq. (22) would explain the commonly observed large scatter in growth measurements at both ends of the growth temperature range. The same could most probably be said about the pH and other factors that have an optimal effect.

As already mentioned, the growth or growth/inactivation model construction does not require knowledge of the mechanisms involved. However, the resulting model parameters ($k_{asymp}$, the $T_c$'s and $m$'s in Eq. (21)) could probably be related to physiological processes at the cellular level, their absolute and relative rates and how they are affected by temperature, pH, and other factors. Such relations, if revealed, could be used to quantify the manifestation of these processes at the population level. The same can be said about the other growth parameters such as the asymptotic growth level and the time to reach the maximum growth rate.

### *The "Gamma Hypothesis"*

Microbial growth rate, however defined, like the rate of biochemical and physiological processes in general, is simultaneously affected by temperature, pH, $a_w$, oxygen tension, etc. Several authors have suggested that these factors act independently and that their combined effect is multiplicative (Zwietering et al., 1992; Ross and Dalgaard, 2004). According to this notion, the combined effect of several factors on the "maximum specific growth rate," $\mu_{\max}$, can be described by:

$$\mu_{\max} = f_T(T) \cdot f_{pH}(pH) \cdot f_{aw}(a_w) \ldots \tag{23}$$

where the $f$'s are different functions of the temperature, pH, $a_w$, etc. Traditionally, these functions have been of the Arrhenius type, or when optimal growth conditions exist, by variants of the Ratkowsky model. According to the "gamma hypothesis," the terms $f_T(T)$, $f_{pH}(pH)$, $f_{aw}(a_w)$ at the right side of Eq. (23) are renamed $\gamma(T)$, $\gamma(pH)$, $\gamma(a_w)$, etc., where the $\gamma$, stands for a ratio that makes them dimensionless and assume a value between zero and one. Examples of gamma terms are, $\gamma(T) = [(T - T_{min})/(T_{max} - T_{min})]^2$, $\gamma(a_w) = (a_w - a_{wmin})/(1 - a_{wmin})$, etc.

The $\mu_{\max}$ which corresponds to the combination of any factors is then calculated from (Ross and Dalgaard, 2004):

$$\mu_{\max} = \mu_{\max\,opt} \cdot \gamma(T) \cdot \gamma(pH) \cdot \gamma(a_w) \ldots \tag{24}$$

where $\mu_{max\,opt}$ is the "maximum specific growth rate" under optimal conditions with respect to all the pertinent factors.

Models of Eq. (23) or Eq. (24) kind have been successfully used to describe experimental $\mu_{max}$ data recorded under a variety of factor combinations (Lambert and Bidlas, 2007a; 2007b). But although the model had a good fit, the generality and utility of the gamma concept which has produced it, remains questionable (Peleg, 2006). As already mentioned several times in the review, the meaning of the "maximum specific growth rate" when considered in isolation is unclear. This might become an especially acute problem when asymmetric growth patterns are involved, in which case the highest momentary growth rate could be observed early or late during the exponential phase as shown schematically in Fig. 5. But even if the compared growth curves were all sigmoid and almost perfectly symmetric, when the maximum growth rate occurs might be just as important as the maximum rate if not more. Also, microbial populations having the same maximum growth rate might reach a small or large overall growth level (Fig. 5), which could be by far more significant when food safety or quality is concerned. Thus the "gamma hypothesis" based on $\mu_{max}$ alone will always be deficient where varying the combination of temperature, pH, $a_w$, and other factors can result in a dramatic change in the overall shape of the growth curve. For example, one cannot exclude an

a priori a scenario where certain temperature, pH, and $a_w$ combinations have qualitatively different effect on the growth at its inception, when resources are still plentiful and the density of the population is low, and later on when the available resources are largely exhausted, the density is high and metabolic pollutants abound. A proper growth model, therefore, must start with the assumption that the growth rate everywhere along the curve depends on the ambient conditions in a manner that need not follow any universal rule, such as that dictated by the "gamma hypothesis." This is regardless of whether the "isolated effect" of each factor is described by a modified version of the Arrhenius equation, a Ratkowsky type model, a "$\gamma$ term" or any other "universal" algebraic expression.

An alternative view to the "gamma hypothesis" is that the whole growth pattern should be modeled, not one or two of its more salient characteristics only. Thus, an ideal growth model, especially for predicting dynamic growth, should be in the form of a differential (rate) equation whose coefficients are functions of all the pertinent factors combined (Peleg, 2006). There is no reason to assume that high or low temperature cannot enhance the inhibitory effect of low pH, high salt content, or added antimicrobial by a factor that exceeds a prediction based on the fact that the effects of the factors are independent. It is also possible that the combined effect of several factors would be lower than expected if for some reason they interfere with each other. All of the above can be said on the combined effect of growth promoting factors and that of growth promoting and suppressing factors. Proper determination of the growth rate equation's three or more coefficients' dependence on several factors simultaneously, especially if written as nested terms such as $\mu_{max}(t) = \mu_{max}[T(pH(t))]$, $Y_{asymp}(t) = Y_{asymp}[T(pH(t))]$ and $t_c(t) = t_c[T(pH(t))]$, see below, will require a large amount of experimental work that might be rightfully considered unfeasible. This is regardless of the type of the basic growth model, that is, Gompertz, logistic, etc. The more parameters the primary growth model has, and the more factors are involved, the more difficult it will be to determine the secondary models. Therefore, rate models with temperature, pH, and $a_w$ dependent coefficients, might only find use in simulations with assumed secondary models, at least in the foreseeable future. Without proper data to determine their coefficients under simultaneously changing temperature, pH, $a_w$ and other ambient conditions, the simulations would not be able to predict actual growth curves. But they might reveal trends and perhaps set theoretical limits on growth that would be of practical value. Such models will allow researchers to generate and predict microbial growth patterns that the "gamma hypothesis" and its cognates might not allow the onset of massive mortality, for example.

