A tutorial on uncertainty propagation techniques for predictive microbiology models : a critical analysis of state-of-the-art techniques

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A tutorial on uncertainty propagation techniques for predictive microbiology models: A critical analysis of state-of-the-art techniques

Simen Akkermans, Philippe Nimmegeers, Jan F. Van Impe

BioTeC, Chemical and Biochemical Process Technology and Control,

Department of Chemical Engineering, KU Leuven, Ghent, Belgium,

OPTEC, Optimization in Engineering Center-of-Excellence, KU Leuven, Belgium,

CPMF^2, Flemish Cluster Predictive Microbiology in Foods - www.cpmf2.be

[simen.akkermans, philippe.nimmegeers, jan.vanimpe] @kuleuven.be

Correspondence to:

Prof. J. F. Van Impe

Chemical and Biochemical Process Technology and Control (BioTeC)

Department of Chemical Engineering, KU Leuven

Gebroeders de Smetstraat 1, B-9000 Ghent (Belgium)

jan.vanimpe@kuleuven.be

Tel: +32-16-32.14.66

Fax: +32-9-265.86.24

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Abstract

Building mathematical models in predictive microbiology is a data driven science. As such, the experimental data (and its uncertainty) has an influence on the final predictions and even on the calculation of the model prediction uncertainty. Therefore, the current research studies the influence of both the parameter estimation and uncertainty propagation method on the calculation of the model prediction uncertainty. The study is intended as well as a tutorial to uncertainty propagation techniques for researchers in (predictive) microbiology. To this end, an *in silico* case study was applied in which the effect of temperature on the microbial growth rate was modelled and used to make simulations for a temperature profile that is characterised by variability. The comparison of the parameter estimation methods demonstrated that the one-step method yields more accurate and precise calculations of the model prediction uncertainty than the two-step method. Four uncertainty propagation methods were assessed. The current work assesses the applicability of these techniques by considering the effect of experimental uncertainty and model input uncertainty. The linear approximation was demonstrated not always to provide reliable results. The Monte Carlo method was computationally very intensive, compared to its competitors. Polynomial chaos expansion was computationally efficient and accurate but is relatively complex to implement. Finally, the sigma point method was preferred as it is (i) computationally efficient, (ii) robust with respect to experimental uncertainty and (iii) easily implemented.

Keywords: Prediction uncertainty, parameter estimation, sigma point method, linear approximation, Monte Carlo method.

1 Introduction

During the last decades, researchers in the field of predictive microbiology have focused on developing and fine-tuning a wide range of mathematical models that contribute to the assessment and prediction of microbial food safety and quality. Currently, there is a wide interest in moving towards mechanistic modelling methods such as individual based models (e.g., Kreft et al., 1998; Tack et al., 2015) or systems biology approaches (e.g., Brul et al., 2008; Vercammen et al., 2017). In practice, however, the state-of-the-art for real life application will remain for a considerable time the use of grey box models. These grey box models are built to deliver a simplified representation of the relevant microbial response (e.g., growth rate, inactivation rate, probability of growth). Grey box models require experimental data to select mathematical model structures and to estimate the most suitable combination of model parameters. As such, building mathematical models in the field of predictive microbiology will remain, for the time being, a data driven science.

The experimental data used to build a mathematical model will influence the choice of the model structure and the estimated values of the model parameters. As such, the experimental data also influences the model predictions that will be obtained. Knowing this, several publications have focused on assessing the quality and validity of the models that are obtained. For example, Ross (1996) developed indices to evaluate the accuracy and bias of models based on the predicted generation time. Apart from the accuracy, also variation plays an important role when modelling microbial responses. The sources of variation in predictive microbiology were distinguished as follows by Van Impe et al. (2001): (i) the type and quantity of microorganisms in the initial microbial load, (ii) the true intrinsic and extrinsic conditions that characterise a food product, (iii) the lack of observations both in the monitoring points and the number of

samples, (iv) random noise which inevitably corrupts measurements. The sources of variation can be categorised as uncertainty or variability. Uncertainty refers to the precision with which a state or parameter is known (e.g., error on an experimental measurement) and variability refers to the natural variation of a variable or process (e.g., microbial growth rate).

Due to the inevitable presence of variation in building predictive models, it is generally deemed important to assess the accuracy of the model predictions. This is often simplified to finding the confidence intervals of the parameter estimates. The confidence intervals of the parameter estimates (or simply the variation of the parameter estimates) can lead to the calculation of the uncertainty on the model prediction. As such, the user of a predictive model can be provided with an estimate of, e.g., a 95% confidence interval of the model prediction. The determination of this uncertainty is indispensable when using predictive models for quantitative microbial risk assessments (Zwietering, 2015). As (the uncertainty on) the estimated values of the model parameters are determined by (the uncertainty on) the experimental data, also the calculated uncertainty on the model parameters and model prediction will be determined by the experimental data. Consequently, it is worth wondering how to ensure that the provided uncertainty is actually reliable.

This research studies how a reliable determination of the prediction uncertainty can be obtained. The focus lies on modelling and predicting the growth of microorganisms as a function of temperature, but the results should be transferable to other conditions and to modelling of microbial inactivation as well. However, further research should be performed to confirm the conclusions of this research for other applications. It is worth noting that an accurate determination of the model prediction uncertainty will become more difficult for more complex models (e.g., in case of

multiple influencing variables and interactions). Two steps in the modelling procedure are investigated with respect to their influence on determining the model prediction uncertainty: (i) the parameter estimation method and (ii) the uncertainty propagation method. These are deemed most influential on the calculation of the prediction uncertainty. For this purpose, a case study was applied in which a mathematical model was built for the effect of temperature on the microbial growth rate and used to predict microbial growth for a temperature profile that is characterised by variability. This research also is meant to serve as a tutorial to uncertainty propagation techniques for scientists working in the field of (predictive) microbiology.

2 Materials and methods

For the current research, data is simulated according to the protocol explained in Section 2.1. The parameters of the predictive model will be estimated according to the methods explained in Section 2.2. This section also explains the method generally used to determine the model parameter accuracy. Finally, Section 2.3 elaborates on the different methods for uncertainty propagation that are tested in this publication to calculate the model prediction uncertainty.

