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Growth curve prediction from Optical Density Data

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Abstract

A fundamental aspect of predictive microbiology is the shape of the microbial growth curve and many models are used to fit microbial count data, the modified Gompertz and Baranyi equation being two of the most widely used. Rapid, automated methods such as turbidimetry have been widely used to obtain growth parameters, but do not directly give the microbial growth curve. Optical density (OD) data can be used to obtain the specific growth rate and if used in conjunction with the known initial inocula, the maximum population data and knowledge of the microbial number at a predefined OD at a known time then all the information required for the reconstruction of a standard growth curve can be obtained.

Using multiple initial inocula the times to detection (TTD) at a given standard OD were obtained from which the specific growth rate was calculated. The modified logistic, modified Gompertz, 3-phase linear, Baranyi and the classical logistic model (with or without lag) were fitted to the TTD data. In all cases the modified logistic and modified Gompertz failed to reproduce the observed linear plots of the log initial inocula against TTD using the known parameters (initial inoculum, MPD and growth rate). The 3 phase linear model (3PLM), Baranyi and classical logistic models fitted the observed data and were able to reproduce elements of the OD incubation-time curves. Using a calibration curve relating OD and microbial numbers, the Baranyi equation was able to reproduce OD data obtained for *Listeria monocytogenes* at 37 and 30^{o}C as well as data on the effect of pH (range 7.05 to 3.46) at 30^{o}C.

The Baranyi model was found to be the most capable primary model of those examined (in the absence of lag it defaults to the classic logistic model). The results suggested that the *modified* logistic and the *modified* Gompertz models should not be used as Primary models for TTD data as they cannot reproduce the observed data.

1 Introduction:

A fundamental aspect of predictive microbiology is the shape of the microbial growth curve. General population growth can be modelled using the logistic model and variations of this model have been used in many diverse areas such as the analysis of fish stocks, forestry management and human population growth (e.g. Alexandrov 2008). The general pattern of growth is sigmoidal, with an apparent slow phase followed by a more rapid increase in numbers followed by a slowing down, finally reaching a maximum population level. In most texts it is noted that the growth of bacteria also follows a similar pattern: a lag before replication, followed by exponential growth and then a period of maximum population density eventually followed by the ‘death-phase’. A major difference is that the microbial growth curve is depicted in terms of log numbers of microbes. The microbial growth curve (as log numbers) has the characteristic sigmoid shape and the varieties of models which are used to fit the curve reflect this sigmoid character. There are two principal *empirical* curves used – the symmetric modified logistic and the asymmetric modified Gompertz (‘modified’ by virtue of using log numbers rather than numbers explicitly). Many models in the microbiological literature are variations on these two themes (Li et al., 2007; Pruitt and Kamau 1993; Zwietering et al. 1990).

The Baranyi model, however, is different to the normal growth models in that it is based on the logistic model of growth, but has an additional function which deals with the presence of lag making it a non-autonomous differential equation (Baranyi et al., 1993, 1993b; Baranyi and Roberts 1994);

$$\frac{dn}{dt} = \alpha(t)\mu(n)n$$

Where $\mu(n)$ is a function of the specific growth rate, n is the numbers of microbes and $\alpha(t)$ is termed the adjustment function. The derived equation uses the idea of Michaelis –Menten kinetics to suggest a lag time during which organisms adapt from one environment (the culture) to the test environment. The function used essentially delays the time before growth occurs.

Baranyi and Roberts (1995) in their paper on the fundamentals of mathematics in predictive microbiology stated that rapid methods such as turbidimetry or conductimetry cannot be used directly to obtain growth parameters such as the specific growth rate if the rescaling function employed has a constant other than zero;

"if the measured quantity is q, then q = f(x), where f is a linear calibration function: f(x) = a .x + ***b.*** If b is different from 0 then neither q nor log q is linearly proportional to log x. Hence, in a strict sense, the rate of change in q should not be used to estimate the viable count specific growth rate unless the proportionality of q (turbidity, conductance, etc.) to the original cell concentration, x, has been established over the complete matrix of environmental variables (temperature, pH, a_w). Nor should the viable count models describing x(t) be directly applied to model q(t). New calibration function, or other considerations, should be taken into account to model q(t) and/or to compare it with the viable count model." (Baranyi and Roberts 1995).

Models used to examine the shape of microbial growth generally require four parameters: the initial and final population levels (I_0 and MPD respectively), the maximum specific growth rate and the time at which this occurred. If three pieces of information are available, e.g. the initial population, the MPD and the specific growth rate, then knowledge of the population at a specific time can be used to reproduce the growth curve simply by substituting the values into the equations and solving for the missing parameter. Herein we show that this seemingly simple hypothesis serves as a "consideration" and also has ramifications on the validity of the modified empirical growth curves, whilst adding value to the interpretation of the Baranyi equation.

2 Methods: Microbes and Models

2.1 MICROBES

From a previously prepared and stored slope on tryptone soya (TSA) of the pure culture of *L. monocytogenes* (Lm 252 an industrial isolate donated by Nestlé), a portion was removed with a sterile loop, transferred into a conical flask containing 80 ml tryptone soya broth (TSB) and incubated with shaking (150 rpm) at 30°C overnight. The resulting culture was split into four portions and centrifuged at 500g for 10 minutes. Two of the resulting pellets were resuspended in TSB (2 ml) and pooled. The resuspended culture (1ml) was transfer into TSB (9ml) in a universal tube and mixed thoroughly; 1ml of this suspension was diluted in TSB to obtain a standard optical density of approximately 0.5 with a 1cm path length at 600nm (M350 Double Beam U.V. Visible Spectrometer). From the standardised culture a series of decimal dilutions were prepared in TSB (labelled 0 to -9).

