CARDINAL TEMPERATURES ESTIMATION VIA OPTIMAL DYNAMIC EXPERIMENT DESIGN FOR PARAMETER ESTIMATION: VALIDATION ON *Z. bailii*

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Abstract. Previously, dynamic temperature profiles were optimized with the technique of optimal experiment design for parameter estimation to obtain unique and accurate identification of a nonlinear, microbial kinetic model enclosing four parameters [13]. *E. coli* K12 MG1655 was selected as a model micro-organism. The dynamic optimization problem was reduced to a series of two-parameter estimation problems. For all combinations of two model parameters, D-optimal dynamic temperature profiles were designed within a temperature region, confined to guarantee practical feasibility and model validity. The optimal experiments were implemented in a computer controlled bioreactor, and the kinetic parameters were identified from the resulting experimental data. Experimental observations underlined the importance of selecting the upper temperature constraint for OED/PE as close as possible to the true maximum growth temperature T_{max}. Realistic and accurate model parameters were obtained.

In this paper, the OED/PE strategy, as constructed for the case study of *E. coli* K12 MG1655, is further exploited for the yeast *Zygosaccharomyces bailii*. Again, accurate and reliable kinetic parameter values are obtained from the series of optimal dynamic bioreactor experiments, confirming the advantage of OED/PE implementation for the identification of kinetic models.

1 Introduction

Mathematical models are the key-stone of optimal design, control and operation of bioprocesses. In the domain of predictive microbiology, models are developed in which the influence of varying environmental factors on the microbial evolution in food products is described. A general condition of the global predictive model is the ability to describe the general characteristics of the investigated microbial behavior. A predictive model structure is mostly developed for an extended range of food-related microorganisms, but the model parameter values need to be improved for each microorganism specificly. Once this model structure is selected, solid implementation asks for accurate estimation of the model parameters such that model predictions closely represent reality. Accurate parameter estimation is, however, often hindered by *(i)* a too small amplitude of process output sensitivities with respect to the model parameters, *(ii)* correlation of model parameters, *(iii)* measurements with limited accuracy and/or small measurement frequency, and *(iv)* a lack of measurements for certain (biologically important) state variables [4].

In predictive microbiology, model parameters are usually determined based on static experiments covering the whole range supported by the model, or a fixed range selected for its high relevance. An accurate determination of parameters asks for an extended range of experimental data so that this working method is time-consuming and labor-intensive. Furthermore, problems can occur when the estimates are transferred to more realistic varying conditions. The use of dynamic experiments is a better alternative as this lowers the experimental load and allows evaluation of the model validity under more realistic, time-varying environmental conditions. Presuming model validity, the mathematical technique of optimal experiment design for parameter estimation (OED/PE) forms an excellent starting point for the selection of highly informative, dynamic experiments, aiming at unique and accurate parameter estimation. OED/PE was already successfully adopted in [13] for the estimation of the four parameters belonging to the Cardinal Temperature Model with Inflection (CTMI) [11], i.e., T_{min}, T_{opt}, T_{max} (the minimum, optimum and maximum growth temperature, respectively) and μ_{opt} (the specific growth rate at T_{opt}). This nonlinear kinetic model describes the effect of temperature on the microbial growth rate in the whole growth region, ranging from the minimum to the maximum temperature for growth. *E. coli* K12 MG1655 was selected as a model organism. Here, the optimization problem was reduced to a problem wherein the parameters are estimated two-by-two, as accurate estimation of the four parameters from a single experiment, taking into account the stringent limitations on the dynamics of a biological system, is doubtful. Starting from the four CTMI parameters, six combinations of two parameters were constructed. For each parameter couple, the D-optimal experiment was designed, while the two other parameters were assumed perfectly known. To guarantee model validity and practical feasibility, optimal experiments were selected in a confined temperature region. Next, the six optimal experiments were performed

in a computer controlled bioreactor and CTMI parameters were identified from the experimental data. Realistic CTMI parameters were obtained, and it was concluded that, in general, the implementation of OED/PE can improve the statistical quality of the kinetic model parameters. However, a major drawback of the use of OED/PE for the identification of the CTMI was uncovered. A reliable and accurate estimate of the maximum growth temperature T_{max} can only be obtained when temperatures at or very close to the true T_{max} are included in the optimal temperature profile. Therefore, a reliable initial estimation of the maximum temperature for growth is required.

