Food Microbiology, 1993, **10**, 43–59

A non-autonomous differential equation to model bacterial growth

J. Baranyi, T. A. Roberts and P. McClure*

AFRC Institute of Food Research, Reading, Laboratory, Earley Gate, Whiteknights Road, Reading RG6 2EF, UK

Received 26 April 1992

In order to describe the dynamics of growing bacterial cultures a non-autonomous differential equation is applied. The model describes the lag phase as an adjustment period and for the lag-parameter a new definition is introduced. Some mathematical aspects of the model are described and, on the basis of more than 500 growth curves, its statistical properties are compared with the Gompertz-approach commonly used in food microbiology.

Introduction

Several approaches to modelling bacterial growth can be found in the microbiological literature. Following the classification of Roels and Kossen (1978) we deal here with unstructured, non-segregated growth models. This means that we suppose that (1) the biomass is homogenous; (2) the mass concentration is the only dependent variable of the system; and (3) environmental parameters like temperature, chemicals, etc. are not involved in the model (the possible dependency on them is expressed through the dependency on the mass concentration).

In food microbiology several basic sigmoid functions (logistic, Gompertz, etc.; see, for example France and Thornley 1984), as empirical models, have become widely used. A comparative study about them was published by Zwietering et al. (1990). However, with the use of more and more exact methods in microbiology, the demand for less empirical growth models is increasing (Whiting 1992).

The commonly used simple growth model of population theory is a first order ordinary differential equation where the growth rate does not depend on time directly (autonomous, non-segregated, unstructured models). As an example one can consider the model of Turner et al. (1976), which is general enough to include the logistic, Gompertz, Richards, Bertalanffy etc. growth functions.

The typical representation of a bacterial batch culture is to plot the logarithm of the cell concentration against time and, in most cases, the result is a sigmoid curve. A possible empirical solution is to fit a basic sigmoid function, augmented with an additive term, to these data. This way was followed by many authors in recent years in food microbiology (among them Gibson et al. 1988, Buchanan and Cygnarowicz 1990, Zwietering et al. 1990). We should keep in mind that in this case these models should not be referred to as the logistic, Gompertz etc. growth models which are meant to apply to the cell concentration and not to its logarithm.

In this paper we show a new way to apply fundamental growth models

*Present address: Unilever Research, Colworth Laboratory, Colworth House, Sharnbrook, Bedford MK44 1LQ, UK.

0740–0020/93/010043 + 17 $08.00/0

generally accepted in population theory. A mathematically formalized approach is shown which assumes that a given environment determines the *potential growth rate* of the culture that is higher than the *actual growth rate* if the time is close to the inoculation. The ratio of the actual and the potential growth rate characterizes the process of adjustment of the cells to the new environment. The most important mathematical theorems on the model have been shown in Baranyi et al. (1992) and are collected in the Appendix.

Before starting an experiment to study bacterial growth in a given environment the cells are normally grown in a favourable, more or less optimal environment, in the so-called subculture, to a get a sufficient amount of cells for inoculation. Denote the pre-inoculation environment (the subculture) by E_1 and the actual (post-inoculation) environment by E_2. Suppose that E_1 is sig-nificantly different (in this case more favourable) from the actual environment, E_2. Our aim is to create a model which describes the lag as the process of adjustment to the new environment. Note that this approach already suggests a non-autonomous model, because it takes a sudden external effect on the system into account.

The mathematical formalization of the above concept can be outlined as follows.

We can consider the time of inoculation as zero time. Suppose that if the effect of E_1 is neglected (or, which is the same, $E_1 = E_2$) then some well-known autonomous differential equation of the form

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

describes the bacterial growth in E_2, where x denotes the cell concentration, $\mu(x)$ denotes the specific growth rate. In this case the growth rate does not depend on time directly, only through the cell concentration. This basic assumption is an implication of the fifth hypothesis of the framework of Frederickson et al. (1967). Multiply the right-hand side of this differential equation by a smooth function (adjustment function) having the property that it is closer and closer to 1 as the experimental time passes. This operation expresses our wish to describe the gradually diminishing effect of the previous environment. The result is a non-autonomous, separable, first order ordinary differential equation. In this way the lag phase (adjustment) is formally separated from the exponential and stationary phase which can be regarded as parts of the potential growth defined by the autonomous model.

It is especially important in food safety investigations to investigate the duration of the lag phase. The classical definition of the lag (Pirt 1975) assumes that the logarithm of the cell concentration forms a sigmoid curve against time and the intercept of the tangent at the inflexion with the lower asymptote is considered as the turning point indicating the end of the lag phase.

By means of the adjustment function we will give a less geometrical definition for the lag. Our lag parameter, as well as the maximum specific growth rate of the new model, will be compared with other definitions of the respective parameters.

In many respects the new model is a generalization of other models already used in the literature.

Theory

In most papers on unstructured, non-segregated models of population dynamics the starting point is the assumption that the growth of a population in a given environment is described by the first order ordinary differential equation

$$\dot{x} = \mu(x)x \quad (0 \le t < \infty; 0 < x) \qquad (1a)$$

with the initial value

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

where x is the number of individuals per

unit (concentration). The quantity $\mu(x)$ is called the specific growth rate and for the classical growth functions it is assumed that

(a) $\mu(x)$ is defined for $x_0 \le x \le x_{max}$ (x_{max} is fixed)
(b) $\mu(x_0) > 0$ and $\mu(x_{max}) = 0$
(c) $\mu(x)$ is continuously differentiable in its domain and $d\mu/dx$ is strictly negative there.

Under these conditions the so-called *initial value problem* defined above (differential equation together with its initial value) has a unique solution, denoted here as $f(t)$. This solution is monotone increasing and converges to x_{max} as $t \to \infty$ (Vance 1990).