2.2 PREPARATION OF MICRO-ARRAY PLATES

The Bioscreen micro-array plates were filled as follows: all wells except column 10 received 200µl of TSB. The wells of column 10 received 400µl of the appropriate serial dilutions (with the highest inoculum (the zero dilution) in well 100. Using a multi-pipette, 200µl were removed from each well of column 10 and transferred into the wells of column 9, the -1 dilution, mixed by repeated syringing, etc. This was repeated across the plate discarding the excess 200µl after final mixing. Theoretically for an initial inoculum of $1x10^9$ cfu/ml, this method will give a range from 9 log_{10} to -2.7 log_{10} cfu/ml. The OD of a sample in the Bioscreen is dependent on the volume used: a standard OD of 0.5 measured in the spectrophotometer has an OD of 0.29 at 600nm for a volume of 200µl in the Bioscreen.

Plates were typically incubated for 1 to 3 days, with the optical density of the wells being read at 600nm every ten minutes.

2.3 EFFECT OF PH

A range of 30 different pH in TSB (pH 7.05 to 3.46 adjusted with filter sterilised HCl (0.01M) in approx. 0.35 pH unit intervals) each with 3 replicates per plate and done in duplicate on separate machines were tested against a single inoculum size of *L. monocytogenes* (LM 252) at 30^{o}C.

2.4 Models and Data analysis

From the resulting Bioscreen OD/time data, the background OD due to the media was removed from each. A time to detection (TTD) criterion of OD = 0.2 was then used on the background corrected data: TTD were found using linear interpolation between OD/time values which straddled the OD = 0.2 value. For a given set of environmental conditions a plot of the log of the initial inoculum size against the time to detection (TTD) gives a straight line relationship with gradient equal to the reciprocal of the specific growth rate (Cuppers and Smelt 1993). In the absence of a lag the line will intersect the log initial inoculum axis at the detection value for the given OD criterion used.

TTD data have approximately constant variance until the initial inoculum level is less than 10^2 cfu/ml, below this level the variance increases (Bidlas *et al.* 2008). To preclude the need for weighted regression or for a data transformation data below this inoculum size threshold were censored in the regression fits.

2.4.1 Modified logistic and Gompertz

The re-parameterised modified logistic and Gompertz models (Zwietering et al. 1990) were rearranged to equate the initial log inoculum with the time to detection of a known number of microbes per ml (N_D), giving equations 1 and 2 respectively.

$$t_D = \lambda - \frac{A}{4\mu_m}\left\{\ln\left[\frac{A}{\log N_D - \log N_0} - 1\right] - 2\right\}$$

(Eq.1, modified logistic)

$$t_D = \lambda - \frac{1}{\mu_m}\left(\frac{A}{e}\right)\left[\ln\left\{\ln\left(\frac{A}{\log N_D - \log N_0}\right)\right\} - 1\right]$$

(Eq.2, modified Gompertz)

2.4.2 Three phase linear Model (3PLM)

The 3-phase linear model is a simplified model of the growth curve. Its simplicity has been regarded by some as its strength and too simplistic by others (Baranyi 1997; Buchanan et al. 1997; Garthright 1997). The 3-phases are given by the following;

log N = log N_0 if $t \le t_\lambda$ else

log N = log N_0 + $\mu(t - t_\lambda)$ if t: $t_\lambda \le t < t_{max}$ else

log N = log N_{max}, when $t \ge t_{max}$

The parameter t_λ is the duration of lag time. This equation can be rearranged to equate the initial log inoculum with the time to detection of a known number of microbes per ml (N_D),

$$t_D = \lambda + \frac{\log N_D - \log N_0}{\mu_m} \qquad \text{(Eq.3)}$$

2.4.3 Baranyi and logistic models

$$\log N = \log N_0 + \frac{y_1}{\ln(10)} - \frac{y_2}{\ln(10)}, \qquad where$$
$$y_1 = \mu_m t + \ln\left[e^{-\mu_m t} - e^{-\mu_m (t + t_{lag})} + e^{-\mu_m t_{lag}}\right], \qquad and$$
$$y_2 = \ln\left[1 + 10^{(\log_{10} No - \log_{10} M)}\left(e^{\mu_m (t - t_{lag})} - e^{-\mu_m t_{lag}}\right)\right] \qquad \text{(Eq.4)}$$

The Baranyi model, which is a non-autonomous equation, cannot be rearranged to obtain time explicitly. In the absence of lag the Baranyi model defaults to the basic logistic model of growth in which the time to detection for a given number of microbes is given by

$$t_D = \frac{1}{\mu} \ln\left\{\frac{\frac{M}{N_0} - 1}{\frac{M}{N_D} - 1}\right\} \qquad \text{(Eq. 5a)}$$

Where M = maximum population density (cfu/ml), N_D = numbers of microbes per ml at the detection value, N_0 = initial inoculum level (cfu/ml) , μ = specific growth rate. In general $N_0 \le N_D$ <M. When $N_0 = N_D$, $t_D = 0$.

A simple, empirical, approximation to the Baranyi equation when a lag exists is given by

$$t_D = \lambda + \frac{1}{\mu}\ln\left\{\frac{\frac{M}{N_0}-1}{\frac{M}{N_D}-1}\right\}$$ (Eq. 5b)

3 Results

3.1 GROWTH RATE OF L. MONOCYTOGENES AT 37°C FROM OD DATA

The optical density/incubation time curves for different initial inocula of *Listeria monocytogenes* in TSB are shown in Figure 1. Each individual curve was essentially congruent with all other curves: there is no decrease in the slope with decreasing initial inocula. The average maximum OD reached was 0.99 (σ = 0.034, se. mean = 0.004). For each curve, the TTD of OD = 0.2 was found using simple linear interpolation between OD/time data which straddled the OD = 0.2 position. The analysis of the TTD of these multiple dilutions of initial inocula (the zero dilution starting culture had a viable count of 1.11 $x10^9$ cfu/ml), showed a simple linear relationship between the initial inoculum and the TTD criterion used (Figure 2). The reciprocal of the gradient gives a growth rate of 0.0092 $\log_{10}$ cfu/ml/min, which equates to a specific growth rate of 1.27 ln cfu/ml/h (Table 1). The intercept of 961 minutes corresponds to the theoretical time taken for a single organism per ml to reach the TTD= 0.2 criterion; when TTD = 0, the regression line cuts the axis at an initial log inoculum of 8.81 $\log_{10}$ cfu/ml (95% CI 8.77-8.86), which was statistically equivalent to the $\log_{10}$ cfu/ml count of the viable count recorded from multiple wells with OD = 0.2. Hence in this case there was no measurable lag; this was also confirmed from an analysis of Figure 1 – the highest inocula examined do not show any lag period.

