Estimating the Parameters of the Baranyi-model for Bacterial Growth

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Abstract

The identifiability properties of the Baranyi-model for bacterial growth were investigated, both structurally and applied on real-life data. Using the Taylor-series approach, it was formally proven that the model is structurally identifiable, i.e. the model parameters can be given unique values in the presence of perfect (noiseless) bacterial growth data. The model also has acceptable practical identifiability properties in the presence of realistic data, although there was a relatively high correlation between two of the model parameter: the maximum specific growth rate μ_{max} and the suitability indicator h_0. An optimal experimental design to improve parameter estimation uncertainty was worked out, using the sampling times of the microbial growth curve as experimental degree of freedom. Using a D-optimal design criterion, it could be shown that the optimal sampling times were concentrated in 4 regions, each providing maximum information on a particular parameter. The uncertainty of the design was assessed with a Monte Carlo simulation to yield the 95 % confidence intervals on the proposed sampling times. Based on these intervals, a design was proposed and experimentally validated. The error on the parameter estimates was more than halved, their correlation diminished and the nonlinearity of the result improved.

Introduction

Predictive modelling of bacterial growth and inactivation is an important research topic among food microbiologists (Buchanan, 1993, Skinner and Larkin 1994, McMeekin et al. 1997). Predictive models allow to estimate the shelf-life of foods, isolate critical points in the production and distribution process and can give insight on how environmental variables affect the behaviour of pathogenic or spoilage bacteria. Whiting (1995) distinguishes three levels among predictive microbiological models: primary level models describe changes of microbial numbers with time, secondary level models summarise the effect of environmental conditions and the tertiary level models combine the two first levels. Although the concepts presented in this paper apply to all types of models, the practical implementation will be restricted to a level 1 model.

Among the most used level 1 predictive models are the modified Gompertz model (Zwietering et al. 1990, McMeekin et al. 1993) and the logistics model (Peleg 1997). Although based on theoretical considerations, these models were not originally developed for modelling bacterial growth and certainly not for modelling the logarithm of the bacterial cell concentration (Baranyi et al. 1993). They should therefore be considered as purely empirical models. Nevertheless, the parameters in the model are given physical meaning whose values are crucial for the deployment and interpretation of simulation results. More recently, Baranyi and coworkers developed a mechanistic model for bacterial growth (Baranyi et

al. 1993, Baranyi and Roberts 1994, 1995). Last years, this model is being more and more adopted over the modified Gompertz model (George et al. 1996, McClure et al. 1997, Sutherland et al. 1997). One of the attractive points of the Baranyi-model, besides its good predictive capabilities, is the fact that it is a truly dynamic model in the sense that it can deal with time varying environmental conditions. In view of the growing attention given to shelf-life prediction of foods and quantitative risk analysis of food production cycles (McMeekin and Ross 1996, Foegeding 1997), this is an indispensable property.

The value of model-based predictions and risk analysis is to a large extent dependent on the reliability of the model parameters. Parameter values are obtained by fitting the model to experimental data. The experimental error, the experimental design and the model bias determine the reliability of estimates. The statistical significance that can be attributed to parameter estimates is generally referred to as the identifiability of the parameters. Problems with the identifiability arise when parameters cannot be significantly estimated or when estimates are strongly correlated. The importance of sufficient and well distributed experimental data has been addressed by Bratchell et al. (1989) and Baranyi et al. (1996) and cannot be overestimated: a good experimental design can overcome many problems with parameter identifiability.

In this paper, the identifiability properties of the Baranyi-model have been examined in detail. The analysis is restricted to the explicit form of the model for constant environment settings only. This was done for two reasons:

- The analysis gets quite complicated when considering the differential equation form of the model, which could conceal the principles of the identifiability analysis.
- Generally, the model is used in the explicit form when the aim is to estimate the parameters. The differential equation form is subsequently used to simulate dynamic environmental conditions (Baranyi et al. 1995). It could be more efficient to estimate parameters directly from data gathered under dynamic environmental conditions with the differential-equation model. Using optimal experimental design, Bernaerts et al. (1998) showed that temperature-dependent parameters could be estimated directly from the differential equation form of the Baranyi-model.

The accuracy of predictions made by predictive microbiological models is dependent on the model parameter estimate errors. The smaller these errors, the more reliable predictions will be. The aim of this paper is to analyse the identifiability properties of the Baranyi model and to establish an efficient method for improving the reliability of the model parameter estimates based on available knowledge.

The model

The reader is referred to Baranyi et al. (1993) for the theoretical derivation of the model. The explicit form of the model is the following (Baranyi and Roberts 1994):

$$\begin{aligned} y(t) =& y_0 + \mu_{max} t + \frac{1}{\mu_{max}} \ln\left(e^{-\nu \cdot t} + e^{-h_0} - e^{-\nu \cdot t - h_0}\right) \\ & - \frac{1}{m} \ln\left(1 + \frac{e^{m\mu_{max} t + \frac{1}{\mu_{max}} \ln\left(e^{-\nu \cdot t} + e^{-h_0} - e^{-\nu \cdot t - h_0}\right)} - 1}{e^{m(y_{max} - y_0)}}\right) \end{aligned} \quad (1)$$

where:

- $y(t) = \ln(x(t))$ with $x(t)$ the cell concentration $\left(\frac{CFU}{ml}\right)$
- $y_0 = \ln(x_0)$, $y_{max} = \ln(x_{max})$, x_0 being the initial and x_{max} the asymptotic cell concentration, respectively
- μ_{max} is the maximum specific growth rate $\left(\frac{1}{h}\right)$
- m is a curvature parameter to characterise the transition from the exponential phase
- ν is a curvature parameter to characterise the transition to the exponential phase
- h_0 is a dimensionless parameter quantifying the initial physiological state of the cells. From that, the lag time λ (h) can be calculated as $\frac{h_0}{\mu_{max}}$.

