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Estimating the Maximum Specific Growth Rate from Microbial Growth Curves: Definition is Everything

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ABSTRACT

The maximum specific growth rate ($\mu_{\text{max}}$) is an important parameter in modelling microbial growth under batch conditions. However, there are two definitions of this growth parameter in current use and some of the comparisons of data made in the literature fail to acknowledge this important fact.

We compared values of $\mu_{\text{max}}$ obtained by applying the Gompertz, logistics and Baranyi-Roberts models to experimental data on the growth of *Listeria monocytogenes* and *Listeria innocua* using both absorbance and viable counts measurements of cell concentration. All three models fitted the experimental data well, however, the values of $\mu_{\text{max}}$ obtained using the Gompertz and logistic models were similar to each other but substantially different from that predicted by the Baranyi-Roberts model. The latter growth model was used to derive a second estimate of $\mu_{\text{max}}$ based on the slope at the inflection point of the growth curve function; this value was in closer agreement with those obtained using the Gompertz or logistic models. Conditions were identified when values of $\mu_{\text{max}}$ based on different definitions would converge towards one another.

INTRODUCTION

There is increasing interest in being able to predict the consequences of microbial growth on foods during storage. If methods can be developed to give realistic predictions, considerable savings can be made in the costs associated with laboratory challenge testing of foods (Baranyi and Roberts, 1994).
The time-dependent increase in the microbial population in a closed system is referred to as a growth curve and fundamental to all predictive methods is a requirement to mathematically model, either partly or fully, growth curves for micro-organisms of particular interest over a range of environmental conditions. Numerous expressions have been proposed including some, which as Grijspeerdt and Vanrolleghem (1999) have pointed out, were never envisaged as having been formulated for this purpose. Zwietering et al. (1990) surveyed a number of commonly used empirical expressions – including the Gompertz and logistic expressions – and ‘re-parameterised’ some of them in an attempt to give physical meaning to their parameters. Approaching the problem from a more mechanistic basis, Baranyi and his co-workers (Baranyi et al., 1993; Baranyi and Roberts, 1994; Baranyi, 1997) have proposed and developed a dynamic growth model that has found widespread acceptance.

Selecting the most appropriate growth model is often a matter of trial and error. Moreover, different criteria for determining the suitability of one particular model over another vary: some authors have relied on mathematical measures of goodness of fit (Buchanan et al., 1999), whilst others have focussed on direct comparisons of particular growth parameters as predicted by the various models (Membrè et al., 1999). The important growth parameter $\mu_{max}$, the maximum specific growth rate, is a case in point. Two definitions for this parameter are in current use. One is based on the inflection of the slope of the growth curve in the exponential phase (Baranyi et al., 1993) whilst the other is taken to be the growth rate at infinite dilution (Zwietering et al., 1990). This can lead to instances where like is not always being compared with like. The situation is further complicated by the fact that different approaches to
the gathering of growth data are in current use. Some workers use viable counts as a measure of cell concentration, whilst others use optically based methods.

In this work we illustrate some of the anomalies that can arise by modelling growth rate data for *Listeria monocytogenes* and *Listeria innocua* using the logistic, Gompertz and Baranyi growth models. Data is obtained for these two organisms both in the form of viable counts and absorbance measurements. *L. monocytogenes* is the cause of a significant number of food poisoning incidents in developed countries (Farber and Peterkin, 1991). *L. innocua* is not pathogenic but is often isolated alongside the latter and some reports suggest that it can mask the presence of *L. monocytogenes* (Cornu et al., 2002).

