A Dynamic Approach to Predicting Bacterial-Growth in Food

Article *in* International Journal of Food Microbiology · December 1994

DOI: 10.1016/0168-1605(94)90157-0 · Source: PubMed

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International Journal
of Food Microbiology

Review Paper

A dynamic approach to predicting bacterial growth in food

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Received 19 August 1993; revised 3 January 1994; accepted 25 April 1994

Abstract

A new member of the family of growth models described by Baranyi et al. (1993a) is introduced in which the physiological state of the cells is represented by a single variable. The duration of lag is determined by the value of that variable at inoculation and by the post-inoculation environment. When the subculturing procedure is standardized, as occurs in laboratory experiments leading to models, the physiological state of the inoculum is relatively constant and independent of subsequent growth conditions. It is shown that, with cells with the same pre-inoculation history, the product of the lag parameter and the maximum specific growth rate is a simple transformation of the initial physiological state. An important consequence is that it is sufficient to estimate this constant product and to determine how the environmental factors define the specific growth rate without modelling the environment dependence of the lag separately. Assuming that the specific growth rate follows the environmental changes instantaneously, the new model can also describe the bacterial growth in an environment where the factors, such as temperature, pH and a_w, change with time.

Keywords: Bacterial growth; Differential equation; Lag; Growth rate

1. Introduction

Among others, food microbiologists have been seeking efficient models of microbial growth that might enable them to predict the microbiological consequences of food storage. Experience has shown that the necessary mathematical models cannot simply be copied from those elaborated over many years in biotechnology and chemical engineering, recently reviewed by Nielsen and Villad-

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SSDI 0168-1605(94)00049-C

sen (1992). The reasons why predictive food microbiology should build its own store of mathematical-statistical tools are numerous and varied.

(1) The goal of food microbiologists is to minimize or prevent microbial growth rather than optimize it, as often occurs in biotechnology. Consequently, effects of the inhibitory environmental factors, like preservatives, have been investigated more intensively.

(2) The cell concentration of interest is much lower than in biotechnology, where it is typically greater than 10^6–10^7 cell/ml. As a consequence, some methods validated at high cell concentration, e.g. turbidity, biomass or conductance measurements, should not be applied directly without establishing the relationship between cell numbers and the measurement at lower bacterial concentration (Baranyi and Roberts, 1992).

(3) In food microbiology, the kinetics of the lag phase is of great importance, while it is less important in a bioreactor. On the other hand, well-known models of the transition from the exponential to the stationary phase, like Monod's model (Monod, 1942), lose their significance in food microbiology because substrate limitation is rarely important until the microbial concentration reaches levels associated with spoilage.

(4) Generally much less information, and often less accurate information, is available about the physicochemical environment of the food in question than, say, in a bioreactor. Therefore the applied mathematical-statistical methods involve various simplifying and empirical elements.

It is usual to classify the applied models into empirical and mechanistic (Roels and Kossen, 1978). In most investigations, however, the situation is somewhere in between and, as more information is gained, that situation can change (Box and Draper, 1987). Attempts, like those of McMeekin et al. (1993), to find a fundamental (in that case, thermodynamic) basis for a model (the square-root model) are important steps towards more mechanistic approaches. Whiting and Cygnarowicz-Provost (1992) and Baranyi et al. (1993a), albeit in different ways, found a less empirical approach to substitute the commonly used Gompertz function describing the growth of a bacterial culture. As those authors agreed, the Gompertz function applied to the logarithm of the cell concentration, as in Gibson et al. (1988), Buchanan and Phillips (1990), Zwietering et al. (1990), is a new model and should be termed a 'modified' Gompertz model. The 'modified' Gompertz model does not have that deep root in population dynamics which was analyzed by Holgate (1989), and no mechanistic derivation is known for it. (Its differential-equation-form, published by Van Impe et al. (1992), is just another form of the same, 'modified', Gompertz model.)

An important feature of a model is how well it can be embedded in other, more general, established theories of the natural world. We suggest a simpler, deterministic version of the framework of Frederickson et al. (1967) which assumes that the kinetics of a homogeneous bacterial cell population can be characterized by the extracellular, physicochemical environment (temperature, atmosphere, substrate, etc.) and the intracellular conditions. The variables built in the mathematical model are divided into three classes:

(1) Extracellular conditions assumed to be unaffected, or negligibly affected, by the growth of the cells that characterize the growth-independent environment which may change with time. The most important components of the growth-independent environment is usually the temperature. Note that since growing cells produce heat, in the strict sense, the temperature should not be considered as a growth-independent variable. However, the small contribution of that heat produced is neglected here.

(2) Extracellular conditions which are changed by the growing culture. These will be called growth-dependent environmental quantities. They include, for example, the concentration of various chemicals such as growth substrates and metabolic products surrounding the cells.

(3) Intracellular concentrations of certain substances, like DNA, RNA, etc. which change during growth and characterize the physiological state of the cells.

It is important to appreciate that the boundary between the growth-independent and growth-dependent quantities is not fixed for all models, and a variable being growth-independent in a particular model may represent a growth-dependent quantity in an other, more sophisticated, model. For example, it is known that the pH level is usually changed by cells growing to high concentrations. Nevertheless, the pH value is often considered to be constant during the growth; partly by disregarding the small increases or decreases in pH, and partly by not concentrating on the dynamics of the culture at high concentrations. With model development, the pH could be treated as a growth-dependent quantity.

In our model, all the extracellular physicochemical quantities are considered to be growth-independent and they are included in an environmental vector-variable, $E(t)$, which possibly changes with time during growth.