Equation (1a) is a so-called autonomous differential equation because its right-hand side depends on the unknown dependent variable itself and it does not depend on time directly. Its general solution takes the form $x(t) = f(t-T)$, where T is a constant characterizing a shift parallel to the t axis (it can be considered as a delay in growth). The value of T can be uniquely defined by fixing an initial value for $x(0)$.

Depending on the choice of $\mu(x)$, different sigmoid functions can be obtained which satisfy (1a). Note that the condition (c) corresponds to the assumption that the larger the population density the lower is its specific growth rate. As can be shown mathematically, this means that the time-derivative of the logarithm of the wanted growth function is strictly monotone decreasing.

Consider the experimental time (i.e. the time elapsed from inoculation) as the independent variable, t. Fix the time of the inoculation as $t = 0$. Our model will be defined for non-negative values of t in the following way.

We postulate that after inoculation the cell concentration of the culture is described by the initial value problem

$$\dot{x} = \alpha(t)\mu(x)x \quad (0 \le t < \infty;\ 0 < x) \tag{2a}$$
$$x(0) = x_0 \quad (0 < x_0 < x_{max}) \tag{2b}$$

where:

(a) $\mu(x)$ is independent of E_1 and satisfies the conditions assumed under (1a) and (1b).
(b) $\alpha(t)$ depends on E_1 and E_2 and
$$0 \le \alpha(t) \le 1 \quad (0 \le t < \infty). \tag{3}$$

Furthermore if $\alpha(t) \to 1$ monotone increasingly as $t \to \infty$ then we say that $\alpha(t)$ is an *adjustment function* from E_1 to E_2.

We say that (1a), (1b) define the *potential growth*, and (2a), (2b) define the *actual growth* in the environment E_2.

The interpretation of these definitions is as follows: The given, actual environment E_2 and the inoculum level, x_0, uniquely determine the potential growth curve, according to which the population would be able to grow if the previous environment had been the same as the present environment ($E_1 = E_2$: no need to adjust, that is $\alpha(t) \equiv 1$). The potential growth of the population is described by the autonomous equation (1a). The actual growth, however, is described by (2a) and (2b) which means that after inoculation (a sudden change in the environment from E_1 to E_2), the cells' actual specific growth rate is heavily influenced by the fact that the time is close to zero. Later, however, the effect of the previous environment diminishes, until some time after the inoculation it has little or no effect and the cells grow essentially at their potential growth rate, $\mu(x)$, defined by the new environment, E_2. Therefore the ratio of the actual and the potential growth rate, i.e. the adjustment function, is expected to increase from zero (no growth because of lagging) to 1 (total adjustment).

Theorem 1 in the Appendix gives the solution $g(t)$ for the actual growth curve by means of the functions f and α. Its form is:

$$g(t) = f(A(t)), \tag{4}$$

where $A(t)$ is the integral function of $\alpha(t)$.

A class of adjustment functions of the form

$$\alpha_n(t) = \frac{t^n}{\lambda^n + t^n}, \qquad (5)$$

where λ and n are positive numbers, proved to be generally very effective when fitting our viable counts data. This adjustment function can be derived in the following way:

Suppose that the growth is controlled by a critical substrate, or product, say P, which is vital to ensure growth in the new environment and it was present in a negligibly small amount in the previous environment. Furthermore suppose that the dependence of growth on this product follows the well-known Michaelis-Menten rule:

$$\dot{x} = \frac{P(t)}{K_p + P(t)} \mu(x)x, \qquad (6)$$

where K_p is the so-called saturation constant. This gives the adjustment function the form:

$$\alpha(t) = \frac{P(t)}{K_p + P(t)}. \qquad (7)$$

After rearrangement:

$$\alpha(t) = \frac{\frac{P(t)}{K_p}}{1 + \frac{P(t)}{K_p}}. \qquad (8)$$

The quantity $P(t)/K_p$ is dimensionless.

Suppose that after inoculation the production of the critical product depends on E_2 and is proportional to the n-th power of time:

$$\frac{P(t)}{K_p} = \left(\frac{t}{\lambda}\right)^n \qquad (9)$$

Here n characterizes the rate at which P is built up around $t = \lambda$ (the larger is n, the faster is the accumulation of P) where λ is the time point were $P = K_p$. Substitute the latter expression in (7) and the adjustment function of the form (5) can be obtained.

The interpretation above explains the next definitions. We call the parameter λ of the adjustment function of the form (5) the *lag parameter*. In what follows we refer to $\alpha_n(t)$ as *adjustment function of order n*.

Note that Theorem 2 in the Appendix gives an estimation of what happens if the adjustment function is derived in a different way. Roughly it can be said that if two adjustment functions are close to each other (this 'closeness' is measured by the integral of their difference), then the respective actual growth curves will also be close to each other.

Some properties of the adjustment function (5) are:

- $\alpha_n(0) = 0$; $\alpha_n(\lambda) = 1/2$
- $\alpha_n(t)$ is strictly monotone increasing
- $\alpha_n(t) \to 1$ $(t \to \infty)$
- if $n > 1$ then
  a/ $d\alpha_n/dt = 0$ at $t = 0$
  b/ $\alpha_n(t)$ has an inflexion point at

$$T_n = \sqrt[n]{(n-1)/(n+1)}\ \lambda. \qquad (10)$$

Generally it simplifies the calculation of the $A_n(t)$ integral function if the next relation is used:

$$A_n(t) = \int_0^t \frac{s^n}{\lambda^n + s^n} ds = \lambda \left(\frac{t}{\lambda} - B_n\left(\frac{t}{\lambda}\right)\right) \qquad (11)$$

where,

$$B_n(t) = \int_0^t \frac{1}{1 + s^n} ds. \qquad (12)$$

Theoretically the integral function $B_n(t)$ can be expressed by elementary functions for a fixed positive integer, n, but for larger values of n the expression

is more and more complicated. For bacteriological data representing a broad range of growth conditions for a variety of organisms, an adjustment function of order 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:

$$B_4(t) = \frac{1}{2\sqrt{2}}\left(\frac{1}{2}\ln\frac{t^2+\sqrt{2}\,t+1}{t^2-\sqrt{2}\,t+1} + \gamma(t)\right) \tag{13}$$

where,

$$\gamma(t) = \begin{cases} \arctan\dfrac{\sqrt{2}\,t}{1-t^2} & (t<1) \\ \pi/2 & (t=1) \\ \arctan\dfrac{\sqrt{2}\,t}{1-t^2}+\pi & (t>1) \end{cases} \tag{14}$$