**MATERIALS AND METHODS**

**GROWTH MODELS**

In their re-parameterisation of empirical expressions applied to growth curves, Zwietering et al. (1990) took $\mu_{\text{max}}$ to be the tangent to the growth curve at its inflection point, and the lag time as the intercept of this tangent at the x-axis. On this basis the Gompertz expression became:-

$$y = D \exp \left( -\exp \left( \frac{\mu_{\text{max}} e^\lambda}{D} (\lambda - t) + 1 \right) \right)$$

(1)

and the Logistic expression:-

$$y = \frac{D}{1 + \exp \left( \frac{4\mu_{\text{max}}}{D} (\lambda - t) + 2 \right)}$$

(2)
In the model proposed by Baranyi et al. (1993), the variation of the cell population with time is described by a first order differential equation and assumes the following relationship:

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

(3)

Baranyi and his co-workers presented the solution to this differential equation firstly with six parameters (Baranyi and Roberts, 1994), but this was reduced to four parameters in Baranyi (1997) as follows:-

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

(4)

where:

$$A(t) = t + \frac{1}{\mu_{\text{max}}} \ln \left(e^{-\mu_{\text{max}}t} + e^{-h_0} - e^{-\mu_{\text{max}}t-h_0} \right)$$

(5)

A figure in the work of Baranyi and Roberts (1994) implies, but does not explicitly state, that $\mu_{\text{max}}$ and the slope of their growth curve function are synonymous. This is clearly in contradiction of the definition for $\mu_{\text{max}}$ given in Baranyi et al. (1993) as the value of $\mu$ at infinite dilution (equation 3). Notwithstanding, we wanted to determine the relationship between $\mu_{\text{max}}$ as predicted by the Baranyi-Roberts model and that of the slope of the growth curve function. To do this the first derivative with respect to time was obtained:

$$\frac{dy}{dt} = \mu_{\text{max}} A' - \frac{e^{\mu_{\text{max}}A(t)} \mu_{\text{max}} A'}{1 + \frac{e^{\mu_{\text{max}}A(t)} - 1}{e^{y_{\\text{max}}-y_0}}} = \mu_{\text{max}} A' \left(1 - \frac{e^{\mu_{\text{max}}A(t)}}{e^{y_{\\text{max}}-y_0} + e^{\mu_{\text{max}}A(t)} - 1} \right)$$

(6)

where:

$$A' = \frac{dA}{dt} = 1 - \frac{e^{-\mu_{\text{max}}t} - e^{-\mu_{\text{max}}t-h_0}}{e^{-\mu_{\text{max}}t} + e^{-h_0} - e^{-\mu_{\text{max}}t-h_0}} = \frac{e^{-h_0}}{e^{-\mu_{\text{max}}t} + e^{-h_0} - e^{-\mu_{\text{max}}t-h_0}}$$

(7)
It was also necessary to obtain the maximum value of the first derivative. Because finding the second derivative and setting it equal to zero would not have yielded an expression explicit in t, we plotted equation (6) for all values of t in order to obtain the maximum.

EXPERIMENTAL

*L. innocua* (NCTC 11288) and *L. monocytogenes* (Scott A) were maintained on Brain Heart Infusion (BHI) agar (Oxoid Ltd, Basingstoke, UK) slopes at 4 °C. Erlenmeyer flasks (500ml) each containing 100 ml of BHI broth were inoculated using a wire loop and then placed in a shaking incubator at 30 °C and 150 rpm. After 24 hours these cultures were used to inoculate fresh Erlenmeyer flasks of BHI broth (1ml per flask) and which were cultured under identical conditions. Samples were taken from the latter and analysed to generate growth curves. Viable counts were performed by spreading 100 μl of appropriately diluted cell culture onto the surface of Tryptone Soya Agar. The plates were incubated at 30 °C for 24 hours and the number of colonies counted. Counts are quoted as the number of colony forming units per ml (cfu/ml). Absorbance determinations were made at 600 nm using a spectrophotometer (UV-1201, Shimadzu (U.K.), Milton Keynes). All growth determinations were performed in triplicate and the average value of each measurement was modelled.

The parameters for all the models were obtained using regression software (DATAFIT 7.1, Oakdale Engineering, USA) and we were able to verify our parameter
estimates for the Baranyi-Roberts model for viable counts data using software made available at http://www2.ifr.bbsrc.ac.uk/MicroFit/.