3.2 FITTING THE MODIFIED LOGISTIC AND GOMPERTZ MODELS TO OD DATA

From a plate count the MPD of the *Listeria monocytogenes* culture was 9.8 $\log_{10}$ cfu/ml, the initial inoculum size for each well was calculated from the plate count of the initial inoculum and the dilution sequence used. From the OD data, the specific growth rate and lag were obtained, hence all the parameters required to reproduce the growth curve using either the modified logistic or Gompertz equations were present. Equations 1 and 2 were used to calculate the TTD for the given initial inocula using the observed parameters. A plot of the calculated TTD (TTD_{calc}) against the initial inocula gave a regression fit of $TTD_{calc} = -125.53 \log N_0 + 1087.7$, $r^2 = 0.999$ and $TTD_{calc} = -144.6 \log N_0 + 1235$, $r^2 = 0.997$ for the modified logistic and Gompertz equations respectively. The gradients were 16% and 33% greater for the modified logistic and Gompertz respectively over that observed. In both cases the plot was a curve rather than the observed linear relationship. The sum of squares between the observed TTD and that calculated using the two equations was minimised by regressing the growth rate and lag, this gave growth rates of 0.0107 and 0.0122 $\log_{10}$ cfu/ml/min with a lag of 17 and 31 mins, respectively, but the gradient of the calculated TTD/ log initial inoculum plots were now equal to the observed (-108.5 $\log_{10}$ cfu/ml/min). Hence there is a discrepancy between the fit of the modified logistic and Gompertz equations with the interpretation of the observed values.

Multiple growth curves were produced *in-silico* using the observed rate, lag and the known initial inocula and MPD. The calculated log numbers were transformed to numbers per ml and plotted against time. If the modified Gompertz equation was an adequate descriptor of the observed data then congruent plots should be observed. However, as the initial inocula decreased the modelled curves became shallower, i.e. they do not reflect the observed OD curves (note a calibrant which transforms the number to an OD will result in the same conclusion). The analyses performed were also carried out using the modified logistic equation, resulting in the same conclusion: the modified logistic model cannot reproduce the congruent shapes of the observed OD curves shown in Figure 1.

3.3 Fitting the Three Phase Linear Model to OD data

The 3PLM (Eq. 3) was fitted to the TTD data by minimising the sum of squares between the observed TTD and the modelled; initial values of μ = 0.0092 and lag = 0 mins were used (note the MPD and the log of the detection numbers are fixed values).The fit gave μ = 0.00922 (95% CI: 0.00914 – 0.00930), and a lag = -8.89 mins (95% CI: -12.84 to - 4.93). A plot of the calculated TTD against the initial inocula gave a regression fit of $TTD_{calc} = -108.5 \log N_0 + 965.6$, $r^2 = 1.00$, i.e. the 3PLM reproduced the observed TTD data and (by definition) was a straight line fit.

Using the full form of the 3PLM, multiple growth curves were produced *in-silico* using the calculated rate, lag and the known initial inocula and MPD. The calculated log numbers were transformed to numbers per ml and plotted against time. In this case the 3PLM produced congruent curves and reproduce the initial shape of the OD curves (data not shown); however, since the model gives only exponential growth until MPD is reached, i.e. there is no slow down in the rate of growth, the discrepancy between the shapes of the observed OD and calculated numbers quickly increases. In this case the ‘simple’ is good enough to fit the TTD data but not ‘enough’ to model the full data.

3.4 FITTING OF THE BARANYI MODEL TO OD DATA

The Baranyi model cannot be used to explicitly obtain the TTD for a given set of parameters, although this can be easily solved numerically. To fit the Baranyi model to the observed TTD data, and obtain a growth rate and lag, the observed TTD was used as the independent variable and the model used to fit the difference between the size of the detection inoculum and the initial inoculum. The estimated growth rate was 0.00918 $\log_{10}$ cfu/ml/min (95% CI:0.00910 – 0.00927), with a lag of -17.3 mins (95% CI: -21.8 to -12.9).

The Baranyi model was also used to generate multiple growth curves *in-silico*, using a growth rate of 0.00922 $\log_{10}$ cfu/ml/min, a lag of zero minutes along with the given MPD of 9.8 and the size of the known initial inocula. The time taken to reach the inoculum detection value of 8.9 for all the simulated growth curves was obtained numerically using a simple linear interpolation procedure. A plot of the log initial inoculum against the calculated TTD gave a straight line fit with a regression fit of $TTD_{calc} = -108.4 \log_{10}N_0 + 956.49$, $r^2 = 0.999$. The multiple growth curves obtained were congruent and have the desirable feature of a slow down in the rate of growth as MPD is approached unlike the 3PLM (Figure 3).

3.5 OD – BARANYI CALIBRATION CURVE

Above the background OD, the Baranyi model and the OD data are 1:1 up to the maximum OD, i.e. there is a unique OD for each cfu/ml. Past the maximum OD, in the cases studied here, there is a reduction in the OD with incubation time, whereas the model stays at a constant MPD. This is a failing of the model as it is a purely growth rather than a growth and decay model. The 1:1 nature of the relationship up to the maximum OD can be used to construct a calibration curve between the optical density at a given time and the number of microbes per ml predicted from the Baranyi model. To construct the calibration curve ten observed OD curves were chosen and a plot of the OD (up to a maximum of 0.85) against the equivalent calculated numbers for the observation time constructed. Simple linear regression was applied (Eq.s 6a and 6b), Figure 4.

$$OD = 1.308\times10^{-10}\,(No.) + 0.0946,\; r^2 = 0.997 \qquad (Eq.6a)$$

$$No. = 7.625\times10^{9}(OD) - 7.172\times10^{8},\; r^2 = 0.997 \qquad (Eq.6b)$$

Where No. are the calculated microbial numbers per ml. Data up to an OD = 0.85 gave a good linear relationship between OD and the calculated cfu/ml; at OD greater than 0.8, the inclusion of the cubic and quadratic terms (No. = $-8.38\text{x}10^{9}(\text{OD})^{3} + 1.23\text{x}10^{10}(\text{OD})^{2} + 2.67\text{x}10^{9}$ (OD) $-7.049\text{x}10^{8}$, $R^2 = 0.999$, $r^2 = 0.997$; OD = $3.217\text{x}10^{-30}$ $(\text{No.})^3 - 2.787\text{x}10^{-20}(\text{No.})^2 + 1.896\text{X}10^{-10}$ (No.) + 0.0831, $R^2 = 0.998$) gave better fits.