(See, for example, Korn and Korn 1973).

Some theoretical aspects of this adjustment function are summarized in Theorems 4–5 in the Appendix. Since the adjustment function, $\alpha_n(t)$, is the derivative of $A_n(t)$, and $\alpha_n(t)$ converges to 1 as the time elapses, $A_n(t)$ plays the role of delayed time; i.e. $A_n(t)$ is closer and closer to the function t-λ and the actual growth function is closer and closer to a delayed potential growth function, $f(t$-$\lambda)$. Therefore, this delay, λ, can be considered as a good approximation of the length of the lag phase in the actual growth.

Now we examine two important classes of autonomous growth as potential growth.

(a) Pure exponential growth as potential growth

When collecting data from experiments, sometimes, for one reason or other, there are no data to indicate the stationary phase of the growth curve. In these cases a computer program fitted a sigmoid curve to the data can easily fail because of the lack of information to estimate the parameter characterizing the stationary phase. Still it is desirable not to waste the results of these experiments. The solution can be either to fix the parameter characterizing the stationary phase or to have a growth function which models only the lag and the exponential phase.

The new approach introduced in this paper is suitable to get a growth function of the latter type. For this purpose consider the pure exponential growth as potential growth and combine it with our adjustment function given in (5). (Although in this case the condition (c) under (1a) and (1b) is not satisfied, according to the Theorems in the Appendix the connection between the potential and actual growth remains the same as above). The respective equations are:

Potential growth curve (solution of (1a)–(1b)):

$$f(t) = x_0 exp\,(\mu_{max} t).$$

Actual growth curve (solution of (2a)–(2b)):

$$g(t) = x_0 exp\,(\mu_{max} A_n(t)).$$

where $A_n(t)$ is defined by (11), (12).

In the study of batch cultures in food microbiology it is more common to consider the logarithm of the cell concentration as the dependent variable. If this is the natural logarithm:

$$y(t) = \ln x(t),$$

then the slope of the tangent of the $y(t)$ curve will be the specific growth rate. Transform the actual growth curve into the y,t plane:

$$y(t) = y_0 + \mu_{max} A_n(t) \tag{15}$$

where $y_0 = \ln x_0$.

By means of the obtained model (15)

we can demonstrate the role of the parameters λ and n also in the following way:

(i) *Second-derivative-concept.* Recently, Buchanan and Cygnarowicz (1990) defined the end of the lag phase as the time point where the second derivative of the logarithm of the actual growth curve has its maximum, i.e. where the third derivative of $A_n(t)$ is zero. Since $\alpha_n(t)$ is the first derivative of $A_n(t)$, the point in question is the inflexion point T_n of the $\alpha_n(t)$ adjustment function. It can be seen from Eqn (10) that in our adjustment function, T_n is very close to λ (for example, for $n = 4$: $T_4 \approx 0{\cdot}9\ \lambda$). In fact from (10) it follows that $T_n \to \lambda$ monotone increasingly as $n \to \infty$. This demonstrates again why we can call out λ parameter the lag parameter. (Note that the classical lag definition by means of the tangent drawn to the inflexion of the growth curve could not be applied here because the growth curve itself has no inflexion!)

(ii) *Step-function-concept.* It can be checked that $\alpha_n(t) \to \alpha_\infty(t)$ as $n \to \infty$, where $\alpha_\infty(t)$ is the step function

$$\alpha_\infty(t) = \begin{matrix} 0 & (t < \lambda) \\ 1/2 & (t = \lambda) \\ 1 & (t > \lambda). \end{matrix} \qquad (16)$$

This function was implicitly used by Kono (1968) to model the lag phase. There the author used a constant factor which was 0 for $t < t_1$ and 1 for $t > t_1$, where t_1 was a 'suitable' time (see also Barford et al. 1982). In our model λ plays the role of t_1. For $\alpha_\infty(t)$ the definite integral function is $A_\infty(t)$, where

$$A_\infty(t) = \begin{matrix} 0 & (t \le \lambda) \\ t - \lambda & (t \ge \lambda) \end{matrix} \qquad (17)$$

(Theorem 4 in the Appendix).

Therefore the actual growth curve of our model converges to the function $\mu_{max}A_\infty(t)$ as $n \to \infty$. The parameter n characterizes the curvature of the growth curve at the transition between the lag and the exponential phase (Fig. 1). If n is large then the transition is practically a breakpoint, just as in the model of Kono (1968).

(b) Logistic growth as potential growth

For another example consider the well-known logistic growth model as potential growth. In this case

$$\mu(x) = \mu_{max}\left(1 - \frac{x}{x_{max}}\right) \qquad (18)$$

where μ_{max} is the limit of $\mu(x)$ as $x \to 0$. The respective equations are:

Potential growth curve (solution of (1a)–(1b)):

$$f(t) = \frac{x_{max}}{1 + \left(\frac{x_{max}}{x_0} - 1\right)e^{-\mu_{max}t}} \qquad (19)$$

Actual growth curve (solution of (2a)–(2b)):

$$f(t) = \frac{x_{max}}{1 + \left(\frac{x_{max}}{x_0} - 1\right)e^{-\mu_{max}A_n(t)}} \qquad (20)$$

where $A_n(t)$ is defined by (11), (12).