## *DYNAMIC GROWTH MODELS*

Rate models like the original logistic (Verhulst) equation and its modified versions can be readily used to generate and predict dynamic growth curves, that is, where the temperature, pH level, or any other influential factors varies with time. Let us consider non-isothermal growth history as an example of dynamic conditions and the generalized logistic (Verhulst) equation (Eq. (12)) with a fixed $N_{asymp}$ as the growth model. In principle, and in practice, the temperature-dependence of its three coefficients $r(T)$, $\alpha(T)$, and $b(T)$ can be determined by fitting isothermal growth curves recorded at different temperatures in a pertinent range. Once the temperature dependence of each of the coefficients has been experimentally determined, it can be described by an ad hoc empirical secondary model, or if possible, by a model derived from mechanistic considerations. For a varying temperature, $T(t)$, the coefficients of the rate equation become functions of time, that is, $r[T(t)]$, $\alpha[T(t)]$ and $b[T(t)]$. Insertion of these terms into the equation of the model yields:

$$\frac{dN\ (t)}{dt} = r\ [T\ (t)] \cdot N\ (t)^{\alpha[T(t)]} \left[1 - \frac{N\ (t)}{N_{asymp}}\right]^{\beta[T(t)]} \tag{25}$$

Equation (25) would have a cumbersome appearance if $r(T)$ were described by the expanded Ratkowsky model (Eq. (20)), for example, and $T(t)$ by a sinusoidal expression that accounts for temperature fluctuations. No doubt that inserting such a $T(t)$ term into the $\alpha[T(t)]$ and $b[T(t)]$ expressions will further complicate the rate equation. Yet, despite these complexities Eq. (25) will remain and Ordinary Differential Equation (ODE), which could be easily solved numerically with advanced mathematical software such as Mathematica®. Examples of such solutions are shown in Fig. 18. The same is true for the coefficients of any other growth rate model. These might be the traditional $\mu_{max}\ [T(t)]$ and $\lambda[T(t)]$, or any other temperature dependent parameter, which will become a function of time too. Neither the solution of the rate model equation nor the quality of its predictions hinges on whether these coefficients had been derived from mechanistic considerations or not. As long as the primary and secondary models faithfully describe the isothermal growth curves and the temperature dependence of their coefficients, very different mathematical models will yield very similar predictions of the dynamic growth curves. This is provided that the secondary models are not used for extrapolation to temperatures beyond the range covered by the experimental data from which they have been derived. All the above also pertains to dynamic conditions with respect to a varying pH, as in isothermal lactic acid fermentation, for example, or the water activity, $a_w$, during a constant temperature regime in drying. However, if two or more factors vary simultaneously, a pH drop under rising or oscillating temperature as in uncontrolled and hence non-isothermal fermentation, say, or a temperature rise and $a_w$ drop during a long drying process, then one has to know how to express the coefficients of the rate equation as a function of time. This, as explained in the previous section might require a large database, because a nested term like $r(t) = r(pH[T(t)])$ or $r(T[pH(t)])$, or $m_{max}(t) = m_{max}(pH[T(t)])$ or $m_{max}(T[pH(t)])$, can only be derived from experimental data. The same applies to each and every other growth parameter, which is affected by the pH-temperature combination. [If the "gamma hypothesis"