A calibration curve was also obtained from a regression analysis of plate counts against optical density in the Bioscreen and gave a fit of OD $=0.997\text{x}10^{-10}$ (No.) + 0.14, $r^2 = 0.956$.

Using the calibration curve derived from the Baranyi-analysis, the calculated microbial numbers were converted to OD values. Figure 5 shows a direct comparison of the predicted OD/time curves with the observed for five selected cases.

3.6 CLASSICAL POPULATION LOGISTIC MODEL

Fitting the classical logistic model (Eq. 5a) to the TTD data gave a straight line fit of $TTD_{calc} = -105.43 \log_{10}N_0 + 943.59$, equating to a growth rate of 0.0095 $\log_{10}$ cfu/ml/min (95% CI: 0.00945 – 0.00955). The addition of a constant lag (Eq. 5b) improved the fit giving a straight line fit of $TTD_{calc} = -108.48 \log_{10}N_0 + 956.56$, equating to a growth rate of 0.00923 $\log_{10}$ cfu/ml/min (95% CI: 0.00915 – 0.00932). A lag of -14.3 minutes (95% CI: -18.4 to -10.2) was obtained; the correlation between lag and growth rate was 0.90, a result very similar to the fitting of the Baranyi equation.

3.7 GROWTH RATE OF *L.* MONOCYTOGENES AT 30°C FROM OD DATA

The TTD from a ten-fold dilution series of an initial standardised inoculum of *L. monocytogenes* incubated at 30°C in TSB were obtained. A regression fit gave $TTD = -127.09 \log_{10}N_0 + 1121.8$, $r^2 = 0.999$; giving a growth rate of 0.00787 $\log_{10}$ cfu/ml/min. The intercept of 8.83 $\log_{10}$ cfu/ml (95% CI: 8.80 – 8.85) suggests the absence of a lag. Initially the Baranyi model was fitted to the TTD data using an MPD of 9.8 and a detection inoculum of 8.9. The specific growth rate obtained was 0.00786 $\log_{10}$ cfu/ml/min (95% CI: 0.00780 – 0.00792) and a lag of -17.5 mins (-22.5 to -12.5 mins). Using the calibration curve found previously (Eq.5a) the calculated numbers were transformed to OD values. An MPD of 9.8 was found, however, to be too low for the maximum OD observed. The MPD was increased to 9.9 and this gave reproducible OD curves. Changing the MPD left the specific growth rate unchanged but the lag increased to -1.0 min.

3.8 EFFECT OF PH

An initial $\log_{10}$ inoculum of 5.4 (determined from plate counts) was used to study the effect of a range of pH (7.05 to 3.46) on growth. No visible growth was observed during the 3 day incubation at 30^{o}C at pH 4.42 or less. As the pH was reduced, the OD maximum was reduced and the rate of change of OD also decreased. An analysis of the numbers per ml at an OD = 0.2 at pH 5.03, 4.95, 4.88 and 4.68 gave an average of 8.8 $\log_{10}$ cfu/ml. There was no statistical difference between the numbers obtained at the lower pH at OD = 0.2 and at the more optimal pH values. The OD data at pH 6.95 were fitted with the Baranyi equation in concert with the calibration equation. Although the initial log inoculum size was determined as 5.4 from plate counts, from the TTD/log initial inoculum calibration curve obtained at 30^{o}C a count of 5.5 was expected. The initial log cfu/ml was held at 5.5 and the specific growth rate, lag and the maximum population density were obtained by regressing the calculated OD against the observed, Figure 6 displays these results. Table 2 gives the parameters obtained; in no case was a significant value for a lag observed (i.e., in all cases the confidence interval for the calculated lags included zero).

4 Discussion

Cuppers and Smelt (1993) described an observed linear relationship between the log of the initial inoculum size and the time taken for the incubating culture to reach a specified turbidometric detection level due to a $10^{6.4}$ cfu/ml culture. They modeled the TTD data using a model based on the presence of a lag and the time taken for the initial culture to grow to the threshold value. Hence the growth rate could be calculated. Essentially this study modeled the underlying growth curve as a 3-phase linear model, ignoring the MPD value.

From the classic logistic equation, the time taken (t_N) to reach a specific population level (N) from a given initial value (N_0) is given by

$$t_N = -\frac{1}{\mu}\ln N_0 + \frac{1}{\mu}\ln\left(\frac{N(M-N_0)}{M-N}\right) \qquad \text{(Eq. 6)}$$

where μ is the growth rate and M is the maximum population density (also known as the carrying capacity).

This is almost in the linear form y = mx + c, especially when M >> N_0, and a plot of the time to the specific level against the natural log of the initial population number gives a gradient from which the growth rate can be found. When $N_0 = 1$, the intercept is obtained – the time taken for one organism to reach the specified detection number. One important point is that the logistic model as applied here has no lag. When we consider the phenomenon of microbial lag, we could simply state that if t < t(lag) then N(t) = N(0) and change the time function to t-t(lag) to account for the change. Physically this makes sense; the logistic equation is devoid of a lag, microbial lag is caused by an event (or sequence of events) before the onset of growth, hence is not part of the original derivation. Mathematically, however, the resulting logistic with lag equation has some undesirable features: the formula is discontinuous at the end of lag. This was, essentially, the equation reported by Jason (1983) and indeed the linear relationship between the log of the initial inocula (of *E. coli* growth measured using conductivity) and the time to reach a specific value was reported then.