2.1 Simulation protocol

Experiments are always simulated at the same 8 temperatures (10, 15, 20, 25, 30, 35, 40, 45°C). At each temperature, the maximum specific microbial growth rate μ_{max} [h^{-1}], which is reached during the exponential phase of growth, is calculated according to the Cardinal Temperature Model with Inflection (CTMI) of Rosso et al. (1993):

$$\mu_{max}(T) = \mu_{opt} \cdot \frac{(T-T_{min})^2 \cdot (T-T_{max})}{(T_{opt}-T_{min}) \cdot [(T_{opt}-T_{min}) \cdot (T-T_{opt}) - (T_{opt}-T_{max}) \cdot (T_{opt}+T_{min}-2T)]} \quad (1)$$

In this equation, T_{min} [°C] and T_{max} [°C] represent the minimum and maximum temperature that allow microbial growth. T_{opt} [°C] is the optimum temperature at which the optimum growth rate μ_{opt} [h^{-1}] is reached, as such $\mu_{opt} = \mu_{max}(T_{opt})$. The value of μ_{max} [h^{-1}] (at any temperature) is then used to simulate a growth curve using the model of Baranyi and Roberts (1994):

$$\frac{dn(t)}{dt} = \frac{1}{1+\exp(-q(t))} \cdot \mu_{max}(T) \cdot [1 - \exp(n(t) - n_{max})] \quad (2)$$

$$\frac{dq(t)}{dt} = \mu_{max}(T) \quad (3)$$

with $n(t)$ [$\ln(CFU/mL)$] the natural logarithm of the population density at a time point t [h], n_{max} [$\ln(CFU/mL)$] the natural logarithm of the maximum population

density and $q(t)[-]$ the natural logarithm of the physiological state of the cell. The initial values of $n(t)$ and $q(t)$ are respectively n_0 and q_0. Nominal values for T_{min}, T_{opt}, T_{max}, μ_{opt}, n_0, q_0 and n_{max} were chosen arbitrarily for a hypothetical microorganism and are listed in Table 1. Growth curves were simulated until the population density reached a value approximating the nominal n_{max}. In these growth curves, 8 samples were taken at equidistant time points. Gaussian noise with zero mean was added to these samples to simulate the variation of the experimental data. The standard deviation of the Gaussian noise was taken equal to $0.28\ \ln(\mathrm{CFU/mL})$ based on the mean squared error of previous (unpublished) parameter estimation results with secondary models for growth. Discrepancy between the model structure and the microbial system under study is not considered in this research. Also the effect of the experimental design was not considered in this research.

The simulations used to compare different methods for assessing the propagation of uncertainty from experimental data to model predictions (Section 3.2) are based on a temperature profile that is characterised by variability as well. An arbitrary temperature profile was selected for these simulations to mimic the food chain of a product that is kept at refrigeration temperatures. The different steps of the temperature profile are listed in Table 2. Fig. 1 illustrates the temperature profile with all parameters at their mean value. The durations of each step was considered to have a uniform distribution. Both the linear approximation and the sigma point method (described in Section 2.3) rely on the mean value and variance for their computations. As such, also the normal distributions that correspond with these uniform distributions are listed. Based on the lower bound (lb) and upper bound (ub) of the uniform distribution, the mean ($1/2(\mathrm{ub} + \mathrm{lb})$) and variance ($1/12(\mathrm{ub} - \mathrm{lb})^2$) of the normal distribution were calculated.

Simulations also took into consideration that the temperature of the food product will change gradually when placed in an environment with a new temperature. As such, the model used to make predictions on the microbial growth is made both more complex and more realistic. The hypothetical food product was given the shape of a cube with edges of 0.1 m. As such, the total surface area of the product, A, is 0.06 m^2. It is packed in low density polyethylene with a thickness, d, of 1 mm and a thermal conductivity, k, of 0.33 W/mK. The heat capacity of the product, C, is chosen to be equal to that of water, i.e., 4 181 J/K. Given that the heat transfer through the product is neglected compared to the heat transfer through the packaging (a single temperature for the entire food product was assumed), the change of temperature T inside the food product is given by the following differential equation:

$$\frac{dT}{dt} = k \cdot A \cdot \frac{\Delta T}{d \cdot C} \quad (4)$$

with k the thermal conductivity, A the surface area of the product, ΔT the difference in temperature between the food product and its environment, d the thickness of the packaging and C the heat capacity. The temperature at $t = 0$ h is taken equal to the temperature of the environment in the first step. The above equation in combination with the temperature profile in Table 2 leads to the temperature of the food product that is the input to the secondary model of Eq. 1.

2.2 Parameter estimation

Parameter estimations were performed using the function *lsqnonlin* of MATLAB R2016a (The Mathworks). As such, the objective of the parameter estimation was the minimisation of the Sum of Squared Errors (SSE) between the measurements and model predictions. Two types of parameter estimations are considered in this publications, i.e., the one-step and two-step method (Akkermans et al., 2016). In case of the one-step

159 method, all primary and secondary model parameters are estimated in a single step
160 using all experimental data (at different temperatures). With the two-step methods, the
161 maximum specific growth rate is first estimated by fitting a primary model to every
162 growth curve and then, the parameters of the secondary model are estimated using the
163 growth rates at different temperatures as a dataset. For the remainder of the explanation
164 of the parameter estimation method, the one-step method is used as an example. The
165 objective function is then formulated as:

166 $$\mathrm{SSE} = \sum_{i=1}^{\nu_m} \left(n_{m,i} - n_{p,i}(\boldsymbol{p}) \right)^2 \quad (5)$$

167 with n_m and n_p the measured and predicted cell densities. ν_m is the total number
168 of measurements and $\boldsymbol{p}$ the vector of model parameters. The 95% confidence interval
169 of every parameter p_i is calculated based on the Student's t-distribution:

170 $$\left[p_i \pm t_{0.975,\nu_m-\nu_p} \cdot \sqrt{\sigma_{p_i}^2} \right] \quad (6)$$

171 where ν_p is the number of parameters and consequently $\nu_m - \nu_p$ is the number of
172 degrees of freedom. $\sigma_{p_i}^2$ is the variance on the model parameter p_i and is found on the
173 main diagonal of the variance covariance matrix V_p, which is approximated as the
174 inverse of the Fisher Information Matrix F (Walter and Pronzato, 1997):

175 $$\sigma_{p,i}^2 = V_p(i,i) \quad (7)$$

176 $$V_p = F^{-1} \quad (8)$$

177 $$F = \frac{1}{\mathrm{MSE}} \cdot J^T \cdot J \quad (9)$$

178 $$\mathrm{MSE} = \frac{\mathrm{SSE}}{\nu_m - \nu_p} \quad (10)$$

179 with J the Jacobian matrix and MSE the mean sum of squared errors.