RESULTS

Figure 1 shows the application of all three models - Gompertz, logistic and Baranyi - Roberts to the experimental absorbance and viable count data for both \textit{L. innocua} and \textit{L. monocytogenes}. Computation of the residual sum of squares (RSS) revealed that no single model gave a consistently better goodness of fit over all the data, a conclusion also reached by Baty and Delignette-Muller (2004). In Table 1 are compared estimates of $\mu_{\text{max}}$ from all three models for both absorbance and viable counts data. Also shown are values of the maxima of the slopes derived from the Baranyi-Roberts model using equation (6). All the models are consistent in predicting $\mu_{\text{max}}$ for \textit{L. innocua} to be greater than that for \textit{L. monocytogenes}.

For both species of bacteria, the values of $\mu_{\text{max}}$ predicted by the logistic and Gompertz models which are based upon viable counts data are higher than those based on absorbance data. This is also the case for the estimate of $\mu_{\text{max}}$ based on the slope of the Baranyi-Roberts model. In contrast, the Baranyi-Roberts model predicts a greater value for $\mu_{\text{max}}$ based on absorbance. The discrepancies between the values for $\mu_{\text{max}}$ based on absorbance and viable counts data have been the subject of previous investigations (Dalgaard et al., 1994; Begot et al., 1996; Dalgaard and Koutsoumanis 2001). The overall consensus appears to be that whilst growth parameters estimated on the basis of viable counts data are generally held to be more reliable, particularly at low cell densities, the use of absorbance methods is valid. The particular appeal of
absorbance measurements is that they can be obtained with relative ease and can even be automated (Begot et al. 1996). The ratios of the estimates for $\mu_{\text{max}}$ by absorbance and viable counts data obtained here vary from 0.89 to 1.3 - well within the ranges determined by Dalgaard and Koutsoumanis (2001) who analysed some 176 data sets. Dalgaard et al. (1994) proposed a function that corrected for non-linearities in absorbance measurements at high cell densities.

A very significant feature of Table 1 is that the values of $\mu_{\text{max}}$ predicted by the Gompertz and logistic models are very similar to each other and similar to the slope of the Baranyi-Roberts model whilst being markedly different to the values of $\mu_{\text{max}}$ predicted by the Baranyi-Roberts model: this applies to both species bacteria irrespective of whether growth was determined by absorbance or viable counts data. Using hypothetical data we fixed the value of $h_o$ as 2.7 and $\mu_{\text{max}}$ as 1.0 in the Baranyi-Roberts model – values similar to those obtained using experimental data for the listeriae – and then calculated the slope of the Baranyi-Roberts model at different values of $y_o$ and $y_{\text{max}}$. This is illustrated in Table 2 where it is seen that the value of the slope approaches 1.0 at high values of $y_o$ and $y_{\text{max}}$. This indicates that convergence is more likely to occur when the estimates are based on viable counts data because in these instances the value of $y_o$ will be relatively high. This effect has been observed for experimental data by Dalgaard and Koutsoumanis (2001) who found that ratios of $\mu_{\text{max}}$ obtained by absorbance and viable counts data tended towards 1.0 at high cell yields – i.e. at high values of $y_{\text{max}}$.

DISCUSSION
The notion that an organism can grow at a maximum specific growth rate is not a difficult one to grasp. The potential for confusion arises because there exists more than one definition of this important parameter. In such circumstances, caution needs to be exercised in comparing values without first establishing the basis upon which each is defined. This seems to have been appreciated in relation to predictions of the duration of the lag phase, \( \lambda \); Zwietering et al. (1992) treated as distinct values of \( \lambda \) obtained using three different definitions of the parameter. However, the same awareness has not always been applied to comparisons of \( \mu_{\text{max}} \). For example, Membre et al. (1999) compared values of \( \mu_{\text{max}} \) obtained for \( L. \) monocytogenes using both the Gompertz and Baranyi-Roberts model. Similarly, Sutherland et al. (1996) in developing a model to predict the growth rate of \( B. \) subtilis at different environmental conditions, obtained values of \( \mu_{\text{max}} \) from growth curves using the Baranyi-Roberts model and then compared their values with literature data, some of which were based on the alternative definition of \( \mu_{\text{max}} \).

