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Modeling bacterial growth responses

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SUMMARY

The main steps of modeling bacterial growth responses are summarized and a new model for growth curves is shown. Its advantages are analyzed from some theoretical and practical points of view. The new model fits better and has more advantageous statistical properties than the Gompertz curve.

INTRODUCTION

A first approach to modeling bacterial growth uses empirical techniques. Those models can be analyzed from certain statistical, numerical and computational points of view, such as goodness of fit, standard errors of the estimated parameters, linearity of the estimator, and so on. Variance analysis can help to identify the main environmental factors (viz. temperature, pH, water activity) controlling bacterial growth.

In a batch culture under constant environmental conditions, the bacterial growth can generally be characterized by a sigmoid curve where the dependent variable is the logarithm of the viable cell concentration. At a given time the slope of that curve gives the instantaneous specific growth rate, which can be considered as the cells' per capita rate of division [6]. One of the most important characteristics of an organism in a given environment is the maximum specific growth rate at the inflexion point of the sigmoid curve. Another important parameter is the length of the lag period, which is usually defined as the intercept of the tangent, drawn to the inflexion, with the lower asymptote of the sigmoid curve [11].

Alternative approaches to measuring the amount of microbial growth use biomass, turbidity, conductance, etc. (or their logarithm) as the time-dependent variable. It is worth noting that the slope of a growth curve of this kind is generally not equivalent to the above defined (specific) growth rate. To compare this approach with the viable count models, it is necessary to find the quantitative connection between the measured quantity and the viable cell concentration.

Fitting growth curves by the Gompertz function is widely used and discussed in the literature [1,4,5,7,8,14] and has proved to be very useful, as a first approach, on a range of organisms and controlling factors. However, it suffers a number of disadvantages. Because the Gompertz function is fitted to the logarithm of the cell concentration, and not to the original number, this is strictly an empirical model and it has no connection with the well-defined Gompertz growth of the cells [9]. As a consequence, the Gompertz curve cannot produce an essentially straight line in the exponential phase. Instead, it has a definite curvature around the inflexion and, therefore, it suggests a maximum specific growth rate that is higher than might be expected.

Another disadvantage originates from the rather geometrical definition of the lag phase. Because the slope of the Gompertz function cannot be zero, the lower asymptote of the sigmoid curve must be below the inoculum level, so for some datasets the estimate of the duration of the lag can give a negative number.

The second stage of developing a model is to fit a response surface to one or more parameters of the sigmoid growth curves applied in the first stage. For example, the environment-dependence of the main parameters of the fitted growth curves (maximum specific growth rate, lag time) can be modeled by multivariate quadratic functions. This makes it possible to predict the growth responses of an organism growing under specified environmental conditions inside the experimental region, including conditions where no experimental data exist.

A new growth function

Here we show a new growth model which aims, first of all, to give a simple, but more mechanistic, definition for the duration of the lag period. The mathematical properties of the model have been published elsewhere [2].

Theory

Our starting point is the assumption that a constant physical environment, E, unambiguously defines a time-dependent growth curve, $x(t)$, described by the first order autonomous differential equation

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$$\dot{x} = \mu(x)x \tag{1a}$$

with the initial value

$$x(0) = x_0 \qquad (0 < x_0 < x_{\max}) \tag{1b}$$

where x denotes the cell concentration, assumed to be homogenously distributed in the living space, and $\mu(x)$ is the so-called specific growth rate where:

$\mu(x)$: $[0,x_{\max}] \to R$
$\mu(x)$ is continuously differentiable on $(0,x_{\max})$
$d\mu/dx$ is strictly negative on $(0,x_{\max})$
$\mu(x_0) > 0$ and $\mu(x_{\max}) = 0$

[13]. As is well-known, under these conditions the above differential equation has a unique solution, and that solution is monotone increasing and converging to $x_{\max}$ as $t \to \infty$.

Turner et al. [12] published a formula for $\mu(x)$, called 'generic equation', which is general enough to include most of the well-known sigmoid functions (logistic, Gompertz, Richards, Bertalanffy, etc.).

A typical experiment, regularly carried out in food microbiology laboratories, would be first to grow the bacteria under favorable environmental conditions, E_1 (in the primary culture), to get an appropriate amount for the inoculation. It is then inoculated and held in a different, but constant, physical environment, E_2, in a batch culture. Before the inoculation, the cells grow exponentially in E_1, then, after a certain lag period, they again grow exponentially in E_2, although often at a different specific growth rate, until they reach the stationary phase.