180

2.3 Uncertainty propagation techniques

In this research, four methods are considered to propagate the variability of the experimental data and the variability of the model inputs to the model predictions. The theoretical explanation of these uncertainty propagation techniques is found below.

Monte Carlo method

When applying the Monte Carlo method, random samples are drawn from all known or estimated distributions of the model parameters and inputs. Based on these random samples, a single sample of the output distribution is calculated (Poschet et al., 2003). By repeating this procedure, a large set of samples of the model output is obtained, and as such, the distribution of the model output is characterized. This method is relatively easy to implement using widely available random number generators (e.g., the function *random* in MATLAB). This method is considered the most accurate method to approximate the distribution of a model output, as no assumptions are made about the probability distributions and the model equations are not simplified (e.g., not limited to normally distributed variables). Consequently, it forms the basis of commonly used risk analysis software products such as @Risk (Palisade).

Linear approximation

An alternative calculation of the uncertainty on the model output is possible by making a linear approximation of the model predictions and assuming normally distributed probabilities (Van Impe et al., 2001). In this case, the variance of the model output can be calculated directly, without the use of an iterative technique. The variance-covariance matrix of a set of model predictions (V_y) is calculated as (Omlin and Reichert, 1999):

206 $$V_y = J \cdot V_p \cdot J' \quad (11)$$

207 The model outputs y for the current case study are equal to the cell densities n.
208 The variance of each measurement is obtained from the main diagonal of V_y. As such,
209 the $(1-\alpha)100\%$ confidence bounds on the model output are calculated similarly to
210 the confidence bounds on the model parameters (Seber and Wild, 2003):

211 $$\left[y_i \pm t_{\left(1-\frac{\alpha}{2},\nu_m-\nu_p\right)}\sqrt{V_y(i,i)}\right] \quad (12)$$

212 The true uncertainty is expected to be even higher than that calculated by the above
213 equation because (i) the variance on the parameter estimates that is calculated, is
214 actually the lower bound of the true variance and (ii) the black or grey box models used
215 here remain an oversimplification of the complex microbiological systems that are
216 studied (Omlin and Reichert, 1999). When making predictions with growth or
217 inactivation models, the true variability also increases in case of lower population
218 densities due to the variability between individual cells (see, e.g., Pin and Baranyi
219 (2006)).

220

221 **Sigma point method**

222 The sigma point method was devised by Julier and Uhlmann (1996) and is aimed
223 at calculating nonlinear transformations of probability distributions. This method is also
224 referred to as the unscented transformation. In the sigma point method, the uncertainty
225 on the model output is calculated based on a specific set of model inputs and parameters,
226 drawn from their distribution. Different from the Monte Carlo method, model inputs
227 and parameters are not generated randomly but chosen in a systematic way. The
228 mathematical notation of this method was based on the work of Telen et al. (2015). In
229 this notation, $\boldsymbol{x}$ will be the vector of both model inputs and parameters with a known
230 variance-covariance matrix V_x. A set of model outputs is calculated as follows:

$$y_0(\bar{\boldsymbol{x}}) \tag{13}$$

$$y_i\left(\bar{\boldsymbol{x}} + \sqrt{3V_{x,i}}\right) \quad \text{with } i = 1, \dots, \nu_x \tag{14}$$

$$y_{i+\nu_x}\left(\bar{\boldsymbol{x}} - \sqrt{3V_{x,i}}\right) \quad \text{with } i = 1, \dots, \nu_x \tag{15}$$

In the above equations $V_{x,i}$ expresses the i^{th} row of the variance-covariance matrix and ν_x is the total number of variable parameters and inputs. As such, the output of the mathematical model has to be calculated $2\nu_x + 1$ times. The mean value of the model predictions is then calculated as:

$$\bar{y} = \frac{1}{3}\left((3 - \nu_x)y_0 + \frac{1}{2}\sum_{i=1}^{2\nu_x} y_i\right) \tag{16}$$

Moreover, the variance-covariance matrix of the model predictions (V_y) is approximated with the following equation:

$$V_y = \frac{1}{3}\left((3 - \nu_x)(y_0 - \bar{y})(y_0 - \bar{y})^T\right) + \frac{1}{3}\left(\frac{1}{2}\sum_{i=1}^{2\nu_x}(y_i - \bar{y})(y_i - \bar{y})^T\right) \tag{17}$$

V_y can then be used in combination with Eq. 12 to calculate the confidence bounds on the model output.

Polynomial chaos expansion

Polynomial chaos expansion (PCE) was first presented by Wiener (1938) and its use in uncertainty quantification has been illustrated in, e.g., Webster et al., (1996), Tatang et al., (1997) and Xiu and Karniadakis, (2002). The PCE method exploits information on the distribution of uncertain variables (assuming that these uncertain variables are independent) to accurately compute the mean and variance of a model response. The PCE of the model output $y(\boldsymbol{x})$ is written as follows:

$$y(\boldsymbol{x}) = \sum_{i=0}^{\infty} a_i \Phi_i(\boldsymbol{x}) \tag{18}$$

Due to the infinite number of terms, this expansion is in practice truncated to a finite number of terms:

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$$y(\boldsymbol{x}) \approx \sum_{i=0}^{L-1} a_i \Phi_i(\boldsymbol{x}) \quad (19)$$

with L the number of terms in the PCE, i a term based index, a_i the PCE coefficients that have to be determined, $\Phi_i(\boldsymbol{x})$ the multivariate orthogonal polynomials and $\boldsymbol{x}$ the vector of both model inputs and parameters which are assumed to be independent, with a known variance-covariance matrix V_x. Note that L depends on the order of the PCE m and the total number of variable (i.e., uncertain) parameters ν_x as follows:

$$L = \frac{(m+\nu_x)!}{m!\nu_x!} \quad (20)$$

The multivariate polynomials $\Phi_i(\boldsymbol{x})$ are derived from the probability distributions of the variable parameters. As the variables are considered to be independent, these multivariate polynomials $\Phi_i(\boldsymbol{x})$ are constructed by deriving univariate orthogonal polynomials $\phi_i(x_i)$ for each variable parameter x_i from the probability distribution functions, see e.g., Nimmegeers et al., (2016).