From a practical point of view, there is a need to be able to predict the course of bacterial growth under a temperature profile that changes with time. The simplest approach supposes that the specific growth rate of the bacterial population responds instantaneously to the temperature changes. Fu et al. (1991) showed that, if the temperature remains within the growth conditions, instantaneous adjustment of the specific growth rate to the temperature changes can be assumed if the cells are in the exponential phase. The model described below makes it possible, among other uses, to predict the course of bacterial growth when the temperature changes in the lag phase during which the cells adjust to a new environment. The idea is similar to that of Srivastava and Volesky (1990) who assumed that there is a bottleneck-substance (RNA, in their paper) that must reach a certain level to induce the growth of the cells, and the rate of the accumulation of that substance changes instantaneously as the temperature changes. We have combined this idea with the growth model of Baranyi et al. (1993a), the mathematical properties of which are analyzed in Baranyi et al. (1993b). The new model gave reliable predictions when tested on experimental data obtained under changing temperature profiles (Baranyi et al., 1994). It provides an explanation, too, why the lag is inversely proportional to the maximum specific growth rate, a relation which is observed or supposed (implicitly or explicitly) by many authors.

In this paper, a new variable, $q(t)$, representing the physiological state of the cells is introduced. From $q(t)$, by a simple transformation,

$$\alpha(t) = \frac{q(t)}{1+q(t)}$$

the value of the so-called 'adjustment function' (Baranyi et al., 1993a) can be calculated. It can be considered as a capacity-type quantity expressing the proportion of the potential specific growth rate (which is determined by the actual environment) that is utilized by the cells. The process of adjustment (lag period) is characterized by the gradual increase of $\alpha(t)$ from a low value towards 1. Another transformation,

$$h(t) = \ln\left(1 + \frac{1}{q(t)}\right) = -\ln\ \alpha(t)$$

proves to be useful from a computational point of view; it can be considered as a statistically stable transformation of $q(t)$ and $\alpha(t)$. The value $h_0 = h(0) = -\ln\ \alpha(0)$ will be the product of the maximum specific growth rate and the lag, therefore this product is constant for different growth curves provided that the physiological state of the cells at inoculation is identical (i.e. the subculturing procedure is carefully standardized).

The environment dependence of the maximum specific growth rate and the lag (or their reparameterized form, see Garthright, 1991; Baranyi, 1992a,b) are usually modelled independently (Gibson et al., 1988; Buchanan and Phillips, 1990) although there is an obvious, high, correlation between them. Due to the construction of the new model, this problem is eliminated and the model is made applicable to a time-dependent environment. The way in which the variance of the growth parameters is decreased by the new model is shown below using a published dataset.

2. Theory

2.1. A generic growth model

Throughout this paper, unless otherwise stated, the logarithm of a quantity, x, is always taken to be its natural logarithm, $\ln x$. Furthermore, a notation $f(t)$ can mean either the f function or its value at the point t.

Let $x(t)$ denote the cell concentration of a bacterial population at the time t. The derivative of $x(t)$ gives the (absolute) growth rate and:

$$\frac{\frac{\mathrm{d}}{\mathrm{d}t}x}{x}$$

is the specific, or relative, growth rate. Because:

$$\frac{\mathrm{d}}{\mathrm{d}t}\ln x = \frac{\frac{\mathrm{d}}{\mathrm{d}t}x}{x}$$

the specific growth rate can be measured as the slope of the curve $\ln x(t)$. Note that if the base of the logarithm is 10 then the slope of the curve $\log_{10}(t)$ is $\ln 10 \approx 2.3$ times less than the specific growth rate.

Our starting point is a growth model well-known from population dynamics. We examine the first-order ordinary differential equation:

$$\frac{\mathrm{d}}{\mathrm{d}t}x = \mu(x)\,x \qquad (0 \le t < \infty;\ 0 < x) \tag{1a}$$

with the initial value

$$x(0) = x_0 \qquad (x_0 > 0) \tag{1b}$$

where $\mu(x)$, the specific growth rate, is supposed to be monotone decreasing (Vance, 1990). If $\mu(x) = \mu_{\max} \equiv$ constant, then (1a) describes the pure exponential (Malthus) growth. If, for example, with m > 0:

$$\mu(x) = \mu_{\max}\left(1 - \left(\frac{x}{x_{\max}}\right)^m\right) \qquad (x_0 \le x \le x_{\max}) \tag{1c}$$

then Richards' family of growth curves can be obtained. The parameter $x_{\max}$ is the maximum population density while m is a curvature parameter. The special case of $m = 1$ is called the logistic or Pearl–Verlhurst growth model. These possibilities, and many others, like Gompertz, von Bertalanffy, etc. are analyzed and unified in a generic growth model by Turner et al. (1976).

2.2. *Inhibition in the end of the exponential phase*

A common feature of the growth models of the form (1a) is that, if $\mu(x)$ is strictly monotone decreasing with $\mu(x_{\max}) = 0$, then the solution, $x(t)$, is a sigmoid function which approximates $x_{\max}$ asymptotically as t increases. The differential equation (1a) can be written in the form:

$$\frac{\mathrm{d}}{\mathrm{d}t}x = \mu_{\max}\,u(x)\,x \qquad (0 \le t < \infty;\ 0 < x) \tag{1d}$$

provided that the specific growth rate, $\mu(x)$, is finite (this constraint does not hold, for example, in the Gompertz-case where $\mu(x)$ can be any large value if x is small enough, but does hold in Richards' model). The function $u(x)$ will be called 'inhibition' function because it ensures the transition of the growth curve to the stationary phase. Its values are between 0 and 1 and it decreases as x increases from 0 to $x_{\max}$.

Food microbiologists prefer using the logarithm of the cell concentration (a

consequence of the differences from biotechnology mentioned in the introduction) so we apply the notation:

$$y(t) = \ln\ x(t) \qquad y_0 = \ln\ x_0 \qquad y_{\max} = \ln\ x_{\max}$$

The above system, in terms of $y(t) = \ln\ x(t)$, can be written as:

$$\frac{\mathrm{d}}{\mathrm{d}t}y = \mu_{\max}\, u(\mathrm{e}^y) \qquad (0 \le t < \infty;\ 0 < y) \tag{2a}$$

$$y(0) = y_0 \qquad (y_0 > 0) \tag{2b}$$

Since the applied transformation keeps the monotonity, $u(\mathrm{e}^y)$ monotone decreases as y increases. Consequently, as can be seen from (2a), the derivative of the logarithm of the cell concentration is monotone decreasing, too, and it cannot provide a sigmoid curve. This is why the logarithm of the classical sigmoid (such as the Gompertz or logistic) functions cannot be used to model the variation of the logarithm of the cell concentration in those situations when a lag phase is observed in the growing culture.