For the curvature parameters, Baranyi (1997) suggests $\nu = \mu_{max}$ and $m = 1$, values that are also adopted in this paper. This decreases the number of parameters by 2, so the model has 4 parameters: μ_{max}, h_0, y_0 and y_{max}.

Baranyi and Roberts (1995) noted that h_0 can be thought of as a suitability indicator of the micro-organism population to the actual environment. If the experimental procedure is standardised, this suitability indicator will be more or less constant which is equivalent to the assumption that the lag and μ_{max} are inversely proportional.

Structural identifiability

Theoretical identifiability is related to the possibility to give a unique value to a parameter of a mathematical model (Vanrolleghem and Dochain 1998). Otherwise stated: are parameters identifiable given a model structure and perfect data of model variables? A structural identifiability analysis can for example

reveal that only certain combinations of parameters are identifiable. The method to analyse the structural identifiability followed here was developed by Pohjanpalo (1978) and is based on the Taylor series expansion of the model. This method consists of examining the successive derivatives to check if they contain information about the parameters to be identified. The Baranyi-model contains 4 parameters, so if it can be shown that the model parameters can be written as a combination of any 4 derivatives of the Taylor series expansion, the structural identifiability of the model is proven.

Let the model be denoted by $f(t)$ and $\frac{d^i f}{dt^i}(0)$ by z_i, then it can be proven that the 4 parameters can be written as a combination of the z_i's:

$$\begin{aligned}
\mu_{max} &= \frac{\sqrt{z_2^4 + 3z_3^2 - 2z_2 z_4}}{z_2} \\
h_0 &= \frac{\ln(2)}{\frac{z_2^2 - z_3}{\sqrt{z_2^4 + 3z_3^2 - 2z_2 z_4}} + 1} \\
y_0 &= z_1 \\
y_{max} &= z_1 - \frac{\ln(2)}{\frac{z_2^2 - z_3}{\sqrt{z_2^4 + 3z_3^2 - 2z_2 z_4}} + 1} - \ln\left(\frac{1}{2} - \frac{z_2^2 + z_3}{2\sqrt{z_2^4 + 3z_3^2 - 2z_2 z_4}}\right)
\end{aligned} \tag{2}$$

This analysis demonstrates that the Baranyi-model is structurally identifiable, showing that this model structure will lead to identifiable parameters in the case of an ideal set of data.

Practical identifiability

Now that we know that the Baranyi-model is structurally identifiable, the question remains what the identifiability properties are when using actual data. Real-life data will always contain a certain level of noise, having its impact on the parameter estimation process. The question to be asked here is if it is possible to give the parameters unique values with actual, experimental data. Some theorectical background on parameter estimation of nonlinear models is necessary to explain the methods used and can be found in the Appendix.

Fitting the Baranyi-model to an experimental dataset

Applying the nonlinear estimation technique outlined in the Appendix, the Baranyi-model was fitted to a *Salmonella enteritidis* growth curve. The environmental conditions remained the same for the curve, which was generated at 30°C in egg yolk. The data consists of 18 data points and is summarised in Table 1.

[Table 1 about here.]

The model was fitted to the data using the Levenbergh-Marquardt algorithm to minimize the objective function (Press et al. 1992). The experimental growth data, together with the fitted Baranyi curve is shown in Figure 1.

[Figure 1 about here.]

From the statistical analysis of the results, it can be seen that the parameters all can be estimated significantly and that the correlation between the estimates is reasonably low. It must be added that this type of analysis is strictly only valid for linear models with normally distributed measurement errors.

The 95% confidence intervals indicate that all parameters can be estimated significantly (Table 2). It is clear that the parameter h_0 has the largest boundaries on the interval, i.e., it has the largest estimation error. Since the lag time λ has to be deduced from h_0, there is quite a large uncertainty on λ, which has been reported before (Baranyi and Roberts 1994, Wijtzes et al. 1995). Using the relationship $h_0 = \lambda\ \mu_{max}$, the calculated lag time is 2.441 hours, with a standard error of 3.970 (as calculated from the general law of variances).

[Table 2 about here.]

The correlation matrix of the parameter estimates shows that the parameter estimates are moderately correlated between one another. The μ_{max} and h_0-estimates show a relatively high correlation of 0.75.

$$\begin{pmatrix} & \mu_{max} & h_0 & y_0 & y_{max} \\ \mu_{max} & 1 & 0.752 & 0.268 & 0.00344 \\ h_0 & 0.752 & 1 & 0.803 & -0.00202 \\ y_0 & 0.268 & 0.803 & 1 & -0.000645 \\ y_{max} & 0.00344 & -0.00202 & -0.000645 & 1 \end{pmatrix}$$

More important than the individual confidence intervals is the joint confidence region, obtained by varying all the parameters simultaneously. As was shown before, this joint probability region is a hyperellipsoid, and can only be graphically represented for maximum 3 parameters. Some insight in the shape of the hyperellipsoid can be obtained with the projections of the probability region on the 2 dimensional parameter subspaces. This gives contour plots showing lines of equal value as shown in Figure 2, summarising all the possible parameter combinations. It can be seen that the confidence region does not show strong nonlinear shapes for any of the parameter combinations. However, for

some combinations the ratio of the large axis to the small axis is relatively far away from one (circular contours). The length of the ellipse axes is proportional to the eigenvalues of the Fisher matrix. The closer the eigenvalue ratios are to one, the better the identifiability properties of the model for the particular dataset.