Our analysis serves to emphasise the caution made above regarding comparisons of values of \( \mu_{\text{max}} \). We used the Baranyi-Roberts growth model to derive two estimates of \( \mu_{\text{max}} \) each based on a different definition of the parameter. Moreover, we identified conditions under which both estimates would tend to converge towards each other. In strictly theoretical terms, values of \( \mu_{\text{max}} \) obtained using the two different definitions will, according to equation (3), only become identical when the cell concentration is zero. However, if the inflection point of the growth curve occurs at very low cell concentrations, the growth rate at that cell concentration as represented by the slope of the growth curve at that point will be close to the value of growth rate at infinite dilution (Dalgaard et al., 1994). This might possibly be achieved using a lower
inoculum concentration than was used here, however, in such circumstances the use of absorbance data may no longer be valid (Ref).

**NOTATION**

\[ D = \text{limit of } \ln \frac{VC}{VC(t=0)} \text{ or limit of } \ln \frac{Abs}{Abs(t=0)} \]

\[ A = \text{integral of adjustment function} \]

\[ h_o = \text{parameter of Baranyi-Robert model} \]

\[ y_o = \ln VC \text{ or } \ln abs \text{ at } t=0 \]

\[ y_{\text{max}} = \text{maximum value of } \ln VC \text{ or } \ln Abs \]

\[ x = \text{cell concentration} \]

\[ x_{\text{max}} = \text{maximum cell concentration} \]

*Greek letters*

\[ \lambda = \text{lag phase duration (hours)} \]

\[ \mu = \text{growth rate (h}^{-1}) \]

\[ \mu_{\text{max}} = \text{maximum growth rate (h}^{-1}) \]

**REFERENCES**


Figure 1. Data and models for *L. monocytogenes* Abs (a), *L. monocytogenes* VC (b), *L. innocua* Abs (c), *L. innocua* VC (d)

(Abs, absorbance data; VC, viable counts data)
<table>
<thead>
<tr>
<th>Bacterium</th>
<th>Abs</th>
<th>VC</th>
<th>Abs</th>
<th>VC</th>
<th>Abs</th>
<th>VC</th>
<th>Abs</th>
<th>VC</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>L. monocytogenes</em></td>
<td>0.682</td>
<td>0.809</td>
<td>0.668</td>
<td>0.793</td>
<td>1.031</td>
<td>0.907</td>
<td>0.655</td>
<td>0.759</td>
</tr>
<tr>
<td><em>L. innocua</em></td>
<td>0.778</td>
<td>0.999</td>
<td>0.754</td>
<td>0.956</td>
<td>1.188</td>
<td>1.090</td>
<td>0.734</td>
<td>0.906</td>
</tr>
</tbody>
</table>

Table 1. Comparison of Estimates of $\mu_{\text{max}}$ Derived from Different Growth Models and the Slope of the Baranyi-Roberts Model

(Abs, estimate based on absorbance data measurements; VC, estimate based on viable count measurements)
<table>
<thead>
<tr>
<th>$y_0 = -2$</th>
<th>$y_0 = 0$</th>
<th>$y_0 = 5$</th>
<th>$y_0 = 10$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$y_{\text{max}}$</td>
<td>slope</td>
<td>$y_{\text{max}}$</td>
<td>slope</td>
</tr>
<tr>
<td>1</td>
<td>0.65</td>
<td>5</td>
<td>0.853</td>
</tr>
<tr>
<td>5</td>
<td>0.94</td>
<td>10</td>
<td>0.986</td>
</tr>
<tr>
<td>10</td>
<td>0.995</td>
<td>15</td>
<td>0.999</td>
</tr>
<tr>
<td>15</td>
<td>0.999</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 2. Effect of the Parameter $y_0$ and $y_{\text{max}}$ on the Slope derived from the Baranyi-Roberts Growth Model