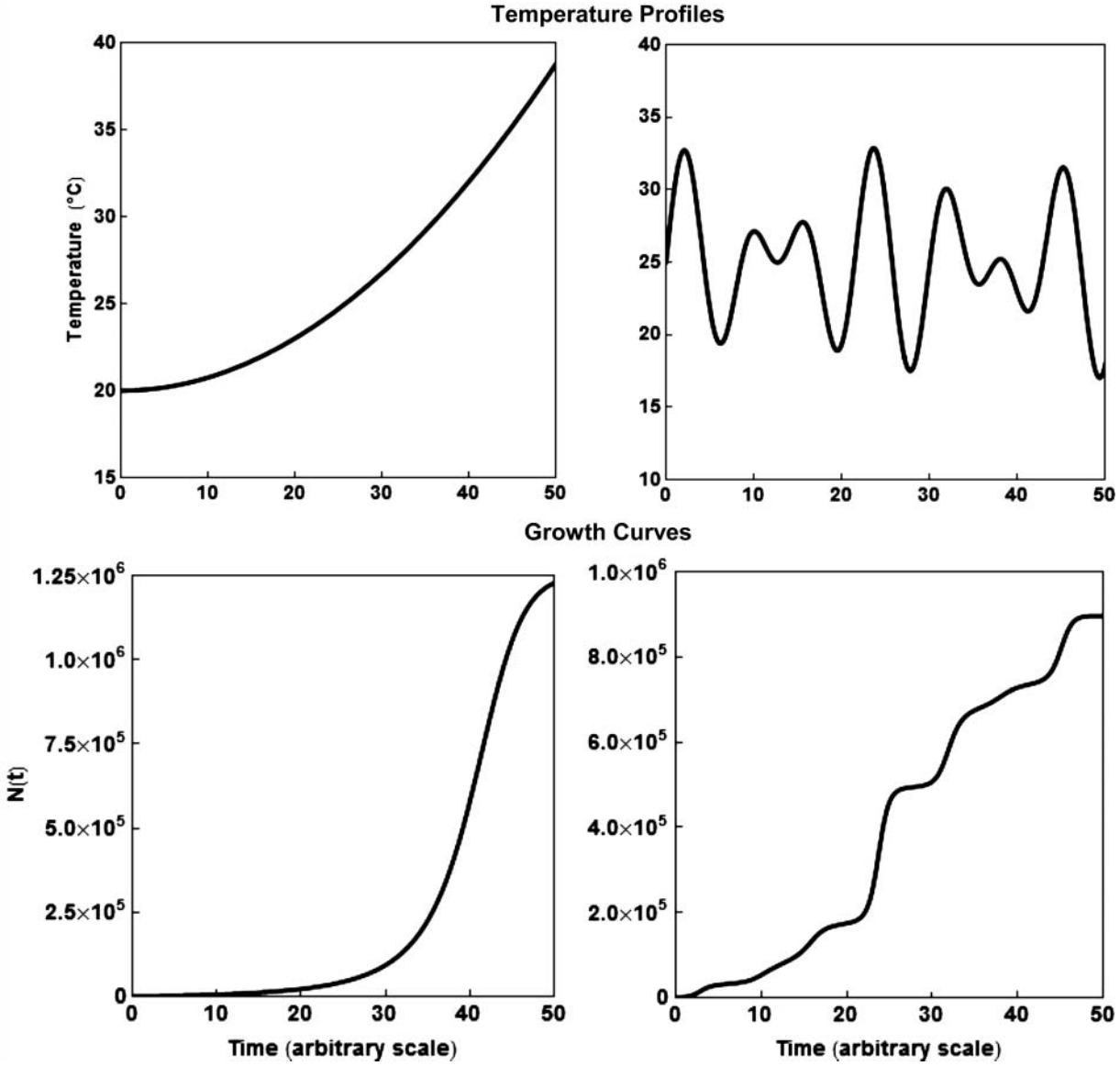


**Figure 18** Simulated dynamic temperature profiles and corresponding growth curves, generated with the generalized Verhulst model (Eq. (25)).

could be confirmed as universal, which is doubtful, replacing the nested terms by an expression of the kind that appears on the right side of Eq. (22) would greatly facilitate the rate model. But until it is demonstrated that models based on the "gamma hypothesis" can correctly predict dynamic growth patterns from experimental data obtained under static conditions, its validity will remain uncertain.]

### *Conversion of Empirical Models into Predictive Rate Models*

Purely empirical algebraic models such as the Gompertz (Eq. 1), "shifted logistic function" (Eq. (6)) or "power type" (Eq. (8)), can be converted into predictive rate models. The conversion, however, does not make them more "mechanistic." It only puts them on equal footing with the traditional rate models, which are based on the logistic (Verhulst) equation, which are not mechanistic either as already explained. The conversion of an algebraic model into a rate equation is based on the same assumption that underlies all the dynamic versions of the logistic equation. Although rarely explicitly stated, all the logistic models are based on the assumption that a momentary ("instantaneous") growth rate of a population under varying temperature, say, is its momentary isothermal growth rate at the momentary temperature at the time that corresponds to its momentary size. Although this seems obvious, there is no theoretical reason why different paths leading to the same time-temperature combination might not affect the momentary rate differently. However, the assumption is testable and so far has been proven valid. There are a considerable number of publications showing that the resulting models can predict dynamic growth curves from isothermal ones (Kreyenschmidt et al., 2010; Juneja et al., 2008; Gospavic et al.,

2008; Mataragas et al., 2006; Corradini and Peleg, 2005; Koutsoumanis, 2001) and even from other dynamic curves (Smith-Simpson et al., 2007).

Consider the conversion of the "shifted logistic" model (Eq. (6)) into a dynamic rate model as an example. According to this model, the momentary isothermal growth rate, $dY(t)/dt$, is:

$$\frac{dY(t)}{dt} = \frac{k(T)\ Y_{asymp}(T)\ \exp\{k(T)[t_c(T) - t]\}}{\{1 + \exp\{k(T)[t_c(T) - t]\}\}^2} \tag{26}$$

where $Y_{asymp}(T)$, $k(T)$ and $t_c(T)$ are these parameters values at any given temperature $T$.

In dynamic growth, the time, $t^*$, that corresponds to the momentary growth ratio $Y(t)$, and hence to the momentary cells number $N(t)$, is:

$$t^*(t) = \frac{1}{k(T)} \times \log_e\left[\frac{\exp[k(T)t_c(T)](Y_{asymp}(T)+Y(t)\{1+\exp[k(T)t_c(T)]\})}{Y_{asymp}(T)\exp[k(T)t_c(T)]-Y(t)\{1+\exp[k(T)t_c(T)]\}}\right] \tag{27}$$

Combining Eqs. (26) and (27) renders the dynamic rate equation:

$$\frac{dY(t)}{dt} = \frac{k[T(t)]\ Y_{asymp}[T(t)]\exp(k[T(t)]\{t_c[T(t)] - t^*(t)\})}{1 + \exp\{k[T(t)]\{t_c[T(t)] - t^*(t)\}\}^2} \tag{28}$$

where $T(t)$ is the non-isothermal time-temperature history and $t^*(t)$ is defined by Eq. (27).

Equation (28) is also an ordinary differential equation (ODE) and can be easily solved numerically by mathematical software and even general-purpose programs like MS Excel® - see below. [The same procedure can be used to develop a dynamic version of the Gompertz model based on Eq. (1) with an added "shift factor." But since it is doubtful that this version of the model will ever be used the equations are not shown.]

An alternative rate model, based on Eq. (8) as a model, is:

$$\frac{dY(t)}{dt} = \frac{a[T(t)]^{m[T(t)]}b[T(t)]^{m[T(t)]}m[T(t)]t^*(t)^{m[T(t)]-1}}{\left[b[T(t)]^{m[T(t)]} + t^*(t)^{m[T(t)]-1}\right]^2} \tag{29}$$

where $t^*(t)$ is:

$$t^*(t) = \left[\frac{b[T(t)]^{m[T(t)]}Y(t)}{a[T(t)]^{m[T(t)]} - Y(t)}\right]^{\frac{1}{m[T(t)]}} \tag{30}$$

The predictive ability and agreement of the shifted logistic and "power" models are demonstrated in Fig. 19. The figure shows that a growth model needs neither to be unique nor "mechanistic" in order to be predictive. It also demonstrates that two simple empirical isothermal models can be converted into dynamic rate models that are just as predictive as the elaborate modifications of the Verhulst model, such as Eqs. (15) or (17).