The Baranyi model (Baranyi et al. 1993, 1993b) can be considered as a well-designed solution to the problem with the application of the Jason model. By invoking a time delay function, based on a biological foundation, the model becomes continuous, and remains biologically interpretable. A major feature of the model has been the assignment of the so-called pre-exponential factor which relates the fitness of an organism to thrive in an environment relative to another. If there is no difference between environments then the theory states that there should be no lag if the organism is transferred from one to the other and therefore the basic logistic model should apply – which is the default for the Baranyi model.

The TTD data produced using the multiple inocula technique described could be well fitted using the 3-PLM, the Baranyi and the logistic (with or without lag), the parameters obtained were consistent between models and reflected the observed gradients well. Further, using a simple conversion between OD and numbers (cfu/ml), the basic features of the OD/time plots could be reproduced with these models. The rescaling functions (Eqs. 6a and 5b) overcome the peculiar problem described by Baranyi and Roberts (1995): that direct fitting of viable count data to turbidity or conductivity data or vice-versa should not be considered without additional information being available. The calibration curves used in this work can be used since they are obtained indirectly from pre-knowledge of the initial inoculum size, the maximum population density and the maximum specific growth rate.

The modified logistic and modified Gompertz equations, however, failed to fit the observed data and could not reproduce the observed OD/time plots. A simple simulation of growth data with a given μ_{max}, lag and MPD for a number of initial inocula was produced using the modified Gompertz (or indeed the modified logistic) equation. A plot of the initial log inocula against the TTD (Eq. 2) for a given detection number (log N_d) gave approximate straight line fits. The gradients of the line, however, were not the reciprocal of the growth rates used. When TTD were obtained for the same initial conditions, but for differing log N_d then these plots did not have the same gradient as was observed, and as log N_d approached the MPD the plot became increasingly curved. From a casual glance at equation 2, this equation cannot give a simple linear fit with respect to the initial log inoculum used.

The 3PLM, the Baranyi and the classical population logistic model were the only models examined which were capable of reproducing the straight line fit observed for the plot of the initial log numbers against the TTD. The 3PLM, however, suffers from the inability to approach the MPD continuously, and although giving the correct TTD for OD = 0.2 for the cultures, it failed to give the approach to the maximum OD observed. The Baranyi and the classical logistic models did not have this problem.

Applying the method of fitting the Baranyi model directly to another set of data (30^{o}C), by simply changing the MPD slightly, a good fit to the OD data was found. Equally, the lack of an apparent lag (either from the fit of the model or from an analysis of the OD/time plot for large initial inocula), suggested that the basic population logistic model would give an equivalent fit. This was indeed found to be the case.

Several reports have suggested that the OD technique is limited as it requires high initial inocula (Baty et al. 2002; Dalgaard et al. 1994; Dalgaard and Koutsoumanis 2001; Perni et al. 2005). The observed data described herein show that this assumption is not valid. If the growth rate of an organism under ideal conditions is obtained using the multiple inoculum dilution method then any subsequent study using non-ideal conditions can use a positive control to set the modelled fit. For example in the study of pH, the growth rate at the ideal growth pH was known. The size of the initial inoculum used could then be either found from the calibration curve (knowledge of the TTD) or from plate counts (or both if confirmation was required). As conditions change (e.g. reducing pH) the fixed parameters of the Baranyi model can be altered to fit the new growth rates and/or lag induction. In the case studied here, the Baranyi model suggested that the growth rate reduced but that lag was not induced over the pH range studied.

In 2002, McKellar *et al.* produced a study of the effect of pH on the growth of *Listeria monocytogenes* (using the strain Scott A) stating that pH had no affect on the initial physiological state. A graph of the initial log inoculum (per well) against the TTD for a range of pH showed multiple linear slopes apparently intercepting the $\log_e$ inoculum axis at 20.086 +/- 1.092: equivalent to a range of 8.25 to 9.2 $\log_{10}$ cfu per well. This range encompasses the 8.37 $\log_{10}$ cfu/well detection threshold found with this work. Further our study of the effect of pH suggests that there was no change in lag– only a change in growth rate. The difference between our interpretation and that of McKellar *et al.* is the presence or not of a lag. However, an important point must be made with respect to the detection value used in McKellar and Knight (2000) and McKellar et al. 2002: if the detection threshold is equivalent to $3.5x10^6$ cfu/well, then all the TTD values of wells containing greater than $3.5x10^6$ cfu/well should be zero; from figure 4 of McKellar et al. 2002 this is clearly not the case.

In McKellar and Knight (2000) the time of lag was calculated as the difference between observed TTD data and the theoretical TTD plot of the log of the initial inoculum based on a detection limit of

3.5×10^6 cfu per Bioscreen well for a given growth rate. Observed data had the theoretical growth rate but had greater TTD values since a lag was present. The observed data at pH 7.2 gave a regression fit of $TTD_{30oC} = -132.84 \log_{10} Io + 1221$, but was quoted in terms of cfu per well, which were filled with 350μl of culture. The growth rate obtained was very similar to that obtained from the data observed in this report (-127.09), but the intercept was higher than was found (in terms of cfu/ml, the intercept was calculated to be 1281 mins, whereas a value of 1122+/- 4.6 was observed in this study). At lower pH, the growth rate reduces and according to McKellar *et al.* since the physiological state is constant the lag increases and this can be obtained from the difference of the observed to the theoretical intercept on the TTD axis. However, if this was the case then at the lower pH values with reduced growth rates, according to the logistic model (eq. 6) a vertical separation between the horizontal inoculum axis and the observed TTD should have been observed and this does not appear to be the case.

We suggest the discrepancy between the work reported here and the interpretation of the observations of these other workers may be due to an inadvertent use of the threshold detection value vs. a TTD based on a specific OD. Figure 2 shows the TTD for different criteria: the OD criteria of 0.09 is slightly above the background, this has a detection value of approximately 8×10^6 cfu/well (recalculated for a volume of 350μl per well). If this calibration curve was considered as the threshold curve, then any of the other calibrants would have a constant time delay (i.e. interpreted as lag) between them and this line. This may explain the difference in interpretation between the studies. We suggest that the work of McKellar shows a constant lag over the pH range used.