[Figure 2 about here.]

Following the approach of Ratkowsky (1983), the linear behaviour of the parameter estimates was assessed by comparing the maximum relative parameter-effects curvature with the 95 % confidence region relative curvature. If the first curvature measurement is small compared to the latter, the estimates are said to behave nearly linear. This is important to evaluate the validity of the statistical tests that are based on the linear approximation of the nonlinear model, such as the standard error of the estimates. The maximum relative parameter-effect curvature had a value of 0.574, while the 95 % confidence region relative curvature had a value of 0.567. The condition of linearity is not met, although the difference is rather small. This means that one has to be careful interpreting the regression statistics.

Sensitivity functions

The output sensitivity functions are the major components of the Fisher matrix, and *a fortiori* of the covariance matrix, so they are key components of practical identifiability analysis. If the sensitivity equations are proportional, the covariance matrix is singular and the model is not practically identifiable (Robinson 1985). If they are nearly proportional, the parameter estimations will be highly correlated. Plotting the sensitivity functions can therefore give a quick indication about identifiability problems. The sensitivity functions of the Baranyi-model are calculated by taking the partial derivatives of the model to the 4 parameters, respectively:

$$\begin{aligned}
\frac{\partial y}{\partial \mu_{max}} &= -\frac{e^{h_0+t\cdot\mu_{max}}\ \left(e^{y_0}-e^{y_{max}}\right)t}{\left(-1+e^{h_0}+e^{t\cdot\mu_{max}}\right)\left(-e^{y_0}+e^{h_0+y_{max}}+e^{y_0+t\cdot\mu_{max}}\right)} \\
\frac{\partial y}{\partial h_0} &= \frac{e^{h_0}\ \left(e^{y_0}-e^{y_{max}}\right)\left(-1+e^{t\cdot\mu_{max}}\right)}{\left(-1+e^{h_0}+e^{t\cdot\mu_{max}}\right)\left(-e^{y_0}+e^{h_0+y_{max}}+e^{y_0+t\cdot\mu_{max}}\right)} \\
\frac{\partial y}{\partial y_0} &= \frac{e^{(h_0+y_{max})}}{-e^{y_0}+e^{(h_0+y_{max})}+e^{(y_0+t\cdot\mu_{max})}} \\
\frac{\partial y}{\partial y_{max}} &= \frac{e^{y_0}\ \left(-1+e^{t\cdot\mu_{max}}\right)}{-e^{y_0}+e^{(h_0+y_{max})}+e^{(y_0+t\cdot\mu_{max})}}
\end{aligned} \tag{3}$$

As illustrated here, for nonlinear models, the sensitivity functions are dependent on the model parameters (Draper and Smith 1981). Figure 3 shows the evolution of the different sensitivity functions with time, using the estimated parameter values.

[Figure 3 about here.]

As stated before, when two or more sensitivity functions are proportional to each other, then the model is not identifiable. In this case $\frac{\partial y}{\partial y_0}$ and $\frac{\partial y}{\partial y_{max}}$ are linearly correlated, but they are not proportional because an intercept exists if a linear regression is performed between them. Visual inspection may be misleading as we are looking for linear combinations of sensitivity functions and they may not appear straightforwardly. Linear analysis may help in this.

The sensitivity functions show at what times the parameters are most sensitive to the measured data. This means that the information content of an experiment can be increased by sampling at times when the sensitivity functions have the highest values. This principle is very important for optimal experimental design, as will be illustrated in the next sections.

Optimal experimental design for parameter estimation (OED/PE)

In the framework of predictive microbiology and quantitative risk analysis, two fields in food microbiology that require extensive use of mathematical models, it is important that the model parameters, which have a clear physical meaning, are known with the most statistical significance. It warrants the best use of experimental resources to produce the most informative experiments.

OED/PE methods use the Fisher information matrix **F** as a starting point because it summarises all information on the preciseness and correlation of the parameter estimates. Several design criteria have been worked out (Godfrey and Di Stefano 1985). The most widely used is the D-optimal or minimum volume design criterion which aims at maximising the determinant of **F**, equivalent to minimising the geometric mean of the identification error.

Optimisation of an experiment can only be done when the experimenter has a certain degree of freedom available to improve the experimental conditions. In this study, only the number of sample points and the timing of the samples are considered. Optimal experimental design then reduces to finding the optimal sampling times to obtain the most reliable parameter estimates. This is equivalent to an optimisation problem with the same dimension as the chosen number of data points.

Optimising the sampling frequency

In order to improve the accuracy of the parameter estimates, the growth curve can be replicated. The aim of the OED/PE method outlined here is to use the available information optimally to do such a replication. There is a need for *a priori* parameter estimates because **F** is dependent on the parameter values. This prior information can be available from previous experiments, literature data, extrapolation

or some other source of data. A change in the chosen experimental degree of freedom, here the sampling times, will result in a different value of **F**. Following the D-optimal criterion the determinant of **F** is then to be maximised as function of the sampling times. The maximisation was done using the multidimensional optimisation algorithm of Brent (Brent, 1973). The only constraint imposed on the optimisation made sure that the sample points could not exceed the preset experimental range. The number of sampling points had to be known in advance. In order to assess the influence of the number of samples, the optimisation exercise was accomplished for several sampling numbers.