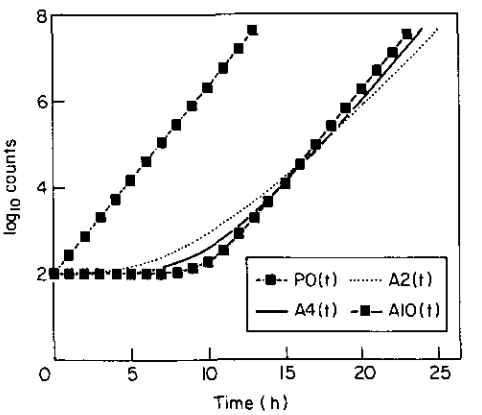

Fig. 1. Influence of the parameter n on the growth curve simulated by the new model. P0(t), Potential growth curve. $Ai(t)$, Actual growth curve at $n = i$ ($i = 2, 4, 10$).

As with (15), we transform the obtained model (20) to the y,t plane, where $y(t) = \ln x(t)$.

We get:

$$y(t) = y_{max} - \ln\left(1 + (e^{-y_{max}-y_0}-1)e^{-\mu_{max}A_n(t)}\right), \quad (21a)$$

where $y_{max} = \ln x_{max}$.

As can be checked, the formula above can be written in another form:

$$y(t) = y_0 + \mu_{max}A_n(t) + \ln\left(1 + \frac{e^{\mu_{max}A_n(t)}-1}{e^{y_{max}-y_0}}\right) \quad (21b)$$

This other form of the same function highlights the connection with the case, when the pure exponential growth was used for potential growth (see Eqn (15)). It is well-known that if $h << 1$ then

$$\ln(1 + h) \approx h$$

therefore the formula (21b) shows that close to the inoculation, where the fraction in (21b) is small, practically there is no difference between (15) and (21a) — that is, the maximum population does not yet influence the growth. In the Discussion we return to the computational consequences of this connection.

Without going into detail, we mention the possibility of choosing the more general Richards-curve (see for example, France and Thornley 1984) as potential growth. It differs from the logistic curve in the sense that it has an extra (positive) parameter, denoted by m here, which characterizes the curvature before the stationary phase. The choice $m = 1$ is equivalent to the choice of the logistic curve. The formulae, respective to (21a) and (21b) can be written as:

$$y(t) = y_{max} - \frac{1}{m}\ln\left(1+(e^{m(y_{max}-y_0)}-1)e^{m\mu_{max}A_n(t)}\right). \quad (22a)$$

$$y(t) = y_0 + \mu_{max}A_n(t) + \frac{1}{m}\ln\left(1 + \frac{e^{m\mu_{max}A_n(t)}-1}{e^{m(y_{max}-y_0)}}\right). \quad (22b)$$

Results

Our concept will be demonstrated with the fourth order adjustment function $\alpha_4(t)$ combined with the logistic growth model above as potential growth curve. Choosing $n = 4$ for the order of the adjustment function has mainly computational advantages. By means of the Eqns (11), (14) it is possible to substitute an explicit expression for the actual growth curve in a curvefitting procedure.

We found that, generally, higher values of n (usually between 6 and 10) give better fit, but either fixing the curvature parameter n at an integer value larger than 4, or fitting its value to a parameter estimating procedure, needs some computational tricks and it is advisable to adjust some well-known codes (for example Press et al. 1990) to solve these problems. These computational aspects of our approach are being summarized presently in a separate paper.

From several points of view Zwietering et al. (1990) found the Gompertz curve the best from a range of growth models. Also Gibson et al. (1988) reported that the Gompertz function was the best fitting curve when analysing their data. This is why we chose the Gompertz function to compare it with one of our family of growth models: the logistic growth combined with fourth order adjustment function. In this case the formula for the logarithm of the cell concentration of a growing culture is given by (21) and (11).

Gibson et al. (1988) published the estimates of the maximum specific growth rate and the lag obtained by fitting four parameter Gompertz curves to a number of *Salmonella*-data. For a numeric

demonstration we selected the first 40 curves of that dataset. Our curvefitting procedure was run on the same datapoints.

Program 1 fitted four parameter Gompertz curves, as suggested by Gibson et ai. (1988), to the measured $\log_{10}$ counts by a standard least squares method using Marquardt's algorithm with single precision arithmetic (Press et al. 1990). The maximum specific growth rate was calculated as the slope of the tangent at the inflexion of the $y_1(t)$ curve where $y_1(t)$ is a fitted Gompertz function, augmented by an additive parameter (see Gibson et al. 1988). The lag was calculated as the intercept of this maximum slope with the lower asymptote of the curve. In what follows, μ_1 and λ_1 will denote the values of these growth parameters as estimated by *Program 1*.

Program 2 fitted our four-parameter model, a logistic potential growth with fourth order adjustment function, using the same Marquardt-method. The estimated growth parameters (μ_{max}, maximum potential growth rate; λ, lag-parameter of the adjustment function) will be denoted by μ_2 and λ_2 respectively.

In Table 1 we list the values which were the datapoints for the first curve, with code = 1, in Gibson et al. (1988). Fig. 2 shows the curves fitted by *Program 1* and *Program 2*.

The main differences can be summarized as follows:

(1) At the time $t = 0$ the slope, produced by the new model, is exactly zero (only non-autonomous models can have that property). This is a consequence of the fact that the value of our adjustment function is zero at the origin;
(2) The new model gave a practically straight line (logarithm of the logistic growth) in the exponential phase. This is a result of the construction: in this phase the potential growth is dominant;

Table 1. The datapoints of the *Salmonellae* growth curve with code = 1 in Gibson et al. (1988).