From the interpretation of the Baranyi model (Baranyi *et al.* 1993b), the physiological state of the cells, denoted as α_0, at t_0 is a measure of the fitness of the cell in one environment to cope with being placed in a new environment. The negative natural log of α_0 is the product of the maximum specific growth rate and the lag. Hence, if there is no lag then $\ln(\alpha_0) = 0$. Both the study described herein and we believe that of McKeller et al. suggest, however, that the automatic link of lag and growth may be globally invalid; growth rates can alter without inducing lags (e.g. the observed data on the change of pH). Conversely, lags can be induced without inducing changes in growth rate (albeit after recovery from injury) as shown by the work of Stephens et al.(1997).

4.1 CONCLUSIONS:

We would simply conclude, therefore, that the Baranyi model is the most capable primary model of those examined, but that the *modified* logistic and the *modified* Gompertz should not be used as Primary models for TTD experiments since they cannot reproduce observed data.

5 References

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6 Tables

Table 1. Parameter estimates from linear regression fits to TTD data for multiple initial inocula of *Listeria monocytogenes* at 37°C in TSB for different time to detection criteria at 600nm.

	Gradient			*Intercept*				
OD criterion	*Coefficient*	*Lower 95%*	*Upper 95%*	*Coefficient*	*Lower 95.0%*	*Upper 95.0%*	r^2	*log ID**
0.09	-104.17	-109.15	-99.19	766.52	740.22	792.81	0.981	7.358
0.1	-104.17	-105.83	-102.50	824.96	816.37	833.54	0.997	7.919
0.2	**-108.49**	**-109.60**	**-107.38**	**956.29**	**950.29**	**962.30**	**0.999**	**8.814**
0.3	-109.20	-110.14	-108.26	996.36	991.23	1001.48	0.999	9.124
0.4	-110.51	-111.54	-109.48	1032.31	1026.56	1038.05	0.999	9.341
0.5	-110.23	-111.31	-109.14	1056.05	1050.08	1062.03	0.999	9.581
0.6	-110.53	-111.63	-109.43	1085.84	1079.78	1091.90	0.998	9.824
0.7	-110.34	-111.75	-108.93	1113.02	1105.24	1120.79	0.997	10.09
0.8	-110.20	-112.44	-107.95	1153.58	1141.18	1165.97	0.994	10.47
0.9	-110.32	-113.38	-107.26	1216.46	1199.52	1233.41	0.988	11.03
0.95	-109.88	-113.23	-106.53	1252.31	1233.60	1271.02	0.987	11.40
1.0	-110.81	-114.12	-107.50	1285.19	1266.28	1304.10	0.993	11.60

*Log ID is the calculated intercept on the log Io axis for the given regression fit; the MPD (from plate counts) was 9.8 log cfu/ml.

Table 2. Parameter Estimates for the Baranyi equation fitting of OD data at various pH values at 30°C

pH	**Mu**	**LCL**	**UCL**	**MPD**	**-95% CI**	**+95% CI**
6.95	**0.01870**	**0.01864**	**0.01875**	**9.771**	**9.769**	**9.773**
5.65	**0.01630**	**0.01625**	**0.01636**	**9.778**	**9.776**	**9.781**
5.51	**0.01472**	**0.01467**	**0.01478**	**9.777**	**9.774**	**9.781**
5.03	**0.01333**	**0.01328**	**0.01338**	**9.780**	**9.776**	**9.784**
4.95	**0.00984**	**0.00980**	**0.00988**	**9.736**	**9.732**	**9.741**
4.88	**0.00812**	**0.00808**	**0.00816**	**9.643**	**9.639**	**9.647**
4.68	**0.00592**	**0.00591**	**0.00594**	**9.566**	**9.563**	**9.569**

Growth rate as ln cfu/ml/min

7 Figures Captions

Figure 1. Optical density-incubation time plot for the growth of multiple initial inocula of *Listeria monocytogenes* (isolate Lm252) at 37^{o}C in TSB. Each curve represents a single initial inoculum ranging from 9.1 $\log_{10}$ cfu/ml to 1 cfu/ml (left to right), wells with no cells present failed to show growth (horizontal OD at background level).

Figure 2. The time to detection of multiple initial inocula of *Listeria monocytogenes* at 37^{o}C in TSB. The TTD criterion was set at OD =0.09 (▲), 0.1 (□), 0.2 (■), 0.4 (○), 0.6 (●). An OD = 0.2 at 600nm was equal to 8.81 $\log_{10}$ cfu/ml.

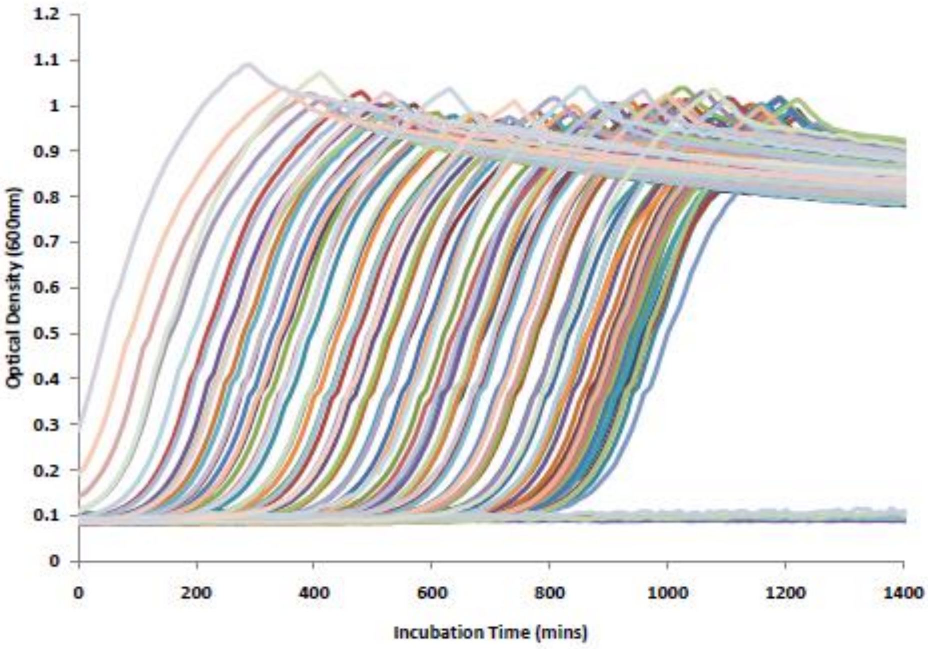
Figure 3. Predicted microbial numbers of *L. monocytogenes* (Lm252) with time from the Baranyi model, with parameters μ=0.00921, Lag = -8.88 mins, MPD = 9.8 $\log_{10}$ cfu/ml, with a range of initial inocula.