One implication of the above, which has largely been overlooked by the designers and users of "time-temperature integrators," is that empirical growth models of the kind described can be used to convert non-isothermal logged time-temperature data into a microbial growth curve in real time. The principle was described by Corradini and Peleg (2005), who also posted on the Internet a freely downloadable MS Excel® program that does the conversion. See http://www-unix.oit.umass.edu/~aew2000/GrowthAndSurvival/GrowthA/MicrobeGrowthModelA.html and http://www-unix.oit.umass.edu/~aew2000/GrowthAndSurvival/GrowthB/MicrobeGrowthModelB.html. For the kinetics to be incorporated, one ought to choose the rate model for the organism of interest and the algebraic terms ("secondary models"), which describe the model's coefficients temperature dependence. In principle at least, the recorded time conversion into a momentary linear or logarithmic growth ratio, and hence to the population's size, can be done simultaneously for several organisms. This would be particularly beneficial where the spoilage of the food or the end of its shelf-life is caused by different organisms under different thermal histories. The temperature can also be monitored at different locations, of course, which will make it possible to map the microbial load of the shipment. This issue is out of the scope of this review and therefore it will not be further discussed.

The same can also be accomplished with the traditional logistic rate models whenever they too fit the isothermal data well, and provided that the issue of assuring that $N(0) = N_0$ could be resolved. The advantage of empirical primary models of Eqs. (6) or (8's) type, is not so much in that they do not have this problem. Perhaps more important is that their successful application might eliminate the widespread but erroneous perception that the predictive ability of the logistic rate models stems from their being "fundamental" or "mechanistic," which they are not. As demonstrated in this section, almost every empirical growth model that can faithfully fit the isothermal data can be converted into a predictive dynamic model, regardless of whether its parameters has mechanistic interpretation or not.

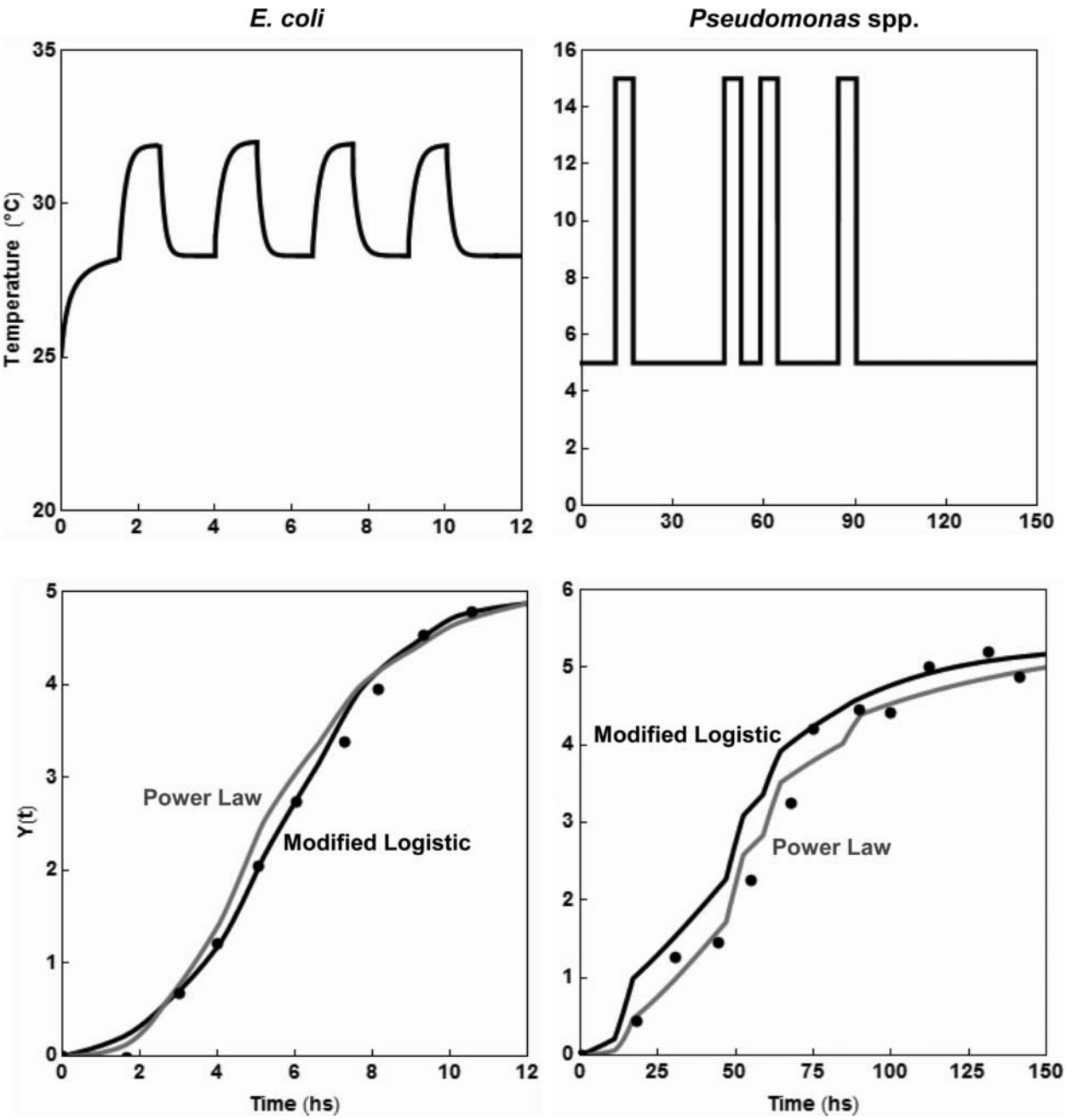


**Figure 19** Demonstration of the ability of the shifted logistic and power models" rate versions (Eqs. (28) and (29), respectively) to predict dynamic growth patterns from isothermal growth data. After Corradini and Peleg (2005). The original experimental growth data are from Koutsoumanis (2001) and Koseki et al. (2004).