No.	Time (h)	Log counts ($\log_{10}$ ml^{-1})
1	0·00	3·39
2	1·17	3·39
3	2·00	3·47
4	2·92	3·46
5	3·92	3·57
6	4·96	3·70
7	5·96	3·98
8	8·08	5·41
9	10·2	4·96
10	13·1	5·74
11	19·8	7·45
12	21·3	7·79
13	22·8	8·10
14	23·8	8·24
15	24·7	8·46
16	26·7	8·69
17	27·7	8·66
18	28·8	8·67
19	29·7	9·16
20	31·3	8·69
21	32·8	8·76
22	49·8	8·78

(3) The curvature before the stationary phase is more pronounced in the new model. This, again, shows the difference

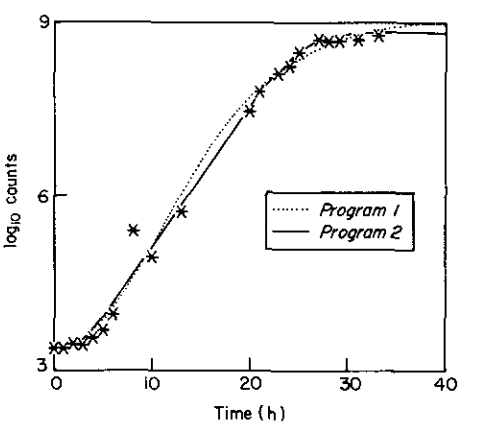

Fig. 2. Comparison of the fits given by *Program 1* and *Program 2* on viable count data (see Table 1). $\lambda_1 = 3{\cdot}78$ and $\mu_1 = 0{\cdot}69$ (estimate of *Program 1*); $\lambda_2 = 2{\cdot}58$ and $\mu_2 = 0{\cdot}56$ (estimate of *Program 2*).

between the curvature of the logarithm of the logistic curve and the curvature of the Gompertz curve before the stationary phase.

Before comparing *Program 1* and *Program 2* on a larger dataset we show an example for the use of the pure exponential growth as potential growth:

Leave out those points from the dataset in Table 1 where the $\log_{10}$ counts are larger than 4 (let only the first seven points remain). Fig. 3 shows a comparison between the results produced by *Program 1* and *Program 3* where *Program 3* is based on pure exponential growth as potential growth combined with fourth order adjustment function.

Here the estimated values, suggested by the Gompertz-fit for the maximum specific growth rate and the lag time, are unrealistically high. The reason is that the inflexion point lies outside the region of the seven experimental data. The new model gives much better estimates of both parameters.

Note that considering the maximum slope inside the experimental region as maximum specific growth rate could improve the results of *Program 1*, but even this cannot overcome the difficulty that it indicates an ill-conditioned problem.

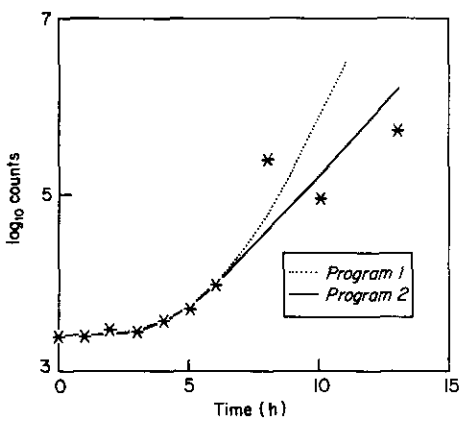

Fig. 3. Comparison of the fits given by *Program 1* and *Program 2* on the first seven datapoints of Table 1. $\lambda_1 = 6{\cdot}08$ and $\mu_1 = 1{\cdot}45$ (estimate of *Program 1*); $\lambda_2 = 4{\cdot}44$ and $\mu_2 = 0{\cdot}81$ (estimate of *Program 2*).

The more general comparison of *Program 1* and *Program 2* below will be based on the following estimates; the subscripts *1* and *2* indicating the Gompertz-curve (*Program 1*) and the new model (*Program 2*), respectively.

length of the lag phase	(λ_1, λ_2)
maximum specific growth rate	(μ_1, μ_2)
residual mean squares	(rms_1, rms_2)
standard error of lag	$(s(\lambda_1), s(\lambda_2))$
standard error of μ	$(s(\mu_1), s(\mu_2),$ respectively)

(see Table 2).

Comparing the outputs, the goodness-of-fit of the new model is generally at least as good as the goodness-of-fit of the Gompertz-curve. The residual mean squares were lower in the case of the new model in 35 cases. The lag and maximum specific growth rate estimated by the new model were slightly lower than in the Gompertz case. One reason for this is that in the exponential phase the Gompertz curve shows a curvature and our model is practically a straight line in this phase (a consequence of the fact the Gompertz curve should be used for the cell concentration rather than for its logarithm!). The estimated standard error of the lag was on average a little lower, and that of the maximum specific growth rate was generally much lower in the output of the new model.