Let us fix the moment of inoculation as zero time. Suppose that before the zero time the environment, E_1, was significantly different from the actual environment, E_2.

We postulate that after inoculation the cell concentration of the culture is described by the following, so-called initial value problem (differential equation with initial values):

$$\dot{x} = \alpha(t)\mu(x)x \quad (0 \le t < \infty; 0 < x) \tag{2a}$$

$$x(0) = x_0 \quad (0 < x_0 < x_{\max}), \tag{2b}$$

where (a) $\mu(x)$ is determined by E_2 and it satisfies the conditions assumed under Eqns 1a and 1b and (b) $\alpha(t)$ depends on E_1 and E_2 and for $0 \le t < \infty$:

$0 \le \alpha(t) \le 1$;
$\alpha(t)$ is monotone increasing; and
$\alpha(t) \to 1$ $(t \to \infty)$.

We call $\mu(x)$ the *potential specific growth rate* and $\alpha(t)\mu(x)$ the *actual specific growth rate*. Furthermore $\alpha(t)$ is called the *adjustment function of* E_2 referring to E_1.

The solution of the autonomous counterpart of Eqns 2a, 2b (i.e. that of Eqns 1a, 1b) is independent of E_1. We call it the *potential growth curve*. Generally the solution of the above initial value problem depends on E_1 as well as on E_2. We call it the *actual growth curve*.

It can be shown mathematically (see [2]) that if $f(t)$ denotes the solution of the initial value problem (Eqns 1a, 1b) and $\alpha(t)$ is an adjustment function, then the $g_\alpha(t)$ solution of the initial value problem (Eqns 2a, 2b) is

$$g_\alpha(t) = f(A(t))$$

where

$$A(t) = \int_0^t \alpha(\tau)\mathrm{d}\tau$$

For practical purposes, a class of adjustment functions of the form

$$\alpha_n(t) = \frac{t^n}{\lambda^n + t^n}$$

where λ and n are (positive) model parameters, was shown to be very effective [3]. This adjustment function can be derived in the following way.

Suppose that there exists a critical substrate or product, say P, which is responsible for the bottleneck of growth. Suppose that the dependence of growth on this product follows the well-known Michaelis–Menten rule:

$$\dot{x} = \frac{P(t)}{K_P + P(t)}\mu(x)x$$

where K_P is the Michaelis–Menten constant.
If $P(t)$ is monotone increasing then

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

can play the role of the adjustment function.

Suppose that $P(t)$ is built up from a negligibly small quantity and around K_P its accumulation is of n-th order according to the following normalized formula

$$\frac{P(t)}{K_P} \approx \left(\frac{t}{\lambda}\right)^n$$

This gives the idea for the approximation:

$$\psi(t) = \frac{P(t)}{K_P + P(t)} \approx \frac{t^n}{\lambda^n + t^n} = \alpha_n(t)$$

We call $\alpha_n(t)$ as n-th order adjustment function.
Let the adjustment function in Eqns 2a, 2b be $\alpha_n(t)$ as introduced above. Let

$$B_n(t) = \int_0^t \frac{1}{1 + t^n}\mathrm{d}t \tag{3}$$

For the solution of Eqns 2a, 2b, we obtain:

$$g_{\alpha_n}(t) = f(A_n(t)) \tag{4}$$

where

$$A_n(t) = \int_0^t \frac{s^n}{\lambda^n + s^n}\,\mathrm{d}s = \lambda\left(\frac{t}{\lambda} - B_n\left(\frac{t}{\lambda}\right)\right) \tag{5}$$

Theoretically the integral function $B_n(t)$ can be expressed by elementary functions for a fixed positive integer, n, but the higher is n the more complicated is $B_n(t)$. For bacteriological data representing a broad range of growth conditions for a variety of organisms, an adjustment function of order $n = 4$ proved to be satisfactory to characterize the transition from the lag to the exponential phase. In this case the expression for $B_4(t)$ is:

$$B_4(t) = \frac{1}{2\sqrt{2}}\left(\frac{1}{2}\ln\frac{t^2 + \sqrt{2}t + 1}{t^2 - \sqrt{2}t + 1} + \gamma(t)\right) \tag{6}$$

where

$$\gamma(t) = \begin{cases} \arctan\dfrac{\sqrt{2}t}{1 - t^2} & (t < 1) \\ \pi/2 & (t = 1) \\ \arctan\dfrac{\sqrt{2}t}{1 - t^2} + \pi & (t > 1) \end{cases} \tag{7}$$

RESULTS

The new growth model has several advantageous features compared with the previous approaches.