### *TRANSLATION EVENTS AT THE CELLULAR LEVEL INTO POPULATION DYNAMICS MODELS*

The starting point of the third class of models is the individual cell, the basic unit of a microbial population. Obviously, an individual cell is not an inert entity and all the cells in a microbial population need not be identical. The variability can be genetic and/or due to differences in the physiological state as a result of differences in the growth history. These differences make cell division a non-isochronous process and the transition between the growth phases (in large populations) smooth rather than abrupt. Buchanan et al. (1997) tried to explain these observations by suggesting that it takes individual cells different times to generate the "energy needed for their division." However, they did not explain if and how this energy can be measured and if indeed stored, where and in what form. They also assumed, probably for the sake of simplicity, that this "energy" remains the same throughout the lag and exponential growth phases. This

would be unlikely even if the concept of the role of "energy" could be accepted. But perhaps more important, any model that starts with processes or events at the cellular level must take into account the possibility that individual cells might die not only during the "lag time" and "stationary" and mortality phases but also the "exponential" growth phase when the population's size continues to grow. The issue of cell mortality, as previously mentioned, is not explicitly addressed by any of the traditional algebraic and rate models. They all deal exclusively with net growth and hence cannot be considered "fundamental" or truly "mechanistic regardless of their mathematical complexity."

### *The Quasi-Chemical Model*

Taub et al. (2003), Doona et al. (2005), and Ross et al. (2005) have produced a dynamic "Quasi-Chemical kinetics model" which allows cell division and mortality to occur simultaneously. A schematic view of the starting point of the model is shown in Fig. 20. As seen in the figure, the rise or fall of a microbial population size is regulated by several processes, whose absolute and relative rates are determined by the organism and the momentary conditions in the habitat. The principal parameters of the model are the physical growth rate of the individual cells prior to their division, the division rate once reaching the appropriate size, and the mortality rates induced by inhibitory metabolites, or other mechanisms that cause "natural death." The Quasi-Chemical model can account for cell mortality prior to, during, and following the observable growth phase. Inactivation according to this model is just a special case where the mortality rate is predominant and the rates of the other processes play a relatively minor role or no role at all. As a result, the Quasi-Chemical kinetic model can describe almost all known microbial growth and inactivation patterns, and transitions between them, at least qualitatively. It can do this without a change of its basic structure.

A certain degree of cell mortality, as mentioned already, can occur at any stage. Thus, at least theoretically, the mortality rate

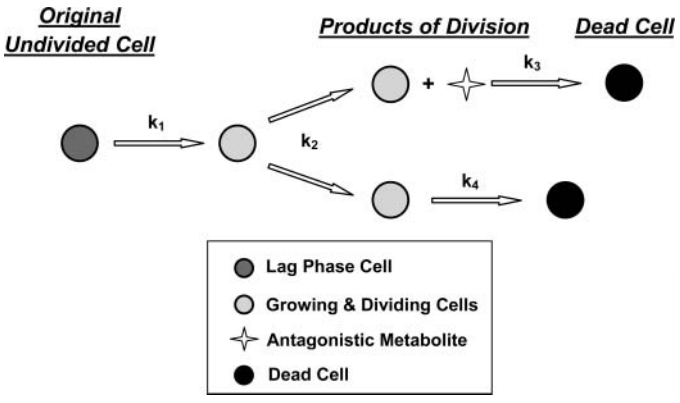


**Figure 20** Schematic view of the cellular events on which the Quasi-chemical model of microbial growth and mortality is based. After Taub et al. (2003) and Doona et al. (2005).

can affect the observed lag time duration and the momentary growth rate at any time. It also determines whether the growth curve will peak during the experiment or observation. Because each underlying rate has its own characteristic dependence on temperature, pH, water activity, etc., the Quasi-Chemical kinetics model can quantify the influence of these factors on microbial growth and mortality through the magnitude of its parameters. The Quasi-Chemical model captures the essence of the growth-mortality duality particularly when a microbial population is exposed to a monotonously rising or falling temperature, for example, where growth might turn into inactivation, or vice versa.

For simplicity, the current version of the Quasi-Chemical kinetics model is based on the assumption that the processes that regulate a cell's fate follow first-, second-, or other fixed order kinetics. But although the individual processes at the cellular and sub-cellular levels have been assumed to follow fixed order kinetics, their manifestation at the population level can be a complex non-linear growth and/or inactivation. Whether the assumption of fixed order kinetics at the cellular and sub-cellular levels needs revision remains to be seen. The issue will most probably be settled as the use of this model to describe and predict microbial growth and mortality patterns will spread, and the quantitative aspects of the mechanisms that control them are better understood.