In this manuscript, the conclusions drawn with respect to the *E. coli* case study are evaluated through the implementation of OED/PE for the identification of the CTMI for *Zygosaccharomyces bailii*. *Z. bailii* is a common spoilage yeast mostly found in food products with low pH values and high sugar concentrations, like apple juice and ketchup. In contrast to *E. coli*, prior knowledge of the impact of temperature on the growth rate of *Z. bailii* is minimal. Optimal experiments are designed and parameters are estimated following the OED/PE solution strategy presented in [13].

2 Materials and methods

2.1 Computational environment

Optimization algorithms. The optimization problem was solved with a hybrid optimization algorithm wherein the stochastic Integrated Control Random Search algorithm (ICRS) [1] was combined with the deterministic NAG routine E04UCF (The Numerical Algorithms Group Ltd). Random values from the normal distribution in ICRS were generated with the NAG pseudo-random generator G05FAF, combined with G05CCF to set the seed to a non-repeatable initial value. Values for the three heuristic ICRS parameters k_1, k_2 and n_e were set at the default values declared in [1]. Differential equations were numerically integrated with the NAG routine D02EJF.

Parameter estimation, uncertainty, confidence intervals and prediction intervals. Model parameters were estimated via the minimization of the global sum of squared errors (SSE_{global}), using the *lsqnonlin* routine of the Optimization Toolbox of Matlab version 6.5 (The Mathworks Inc.). The Matlab routine *ode23s* was used for the integration of ordinary differential equations.

Parameter variances (s_i^2) were obtained from the main diagonal of the parameter variance covariance matrix $\mathbf{P}$. $\mathbf{P}$ was calculated as $\left\{ \mathbf{J}^T \cdot \mathbf{J} \right\} \cdot MSE_{global}$ with $\mathbf{J}$ the Jacobian matrix, calculated by *lsqnonlin*, and MSE_{global}, the global mean sum of squared errors. The asymptotic 95% confidence interval on the best parameter estimate $\hat{p}_i$ was calculated as follows

$$\left[\hat{p}_i \pm t_{(1-\frac{\alpha}{2}, n_t - n_p)} \cdot \sqrt{s_i^2} \right] \tag{1}$$

with $\hat{p}_i$ the i^{th} parameter estimate, t the *Student t* distribution value, α the significance level (α = 0.05), n_t the total number of experimental data in N experiments, n_p the total number of parameters, and $n_t - n_p$ the degrees of freedom. Given the parameter variances, confidence limits on the model output were calculated as follows [14]

$$\left[n(t_i, \hat{\mathbf{p}}) \pm t_{(1-\frac{\alpha}{2}, n_t - n_p)} \cdot \sqrt{s^2_{n(t_i,\hat{\mathbf{p}})}} \right] \tag{2}$$

with $n(t_i, \hat{\mathbf{p}})$ the model prediction at time t_i, t the *Student t* distribution value and $\hat{\mathbf{p}}$ the vector of best parameter estimates. The variance $s^2_{n(t_i,\hat{\mathbf{p}})}$ was derived from

$$s^2_{n(t_i,\hat{\mathbf{p}})} = \left[\frac{\partial n(t_i, \mathbf{p})}{\partial \mathbf{p}} |_{\mathbf{p}=\hat{\mathbf{p}}} \right] \mathbf{P} \left[\frac{\partial n(t_i, \mathbf{p})}{\partial \mathbf{p}} |_{\mathbf{p}=\hat{\mathbf{p}}} \right]^T \tag{3}$$

Prediction limits, taking into account measurement errors, are given by

$$\left[n(t_i, \hat{\mathbf{p}}) \pm t_{(1-\frac{\alpha}{2}, n_t - n_p)} \cdot \sqrt{s^2_{n(t_i,\hat{\mathbf{p}})} + s_n^2} \right] \tag{4}$$

with s_n^2 set equal to the MSE_{global}.

2.2 Experimental protocol

Microorganism. A culture of the yeast *Z. bailii* (No. 174, stored at -75°C) was obtained from the Laboratory of Food Microbiology and Food Preservation (LFMFP, UGent, Belgium). The yeast is grown in Sabouraud liquid medium (SAB, CM147, Oxoid) which has a glucose concentration of 20g/L (2% w/v). The inoculum was prepared by transferring a loop of *Z. bailii* to a tube containing 6mL of SAB which was subsequently stored at 30°C for 24h.