Hence, only the L PCE coefficients a_i are unknown and need to be determined. Different methods exist to compute these coefficients: intrusive (Ghanem et al., 1991; Debusschere et al., 2004) and non-intrusive sampling-based methods (Tatang et al., 1997; Fagiano and Khammash, 2012; Nimmegeers et al., 2016). Intrusive methods use Galerkin projection to compute the coefficients. Non-intrusive sampling-based methods on the other hand, repetitively evaluate the model equations in so-called collocation points x_j to calculate the coefficients as a weighted sum of the model responses evaluated in the different ν_c collocation points. Assuming that the truncation error of the PCE is sufficiently low, the following linear system in the PCE coefficients a_i has to be solved:

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$$\begin{matrix} y(x_1) = \sum_{i=0}^{L-1} a_i \Phi_i(x_1) \\ \vdots \\ y(x_j) = \sum_{i=0}^{L-1} a_i \Phi_i(x_j) \\ \vdots \\ y(x_{\nu_c}) = \sum_{i=0}^{L-1} a_i \Phi_i(x_1) \end{matrix} \quad (21)$$

Note that the number of potential collocation points (following from the combination of these roots) is typically higher than the number of unknown PCE coefficients. In this article, stochastic collocation (Tatang et al., 1997) is used such that the number of collocation points equals the number of unknown coefficients (i.e., $\nu_c = L$) and the system in Eq. (21) has a unique solution. For the collocation points, sets that span the high probability regions of their distributions are selected. Since normalized univariate orthogonal polynomials $\phi_i(x_i)$ have been used for the PCE, the mean and variance of the model output can be calculated as follows:

$$\bar{y} = a_0 \quad (22)$$

$$V_y = \sum_{i=1}^{L-1} a_i^2 \quad (23)$$

As the PCE coefficients are a weighted sum of model output evaluations in the collocation points (i.e., the solution of Eq. (21)), the mean and variance are calculated as a weighted sum of the model output evaluations in the collocation points.

3 Results and discussion

Both the choice of the parameter estimation method and the method to approximate the model prediction uncertainty is studied here. In both cases, the influence of the experimental uncertainty on the accuracy of the model prediction uncertainty is taken into account by applying a Monte Carlo method with randomly generated experimental measurements. Once the parameter estimation method is selected in Section 3.1, this method is applied for all simulations in Section 3.2.

3.1 Assessing the parameter estimation method

In this section, the influence of the parameter estimation method on the parameter estimation and model prediction uncertainty (and its accuracy) is assessed. The two methods compared here are the one-step and two-step parameter estimation method. For this purpose, Monte Carlo simulations with 5000 iterations were performed. In each iteration (i) experimental data was generated, (ii) parameter estimations were performed with both methods on this data, (iii) uncertainty on the model parameters was calculated, (iv) model predictions were made over a temperature range of 10 to 45°C and (v) the uncertainty on these model predictions was determined. For this case study, the quality of the parameter estimation method was only assessed using the Monte Carlo method (5000 iterations, used as a benchmark) and the linear approximation for both the model parameter and model prediction uncertainty. Due to the high number of iterations, the Monte Carlo simulation is expected to present a measure of the true variability. The determination of the uncertainty on the model parameters and model prediction according to the linear approximation is also calculated in the Monte Carlo method, which results in a distribution of these uncertainties. Based on these distributions, 95% confidence bounds of the uncertainties are determined empirically

by ordering the data and determining the values that separate the 2.5% lowest and highest values. As such, no assumption is made on the distribution of these values.

The model predictions used in this section are estimates of the doubling time as a function of temperature. Doubling time was chosen here instead of the growth rate as it is considered more relevant for microbial food safety/quality to accurately assess the time needed for a certain increase of the microbial population than to assess the microbial increase in a given period of time (because simulation times will be longer in case of lower growth rates). In essence, the assessment of the model prediction uncertainty on the doubling time corresponds to the assessment of the model prediction uncertainty on the time required to reach a threshold on the microbial load. The doubling time (t_d) is calculated as:

$$t_d = \frac{\ln(2)}{\mu_{max}(T)} \quad (24)$$

For both parameter estimation methods, it was found that the average values of the parameter estimates approximated the nominal (given) values. However, clear differences were found between the two methods with respect to the uncertainty on the parameter estimates. The true variation of the parameter estimates is illustrated with the Monte Carlo methods in Fig. 2 for the (a) one-step and (b) two-step method. The calculation of the 95% confidence bounds with the linear approximation is provided in the same figure as well. The Monte Carlo methods demonstrated that the uncertainty on the parameter estimates was (slightly) higher for the one-step method. This is probably due to the fact that the growth rates, used as intermediate parameters in the two-step method, were estimated with relatively good accuracy. By using these estimates as inputs for the second parameter estimation, the two-step method led to lower uncertainty on the estimated values of the secondary model parameters. However, the one-step method performed much better with respect to the linear approximation.

342 The variation on the 95% confidence bounds calculated with the linear approximation
343 was much lower for the one-step than for the two-step method. Moreover, in case of the
344 two-step method, the average values of the 95% confidence bounds for the linear
345 approximation were much higher than those calculated with the Monte Carlo method.
346 This means that the two-step method results in less precise and less accurate
347 calculations of the 95% confidence bounds than the one-step method when applying
348 the commonly used linear approximation. This is due to the fact that information on the
349 variability of the model parameters is lost by making the intermediate step in the two-
350 step method.

351 Fig. 3 contains 95% confidence bound on the predicted doubling time according
352 to the Monte Carlo method for both the (a) one-step and (b) two-step method. This
353 figure also contains the linear approximation of the 95% confidence bound with a 95%
354 errors to indicate the variation on this approximation. Comparing the results of the one-
355 step and two-step parameter estimation method shows that the variation of the
356 prediction (as calculated with the Monte Carlo method) is almost identical for both
357 methods. On average, the one-step method leads to a good linear approximation of the
358 95% confidence bounds on the prediction with only limited variation. On the other
359 hand, the two-step method results in the prediction of much wider confidence bounds
360 and has high variation on this prediction. Similar as for the confidence bounds on the
361 model parameters, the two-step method leads to a less precise and less accurate linear
362 approximation of the prediction uncertainty.