Results

Although the optimisation of the D-optimal criterion is in essence a nonlinear optimisation problem, there was no evidence of local optima in the response surface of the objective function. The optimisation results are summarised in Table 3. It can be seen that the criterion has steadily larger values for larger number of data points, which is perfectly logical as the information content is a sum of squares ($\mathbf{J}^T\mathbf{J}$). It is obvious that there are 4 optimal sampling times emerging from the optimisation, irrespective of the number of sample points. Looking to Figure 3, we can see that they roughly coincide with the maximum absolute values of the sensitivity functions for the 4 parameters. The D-optimal criterion points to experiments with sampling on only 4 positions, with replacements to increase the accuracy.

[Table 3 about here.]

Let's denote the 4 optimal sampling times as sample point 1, 2, 3 and 4. It can be seen from Table 3 that points 1, 3 and 4 are quite consistent over the total number of observations, while point 2 is more spread over time, albeit the spreading is rather limited. Point 2 is 'related' to h_0, which showed the largest uncertainty after fitting.

An interesting observation can be made when looking more closely to the optimisation results. The number of samples at each optimal sample point are summarised in Table 4. This gives a feel of the importance of each sample point in terms of experimental information.

[Table 4 about here.]

Confidence limits on the optimal sample points

It is dangerous to rely completely on sampling at one time with replacement, because the design is dependent on the *a priori* parameter values obtained by preliminary experiments. These *a priori* parameter values are considered as being the best available by the design criterion. In the OED/PE methodology, no direct procedure exists for inclusion of parameter uncertainty in designing an experiment. The

robustness of the optimal design of Table 3 to parameter uncertainty was assessed using Monte Carlo simulation on the 4 model parameters (Weijers and Vanrolleghem 1998). Conceptually, this means that synthetic data sets are constructed with exactly the same number of measurements as the actual data set by using the Baranyi-model (equation 1). If there are sufficient synthetic data sets, the distributions for the optimal sample positions can be obtained and confidence limits can be subsequently calculated.

Two series of simulations were done. For both series, the parameter estimates were assumed to follow a normal distribution, truncated at 0 because the parameters must be positive by definition. For the first series, the statistical parameters obtained from the preliminary estimation (Table 2) were used. This is a case where the *a priori* parameter values are of good quality. For the second series, the errors on the parameter estimates were doubled which makes the prior estimates of poor quality. Note that this would imply that the h_0 estimate would be not significantly different from 0. The *a priori* estimates distributions were sampled using the Latin Hypercube scheme (LHS) (Vose 1995).

For a large number of iterations, Monte Carlo simulations can reproduce the true distribution of parameters of interest, in this case the optimal sampling times. Because 4 sampling times emerged as optimal, irrespective of the total number of samples, the simulations were done for the case of 4 sampling times. The number of iterations was 1000, sufficient for reliable results. The sampling points 1 and 4 showed no variation at all, so it is pretty safe to sample the beginning and the end of the growth curve at fixed times. The histograms for optimal sample points 2 and 3 are shown in Figure 4. The particular shape of the distributions give an indication what sample times are most sensitive towards the quality of the *a priori* estimates. From these histograms, the confidence intervals can be calculated using numerical integration. The 95 % confidence intervals for the optimal sampling times are shown in Table 5. As could be expected, the confidence interval is largest for x_2, i.e., the point contributing the information on h_0. Logically, the confidence limits are wider when the *a priori* estimate errors are larger.

[Figure 4 about here.]

[Table 5 about here.]

Experimental validation of the experimental design

Based on the results of the OED/PE calculations, the original growth curve was repeated using the same number of sample points (Table 6).

[Table 6 about here.]

Following the results shown in Table 4, 2 points were measured at sampling time 1, 5 at sampling time 2, 6 at sampling time 3 and 5 at sampling time 4. The Baranyi-model was fitted to this data using

the procedure described before (Figure 5). The estimation results clearly indicate better properties for the parameter estimates (Table 7).

[Figure 5 about here.]

[Table 7 about here.]

The standard error was reduced by more than half for all the parameters. The parameter estimates are somewhat less correlated, as can be seen from the correlation matrix.

$$\begin{pmatrix} & \mu_{max} & h_0 & y_0 & y_{max} \\ \mu_{max} & 1 & 0.634 & 0.08684 & -0.326 \\ h_0 & 0.634 & 1 & 0.733 & -0.108 \\ y_0 & 0.0868 & 0.733 & 1 & -0.00409 \\ y_{max} & -0.326 & -0.108 & -0.00409 & 1 \end{pmatrix}$$

Not only are the estimation errors reduced, the parameter estimates show much more linear behaviour as can be seen from the curvature measurements (maximum parameter-effects relative curvature=0.370, 95 % confidence region relative curvature=0.566). This means that the regression statistics can now be more confidently used.

This example clearly shows that, although the *a priori* parameter estimates are of good quality, they can be further improved by using the OED/PE procedure to do a replicate. Although the number of sample points is the same as for the original curve, the experimental effort is considerably less, because sampling is clustered at 4 time intervals only. Even when the prior information is of poorer quality, the Monte Carlo simulation procedure should give the best possible guarantee that the most interesting time zones are sampled while taking the initial uncertainty into account.