Bioreactor experiments. Dynamic experiments were performed in a computer controlled bioreactor (BioFlo 3000, New Brunswick Scientific). The reactor vessel was filled with 3.5L of SAB. Reactor contents were stirred at 700 rpm and the aeration flow was set at 4L/min. Temperature profiles were programmed and controlled by the Advanced Fermentation Software system (New Brunswick Scientific). Temperatures beyond room temperature

Table 1: Cardinal Temperature Model with Inflection, with T_{min}, T_{opt} and T_{max} respectively the minimum, optimum and maximum temperature for growth [°C], and μ_{opt} the maximum specific growth rate at the optimum growth temperature [1/h].

	μ_{max}	=	$\mu_{opt} \cdot \gamma(T)$
$T < T_{min}$	$\gamma(T)$	=	0
$T_{min} \leq T \leq T_{max}$	$\gamma(T)$	=	$\frac{(T-T_{min})^2(T-T_{max})}{(T_{opt}-T_{min})((T_{opt}-T_{min})(T-T_{opt})-(T_{opt}-T_{max})(T_{opt}+T_{min}-2T))}$
$T > T_{max}$	$\gamma(T)$	=	0

could be attained by the use of a recirculation chiller (CFT-33, Neslab Instruments). The bioreactor was inoculated at approximately 7-9 ln(CFU/mL). After inoculation, cells were allowed to adapt to the new temperature for minimally 2h prior to the implementation of the temperature profile $T_{input}(t)$ and sampling, in order to minimize the initial adaptation phase.

Cell density was determined based on plate counts. After serially diluting the sample in physiological salt solution (8.5g/L NaCl and 1g/L peptone), the appropriate dilutions were plated on TSA supplemented with 15% w/v glucose (Sigma) using a spiral plater (Eddy Jet, IUL Instruments s.a.). For each sample, at least two dilutions were plated. Each cell count shown is an average of countable plates. The average time between sampling and plating was less than 10 min which is smaller than the generation time. Plates were counted after incubation at 30°C for 48h.

3 Optimal experiment design for parameter estimation

3.1 Theory

The information content of an experiment with continuous measurement of the model output $\mathbf{y}$ and duration t_f can be quantified by the Fisher information matrix (see e.g., [15]):

$$\mathbf{F} \triangleq \int_0^{t_f} \left(\frac{\partial \mathbf{y}}{\partial \mathbf{p}}|_{\mathbf{p}=\mathbf{p}^\circ} \right)^T \mathbf{Q} \left(\frac{\partial \mathbf{y}}{\partial \mathbf{p}}|_{\mathbf{p}=\mathbf{p}^\circ} \right) dt \tag{5}$$

with $\partial \mathbf{y}/\partial \mathbf{p}$ the sensitivity matrix which quantifies the sensitivity of the model output to small variations in the model parameters $\mathbf{p}$, and $\mathbf{Q}$ the errors on the output measurements which is typically taken equal to the inverse of the measurement error variance matrix. For nonlinear models, the Fisher information matrix depends on the unknown parameters $\mathbf{p}$. $\mathbf{F}$ is therefore computed for $\mathbf{p} = \mathbf{p}^\circ$, with $\mathbf{p}^\circ$ an initial guess for the unknown model parameters (nominal parameter vector) obtained from literature or preliminary experiments. Optimal experiment design results from the minimization or maximization of a scalar function of the Fisher information matrix. The selected scalar function determines the focus of the design. D-optimal design aims at the minimization of the joint confidence region on $\mathbf{p}$ via the maximization of the determinant of $\mathbf{F}$.

3.2 Application to the case study

The evolution of cell density in time is described by the growth model of Baranyi and Roberts [2]:

$$\begin{aligned} \frac{dn(t)}{dt} &= \frac{Q(t)}{Q(t)+1} \cdot \mu_{max}(T(t)) \cdot [1 - \exp(n(t) - n_{max})] \\ \frac{dQ(t)}{dt} &= \mu_{max}(T(t)) \cdot Q(t) \end{aligned} \tag{6}$$

with $n(t)$ [ln(CFU/mL)] the natural logarithm of the cell density at time t, $n(0)$ the initial and n_{max} the maximum value for $n(t)$, $Q(t)$ [-] the physiological state of the cells, and μ_{max} [1/h] the maximum specific growth rate. The duration of the initial adaptation phase, modeled via the parameter $Q(t)$, is determined by the experimental conditions and is not accurately predictable. Hence, a reduced form of the model of Baranyi and Roberts (Equation (6)), in which the state variable $Q(t)$ is omitted, was used to design the optimal experiments. As such, this model coincides with a logistic growth model consisting of exponential growth followed by a stationary phase. The evolution of μ_{max} as function of temperature is incorporated in Equation (6) by the Cardinal Temperature Model with Inflection (CTMI) [11]. The model equation is given in Table 1.