363 Based on these results, it can be said that the use of the one-step method was most
364 suitable with respect to the calculation of model prediction uncertainty. Even though
365 the experimental data was limited (i.e., only 8 experiments, each containing 8 sampling
366 points), the one-step method resulted in low variation on the calculated 95% confidence

bounds on the model prediction through the linear approximation. Taking into account that the linear approximation is the most commonly used method to calculate the model prediction uncertainty, this parameter estimation method is preferred. It is worth nothing that in practice other sources of variation (e.g., strain variability) that lead to uncertainty on the model predictions have to be taken into account as well (Den Besten et al., 2017).

3.2 Assessing the uncertainty propagation method

After determining the effect of the parameter estimation method, the different methods to estimate the model prediction uncertainty are compared, taking into account the uncertainty on the experimental measurements. For this comparison, Monte Carlo simulations with 5000 iterations were performed with an assessment of the model prediction uncertainty in each iteration with all four techniques listed in Section 2.3. These simulations start again from randomly simulated experimental data. As such, the variation in the results obtained with these techniques due to the experimental uncertainty is studied. Unlike the previous section, in these simulations the model prediction uncertainty was assessed for a specific temperature profile of a hypothetical food product as explained in Section 2.1. The simulations were performed using an initial cell density n_0 of $0\ \ln(\mathrm{CFU/mL})$ and the lag phase and stationary phase were omitted.

Linear approximation

The first method discussed here is the linear approximation. This method resulted in an accurate determination of the model prediction uncertainty when used in combination with the one-step parameter estimation method in the previous section.

392 The main advantage of this method is the relatively low computational burden required.
393 For the current case study, the Jacobian matrix (J) of the vector of model outputs ($\boldsymbol{n}$)
394 was calculated numerically using the symmetric derivative. Numerical differentiation
395 was chosen as it is easy to implement. As such, a single column of J (J_i) is approximated
396 as follows:

397 $$J_i = \frac{\partial \boldsymbol{n}}{\partial x_i} \approx \frac{n(x_i+h_i)-n(x_i-h_i)}{2h_i} \quad (25)$$

398 In the above equation, the model output sensitivity is calculated with respect to
399 the model parameter or input (x_i) by changing it with a finite difference (h_i). In the
400 current case study, h_i was 1/1000 times the nominal parameter value. To calculate the
401 model output, 13 parameters are required, i.e., 4 parameters of the secondary model and
402 9 parameters of the temperature profile. As such, the numerical differentiation required
403 that the model output was calculated 26 times. Including the calculation of the model
404 output at the nominal values of all parameters, the model output was calculated 27 times
405 for the linear approximation. It should be noted that when using a left or right hand
406 difference quotient (instead of the symmetric difference quotient) the required number
407 of calculations of the model output could be reduced to a total of 14. However, the
408 symmetric difference quotient was preferred as it is more accurate than the left and right
409 hand difference quotients.

410 The results of the linear approximation method are presented in Fig. 4a. Overall,
411 the linear approximation resulted in an accurate calculation of the model prediction
412 uncertainty. Moreover, the calculation only shows limited variation due to the
413 experimental uncertainty, as indicated by the narrow 95% error bands around the 95%
414 confidence bounds. However, the 95% confidence bounds have a peak at about 104
415 hours during the simulation. Comparing the model prediction uncertainty according to
416 the linear approximation to that of the other methods (Fig. 4) shows that this peak is an

anomaly. The high uncertainty at this point in the simulation is caused by the high sensitivity of the temperature (and consequently of the model output) to the duration of all steps of the profile and the temperature during transport to the customer's home. When using the linear approximation, the uncertainty that is calculated is an extrapolation of these sensitivities and as such, an overestimation of the true uncertainty.

Based on the current results, it can be said that the linear approximation can give good results for a variety of cases but can also lead to large errors in other cases. Even though the error of the current simulation can be exceptional, the linear approximation is not seen as a reliable method.

Sigma point method

For the sigma point method, 27 combinations of model parameters were calculated and used to calculate the model output. As such, the computational load of the sigma point method is similar to that of the linear approximation (when using the symmetric derivative). The results of the sigma point method are illustrated in Fig. 4b. The sigma point method resulted in low variation in the (mean) predicted values and prediction uncertainty for the full range of the simulation. The method appears to give a robust approximation of the prediction uncertainty with respect to the uncertainty on the experimental measurements. This finding is in agreement with the work of Telen et al. (2014) who noted that the calculation of a variance-covariance matrix through the sigma point method was robust. The robustness of this method is considered to be a significant advantage, definitely when considering that mathematical models will often be much more complex than the model used here (e.g., multiple influencing environmental conditions). It is also important to note that this method did not result in an

442 overestimation of the uncertainty as seen with the linear approximation (peak in Fig.
443 4a) and can therefore be considered as more reliable.
444
445 **Monte Carlo method**
446 A common difficulty when working with Monte Carlo methods is that the user
447 needs to determine the number of iterations that is required to obtain an *accurate*
448 estimate of the model output distribution. Several publications (e.g., Bukaçi et al. 2016)
449 explain methods for calculating the required number of iterations in a Monte Carlo
450 method. These methods rely on the calculation of the variance of the mean model output
451 ($\sigma_{\bar{y}}^2$) through a known variance of the model output (σ_y^2) for a number of iterations ν_i:

452 $$\sigma_{\bar{y}}^2 = \frac{\sigma_y^2}{\nu_i} \quad (26)$$

453 However, as $\sigma_{\bar{y}}^2$ is generally not known, these calculations are not useful in
454 practice. Consequently, in the current research the effect of the number of iterations
455 was taken into account when assessing the Monte Carlo method. Initially, the Monte
456 Carlo method was carried out with 27 iterations, the same number as required for the
457 linear approximation and sigma point method. The results of this simulation are
458 presented in Fig. 4c. It is clear that the results of the Monte Carlo simulation with just
459 27 iterations are characterised by high variation. This means that the results of the
460 Monte Carlo method with a limited number of iterations is markedly dependent on the
461 experimental measurements (or their uncertainty). Comparing Fig. 4b and Fig. 4c
462 demonstrates that, even though the same number of calculations of the model response
463 were used, the Sigma Point method is clearly influenced less by the experimental
464 uncertainty than the Monte Carlo method. Increasing the number of iterations of the
465 Monte Carlo method will make the results more robust. Consequently, the effect of the
466 number or iterations on the width of the 95% errors was tested, starting from 50

467 iterations and increasing in steps of 50 (results not shown). It was found that over 500
468 iterations were needed for the Monte Carlo method to reduce the variation to the same
469 level as that of the sigma point method.
470
471 *Polynomial chaos expansion*
472 The final method tested here is PCE. The first order PCE required just 14
473 calculations of the model output. As such, the computational load is about half of that
474 of the sigma point method. The resulting model prediction uncertainty and its variation
475 is presented in Fig. 4d. Comparing Fig. 4b with Fig. 4d demonstrates that there is only
476 minor difference between the model prediction uncertainty calculated by the sigma
477 point method and the first order PCE. As such, PCE delivered good accuracy
478 calculations of the model prediction uncertainty at a low computational cost. However,
479 the main downside experienced when applying this method was that it is by far the most
480 complex to implement, out of the four techniques compared here. As such, PCE is
481 regarded as a beneficial technique when implemented in, e.g., a software package. On
482 the other hand, when the algorithms are implemented *manually* for specific case studies,
483 the sigma point method would be preferred in the field of predictive microbiology.