Discussion

The aim of this paper was to study the identifiability of the model of Baranyi for bacterial growth. It could be shown that the model has attractive identifiability properties, both structurally and practically. However, the relatively high correlation between μ_{max} and h_0 and the larger variability of the h_0-estimate for the *Salmonella enteritidis* growth curve shows potential identification pifalls. The variability of h_0 (and thus λ) has been reported in the literature, and seems to be difficult to avoid. Besides the inherent variability of microbial growth, the lag time is dependent on the history of the culture under study, a history that is sometimes very difficult to assess and quantify. Only for very controlled experiments can

this aspect be expected to be kept within bounds. For real-life cases such as used for quantitative risk analysis, the variability of the lag phase (estimation) should be accounted for in the analysis.

The D-optimal design criterion proved to be an efficient way to improve the reliability of microbial growth parameter estimates and hence of the predictions made by the model. The criterion consistently pointed towards 4 important time regions, each corresponding with one of the 4 model parameters. The robustness of the design to the preliminary parameter estimates was quantified with a Monte Carlo simulation, using Latin Hypercube sampling of the model parameters. This made it possible to construct 95 % confidence intervals of the optimal sample points. It was not possible to determine the optimal number of data points, but this should be based on the uncertainty of the *a priori* measurements and the expected variability of the measurements which is reflected in the Monte Carlo distributions.

Optimal experimental design can help to use resources more optimally. Of course, preliminary parameter estimates are necessary for experimental design, so that the example shown here using the sampling scheme as only degree of freedom seems somewhat redundant. There are situations, however, where the principles shown in this paper could be very useful. When the identification process is giving difficulties with parameter estimates and replication experiments have to be done or when preliminary information is available on parameter values it could be very beneficial to have an idea when to measure the growth curve. It has also to be kept in mind that other degrees of freedom could be chosen. If the influence of environmental conditions, such as temperature or water activity, is investigated, the above outlined principles can be applied as well. Current practice in predictive microbiology is to measure microbial growth curves at constant environmental conditions, and change e.g. the temperature on a per curve basis. The influence of the environmental conditions on the model parameters is then modeled separately from the growth model, such as the temperature square root model (Ratkowsky et al. 1983). It is clear that this requires considerable experimental effort (McMeekin et al. 1993), and maybe it could be more beneficial to estimate the environmental related parameters directly using the differential equation form of the Baranyi-model. Van Impe et al. (1992) suggested an experimental procedure for estimating the model parameters of a Gompertz-type model on dynamic data. Further research on the identifiability properties of the model could point in the right direction, whereby it has to be pointed out that it is no longer possible to calculate the sensitivity functions analytically, which complicates the calculations. Promising results in this field were obtained by Bernaerts et al. (1998) and Versyck et al. (1998), who used optimal experimental design to improve the identifiability properties of temperature dependent models.

Identifiability properties of predictive microbiological models have not always got the attention they surely deserve. The example presented here show that identifiability analysis can help in optimizing

experimental efforts and reduce parameter variability. Although the paper focussed on the Baranyi-model, the principles are generally valid and it seems advisable that a similar investigation is done on other popular microbial growth models, such as the modified Gompertz-model.

Nomenclature

Roman letters

b Vector of model parameter estimates

F The Fisher information matrix

h_0 The product of the lag time λ and μ_{max}

J The Jacobian matrix

m curvature parameter to characterise the transition from the exponential phase

s^2 Estimate of the experimental error variance

V The covariance matrix of the parameter estimates

x The cell concentration $\left[\frac{CFU}{ml}\right]$

x The vector of independent variables

y The natural logarithm of the cell concentration $\left[\ln\left(\frac{CFU}{ml}\right)\right]$

y_0 The natural logarithm of the initial cell concentration $\left[\ln\left(\frac{CFU}{ml}\right)\right]$

y_{max} The natural logarithm of the asymptotic cell concentration $\left[\ln\left(\frac{CFU}{ml}\right)\right]$

Greek letters

$\boldsymbol{\beta}$ The vector of model parameters

ϵ The experimental error

λ The lag time $[h]$

μ_{max} Maximum specific growth rate $\left[\frac{1}{h}\right]$

ν Curvature parameter

σ^2 The experimental error variance

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Appendix: Parameter estimation of nonlinear models

Consider the following general notation of a nonlinear model:

$$y = f(\mathbf{x}, \boldsymbol{\beta}) + \epsilon \tag{4}$$

With

- y the measured response variable
- f the nonlinear model function
- $\mathbf{x}$ the vector of independent variables
- $\boldsymbol{\beta}$ the vector of p model parameters
- ϵ the experimental error

Suppose n experiments are available. When the experimental error has zero mean and constant variance and is independently distributed, an unbiased estimate $\mathbf{b}$ for the parameters $\boldsymbol{\beta}$ is determined by minimisation of the sum of squares of residuals:

$$\sum_{i=1}^{n} [y_i - f(\mathbf{x}, \boldsymbol{\beta})]^2 \xrightarrow{\boldsymbol{\beta}} \min \tag{5}$$

The minimisation is done in an iterative way and yields the vector of parameter estimates:

$$\mathbf{b} = \left(\mathbf{J}^T\mathbf{J}\right)^{-1}\mathbf{J}^T y \tag{6}$$

With $\mathbf{J}$ the Jacobian matrix, containing the derivatives of the model to the parameters evaluated in the n experimental points.