### *A Fully Probabilistic Model*

A research group at the University of Massachusetts has recently proposed a fully stochastic approach to microbial growth and mortality modeling (Horowitz et al., 2010). It is based on the notion that after a short time interval, a living cell can be in one of the three states: divided, alive but undivided or dead, as shown schematically in Fig. 21. [Situations where injury, recovery from injury, and adaptation might also play a role have not been considered in the current version of the model.] According to the model the probability of finding a living cell divided after a time lapse $\Delta t$ is $P_d(t)\Delta t$, where $P_d(t)$ is the division probability rate function, that is,, the probability of division per unit time. Similarly, the probability of finding this cell dead after the same time interval is $P_m(t)\Delta t$, where $P_m(t)$ is the mortality probability rate function, that is,, the probability of the cell dying per unit time. The probability of finding the cell alive but undivided is therefore 1- $(P_d(t)+ P_m(t))\Delta t$. Thus, while $P_d(t)\Delta t$, $P_m(t)\Delta t$, and 1- $(P_d(t)+ P_m(t))\Delta t$ cannot exceed the value of one, the probability rate functions $P_d(t)$ and $P_m(t)$ do not have this restriction. Notice that $P_d(t)$ and $P_m(t)$ are both functions of time and unlikely to remain constant throughout the growth, except perhaps for a limited time. In a typical growth situation that results in a sigmoid growth curve with a noticeable "lag," both $P_d(t)$ and $P_m(t)$ are expected to be initially very low and then at least $P_d(t)$ will start rising as demonstrated in Fig. 22. The figure also shows that in the "exponential growth phase" $P_d(t)$ is much larger than $P_m(t)$ and hence the net growth. In the

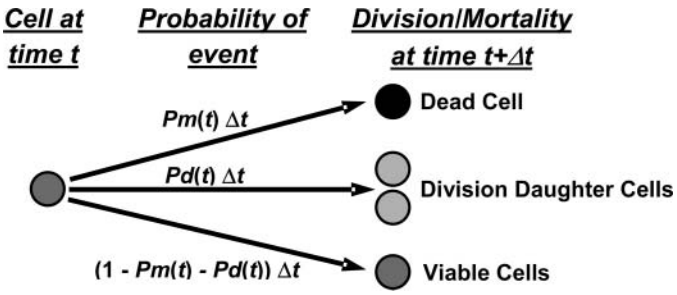


**Figure 21** The three states of a microbial cell according to the probabilistic model and the different growth and mortality patterns that they can produce. After Horowitz et al. (2010). Notice that in its simplified version, the model does not account for injury.

"stationary phase," the division and mortality probabilities must be equal, and when $P_m(t)$ surpasses $P_d(t)$, the population size decreases. According to this model, inactivation will be observed when $P_m(t) > 0$ and $P_d(t) = 0$ or when $P_d(t)) \ll P_m(t)$. Like the Quasi-chemical model, the probabilistic model can describe all the common growth and mortality patterns and transitions between them as shown in the figure, at least qualitatively.

The probabilistic model comes in two versions: one fully stochastic and discrete and the other fully deterministic and continuous. The first is used to follow the state of individual cells and their progenies. After each time interval, $\Delta t$, the fate of each living cell is decided by drawing a random number from a pool with a uniform distribution, $R_n$ ($0 \leq R_n \leq 1$). If $R_n$ falls below the value of $P_m(t)\Delta t$, the cell dies and its "lineage" comes to an end. If $R_n$ falls in the range $P_m(t)\Delta t < R_n \leq (P_m(t) + P_d(t))\Delta t$, the cell divides, otherwise it remains alive but undivided. The number of living cells after each $\Delta t$ is recorded to construct the growth, or growth/mortality curve of each individual cell's "lineage." This is done by repeating the procedure with new $P_m(t)$ and $P_d(t)$ which correspond to the iteration's time. The same process is repeated with other cells. The growth curve of the group is created by adding the number of living progenies of each cell after each iteration. As demonstrated in Fig. 23, this model version is particularly suitable for simulating the growth, growth/mortality, or mortality patterns of very small groups of bacteria. These can be of a highly virulent pathogen that in food might be present in small numbers, the few survivors of a marginal heat treatment, etc.