An example for the $u(x)$ inhibition function, values of which are decreasing in the (0, 1) interval, can be read from the (1c) form of Richards' model. Another, well-known example is Monod's model, where

$$u(x) = \frac{S}{K_S + S}$$

Here S denotes the substrate concentration, K_S is the so-called Michaelis–Menten constant, and it is supposed that

$$\frac{\mathrm{d}}{\mathrm{d}x}S = -Y$$

where Y is a positive constant (see, for example, in Roels and Kossen, 1978). Considering that, as long as $S > 0$,

$$\frac{\mathrm{d}}{\mathrm{d}x}u = \frac{\mathrm{d}}{\mathrm{d}x}\frac{S}{K_S + S} = -Y\frac{K_S}{(K_S + S)^2} < 0$$

we can conclude that the Monod model, too, is a special case of (1d), with the above, substrate-dependent, inhibition function. However, because of the aims of predictive food microbiology mentioned in the introduction, we suggest that a simple, empirical inhibition function, like that of Richards, is suitable.

2.3. Inhibition before the exponential phase – the form of the new growth model

In what follows, we investigate how an 'inhibition' function, similar to $u(x)$, can be derived for the beginning of the growth curve (lag phase).

A scheme of a typical batch culture experiment, regularly carried out in food microbiology laboratories, is shown on Fig. 1. The bacterial population is first cultured under more or less optimal conditions (environment E_1) then inoculated

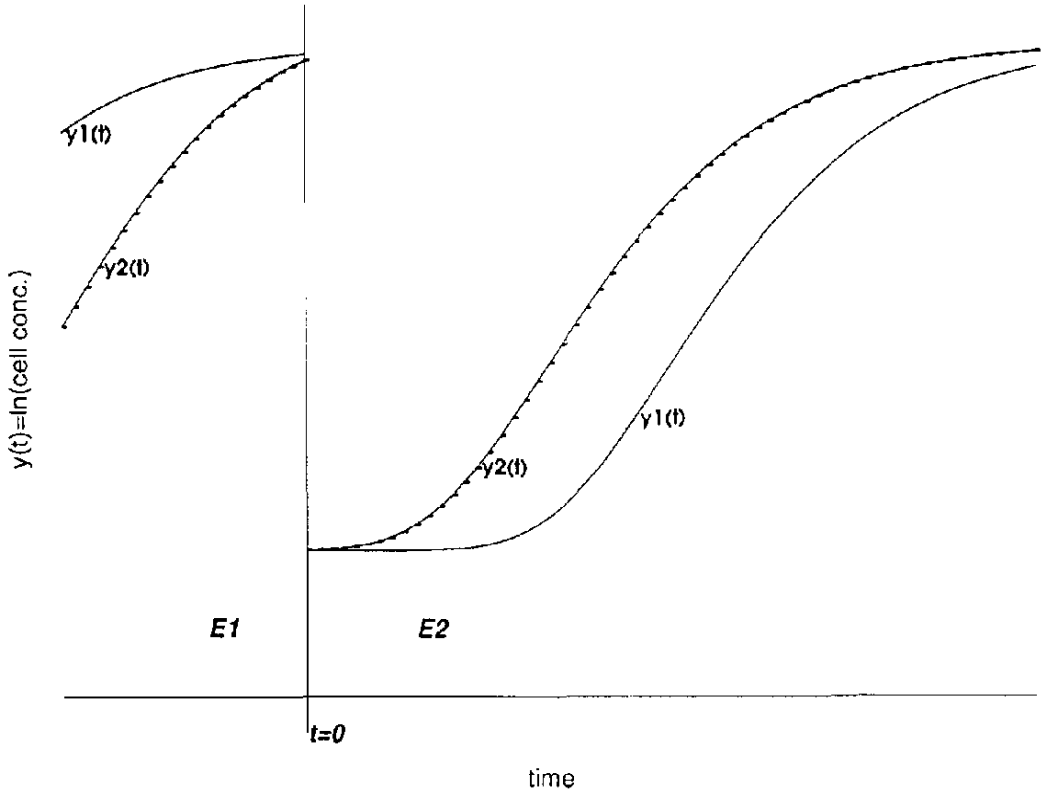

Fig. 1. Effect of metabolic activity at inoculation on subsequent lag time. Low metabolic activity at inoculation causes lag in the growth of the culture. The lag in the post-inoculation environment, E_2, is longer if the cells are closer to the stationary phase in the pre-inoculation environment, E_1.

and grown in the actual environment, E_2, where the logarithm of the cell concentration changes according to a well-known sigmoid pattern, provided that the environment E_2 is kept constant. In what follows, we refer to the time of inoculation as the zero time, $t = 0$. At the end of E_1, just before $t = 0$, the cells are either in the exponential phase or close to the stationary phase and the physiological state at $t = 0$ affects the length of the lag period in E_2 (Pirt, 1975).

It is widely accepted that the maximum specific growth rate of a given population, μ_{max}, in a constant environment, is an intrinsic parameter of that population (Rubinow, 1984). Therefore it is reasonable to suppose that μ_{max} is determined by the variables of E_2 independently of the pre-inoculation environment, E_1. Alternately, instead of μ_{max}, the doubling time can be modelled, using the formula: doubling time $= \ln 2/\mu_{max}$.