A demonstrative way to represent the results of similar comparisons on a large number of growth curves is shown below. We ran the two programs mentioned above on more than 500 sets of growth data obtained from batch cultures of various kinds of micro-organisms (*Listeria, Salmonella, Yersinia, Escherichia coli*). The order of magnitude of the measured cell populations

Table 2. Comparison of the Gompertz-function (*Program 1*) and the new model (*Program 2*) using the first forty growth curves of the salmonellae data of Gibson et al. (1988).

code	rms_1	rms_2	λ_1	λ_2	$s(\lambda_1)$	$s(\lambda_2)$	μ_1	μ_2	$s(\mu_1)$	$s(\mu_2)$
1	0·24	0·20	3·8	2·6	1·28	0·82	0·69	0·56	0·054	0·026
2	0·12	0·10	14·	12·	1·49	1·06	0·27	0·25	0·031	0·007
3	0·26	0·24	4·9	4·0	0·69	0·84	1·18	1·02	0·157	0·116
4	0·19	0·19	75·	60·	12·8	12·3	0·04	0·04	0·005	0·003
5	0·13	0·09	7·4	5·5	0·76	0·52	0·58	0·49	0·043	0·013
6	0·18	0·13	7·3	5·6	1·05	0·74	0·57	0·49	0·057	0·019
7	0·09	0·08	7·6	5·7	0·49	0·46	0·60	0·51	0·030	0·013
8	0·10	0·10	7·4	5·5	0·55	0·54	0·59	0·50	0·034	0·015
9	0·11	0·10	7·0	4·9	0·67	0·55	0·58	0·49	0·037	0·013
10	0·12	0·09	7·2	5·3	0·68	0·49	0·58	0·49	0·040	0·013
11	0·12	0·08	7·8	6·0	0·65	0·49	0·58	0·50	0·040	0·014
12	0·12	0·08	6·8	5·0	0·72	0·42	0·57	0·49	0·040	0·011
13	0·17	0·10	2·8	1·9	0·41	0·25	1·45	1·20	0·135	0·043
14	0·37	0·35	38·	36·	5·32	6·02	0·15	0·15	0·031	0·018
15	0·26	0·22	7·0	6·0	1·10	1·05	0·74	0·66	0·120	0·051
16	0·16	0·14	17·	14·	1·01	1·12	0·36	0·32	0·036	0·015
17	0·33	0·30	84·	81·	9·00	11·3	0·08	0·08	0·008	0·010
18	0·28	0·28	8·7	6·2	3·90	2·67	0·26	0·22	0·040	0·013
19	0·19	0·16	2·5	1·3	0·63	0·48	1·17	0·95	0·127	0·052
20	0·39	0·32	2·0	1·3	1·69	0·98	0·97	0·81	0·218	0·064
21	0·17	0·16	5·7	5·8	5·18	2·64	0·20	0·18	0·033	0·008
22	0·42	0·30	10·	8·5	2·26	1·74	0·69	0·62	0·158	0·052
23	0·55	0·43	13·	12·	2·41	2·72	0·70	0·65	0·189	0·104
24	0·57	0·62	6·3	4·3	2·46	2·81	0·84	0·69	0·215	0·116
25	0·20	0·13	45·	40·	29·2	11·1	0·04	0·03	0·006	0·001
26	0·58	0·49	24·	18·	8·73	8·26	0·23	0·20	0·055	0·022
27	0·19	0·12	7·2	4·4	1·29	0·84	0·57	0·47	0·057	0·015
28	0·31	0·32	5·0	3·4	1·07	1·44	1·17	0·91	0·183	0·115
29	0·772	0·64	5·6	3·9	1·18	1·74	2·29	1·70	0·816	0·387
30	0·49	0·40	26·	17·	6·05	6·25	0·27	0·22	0·056	0·019
31	0·26	0·26	5·1	3·0	0·97	1·23	1·02	0·79	0·124	0·069
32	0·33	0·31	43·	29·	38·7	22·5	0·04	0·03	0·009	0·002
33	0·33	0·25	8·9	5·6	2·01	1·93	0·67	0·52	0·122	0·045
34	0·69	0·53	5·9	5·0	1·09	1·54	2·34	2·05	0·809	0·506
35	0·47	0·42	23·	15·	5·66	5·92	0·27	0·22	0·048	0·017
36	0·15	0·10	5·7	4·1	0·50	0·47	1·20	0·96	0·092	0·041
37	0·63	0·60	71·	34·	23·7	28·7	0·11	0·08	0·039	0·016
38	0·37	0·32	22·	14·	4·76	4·87	0·26	0·21	0·043	0·016
39	0·34	0·22	8·8	5·8	1·69	1·35	0·74	0·60	0·124	0·035
40	0·28	0·28	4·7	3·4	0·97	1·19	1·15	0·97	0·158	0·104

were between 2 and 10 in terms of 10-based logarithm. During the generation of these data, the subculture conditions (E_1) were deliberately close to the optimum pH value and temperature for growth with no added sodium chloride to reduce the water activity. Conditions under which the organisms subsequently multiplied (E_2) generally differed with respect to at least one of those factors controlling growth.

In Fig. 4 the y coordinate is the difference between the rms values:

$$y(\text{Fig. 4}) = rms_1 - rms_2$$

To be able to compare the estimated standard errors of the different lag and specific growth rate values, in Figs 5 and 6 we took the difference between the relative errors of the respective estimates as the y coordinate:

$rl_i = s(\lambda_i)/\lambda_i$
$rm_i = s(\mu_i)/\mu_i$
(i = 1,2 respectively to *Program i*)

$y(\text{Fig. 5}) = rl_1 - rl_2 = s(\lambda_i)/\lambda_i - s(\lambda_2)/\lambda_2$
$y(\text{Fig. 6}) = rm_1 - rm_2 = s(\mu_1)/\mu_1 - s(\mu_2)/\mu_2$

In all these plots (Figs 4, 5, 6) the estimated values of μ_1 are taken for the x-coordinate.

These plots demonstrate that for very slow curves, when the growth rate is less than about 0·1 h^{-1}, there is no significant difference between the two fits, but at higher growth rates the new model is generally better in terms of goodness-of-fit.