4 Conclusions

The current research discusses the selection of methods for parameter estimation and uncertainty propagation for building secondary models in predictive microbiology to obtain reliable calculations of the model prediction uncertainty. The results demonstrated that the one-step parameter estimation method was more suitable than the two-step method to obtain precise and accurate calculations of the model prediction uncertainty. The linear approximation was found to be susceptible to extrapolations of the sensitivity equations and will therefore not always lead to reliable results. The sigma point method gave overall good results with a low computational effort. The Monte Carlo method is the most basic method and therefore easy to implement. However, it was found to be very computationally intensive compared to the other methods. It can be said that the systematic selection of model inputs in the sigma point method gives it a significant advantage over the basic Monte Carlo random sampling. Finally, polynomial chaos expansion resulted in a robust calculation of the output uncertainty with respect to the experimental uncertainty at even lower computational effort than the sigma point method. The main disadvantage of this method was that is more complex to implement, mostly with respect to the calculation of the collocation points. As such, polynomial chaos expansion is not seen as an appropriate technique for the application and target audience considered for research. To conclude, the sigma point method is the most attractive method for the application studied in this publication because (i) it is computationally efficient, (ii) is robust with respect to experimental uncertainty and (iii) is easily implemented.

5 Acknowledgements

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6 References

Akkermans S., Logist F., Van Impe J., 2016. Parameter estimations in predictive microbiology: How to build statistically sound secondary models. Proceedings of FOODSIM 2016. Ghent, 4-7 April 2016.

Baranyi, J., Roberts, T.A., 1994. A dynamic approach to predicting bacterial growth in food. *International Journal of Food Microbiology* 23(3-4), 277-294.

Brul, S., Mensonides, F.I.C., Hellingwerf, K., Texeira de Mattos, M.J., 2008. Microbial systems biology: New frontiers open to predictive microbiology. *International Journal of Food Microbiology* 128(1), 16-21.

Bukaçi, E., Korini, T., Periku, E., Allkja, S., Sheperi, P., 2016. Number of iterations needed for Monte Carlo method using reliability analysis for tunnel supports. *International Journal of Engineering Research and Applications* 6(6), 60-64.

Debusschere, B., Najm, H., Pébay, P., Knio, O., Ghanem, R., Maître, O.L., 2004. Numerical challenges in the use of polynomial chaos representations for stochastic processes. *SIAM Journal on Scientific Computing* 26(2), 698-719.

den Besten, H.M.W., Berendsen, E.M., Wells-Bennik, M.H.J., Straatsma, H., Zwietering, M.H., 2017. Two complementary approaches to quantify variability in heat resistance of spores of *Bacillus subtilis*. *International Journal of Food Microbiology* 253: 48-53.

Fagiano, L., Khammash, M., 2012. Nonlinear stochastic model predictive control via regularized polynomial chaos expansions. In: IEEE 51st Annual Conference on Decision and Control (CDC). pp. 142-147.

Ghanem, R., Spanos, P., 1991. Stochastic finite elements: A spectral approach. SpringerVerlag.

536 Julier, S., Uhlmann, J., 1996. A general method for approximating nonlinear trans-
537 formations of probability distributions. Robotics Research Group, Department of
538 Engineering Science, University of Oxford.
539 Kreft, J.-U., Booth, G., Wimpenny, J.W.T., 1998. BacSim, a simulator for individual-
540 based modelling of bacterial colony growth. *Microbiology* 144(12), 3275-3287.
541 Nimmegeers, P., Telen, D., Logist, F., Van Impe, J., 2016. Dynamic optimization of
542 biological networks under parametric uncertainty. *BMC Systems Biology* 10 (1),1-
543 20.
544 Omlin, M., Reichert, P., 1999. A comparison of techniques for the estimation of model
545 prediction uncertainty. *Ecological Modelling* 115(1), 45–59.
546 Pin, C., Baranyi, J., 2006. Kinetics of single cells : Observation and modeling of a
547 stochastic process. *Applied and Environmental Microbiology* 72, 2163–2169.
548 Poschet, F., Geeraerd, A.H., Scheerlinck, N., Nicolaï, B.M., Van Impe, J.F., 2003.
549 Monte Carlo analysis as a tool to incorporate variation on experimental data in
550 predictive microbiology. *Food Microbiology* 20: 285-295.
551 Rosso, L., Lobry, J.R., Flandrois, J. P., 1993. An unexpected correlation between
552 cardinal temperatures of microbial growth highlighted by a new model. *Journal of*
553 *theoretical Biology* 162(4), 447-463.
554 Seber, G.A.F., Wild, C.J., 2003. Nonlinear Regression. John Wiley & Sons, Hoboken,
555 USA.
556 Tack, I.L.M.M., Logist, F., Noriega Fernandez, E., Van Impe, J.F.M. 2015. An
557 individual-based modelling approach to simulate the effects of cellular nutrient
558 competition on *Escherichia coli* K-12 MG1655 colony behaviour and interactions
559 in aerobic structured food systems. *Food Microbiology* 45, 179-188.