This approach has been followed by many authors working in predictive microbiology (Gibson et al., 1988; Buchanan and Phillips, 1990; McClure et al., 1993). In addition, another parameter of the actual growth curves, the lag time, is frequently modelled. However, the duration of lag depends not only on E_2 but also upon the previous history of the cells. If, for example, the cells are in high metabolic activity at $t = 0$, as when inoculation is made from the exponential phase of E_1, then the lag will be shorter than if this activity is low, as occurs when inoculation is made from the stationary phase in E_1 (see Fig. 1). One reason why it

is more difficult to predict the length of the lag period than the maximum specific growth rate (or the doubling time) is that the cells' history can be significantly different for the same inoculum concentration.

In our system, the instantaneous growth rate at the time t is determined by the cell concentration, $x(t)$, the extracellular environment, E_2, and the physiological state of the cells, characterized by just one variable, $q(t)$, in what follows.

We postulate that the cell concentration of a bacterial batch culture is described by the differential equation

$$\frac{\mathrm{d}}{\mathrm{d}t}x = \mu_{\max}\,\alpha(t)\,u(x)\,x \qquad (0 \le t < \infty;\ 0 < x) \tag{3a}$$

with the initial value

$$x(0) = x_0 \qquad (x_0 > 0) \tag{3b}$$

The constraints for the inhibition function, $u(x)$, are the same as under (1a). The factor $\alpha(t)$ is called adjustment function. It is monotone increasing for $t \ge 0$ describing the adjustment of the culture to the new environment. The 'damping' role of $\alpha(t)$ is similar to that of $u(x)$ (the values of both factors are from the [0, 1] interval), but $\alpha(t)$ will affect the course of growth before the exponential phase.

The so-called initial value problem (3a), (3b) has been analyzed by Baranyi et al. (1993b). Richards' form of $u(x)$ (see (1c)) was suggested in Baranyi et al. (1993a). With that form of the inhibition function and under the constraint that the actual environment, E_2, and therefore $\mu_{\max}$, is constant, an explicit solution of (3a), (3b) was given in the latter paper. It is worth recalling this solution, not only because of the unfortunate misprints in the original paper, but also because of its flexibility in the fitting procedure, which will be analyzed later in Section 3.2: Numerical properties.

As already stated, the slope of the curve $\ln x(t)$ will be the specific (*per* cell) growth rate. If E_2 is constant then the logarithm of the solution of the above differential equation, $y(t) = \ln x(t)$, can be expressed as

$$y(t) = y_{\max} - \frac{1}{m}\ln\left(1 + \frac{\mathrm{e}^{m(y_{\max} - y_0)} - 1}{\mathrm{e}^{m\mu_{\max}A(t)}}\right) \tag{4a}$$

or, after rearrangement:

$$y(t) = y_0 + \mu_{\max}\,A(t) - \frac{1}{m}\ln\left(1 + \frac{\mathrm{e}^{m\mu_{\max}A(t)} - 1}{\mathrm{e}^{m(y_{\max} - y_0)}}\right) \tag{4b}$$

where

$$A(t) = \int_0^t \alpha(s)\,\mathrm{d}s \tag{4c}$$

(s is an integral variable running from 0 to t).

Suppose that the per cell concentration of a critical substance, $P(t)$, is the bottle-neck in the process of growth, and the specific growth rate is influenced by $P(t)$ according to the well-known Michaelis–Menten kinetics:

$$\alpha(t) = \frac{P(t)}{K_P + P(t)}$$

where K_P is the Michaelis–Menten constant. Assuming that $P(t)$ is proportional to the nth power of time, a flexible sigmoid function could be obtained which is suitable to fit growth data (Baranyi et al., 1993a). Here we show another form of $P(t)$ which allows that the environment may be changing during the growth.

Suppose that, after the inoculation, the rates of the enzymatic reactions playing a primary role in producing $P(t)$ adjust instantaneously to the new environment, which may change during the growth, and, in the lag phase, $P(t)$ follows a first-order kinetics:

$$\frac{\mathrm{d}}{\mathrm{d}t}P = \nu P$$

where the rate, ν, is characteristic of the actual environment: $\nu = \nu(E_2)$. In this way the adjustment function is influenced by the entire given environment profile, $E_2 = E_2(t)$, and not just by a single parameter.

It is not easy to decide whether, and how, the Michaelis–Menten constant, K_P, is influenced by E_2. The answer obviously depends on how the critical substance is affected by the factors defining E_2. We return to this question later. For the time being, suppose that, even if E_2 changes during the growth, K_P remains constant and therefore the adjustment function depends on the ratio $P(t)/K_P$. This is the quantity we will use to characterize the physiological state of the cells: let $q(t) = P(t)/K_P$.

2.4. Lag in a constant environment

If the actual environment, E_2, is constant then $P(t)$ grows exponentially at a constant specific rate, ν, and our new adjustment function can be obtained in the form:

$$\alpha(t) = \frac{P(t)}{P(t) + K_P} = \frac{q(t)}{1 + q(t)} = \frac{q_0}{q_0 + e^{-\nu t}} \tag{5a}$$

where $q_0 = q(0) = P(0)/K_P$. As can be seen from the formula, the process of adjustment depends on the initial value of $q(t)$, which characterizes the physiological state of the inoculum, and on the rate ν. The integral function of $\alpha(t)$, denoted by $A(t)$ in (4c), can be expressed explicitly:

$$A(t) = t + \frac{1}{\nu}\ln\left(\frac{\mathrm{e}^{-\nu t} + q_0}{1 + q_0}\right) \tag{5b}$$