Figure 4. Plot of the observed OD against the calculated numbers/ml from the associated Baranyi equation (diamonds), the solid line is the regression fit used in this study. The calibration curve obtained from plate counts (squares) is also displayed.

Figure 5. Comparison of the observed optical density incubation time plot (symbols) and the calculated (solid lines) for *Listeria monocytogenes* incubated at 37^{o}C with initial $\log_{10}$ inocula of (from left to right) 8.789, 7.789, 6.585, 5.09, and 3.284 respectively.

Figure 6. Comparison of the observed optical density incubation time plot (symbols) and the calculated (solid lines) for *Listeria monocytogenes* incubated at 30^{o}C with initial inocula of 4.97 $\log_{10}$ cfu/ml, at pH 6.95, 5.65, 5.51, 5.03, 4.95, 4.88, 4.68 from left to right respectively, pH 4.42 failed to show any visible growth during the period of incubation (constant OD = 0.088).

Figure 1.



1.2
1.1
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
Optical Density (600nm)
0
200
400
600
800
1000
1200
1400
Incubation Time (mins)

Figure 2

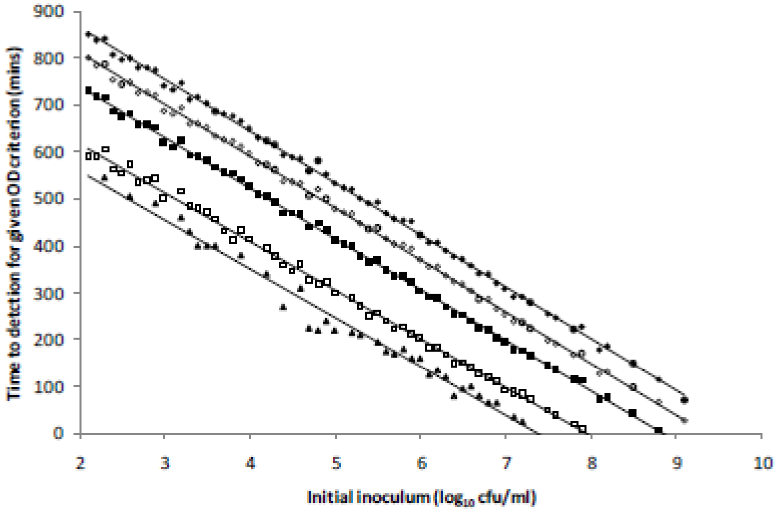

Time to detction for given OD criterion (mins)
900
800
700
600
500
400
300
200
100
0
2
3
4
5
6
7
8
9
10
Initial inoculum ($\log_{10}$ cfu/ml)