An estimate of the covariance matrix $\mathbf{V}$ of the parameter estimates is calculated from (Froment and Bisschoff, 1990):

$$\hat{\mathbf{V}} = \left(\mathbf{J}^T\mathbf{J}\right)^{-1}\sigma^2 \tag{7}$$

The experimental error variance σ^2 is estimated by:

$$s^2 = \frac{\sum_{i=1}^{n} (y_i - \hat{y}_i)^2}{n - p} \tag{8}$$

The matrix $\mathbf{J}^T\mathbf{J}$ is called the Fisher information matrix (Vanrolleghem and Dochain 1998) and is central in the analysis of the practical identifiability. The joint confidence region of the parameter estimates is given by (Froment and Bisschoff 1990):

$$(\boldsymbol{\beta} - \mathbf{b})^T\mathbf{J}^T\mathbf{J}\,(\boldsymbol{\beta} - \mathbf{b}) \leq \delta \tag{9}$$

with δ depending upon the probability level and the number of degrees of freedom. In the case of a linear model, this formula represents a closed hyperellipsoidal surface centred at $\mathbf{b}$. Using a translation of coordinates and an orthogonal transformation $\mathbf{U}\mathbf{v} = \boldsymbol{\beta} - \mathbf{b}$, the hyper-ellipsoid can be rewritten as:

$$\mathbf{v}^T\left(\mathbf{U}^T\mathbf{J}^T\mathbf{J}\ \mathbf{U}\right)\mathbf{v} \leq \delta \tag{10}$$

$\mathbf{U}$ is an orthogonal matrix with columns that are the eigenvectors of $\mathbf{J}^T\mathbf{J}$. The matrix $\mathbf{U}^T\mathbf{J}^T\mathbf{J}\mathbf{U}$ is a diagonal matrix with the eigenvalues of the Fisher information matrix on its main diagonal. This implicates that the axes of the hyperellipsoid, indicating the error on the parameter estimates, are proportional with the eigenvalues of $\mathbf{F}$. This shows why $\mathbf{F}$ is central in any practical identifiability analysis and optimal experimental design.

List of Tables

Time (h)	$\ln\left(\frac{CFU}{ml}\right)$
0	2.833213
2	2.079441
3	3.135494
4	4.49981
5	6.46614
6	4.66343
7	7.91935
8	7.80384
9	9.48797
10	11.1941
10	11.9703
12	13.1283
13	14.2792
14	13.641
15	16.3412
26	21.2885
27	21.0009
28	21.0009

Table 1. *Salmonella enteritidis* growth curve in egg yolk at 30°C

	Estimate	Standard error	Coefficient of variation	95 % confidence limits
μ_{max}	1.089	0.064	0.252	{0.951,1.228}
h_0	2.657	1.051	1.678	{0.404,4.915}
y_0	2.364	0.646	1.159	{0.979,3.740}
y_{max}	21.097	0.446	0.0897	{20.141,22.052}

Table 2. Parameter estimates and statistical properties

Nr. of samples	4	7	10	12	14	18
$\det(\mathbf{F})$	115.3	922.3	3108.2	6912	13823.3	34541.8
x_1	0	0	0	0	0	0
x_2	4.8	4.74	4.56	0	0	0
x_3	17.31	4.8	4.79	4.65	4.62	4.53
x_4	28	17.31	4.83	4.78	4.77	4.67
x_5		17.31	17.3	4.81	4.8	4.79
x_6		28	17.3	17.3	17.3	4.82
x_7		28	17.3	17.3	17.3	4.82
x_8			28	17.3	17.3	17.3
x_9			28	17.3	17.3	17.3
x_{10}			28	17.3	17.3	17.3
x_{11}				28	28	17.3
x_{12}				28	28	17.3
x_{13}					28	17.3
x_{14}					28	28
x_{15}						28
x_{16}						28
x_{17}						28
x_{18}						28

Table 3. The optimal sampling times x_i according to the D-optimal design criterion

Nr. of samples	Sample point 1	Sample point 2	Sample point 3	Sample point 4
4	1	1	1	1
7	1	2	2	2
10	1	3	3	3
12	2	3	5	2
14	2	3	5	4
18	2	5	6	5

Table 4. The distribution of the samples over the 4 sample times as calculated by the D-optimal criterion

Sample point	95 % confidence interval	
1	{0,0}	{0,0}
2	{3.2,8.5}	{3.6,10.6}
3	{16.1,23}	{15.5,23.1}
4	{28,28}	{28,28}

Table 5. The 95% confidence limits on the optimal sampling times

Time (h)	$\ln\left(\frac{CFU}{ml}\right)$
0	3.638
0	3.664
3.8	5.247
4.3	6.116
4.7	5.652
5.6	7.012
6.0	7.832
16.9	19.218
17.1	19.258
17.2	19.734
17.4	19.704
17.6	20.271
17.8	19.662
20	20.060
25	20.120
26	21.001
27	21.060
27	20.760

Table 6. Growth curve sampled at the times indicated by the OED/PE

	Estimate	Standard error	Coefficient of variation	95 % confidence limits
μ_{max}	1.110	0.0242	0.0926	{1.059,1.163}
h_0	2.781	0.381	0.579	{1.966,3.596}
y_0	3.649	0.244	0.284	{3.124,4.173}
y_{max}	20.635	0.158	0.0325	{20.296,20.975}