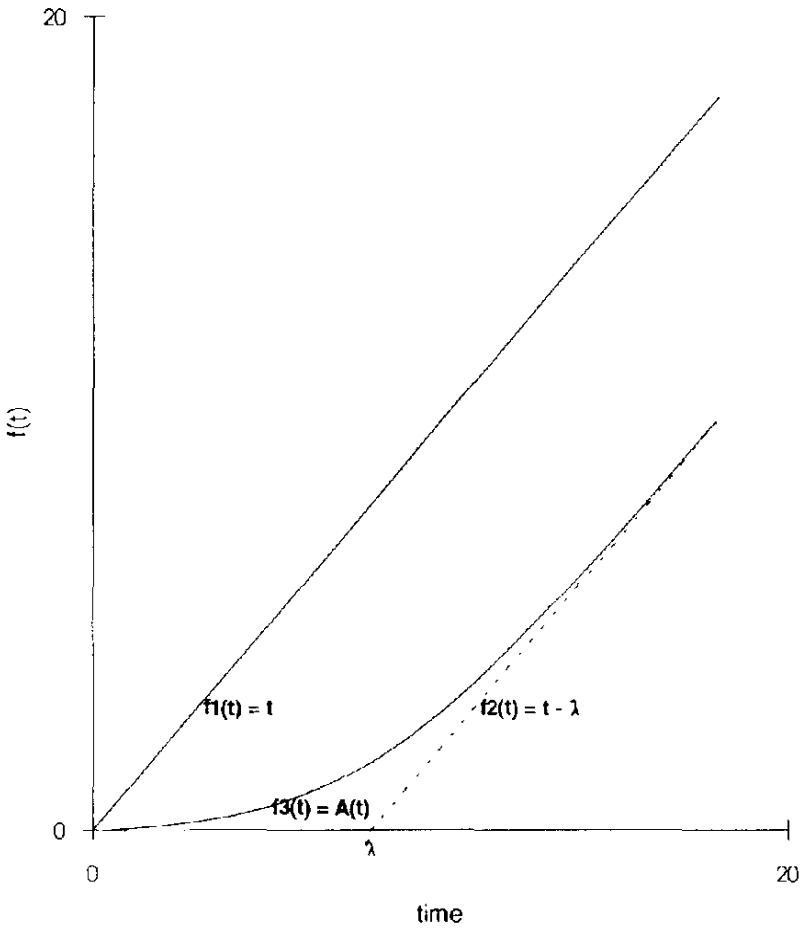

Fig. 2. Relation of the $A(t)$ integral of the adjustment function to $f_1(t)=t$ and $f_2(t)=t-\lambda$. $f_3(t)=A(t)$ can be considered as a rescaling of time. It converges to $f_2(t)=t-\lambda$ which is a delayed version of $f_1(t)=t$. This gradual delay in time is utilized in creating the sigmoid growth function.

The above expression, substituted in the formula (4b), gives a sigmoid function with the parameters, y_0, y_{max}, μ_{max}, q_0, ν and m.

For convenience, if the environment is constant, a lag parameter can also be derived in our model. Consider the formula (5b) given for $A(t)$. As is demonstrated in Fig. 2, $A(t)$ approximates the function $t-\lambda$ more and more as t increases, where

$$\lambda = \frac{\ln(1+1/q_0)}{\nu} \tag{6}$$

Baranyi et al. (1993b) proved mathematically that, if the asymptote of $A(t)$ is a function of the form $t-t_0$, where t_0 is a constant, then the classical definition of the lag (Pirt, 1975) is very close to t_0. Therefore it is reasonable to define our lag-parameter by the formula (6).

The parameter q_0 and the rate ν determine the process of adjustment. The quotient $q_0=P_0/K_P$expresses the physiological state of the inoculum; if q_0 is small then λ is large. The rate ν determines the quickness of the transition from the lag to the exponential phase. If ν is small then the lag phase can be long.

2.5. A special case of the growth model

The specific growth rate of the bacterial culture is a result of certain enzymatic reactions. As a general principle, we assume that this rate is not higher than the rate of the slowest of these reactions, causing the bottle-neck in the growth. This suggests that the specific rate of production of the critical substance, ν, should be equal to that of the bacterial culture, which rate is characteristic of the actual environment. That is $\nu(E_2) = \mu_{max}(E_2)$ and the model for the logarithm of the cell concentration can be described as

$$\frac{d}{dt}y = \frac{1}{1 + e^{-Q(t)}}\mu_{max}(E_2)(1 - e^{m(y - y_{max})}) \tag{7a}$$

$$\frac{d}{dt}Q = \mu_{max}(E_2) \tag{7b}$$

$$y(0) = y_0 \qquad Q(0) = \ln q_0 \tag{7c}$$

where $Q(t) = \ln q(t)$.

The specific growth rate depends on time through the actual, time-dependent, environment in the following manner: the functions $E_2(t)$ and $\mu_{max}(E_2)$ should be provided by the modeller, where $E_2(t)$ is the environment-profile of the culture after the inoculation, and $\mu_{max}(E_2)$ is a suitable function to describe the relationship between the environment and the maximum specific growth rate as in the Arrhenius- or Square-root models, if the environment is defined by the temperature only – see McMeekin et al. (1993). The other parameters and variables have been defined above.

3. Results and discussion

3.1. Biological interpretations

The course of the growth, $y(t)$, is affected by the initial bacterial cell concentration, y_0 as well as the physiological state of the inoculum, q_0. For cultures having identical physiological states at inoculation and being cultivated under constant (but different) temperatures, the product of the lag time and the maximum specific growth rate will be a transformed version of q_0 (see the formula (6) with $\nu = \mu_{max}$) so it is a consequence in our model that the lag is inversely proportional to the maximum specific growth rate. This phenomenon has been observed, for example, by Cooper (1963). Note that several authors (Smith, 1985; Mackey and Kerridge, 1988) use the same model for the specific growth rate as for the reciprocal of the lag time, which implicitly assumes that these two parameters are inversely proportional.