Figure 3

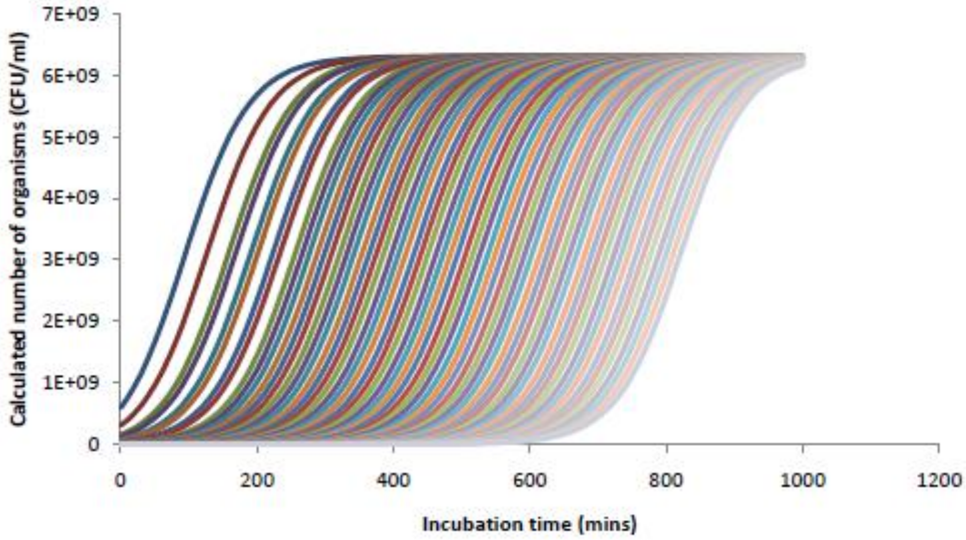
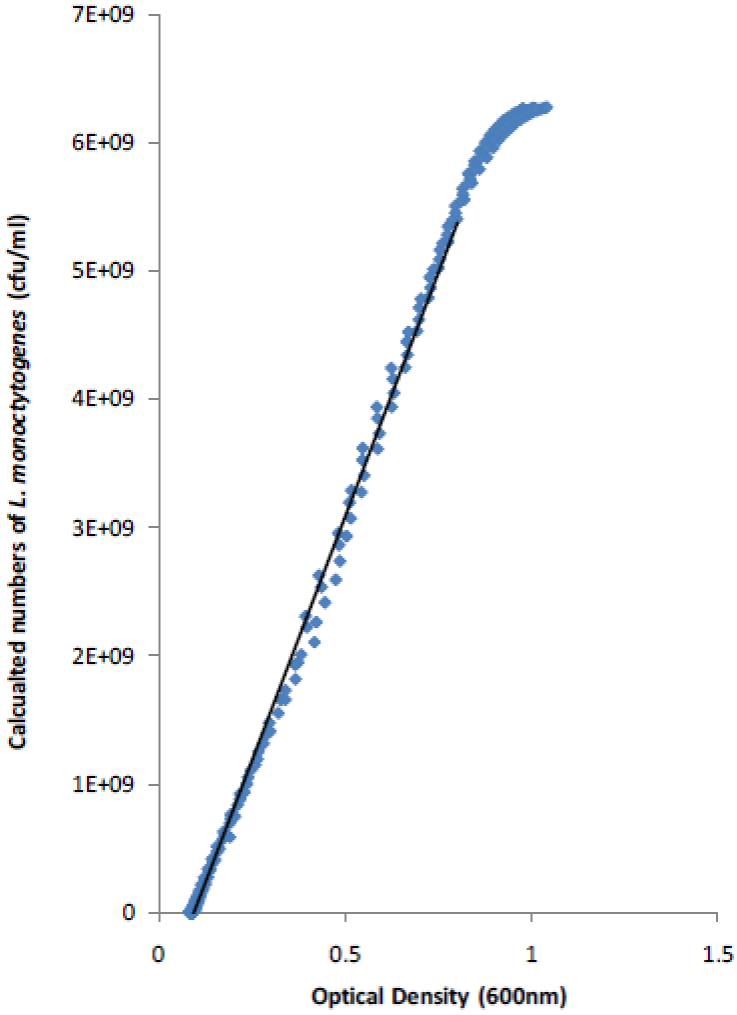
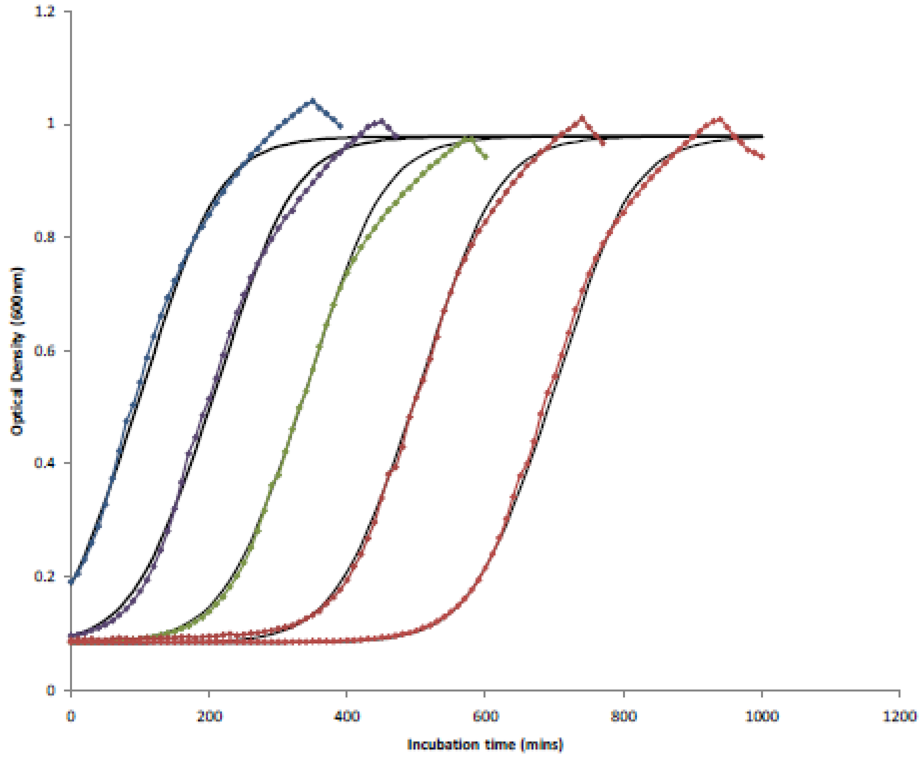

7E+09
6E+09
5E+09
4E+09
3E+09
2E+09
1E+09
0
0
200
400
600
800
1000
1200
Incubation time (mins)
Calculated number of organisms (CFU/ml)

Figure 4



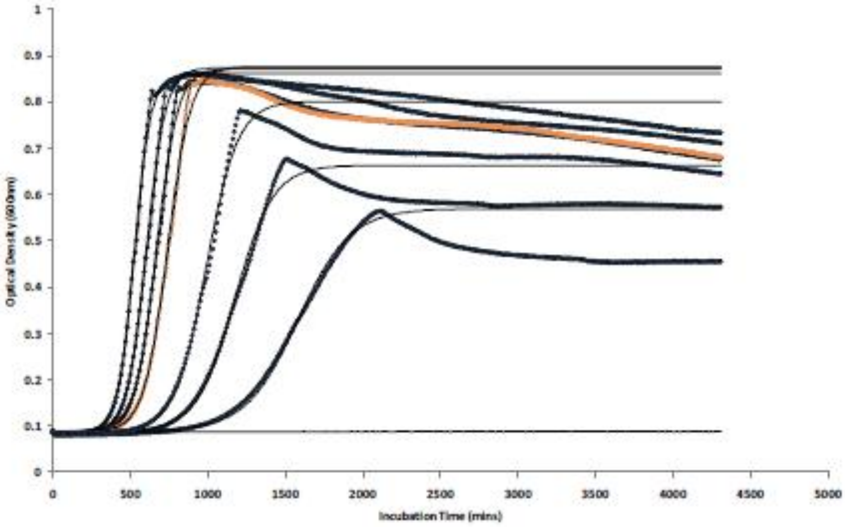
583
584

Figure 5



585
586

Figure 6


1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
Optical Density (600nm)
0
500
1000
1500
2000
2500
3000
3500
4000
4500
5000
Incubation Time (mins)

Growth curve prediction from optical density data

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2012-03-15T00:00:00Z

I Mytilinaios, M. Salih, H.K. Schofield and R.J.W. Lambert. Growth curve prediction from optical density data. International Journal of Food Microbiology, Volume 154, Issue 3, 15 March 2012, Pages 169-176.

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