(1) If we know the explicit solution of the autonomous part which describes the potential growth, then we can also derive the explicit solution for the actual growth, so it is not necessary to solve the differential equation numerically. The simplest case is when the potential growth is the pure exponential growth. If this is combined with our adjustment function then the following model can be obtained:

$$y(t) = y_0 + \mu_{\max} A_n(t)$$

where $y(t) = \ln x(t)$ and $A_n(t)$ is given by Eqns 3 and 5. Therefore the model has the following parameters:

- y_0: logarithm of the initial cell concentration ($= \ln x_0$)
- $\mu_{\max}$: maximum specific growth rate
- λ: lag-parameter
- n: curvature-parameter

This model describes only the lag and the exponential phase. This can be useful when there are no data in the stationary phase. To estimate the maximum growth rate and the lag, it is not necessary to accumulate data in the stationary phase. This is a considerable advantage over using a sigmoid function which is noticeably dependent on data points around the upper asymptote.

Fitting curvature parameter, n, can cause computational difficulties. For the sake of simplicity its value can be fixed as $n = 4$ which has proved to be a good compromise between the goodness-of-fit and convenience. In this case $A_4(t)$ is given by Eqns 5, 6 and 7. The situation is similar to that of the Richards curve after the exponential phase [12]. There the value $m = 1$ is the most common choice for the curvature parameter which corresponds to the logistic curve. Here the curvature parameter $n = 4$ is suggested as a simple but well fitting choice. However, if the culture shows a sudden transition after the lag period then a higher (but fixed) curvature parameter may be needed. Even in this case it is not necessary to integrate the $\alpha_n(t)$ adjustment function numerically in every step of the curve fitting procedure. For $n > 1$ the $B_n(t)$ integral in Eqn 3 is bounded and it is sufficient to calculate some of its values only once and then use those values for interpolation.

(2) Choosing the logistic growth as potential growth and the fourth order adjustment function ($n = 4$), the following explicit growth function can be derived:

$$y(t) = y_{\max} - \ln\left(1 + (e^{y_{\max} - y_0} - 1)e^{-\mu_{\max} A_4(t)}\right)$$

with the notations of Eqns 5, 6 and 7. The parameters of this model are:

- y_0: logarithm of the initial cell concentration ($= \ln x_0$)
- $\mu_{\max}$: maximum specific growth rate
- λ: lag-parameter
- $y_{\max}$: logarithm of the maximum population density ($= \ln x_{\max}$).

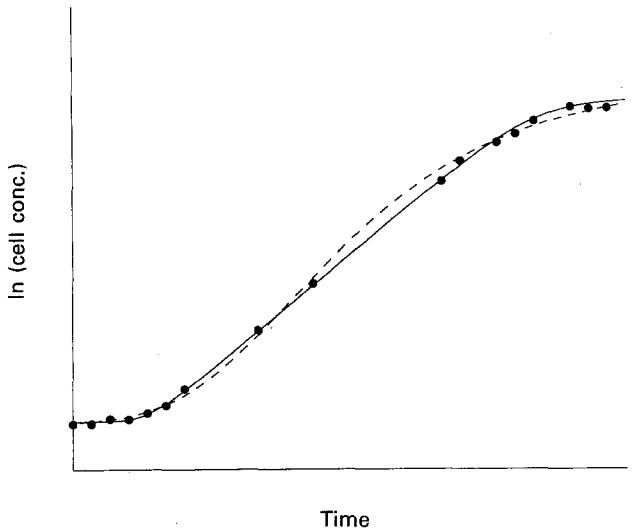

Fig. 1. A salmonellae growth curve (code = 1 in [8]) fitted by the Gompertz-function (- - - - -) and by the new model(————).

An example of this growth curve is shown in Fig. 1. (A more complicated version of the new model could be