If the adjustment function is equal to 1, $\alpha(t) \equiv 1$, then the solution of (7a), (7b), (7c), denoted by $p(t)$ here, is independent of q_0. The function $p(t)$ is called the potential growth in Baranyi et al. (1993a) because it would be the course of the

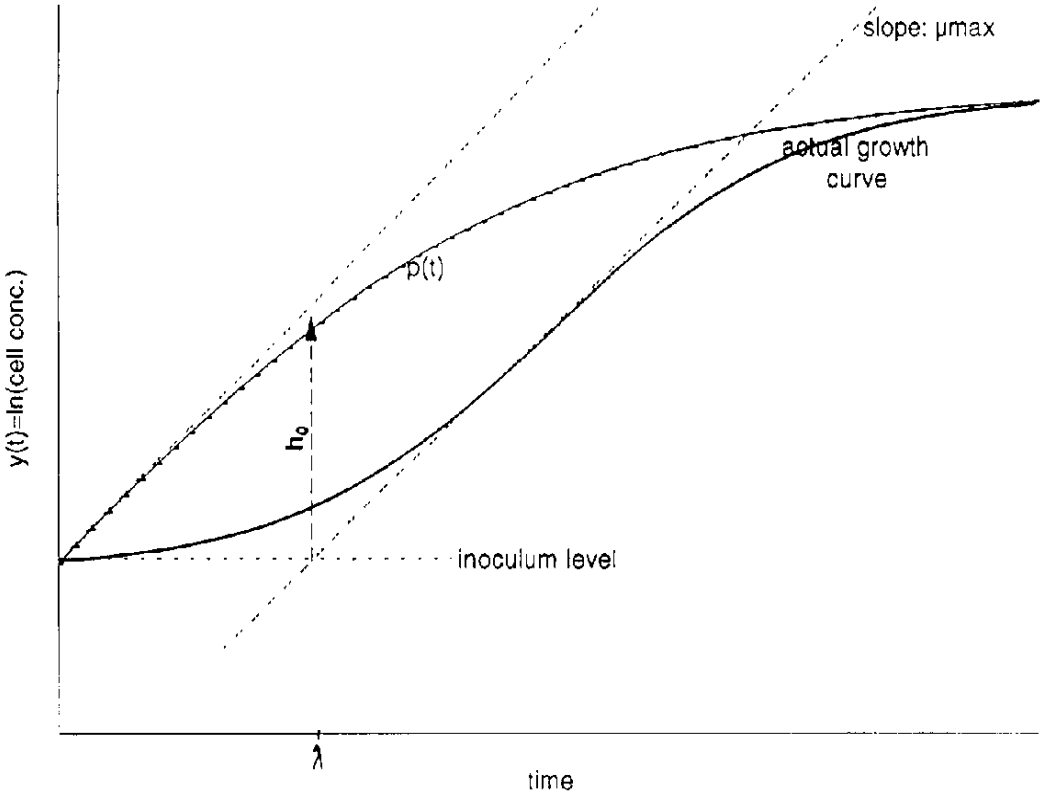

Fig. 3. Role of the potential growth when interpreting the product of the lag and the maximum specific growth rate geometrically. The potential growth, $p(t)$, responds to environmental changes instantaneously, thus reflecting the behaviour of the critical product, $P(t)$. The geometrical meaning of h_0, the product of the maximum specific growth rate and the lag, can be read from the plot by means of the potential growth.

growth curve if the cells had been inoculated from the exponential phase. Because the maximum specific rate of the potential growth is μ_{max}, the formula (6) also means that, from its initial value, an increase of

$$h_0 = \mu_{max}\lambda = \ln\left(1 + \frac{1}{q_0}\right) = -\ln \alpha_0 \tag{8}$$

must be reached by the logarithm of the potential growth by the end of the lag phase (Fig. 3). In the lag phase, the potential growth, $p(t)$, can be interpreted as a variable, proportional to the quantity $q(t)$, representing the actual physiological state of the cells.

Note that, according to the model, $q(t)$ grows to infinity, which is biologically impossible. But for our modelling purposes it is enough to say that, in the adjustment period, $q(t)$ grows according to first-order kinetics, the instantaneous rate of which, $\mu_{max}(t)$, is influenced by the actual environment. Later, after the exponential phase, there is a certain 'surplus' in $q(t)$, but this does not influence the bacterial concentration because the cells cannot grow faster than dictated by their potential specific growth rate. See more details on this question in Baranyi et al. (1994) who analyse whether, and to what extent, the actual decrease of $q(t)$ at cell division can be neglected in the model.

In our model there is no independent parameter for the lag. This expresses our view that the lag time is not interpretable, as a single number, if the temperature changes during the growth. The lag period is a process of adjustment described by the adjustment function, $\alpha(t)$. If the post-inoculation environment is constant, and only in that case, this function can be defined by a single parameter. That parameter may express a lag-type duration, as in Baranyi et al. (1993a), where the $\alpha(t)$ adjustment function depends on a lag-parameter, λ, or may measure the physiological state of the inoculum as in the above model, where $\alpha(t)$ depends on the physiological state of the inoculum, q_0, and the lag depends on q_0 as well as on μ_{max}, the potential maximum specific growth rate in E_2.

However, if the actual environment changes during the lagging period then the adjustment function should be influenced by the entire environmental profile, $E_2(t)$, as well as by q_0. This concept is formalized in the differential equation of Eq. (7b).

The adjustment function, as given in (5a), can be considered as a transformation of the quantity $q(t)$ and expresses the same: the 'readiness' of the cells for the actual environment. Hence, by means of $\alpha(t)$, the ratio of the actual and the potential specific growth rate, we have obtained another indicator of the physiological state of the cells. Also the quantities $h_0 = \ln(1 + 1/q_0) = -\ln \alpha_0$ and $\alpha_0 = q_0/(1 + q_0)$ are just different transformations of q_0, but they all measure, in some way, the physiological state of the inoculum.

3.2. Some numerical properties

The model variables represent rates and concentrations; a lag-parameter can be interpreted under constant conditions only. As can be seen from (6), an estimated lag value cannot be negative for any dataset (unlike in the case of the modified Gompertz function as suggested by Gibson et al., 1988).