Unknown parameters are the four parameters of the CTMI: $\mathbf{p} = [T_{min}\ T_{opt}\ T_{max}\ \mu_{opt}]^T$. The measured model output equals $n(t)$, which is considered as a continuous measurement. For the estimation of the four CTMI parameters, the optimization problem was reduced to a combination of optimization problems of lower complexity [13]. The problem is reformulated as a series of two-parameter estimation problems. An optimal experiment is designed

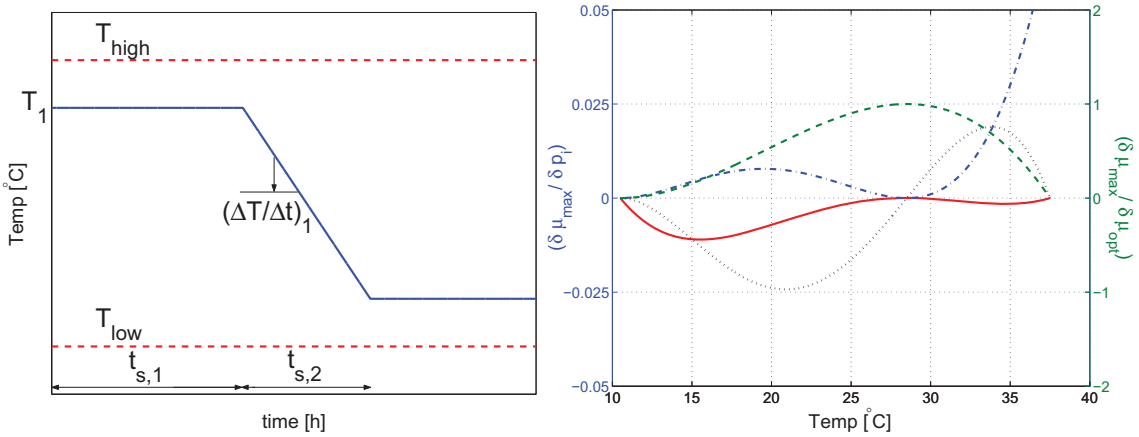

Figure 1: (Left) Representation of the parameterized temperature profile, which is characterized by four degrees of freedom: T_1 the initial temperature, $t_{s,1}$ the time at which the increase or decrease in temperature starts, $(\Delta T/\Delta t)_1$ the rate of temperature change and $t_{s,2}$ the duration of the temperature change. (Right) Sensitivities of the CTMI with respect to its parameters (-),(- ·), (· ·) and (- -) corresponding to T_{min}, T_{opt}, T_{max} and μ_{opt}, respectively. The figure is constructed given the nominal CTMI values displayed in Table 3.

for each two-parameter combination, based on common nominal values. Next, all six optimal experiments are implemented and new parameter values are globally estimated from all experimental data sets. The advantage of this global OED/PE strategy is that the error in one (or more) nominal values is not spread over all designs, i.e., if a nominal value is far from its true value, not all designs are corrupted. Moreover, the advantage of taking into account several experiments is that the variability of the system is included in the parameter uncertainty.

Realistic nominal parameters for *Zygosaccharomyces bailii* are derived from static experiments and literature: T_{min} = 10.50°C, T_{opt} = 28.50°C, T_{max} = 37.50°C, and μ_{opt} = 0.2800 1/h. As an example, the initial and maximum cell density are: n_0 = 7.000 ln(CFU/mL) and n_{max} = 19.20 ln(CFU/mL). The weighting matrix $\mathbf{Q}$ reduces to a single value, namely the inverse of the measurement error variance, which is taken equal to 3.27×10^{-2}.