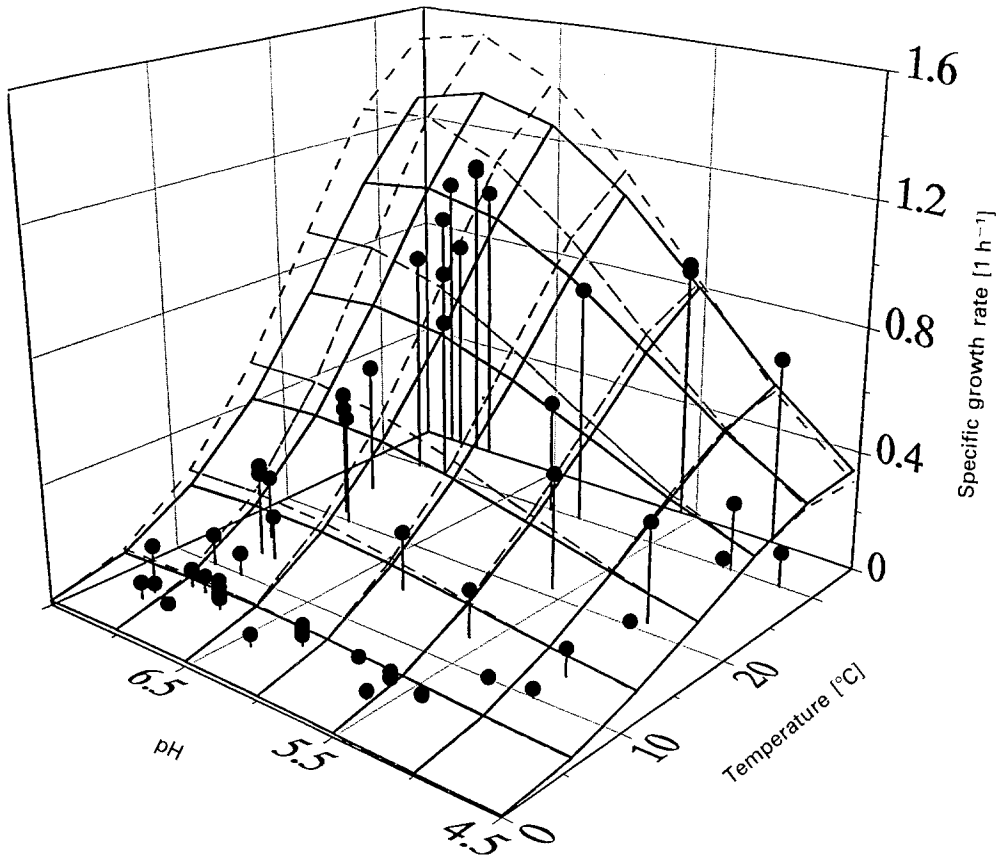

Fig. 2. Response surfaces if the Gompertz-function was applied to determine the maximum specific growth rate (– – – –), or the new model was fitted to experimental growth curves (————). The closed circles represent growth rates collected from the publications of different authors.

obtained by choosing some other function of Turner et al. [12], as potential growth.)

The above model can be compared with the earlier Gompertz approach. Both are four-parameter models. It has been shown that the goodness-of-fit and the standard errors of the estimates are generally better than the respective statistical characteristics of the Gompertz curve fitting [3].

(3) The new concept provides an explanation why it is more difficult to model the lag than the maximum specific growth rate. First, in the new model the duration of the lag depends, in part, on the previous growth environment. Secondly, the estimation of the lag is equivalent to the estimation of the time when the critical product reaches its Michaelis–Menten constant, K_P. As is well-known, the identification of K_P frequently leads to ill-conditioned problems [10] and this property is transferred to the adjustment function.

(4) It can be proved mathematically [3] that our λ parameter is very close to the time where the second derivative of the growth function is at maximum. A definition for lag period, as the time during which the second derivative of the growth curve reaches its maximum, was recently proposed [4]. Now we suggest a more mechanistic definition of the lag: the end of the lag period is the time when the critical product reaches its Michaelis–Menten constant, K_P.

(5) Because the adjustment function is zero at the inoculation, it is impossible to obtain negative estimates for the lag when using the new model.

(6) In the exponential phase the growth is represented essentially by a straight line (Fig. 1). This is why it estimates a lower maximum specific growth rate than the Gompertz function, which has a pronounced curvature around the inflexion.

The consequence is that the response surface fitted to the Gompertz-type maximum specific growth rate, is generally above the response surface fitted to the respective parameter of the new model. In Fig. 2, the dashed surface is the response surface when the Gompertz-approach was used to model the growth of *Listeria monocytogenes* and the surface defined by the continuous line was obtained using the new model. The closed circles represent growth rates collected from different literature data and they are independent of our experiments. It can be seen that the exaggerated predicted values for the growth rate were the result of using the Gompertz function. The new model generally predicts growth rates c. 10% lower, which bring the predictions very close to the growth rates estimated by other authors.

In view of its computational and statistical advantages it is recommended that the new model be used in fitting growth curves from viable count data in preference to the Gompertz function.

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