The environmental profile, $E_2(t)$, has its role in (7a). The system of differential equations (7a), (7b) does not have an explicit solution for arbitrary $E_2(t)$ and $\mu_{max}(E_2)$ functions. Solving and, especially, fitting a differential equation are much more labour intensive than an explicit function, although codes can be compiled for this purpose (Press et al., 1990). It is a purely computational investigation, whether, and under what conditions, an explicit solution is available. One obvious example is when the actual environment, $E_2(t)$, and therefore μ_{max}, are constant and (4a), (4b) can be obtained. According to our experience, from the forms (4a) and (4b), which are theoretically equivalent, the latter should be used in numerical calculations, especially in the lag and the exponential phase. Application of (4b) also has a flexible property: namely, if there are no data indicating a stationary phase, then the last, logarithmic, term (with y_{max} in it) can simply be omitted and the number of parameters to be estimated decreases by one. The potential growth of the obtained growth function will be the pure exponential growth. This 'reducible' feature of our family of growth functions was used efficiently in Gibson et al. (1994, this issue). The practical procedure is similar to omitting a term from a linear regression model (Box and Draper, 1987): if the t-value of the estimated y_{max} parameter is below a certain limit, depending on the required confidence

level, then the upper asymptote is not well-defined by the dataset, so the curve is fitted again with the formula (4b) but omitting the logarithmic term.

3.3. A practical guide

Modelling the growth curve after the exponential phase is less significant in the microbiological safety of food and m plays a role only in the transition from the exponential to the stationary phase. Therefore, an empirical value, $m = 1$ is suggested for this curvature parameter (logistic potential growth).

As has been mentioned, it is not easy to fit these differential equations without skilled mathematical help. This would be the task to carry out if one wanted to use the system (7a), (7b), (7c) to fit experimental data directly. This is why we suggest an alternative below.

If the subculturing procedures are carefully standardized then the physiological state of the inoculum, therefore the parameters q_0 and h_0 (which are only transformations of each other), should be constant for the different growth curves. Rather than estimating q_0 directly, our experience is that estimation of h_0 has the best statistical properties, so this parameter should be fitted from the individual growth curves and then an average of the estimated h_0 values, h_{av}, should be substituted in (8) to calculate q_0. With the obtained q_0 value, (7a), (7b), (7c) can be simulated on a computer to predict the bacterial growth under isothermal conditions or time-varying temperature. This will be demonstrated below on the dataset of McClure et al. (1993) which measured the growth of *Brochothrix thermosphacta* under different environmental conditions.

The subculturing process in those experiments was relatively standardized and at the time just before inoculation the cells were in a stationary phase. This is important if one wants to model the acceleration from the passive to the active physiological state. It is also necessary that the inoculum level should be around the same, relatively low, value, which was $\approx 10^3$ cfu/ml in our case. We have fitted the growth curves by the explicit, four-parameter function defined by (4b) and (5b). The only difference between these curve fittings and those described as *Program 2* in McClure et al. (1993) is the form of the adjustment function. Instead of the lag time estimated in that paper, we now estimate the parameter $h_0 = \ln(1 + 1/q_0)$ for each growth curve. This parameter, which can be considered as the product of the lag time and the maximum specific growth rate, was close to a constant, similarly to the observation of Cooper (1963), although in McClure et al. (1993) not only the temperature but also the pH and a_w were different for different growth curves. The average of h_0 was $h_{av} = 3.2$ with a standard error of 1.4. Note that in McClure et al. (1993), with different sigmoid functions, the quantity $\mu_{max}\lambda$ showed much higher variance.

After fitting the curves individually by the four parameter sigmoid function given by (4b) and (5b), we fixed the value of q_0 as $q_0 = q_{fix}$, where

$$q_{fix} = \frac{1}{\exp(h_{av}) - 1}$$

The result was $q_{fix} = 0.0425$. Substituting this into (5a) fixed the adjustment function. The curves were fitted again with that fixed adjustment function, but now these were three-parameter curve fitting procedures. The newly obtained μ_{max}-values were then used when modelling the dependence of the maximum specific growth rate on the environmental factors.

For the sake of comparison, a quadratic response surface was applied to the logarithm of the newly fitted μ_{max}-values, as in the original paper. In McClure et al. (1993) the standard error of this quadratic fitting was 0.29 and 0.27, depending on which of the two sigmoid functions of that paper was used. With our procedure above, this error fell to 0.22. The improvement is evidently the result of the variance-damping effect of the assumption $h_0 = \text{constant}$. Moreover, it is not necessary to estimate the lag-parameter, if the same initial physiological state is assumed for the cells as in the experiments, because of $\lambda = h_{av}/\mu_{max}$. This is not the situation when other sigmoid curves are used to fit the individual growth curves. It is common experience that estimates of the lag parameter usually show higher variance than those of the maximum specific growth rate. For example, in McClure et al. (1993), the logarithm of the reported lag values varied around the fitted quadratic surface with a standard error of 0.43, which was almost twice as much as that of $\ln(\mu_{max})$.

In Fig. 4, by way of demonstration, the experimental data of the growth curve with code = 1 of McClure et al. (1993) are plotted together with two curves $y_1(t)$ and $y_2(t)$. The curve $y_1(t)$ was predicted by the method of *Program 1* of that paper which fitted a Gompertz function to each growth curve and then a quadratic response surface to the logarithm of the derived growth parameters, the maximum specific growth rate and the lag time, independently of each other. The predicted curve was obtained by taking the values of the maximum specific growth rate and the lag time from the quadratic response surface and producing the desired Gompertz curve by considering the inoculum level for the lower asymptote and a constant for the upper asymptote. With three environmental factors, this means $2 \times 10 + 1 = 21$ model-coefficients. The curve $y_2(t)$ was predicted by the method described above, with only 12 coefficients, because the modelling of lag is replaced by modelling the product of μ_{max} and the lag by a constant. The maximum population density was taken to be $10^{8.5}$ cell/ml, in both cases. As can be seen in the plot, both predictions are acceptable as far as the maximum specific growth rate is concerned ($\mu_{max1} = 0.05$ and $\mu_{max2} = 0.058$, respectively), but $y_1(t)$ underestimates the lag. The larger error in estimating the lag reflects that the average multiplicative error (0.43) of the lag-prediction of *Program 1* is much higher than that of our model where it must be the same as that of μ_{max}, i.e. 0.22. Therefore our re-definition of lag has also eliminated another problem because the lag and the maximum specific growth rate (or their reparameterized versions, see Garthright, 1991) are generally modelled independently of each other, which contradicts the commonly observed phenomenon that they are more or less inversely proportional for cultures when the physiological state of the inoculum is identical. For example, when using the independent lag- and growth rate models to predict a growth curve then it is possible that the lag is underestimated while the