560 Tatang, M., Pan, W., Prinn, R., McRae, G., 1997. An efficient method for parametric
561 uncertainty analysis of numerical geophysical models. *Journal of Geophysical*
562 *Research: Atmospheres* 102 (D18), 21925-21932.
563 Telen, D., Vercammen, D., Logist, F., Van Impe, J., 2014. Robustifying optimal
564 experiment design for nonlinear, dynamic (bio)chemical systems. *Computers and*
565 *Chemical Engineering* 71, 415-425.
566 Telen, D., Vallerio, M., Cabianca, L., Houska, B., Van Impe, J., Logist, F., 2015.
567 Approximate robust optimization of nonlinear systems under parametric uncertainty
568 and process noise. *Journal of Process Control* 33, 140-154.
569 Van Impe, J.F., Bernaerts, K., Geeraerd, A. H., Poschet, F., Versyck, K. J., 2001.
570 Modelling and prediction in an uncertain environment. In: Tijskens, L. M. M.,
571 Hertog, M.L.A.T.M., Nicolaï, B.M. (eds.), *Food process modelling*. Woodhead
572 Publishing Limited, England, 156-179.
573 Vercammen, D., Telen, D., Nimmegeers, P., Janssens, A., Akkermans, S., Noriega
574 Fernandez, E., Logist, F., Van Impe, J. 2017. Application of a dynamic metabolic
575 flux algorithm during temperature-induced lag phase. *Food and Bioproducts*
576 *Processing*, 102: 1-19.
577 Walter, E., Pronzato, L., 1997. *Identification of parametric models from experimental*
578 *data.* Springer, Germany, Berlin.
579 Webster, M., Tatang, M., McRae, G., 1996. Application of the Probabilistic Collocation
580 Method for an Uncertainty Analysis of a Simple Ocean Model. Report MIT Joint
581 Program on the Science and Policy of Global Change.
582 Wiener, N., 1938. The homogeneous chaos. *American Journal of Mathematics*, 60(4),
583 897 - 936.

584 Xiu, D., Karniadakis, G.E., 2002. The Wiener-Askey polynomial chaos for stochastic
585 differential equations. *Siam Journal on Scientific Computing*, 24(2): 619-644.
586 Zwietering, M.H. 2015. Risk assessment and risk management for safe foods:
587 Assessment needs inclusion of variability and uncertainty, management needs
588 discrete decisions. *International Journal of Food Microbiology*, 213: 118-123.

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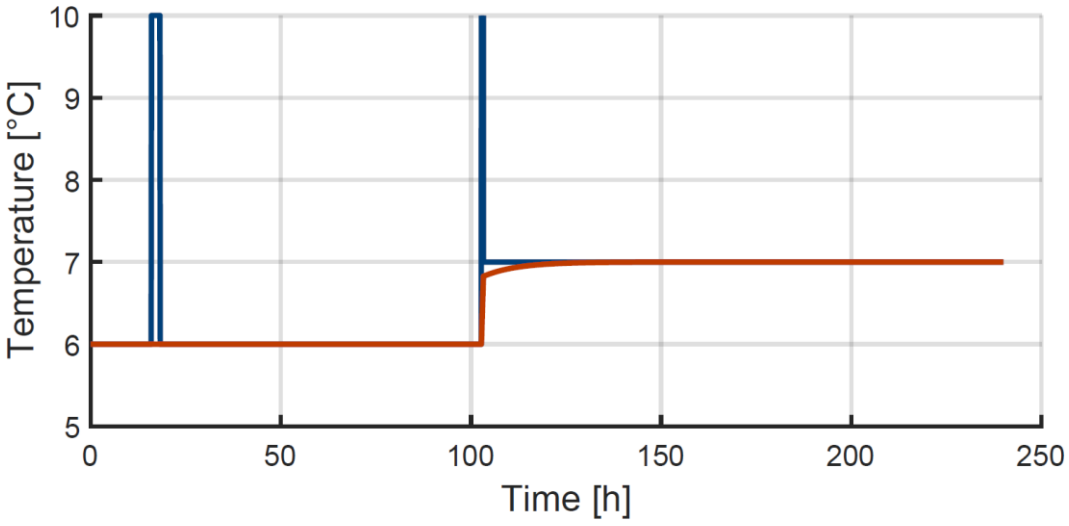
589 # Figures

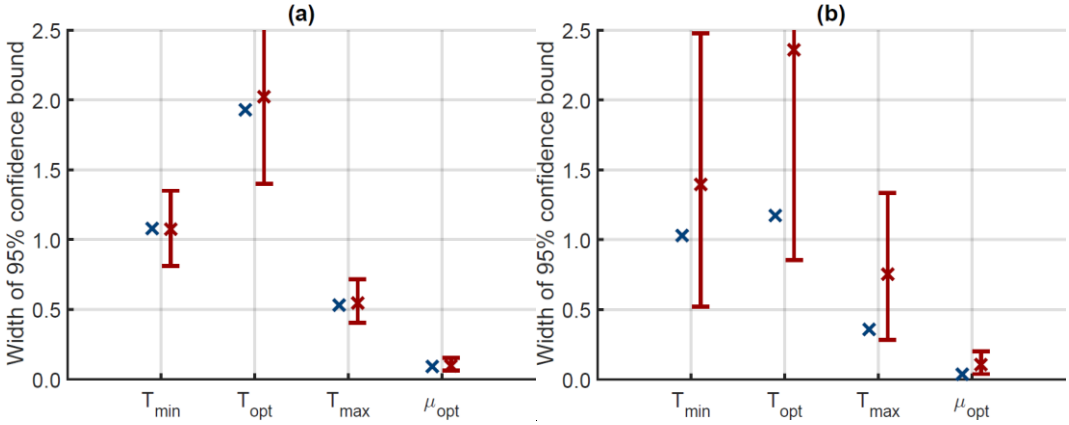


590

591 **Fig. 1:** Temperature profile of the environment (—) and the food product (—

592) resulting from the mean values of the parameters in Table 2 and Eq. 4.