As far as the standard errors of the estimated growth parameters are concerned the properties are different at growth rates less than about 0·3 h^{-1} and at higher growth rates. For slower growth curves the standard error of μ is less if we use the Gompertz curve, but at faster growth curves the new model is more advantageous. However, at slower growth rates the standard error of the lag parameter is much better if we use the new model and at higher rates there is no significant difference between the two models in this respect.

Discussion

Apart from certain statistical and

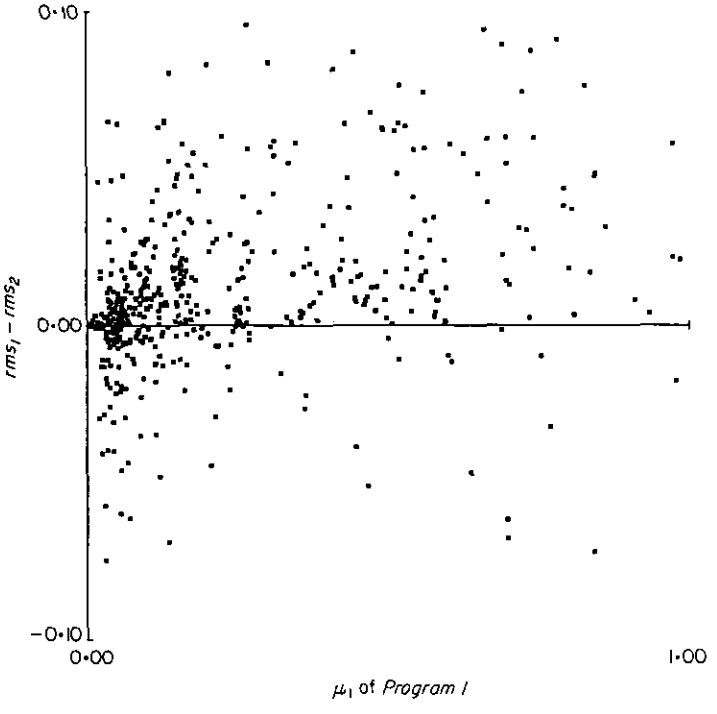

Fig. 4. Comparison of the goodness-of-fit of the Gompertz function (*Program 1*) and the new model (*Program 2*) on more than 500 growth curves. The comparison is characterized by the difference in the residual mean square: $rms_1 - rms_2$. The points above the horizontal axis represent those cases when the fit of the new model was better.

Table 3. Viable count data on *C. jejuni* when the environment in the subculture did not differ from the actual environment.

No.	Time (h)	Log counts ($\log_{10}$ ml^{-1})
1	0·00	3·08
2	2·98	3·64
3	5·08	4·36
4	7·00	4·43
5	8·87	4·63
6	15·53	5·00
7	19·10	5·63
8	21·82	5·81
9	41·28	7·08
10	45·10	7·35
11	66·72	7·78
12	93·02	8·47

an instance when the Gompertz-fit gives a negative value for the lag.

As a simplest case with explicit formulae we suggested the logistic growth for the potential growth and a Michaelis-Menten type relation to create an adjustment function with two parameters: λ, which can be considered as lag-parameter and n which characterizes the curvature after the lag phase. Based on our experience and for computational convenience we do not suggest the fitting of n but choosing its value at $n = 4$. Even in this case one must be careful at programming the formula (21a) or (21b), because, when fitting data, overflow error can occur even if the final results are ordinary numbers of usual magnitude. This situation can be avoided if for small values of $A_n(t)$ (especially at $t = 0$ where $A_n(t) = 0$), the (21b) form of the model is used instead of (21a) and then the logarithm term can be omitted, as it is discussed under (21b). For large values of $A_n(t)$, however, the form (21a) is suggested, where, again, the logarithm term can be omitted and the value of the dependent variable is practically y_{max}. Here the definition of the terms 'small' and 'large' depends on the required accuracy and

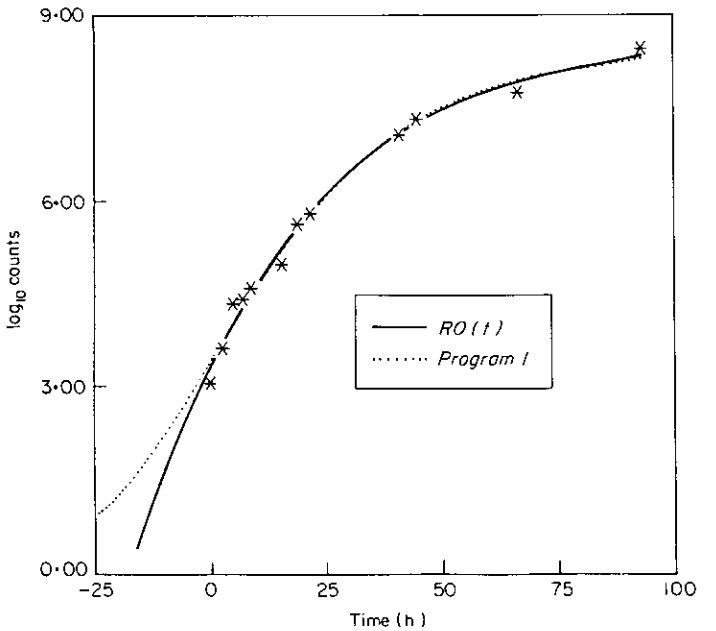

Fig. 7. Applying the Gompertz function (*Program 1*) and Richards' growth function as potential growth without adjustment function (*RO(t)*) to the datapoints of Table 3.

on the limit of number-representation of the given computer.

A more complicated version of the new model can be obtained by (22a) and (22b), which contains two curvature parameters, n and m. However, the fitting of these curvature parameters is not easy and can cause computational problems. This is why we fixed their value as $n = 4$ and $m = 1$ which seemed to be a good compromise between the goodness-of-fit and convenience. This simplest version of our model contains the following parameters:

logarithm of the inoculum, y_0;
lag-parameter, λ;
potential maximum growth rate, μ_{max};
logarithm of the maximum population density, y_{max}.

which represent the main characteristics of a sigmoid curve.