Table 7. Parameter estimates and statistical properties for the resampled growth curve

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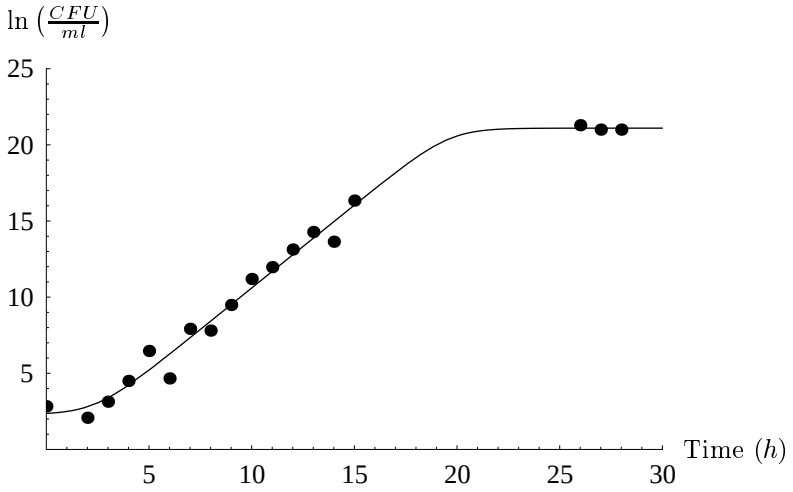

Figure 1. The fitted Baranyi-model to the *Salmonella enteritidis* growth curve

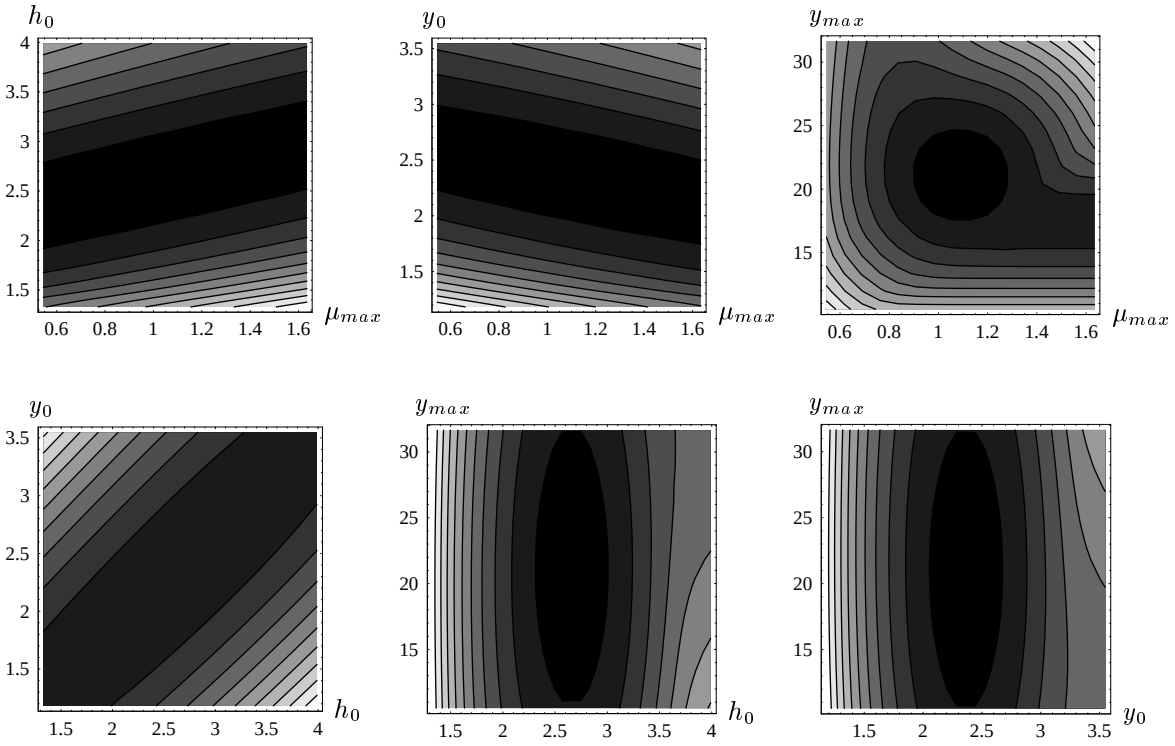

Figure 2. Contour plots of the objective function as function of the model parameters