593



594

595 **Fig. 2:** Illustration of the uncertainty on the parameters of the CTMI as

596 estimated with the (a) one-step and (b) two-step method. The 95% confidence

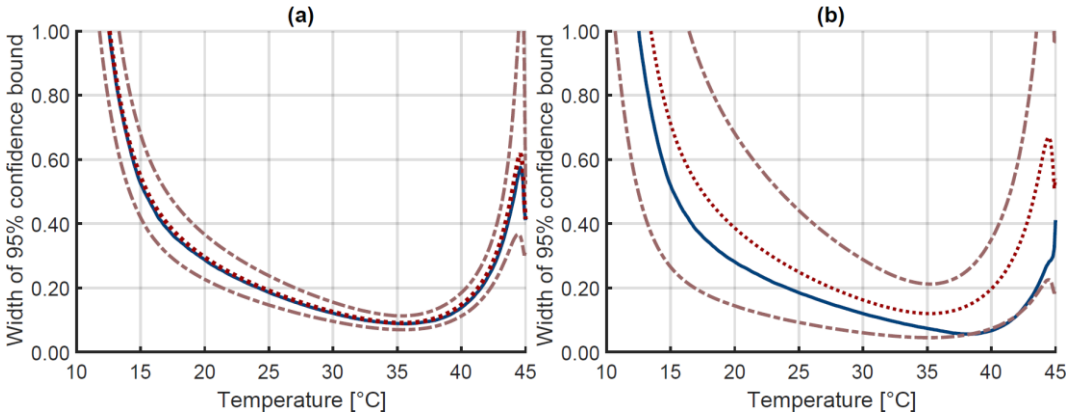
597 bounds determined with the Monte Carlo method (**X**) are considered to represent

598 the real variability of the parameter estimates. The 95% confidence bounds

599 calculated through the linear approximation (**X**) are provided with 95% error

600 bars. For the parameters T_{min}, T_{opt} and T_{max} the confidence bounds are expressed

601 in °C and for the parameter μ_{opt} they are expressed in h^{-1}.



602

603 **Fig. 3:** Prediction uncertainty of the doubling time [h] calculated with the
604 CTMI is presented as the width of its 95% confidence bounds as estimated with
605 the (a) one-step and (b) two-step method. The 95% confidence bounds on the
606 predictions determined with the Monte Carlo method (—) is considered to
607 represent the real variability of the predictions. The 95% confidence bounds
608 calculated through the linear approximation (---) are provided with 95% errors
609 (···).

610

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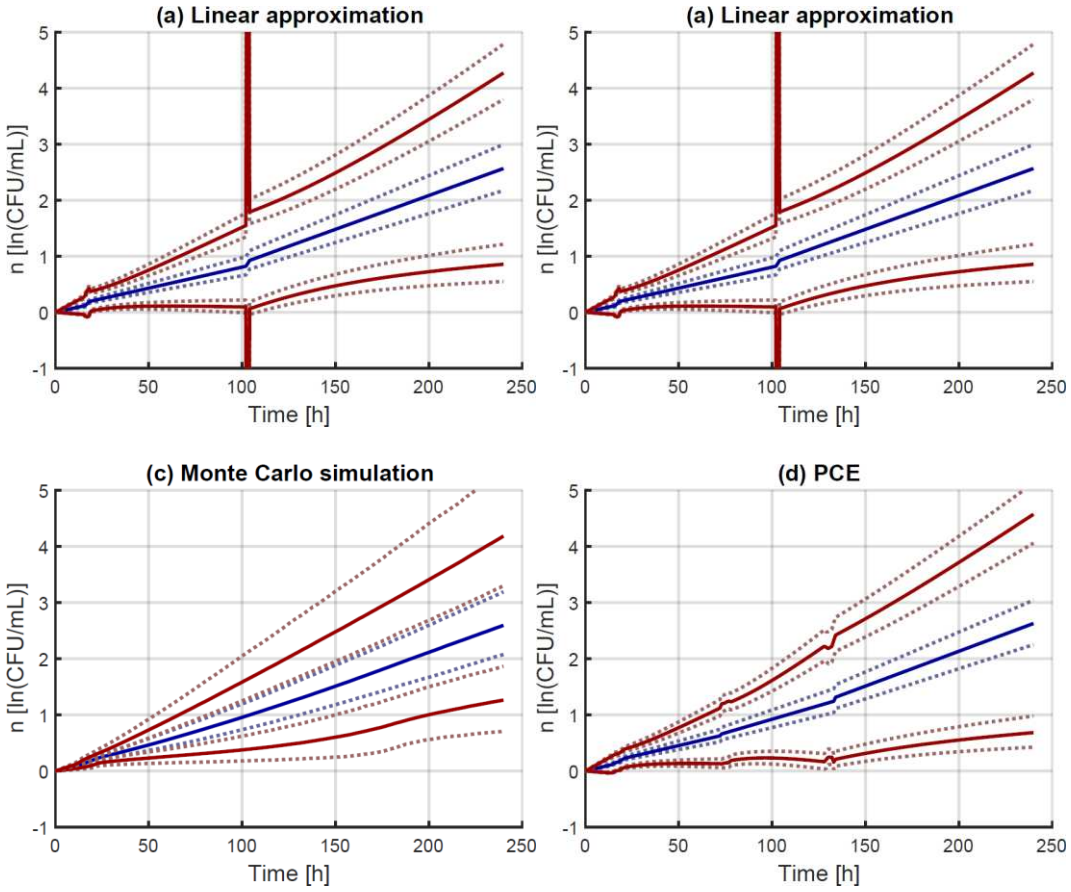

Fig. 4: Model predictions (—) with average 95% confidence bounds (—) for the temperature profile in Table 2 according to: (a) the linear approximation, (b) the sigma point method, (c) a Monte Carlo simulation with 27 iterations and (d) a first order polynomial chaos expansion. 95% errors caused by experimental uncertainty are provided for both the model predictions (···) and confidence bounds (···).

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Tables

Parameters	Values
T_{min} [°C]	2.3
T_{opt} [°C]	40.6
T_{max} [°C]	45.5
μ_{opt} [h^{-1}]	0.623
n_0 [ln(CFU/mL)]	7.00
q_0 [-]	-1.00
n_{max} [ln(CFU/mL)]	22.55

Table 1: Nominal parameter values of the CTMI and the model of Baranyi and Roberts (1994).

Description	Temperature [°C]	Time [h], uniform distribution	Time [h], approximate normal distribution
Storage after production	$N(6.0, 1.5)$	$U(10.00, 22.00)$	$N(16.00, 3.46^2)$
Transportation to shops	$N(10.0, 1.0)$	$U(0.50, 4.00)$	$N(2.25, 1.01^2)$
Storage in shops	$N(6.0, 1.0)$	$U(1.00, 168.00)$	$N(84.50, 48.21^2)$
Transport to customer's home	$N(20.0, 2.0)$	$U(0.08, 1.00)$	$N(0.54, 0.26^2)$
Storage at home	$N(7.0, 1.0)$	Remaining time of total 240 h	

Table 2: Five different steps of the temperature profile used to simulate microbial growth as a function of time with prediction uncertainty. Normal distributions are marked with their mean and variance and uniform distributions with their lower and upper bound.