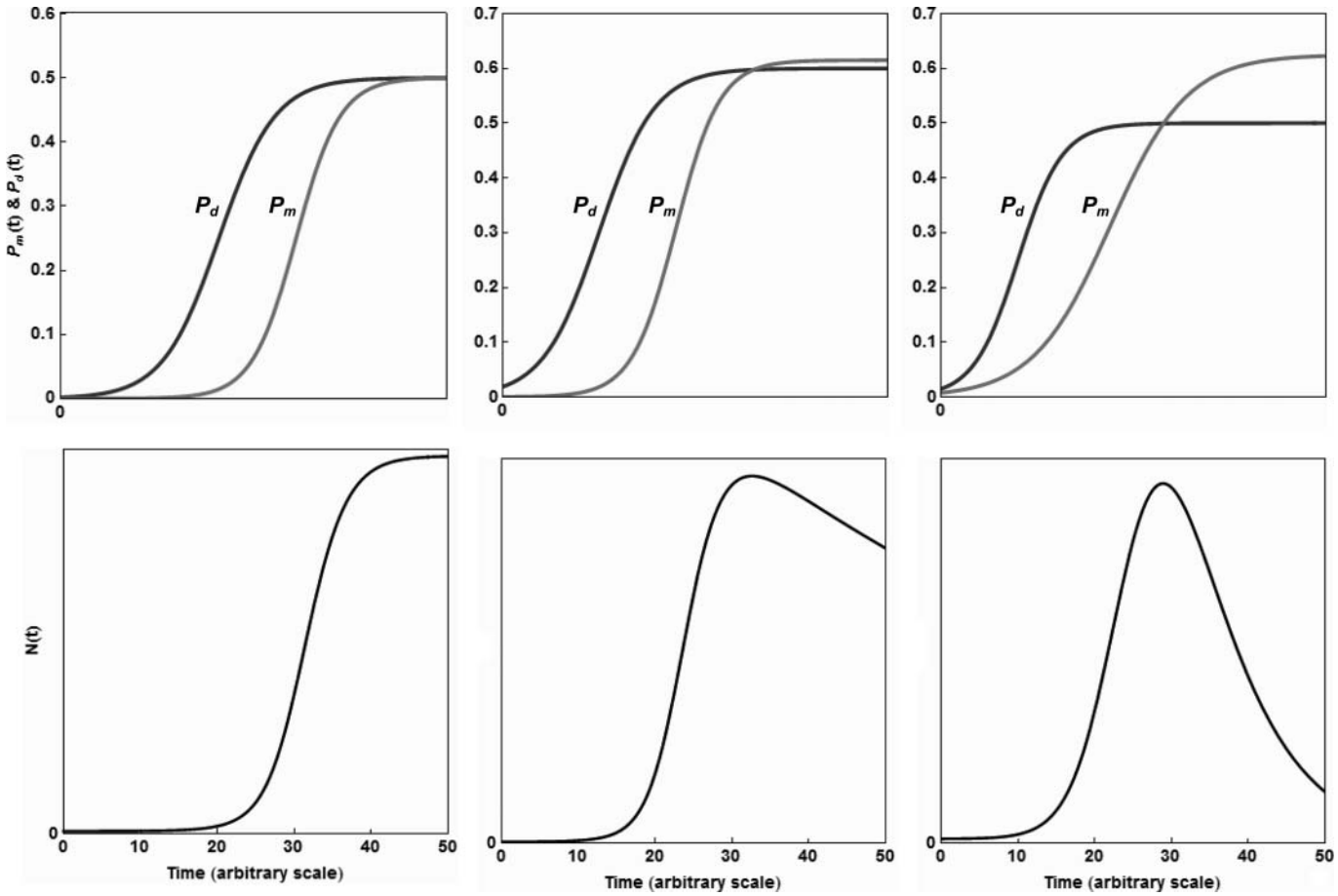


**Figure 22** Examples of simulated growth-mortality curves generated with continuous (deterministic) version of the probabilistic model. Notice that the cross over of the probability rate function $P_d(t)$ and $P_m(t)$ results in a transition between grown and mortality, or vice versa. In a region where $P_d(t) = P_m(t)$, the number of cells remains constant. This is regardless of whether the curve is recorded under static or dynamic conditions. After Horowitz et al. (2010).

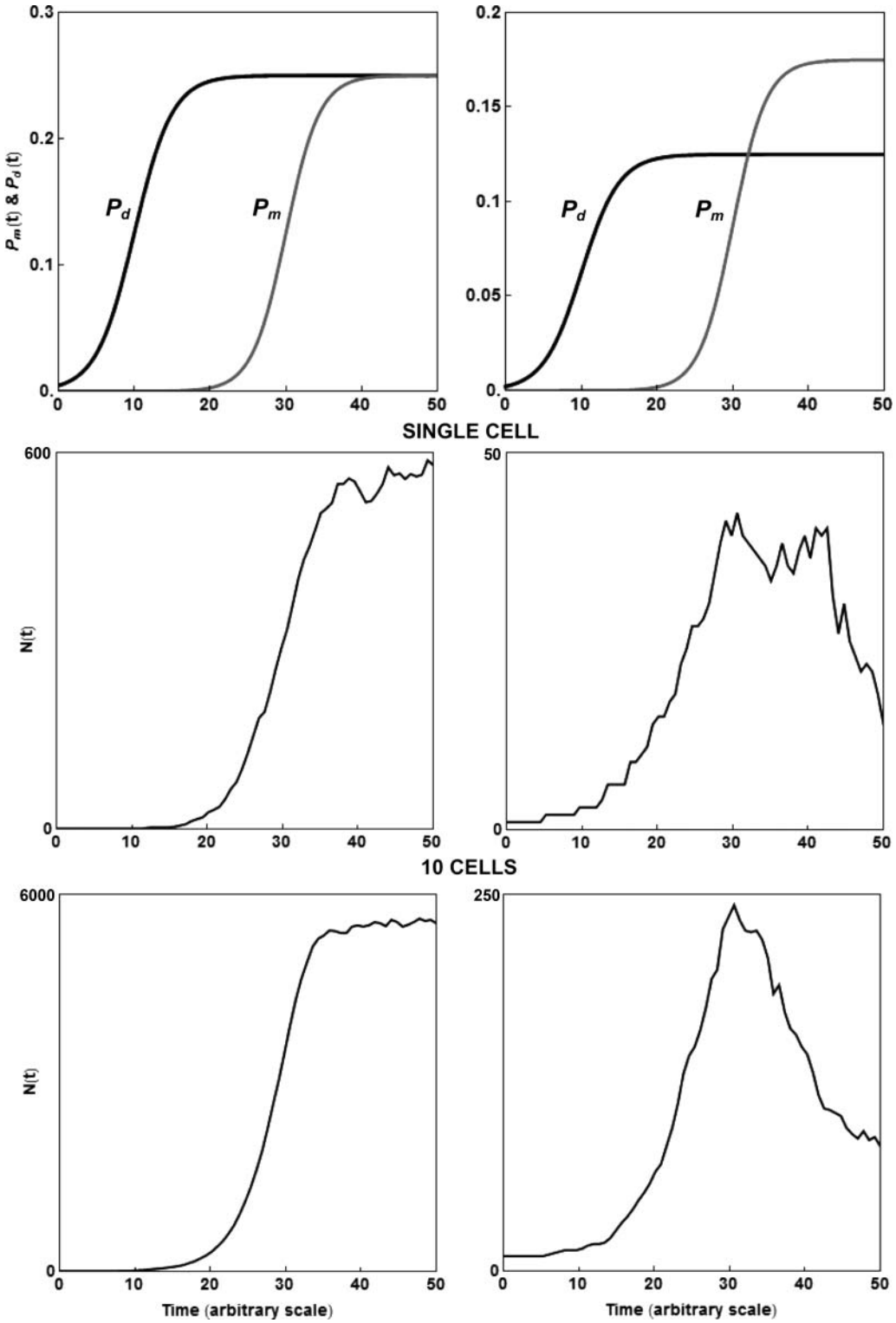


**Figure 23** Examples of simulated growth-mortality curves of individual and small groups of cells. In principle, the underlying probability rate functions, $P_d(t)$ and $P_m(t)$ can be calculated from the experimental growth or growth/mortality curves of large populations, but the result would still need independent verification. Notice that as the group size grows, the growth-mortality curves becomes smoother and more deterministic. After Horowitz et al. (2010).