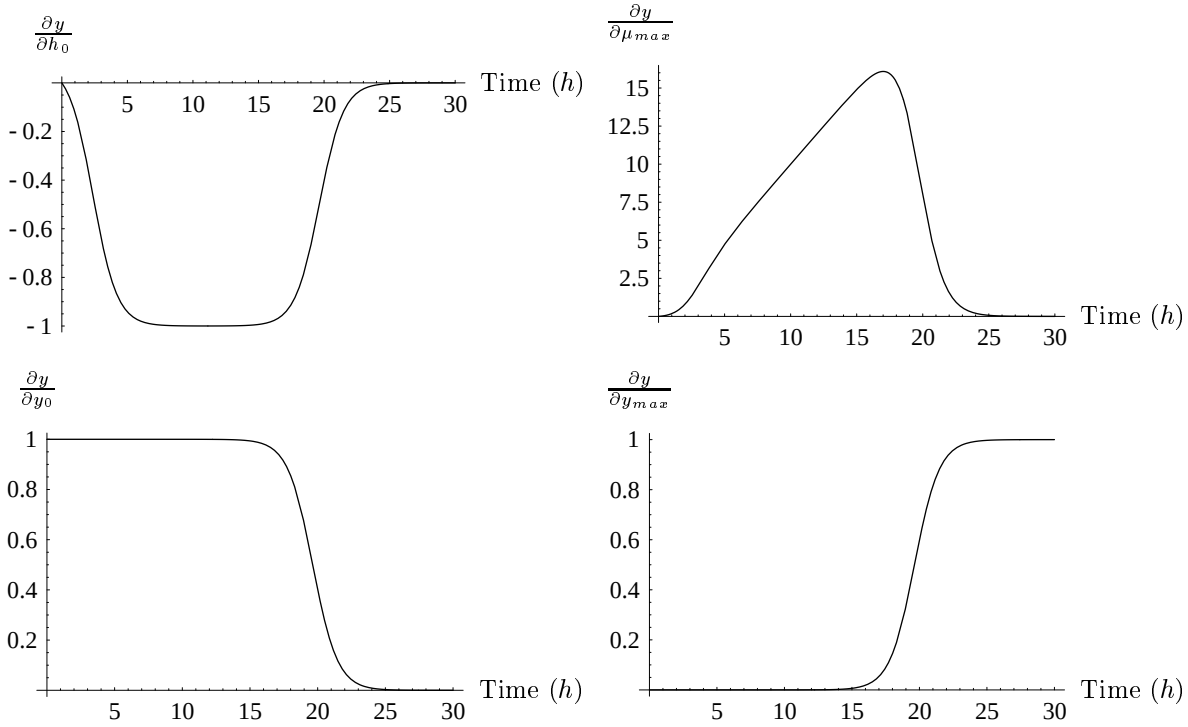

Figure 3. Sensitivities as function of time

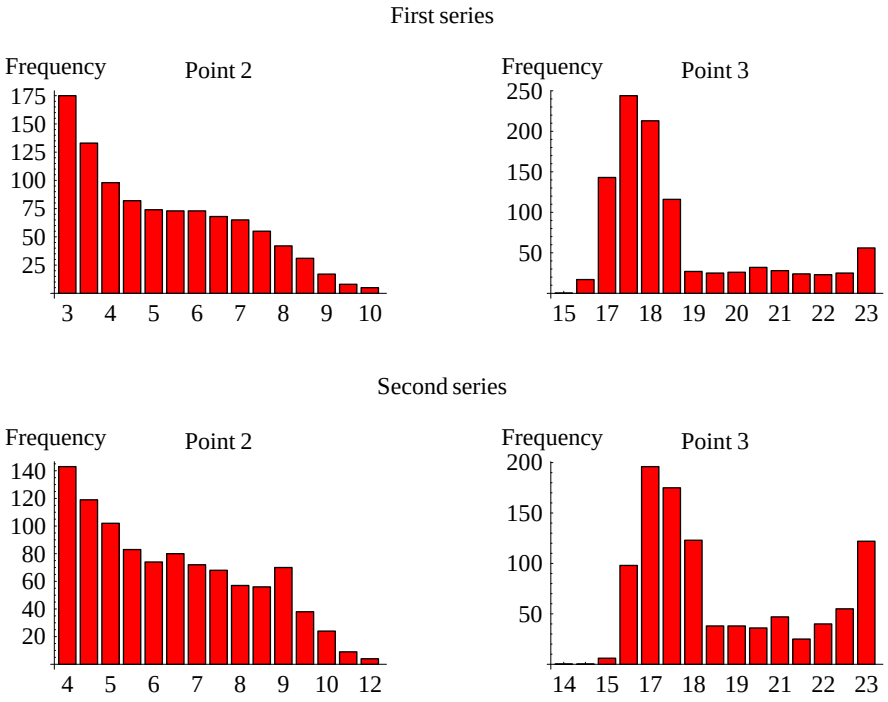

Figure 4. The histograms for the optimal sample points 2 and 3 as determined by the Monte Carlo simulation for the 2 series

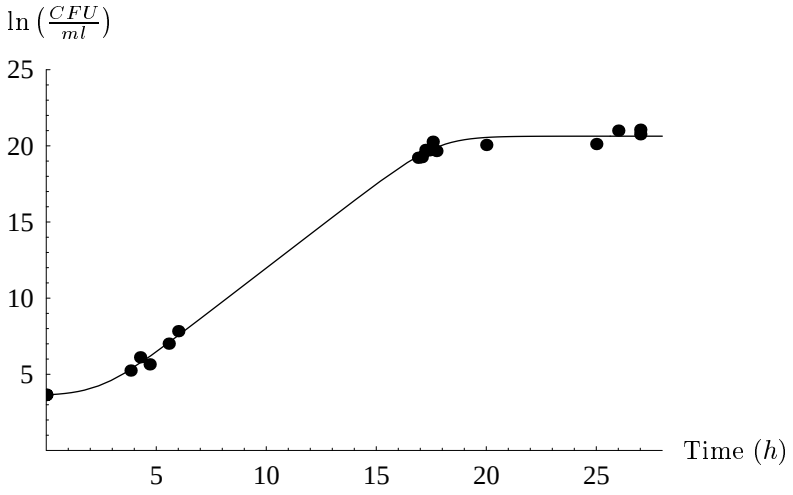

Figure 5. The Baranyi-model fitted to the *Salmonella* growth curve sampled according to the optimal experimental design