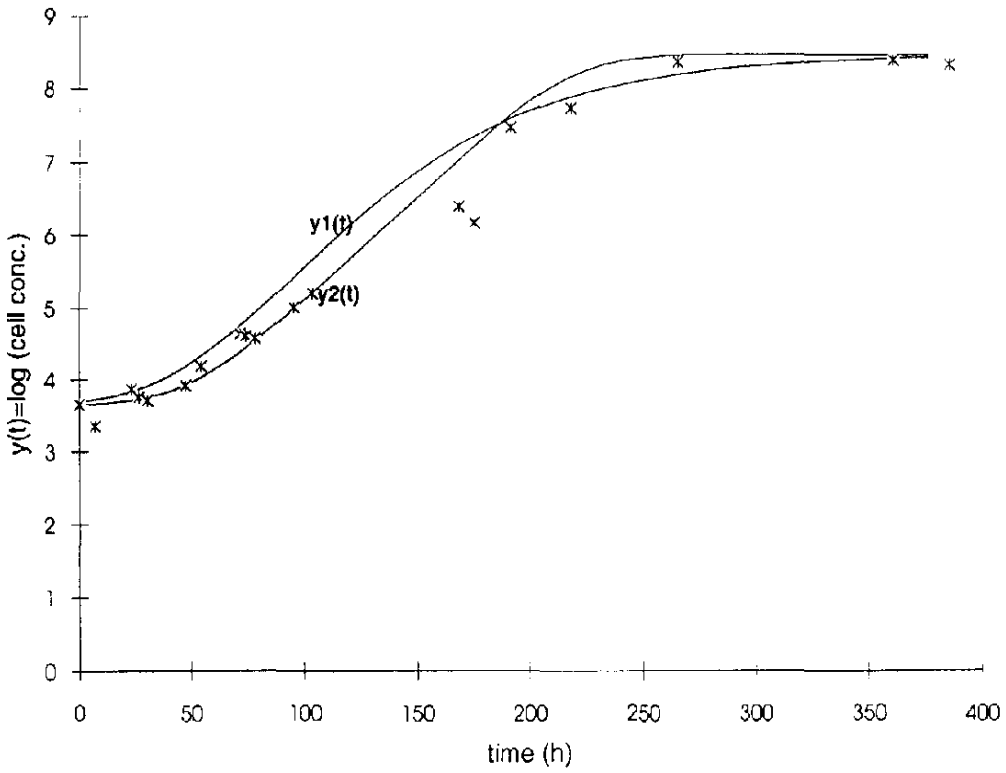

Fig. 4. Experimental data and growth curves produced by different models. Data (*) are those of curve No. 1 in McClure et al., 1993: *Brochothrix thermosphacta*, at temperature = 1°C, pH 5.6, salt 0.7%. When constructing the curve $y_1(t)$, the environment dependence of the lag and the maximum specific growth rate were modelled independently of each other (21 model coefficients). Curve $y_2(t)$ is generated by the new model, where the product of the lag and the maximum specific growth rate is constant, independently of the environment (12 model coefficients).

maximum specific growth rate is overestimated, because the correlation between them is not taken into account in the model. The accumulated effect can result in predicted growth being much faster than the experimental curve. In our approach, in constant environment, the lag is per se inversely proportional to the maximum specific growth rate (see formula (8)) which defines the correlation between them.

It is worth noting that also the average difference between the growth rates predicted by the new model and those from independent literature data, tabulated in McClure et al. (1993), decreased by about 20%.

The questions, how well the model can predict the course of growth when E_2 is varying with time, and to what extent factors may change but the model still provide acceptable predictions, are published in Baranyi et al. (1994). In that paper, the necessary model parameters are estimated from data collected in constant-temperature-experiments then, by means of those parameters, growth curves are predicted for the situation when the temperature changes with time during the growth. The predictions are then compared with experimental data.

3.4. Possibilities of further developments

One of the crucial assumptions in this paper is that the Michaelis–Menten constant of the critical substance, K_P, is independent of the actual environment,

E_2, so the physiological state of the cells can be characterized by just one variable, $q(t) = P(t)/K_P$. This is obviously an oversimplification and is likely to be modified as the model develops further. For example, one consequence of that simplifying assumption was that, if the actual environment, E_2 is constant, the product of the lag and the maximum specific growth rate, h_0, is independent of E_2. Therefore an important area for further research is to decide in what region of environmental factors the lag and the maximum specific growth rate are inversely proportional, i.e. the value of K_P is constant.

The $\mu_{max}(E_2)$ function has a central role in the system (7a), (7b), (7c). It is outside the scope of this paper to investigate what model should be used for that function. If E_2 involves the temperature only, then the Arrhenius- and the Square-root model are the best known relationships. These, and several alternatives to modelling the combined effects of temperature, pH and a_w, are listed in McMeekin et al. (1993). The quadratic response surface applied for the dataset of McClure et al. (1993) is virtually a simple representation of the collected data and it is appropriate to analyze the 'smoothness' of those data. This is why we can say that, among other uses, our model could 'damp' the variance of the measured data. Nevertheless, the improvement of the above dynamic approach depends very much on the quality of the $\mu_{max}(E_2)$ model.

One advantage of mechanistically derived models is that it is easier to develop them further as the quantity and quality of the information on the analyzed system increases. The present paper, derived from a fundamental growth model of population dynamics, supports this statement.

Acknowledgement

This paper was supported in part by the FLAIR Project AGRF-CT91-0047.

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