The continuous deterministic version is a limiting case of the stochastic model where the number of cells is large and the time intervals $\Delta t \rightarrow 0$. It is therefore only suitable for large populations. However, as suggested by Horowitz et al. (2010), this model version can be used to determine the probability rate functions $P_d(t)$ and $P_m(t)$ and calculate their coefficients from experimental growth or growth/mortality data of large populations, that is, by the conventional method of recording growth and/or mortality curves. Once determined in this way, these probability rate functions can be incorporated into the stochastic-discrete model to study the risks associated with low levels of microbial contamination.

The two versions of the models are equally applicable to isothermal and non-isothermal growth and growth/mortality patterns. The difference is just in the temporal values of $P_d(t)$ and $P_m(t)$, but not in the formulation of the model. The same can be said about static and dynamic growth conditions in general, that is, constant versus varying, pH, $a_w$, oxygen tension, a disinfectant or antimicrobial concentration, etc. The influence exerted by these factors, alone or in concert, will be solely reflected in how the probability rate functions $P_d(t)$ and $P_m(t)$ will vary with time.

The starting point of the probabilistic modeling approach is the admission that the sub-cellular processes, which regulate the fate of an individual cell, are not known in sufficient detail to construct a truly mechanistic model. Apart from being a viable alternative to modeling the life history of small microbial populations, the probabilistic modeling approach also offers a way to describe and explain microbial growth and mortality patterns that does not require any kinetic assumptions. But except for small populations, the mentioned probabilistic models have not been intended to replace current phenomenological models in practical applications. Prediction of non-isothermal growth patterns from isothermal data, and translation of the temperature history of a food into a microbial growth curve can be done very effectively by the empirical models discussed earlier. They too are constructed without any detailed knowledge of the mechanisms that operate at the cellular level but are much simpler mathematically and by far more convenient to use.

## *CONCLUDING REMARKS*

The existing growth models have been developed in light of different considerations and on the basis of different assumptions. This is reflected in their mathematical structure and the number of adjustable parameters that they have. Almost invariably, the same experimental growth data can be described and even be predicted by more than one model type, an indication that a growth model need not be unique in order to be useful. The shape of a microbial growth curve is a reflection of events at the cellular level that are regulated by biochemical and biophysical processes inside and outside the individual cells. However, the shape of the growth curve does not contain sufficient information to identify them all, let alone assess their relative contributions quantitatively. Thus the notion that a parameter or set of parameters of a kinetic growth model is the exclusive representative of the cells "physiological state" must be supported by independent evidence. Otherwise, one can almost always come up with alternative explanation of the shape of the growth curve or that of any of its parts. Preferably, the independent verification of a mechanistic interpretation of a model should come from experiments especially devised to confirm or refute it. Until such experimental confirmation is presented, a claim that a particular kinetic model is more "fundamental" or "mechanistic" than others cannot be sustained. All the logistic (Verhulst) model's variants, including the Baranyi-Roberts model, do not take into account the possibility that some cells can die while the population size is still growing. Therefore, none of them can be considered truly "fundamental" for this reason alone. Theoretically, during the "lag time" and "stationary phase," the number of dying cells must equal that added by division, which includes the possibility that no cell is dividing or dying. But this equality tells us nothing about the division and mortality rates. Similarly, a change in the growth rate at the "exponential phase" can be the result of a rising or falling division rate alone or accompanied by a falling or rising mortality rate, for example. What actually happens, and what the changing momentary cell division and mortality rates are, is not revealed by any kinetic model that is solely based on the growth. For this very reason, the probabilistic model described in the previous section, which does take mortality into account, still needs experimental validation.

Let us repeat that there is nothing wrong in writing a phenomenological rate model for net growth. On the contrary, several rate models, being modified versions of the logistic equation or purely empirical, have been able to predict microbial growth correctly under dynamic temperature conditions from isothermal and even from other non-isothermal growth (Smith–Simpson et al. 2007). But despite this success, it is doubtful that any of these models can be directly linked to microbial cells physiology without taking mortality into account. If a kinetic model is primarily judged by its predictive ability, none of the currently available growth models is inherently superior, all claims to the contrary notwithstanding. Consequently, a growth model choice should be solely based on utilitarian considerations, such as mathematical simplicity and having parameters with an intuitive meaning.

The quasi-chemical and probabilistic models of the kind described in the previous section offer two different ways to link events at the cellular level to their manifestation at the population level. However, they too are based on assumptions whose validity is yet to be confirmed experimentally. Hopefully, future research will prove them correct. But even if validated experimentally, it is doubtful that they will replace the currently available empirical kinetic rate models any time soon. This need not be due to any fault in their underlying concept or formulation but because they might require a large number of experiments to determine their parameters. The same might be said on the attempts to anchor conventional secondary models in the laws of thermodynamics, and for the same reason.

Although a way to derive them from basic principles might be eventually developed, the amount of experimental work needed for their confirmation would almost certainly be judged unfeasible unless a shortcut method is discovered or invented. Two more urgent challenges to researchers in the field would be to develop and validate practical models to predict dynamic growth where the temperature, pH, $a_w$, and/or other factors vary simultaneously and to apply them successfully to microbial growth in non-homogenous habitats and in situations where heat and mass transfers also play a role.

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