Examination of non-autonomous growth models is a developing field in population theory (see for example, Vance 1990). They seem especially useful when modelling the adjustment of the population to changing (or new) environment (Coleman 1978). In this paper we showed a possible way to apply this theory in food microbiology.

Acknowledgement

The authors thank the referees for several helpful suggestions.

References

Baranyi, J., Roberts, T. A. and McClure, P. (1992). Theorems on a non-autonomous growth model describing the adjustment of the bacterial population to a new environment. *Sixth IMA Conference on the Mathematical Theory of the Dynamics of Biological Systems*, Oxford, 1–3 July, 1992.

Barford, J. P., Pamment, N. B. and Hall, R. J. (1982). Lag phases and transients. In *Microbial population dynamics*. (Ed Bazin, M.). CRC Press, pp. 55–91. Boca Raton, FL.

Buchanan, R. L. and Cygnarowicz, M. L. (1990) A mathematical approach toward defining the duration of the lag phase. *Food Microbiol.* **7**, 237–240.

Coleman, B. D. (1978) Nonautonomous logistic equations as models of the adjustment of populations to environmental change. *Math. Biosci.* **45**, 159–173.

France, J. and Thornley, J. H. M. (1984) *Mathematical models in agriculture*. Butterworth. Oxford.

Frederickson, A. G., Ramkrishna, D. and Tsuhiya, H. M. (1967). Statistics and dynamics of procaryotic cell populations. *Math. Biosci.* **1**, 327–374.

Gibson, A. M., Bratchell, N. and Roberts, T. A. (1988). Predicting microbial growth: growth responses of salmonellae in a laboratory medium as affected by pH, sodium chloride and storage temperature. *Int. J. Food Microbiol.* **6**, 155–178.

Kono, T. (1968) Kinetics of microbial cell growth. *Biotech. Bioeng.* **10**, 105–131.

Korn, G. and Korn, T. (1973). *Mathematical handbook for scientists and engineers*. New York. McGraw-Hill.

MacDonald, N. (1978). *Time lags in biological models*. Berlin. Springer.

Pirt, S. J. (1975). *Principles of microbe and cell cultivation*. London. Blackwell.

Press, W. H., Flannery, B. P., Teukolsky, S. A. and Vetterling, W. T. (1990) *Numerical recipes*. Cambridge. Cambridge University Press.

Roels, J. A. and Kossen, N. W. F. (1978). On the modelling of microbial metabolism. In *Progress in industrial microbiology*, vol. 14. (Ed. Bull, M. J.), pp. 95–203. Amsterdam. Elsevier.

Turner, M. E., Bradley, E. L., Kirk, K. A. and Pruitt, K. M. (1976). A theory of growth. *Math. Biosci.* **29**, 367–373.

Vance, R. R. (1990) Population growth in a time-varying environment. *J. Theor. Biol.* **37**, 438–454.

Vance, R. R. and Coddington, E. A. (1989) A nonautonomous model of population growth. *J. Math. Biol.*, **27**, 491–506.
Whiting, C. (1992) Letter to the editor. *Food Microbiol.* **9**(2), 173–174.
Zwietering, M. H., Jongenburger, I., Rombouts, F. M. and van't Riet, K. (1990) Modelling of the bacterial growth curve. *Appl. Environ. Microbiol.* **56**, 1875–1881.

APPENDIX

Below we summarize some mathematical theorems on the new model. These theorems were presented in Baranyi et al. (1992).

Consider the initial value problem (1a), (1b). The theorems below are valid also if we consider the case of limited growth rate, so instead of condition (c) under (1a), (1b) we suppose the much weaker constraint:

$$d\mu/dx \text{ is limited} \quad (0 \le x \le x_{max}).$$

Theorem 1

Let $\alpha(t)$ be an adjustment function as defined under (2a), (2b). Then the solution of the initial value problem (2a) and (2b) is

$$g(t) = f(A(t)),$$

where

$$A(t) = \int_0^t \alpha(s)ds$$

Theorem 2

Let $\alpha(t)$ and $\beta(t)$ adjustment functions and let $g_\alpha(t)$, $g_\beta(t)$ denote the respective solutions of (2a), (2b).

If

$$\left| \int_0^t (\alpha(s) - \beta(s))ds \right| < \epsilon,$$

then

$$|g_\alpha(t) - g_\beta(t)| < \epsilon |\mu(x)x|_{max} < \epsilon \mu(x_0)x_{max}.$$

Let the adjustment function have the form

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

for some $\lambda, n > 0$. In this case

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

where

$$B_n(t) = \int_0^t \frac{1}{1 + s^n} ds.$$

Theorem 3

If $n > 1$ then the improprius integral

$$B_n = \lim_{t \to \infty} B_n(t)$$

exists. Moreover if $n \to \infty$ then $B_n \to 1$.

Theorem 4

If $n \to \infty$ then $B_n(t) \to B_\infty(t)$ uniformly on $(0,\infty)$ where

$$B_\infty(t) = \begin{matrix} t & (t \le 1) \\ 1 & (t \ge 1) \end{matrix}.$$

Consequence: if $n \to \infty$ then $A_n(t) \to A_\infty(t)$ uniformly on $(0,\infty)$, where

$$A_\infty(t) = \begin{matrix} 0 & (t \le \lambda) \\ t-\lambda & (t \ge \lambda) \end{matrix}.$$

Theorem 5

Using the notations above: if $n \to \infty$ then

$$f(A_n(t)) \to f(A_\infty(t)) \text{ uniformly on } (0,\infty).$$