The control input is the time-varying temperature $T_{input}(t)$ that is parameterized to obtain a finite dimensional dynamic optimization problem. The structure of $T_{input}(t)$ is drafted in Figure 1 (Left). After an initial period at constant temperature, temperature linearly decreases or increases to reach a final phase of constant temperature. Four degrees of freedom characterize this temperature profile: T_1 [°C] the initial temperature, $t_{s,1}$ [h] the time at which the increase or decrease in temperature starts, $(\Delta T/\Delta t)_1$ [°C/h] the rate of temperature change, and $t_{s,2}$ [h] the duration of the temperature change. The four degrees of freedom implicitly determine the final temperature T_2 [°C]. To guarantee validity of the models and practical feasibility of the optimal experiments at all time, two constraints are imposed on the temperature input profile. (*i*) Only very moderate temperature gradients can be applied as rapid changes can induce an intermediate adaptation phase and violate the model structure (see, e.g., [12]). Analogously to the case study of *E. coli* where the maximum rate of temperature change was set at 5°C/h ($\leq 2\times \mu_{opt}$), the maximum $(\Delta T/\Delta t)$ for *Z. bailii* was chosen at 0.5°C/h. (*ii*) The dynamic temperature profiles are confined between T_{low} and T_{high}, here taken equal to 12°C and 37°C, respectively, ensuring smooth growth curves and measurable growth rates at all times. From the results presented for *E. coli*, it was stated that T_{max} can only be estimated accurately when the nominal T_{max}, and also the upper temperature constraint T_{high}, are close to the true T_{max}. Therefore, the upper temperature limit T_{high} was chosen very close to the nominal T_{max}. The duration of the designed experiments is fixed a priori at 60h from a practical point of view.

4 Results and discussion

4.1 Design of optimal temperature profiles: results and interpretation

The resulting OED/PE approach can be summarized as finding the optimal values for the four DOF of the parameterized temperature input that maximize the information embedded in the model output, i.e., cell density as function of time. The input profile is subject to

$$\begin{array}{rcccl} 12°\mathrm{C} & \leq & T_{input}(t) & \leq & 37°\mathrm{C} \\ -0.5°\mathrm{C}/h & \leq & (\Delta T/\Delta t) & \leq & 0.5°\mathrm{C}/h \end{array} \tag{7}$$

D-optimal experiments for all six parameter couples are calculated with the nominal T_{min}, T_{opt}, T_{max} and μ_{opt} chosen at 10.50°C, 28.50°C, 37.50°C and 0.2800 1/h, respectively.

Table 2: D-optimal temperature profiles for all two-parameter combinations. The parametrization of the temperature profile is illustrated in Figure 1 (Left). Temperature is bounded within [12°C, 37°C] and t_f is fixed at 60h. The global optimum of the adopted criterion is presented in bold. (Letter codes refer to graphs of the performed experiments.)

Negative slope: $\Delta T/\Delta t \in$ [-0.5°C/h, 0°C/h]							
	T_1	$t_{s,1}$	$(\Delta T/\Delta t)_1$	$t_{s,2}$	T_2	det(**F**)	
(T_{max}, μ_{opt})	37.00	22.25	-0.5000	17.00	28.50	4.408×10^{8}	
(T_{max}, T_{min})	37.00	9.001	-0.5000	43.50	15.25	$\mathbf{6.387\times10^{3}}$	(B)
(T_{max}, T_{opt})	37.00	37.00	-0.5000	7.083	33.46	1.570×10^{5}	
(T_{min}, μ_{opt})	28.50	8.723	-0.5000	26.78	15.11	$\mathbf{1.470\times10^{7}}$	(D)
(T_{min}, T_{opt})	22.32	16.33	-0.5000	16.07	14.29	$\mathbf{6.706\times10^{3}}$	(E)
(T_{opt}, μ_{opt})	33.73	8.537	-0.5000	30.11	18.67	$\mathbf{8.678\times10^{7}}$	(F)
Positive slope: $\Delta T/\Delta t \in$ [0°C/h, 0.5°C/h]							
	T_1	$t_{s,1}$	$(\Delta T/\Delta t)_1$	$t_{s,2}$	T_2	det(**F**)	
(T_{max}, μ_{opt})	28.50	11.94	0.5000	17.00	37.00	$\mathbf{4.622\times10^{8}}$	(A)
(T_{max}, T_{min})	15.29	1.511	0.5000	43.43	37.00	6.243×10^{3}	
(T_{max}, T_{opt})	33.44	20.06	0.5000	7.127	37.00	$\mathbf{1.577\times10^{5}}$	(C)
(T_{min}, μ_{opt})	14.96	15.98	0.5000	27.08	28.50	1.374×10^{7}	
(T_{min}, T_{opt})	14.28	17.48	0.5000	16.02	22.29	6.679×10^{3}	
(T_{opt}, μ_{opt})	19.82	9.244	0.5000	30.22	34.93	8.088×10^{9}	

Design of optimal temperature profiles. The resulting optimal temperature profiles are listed in Table 2. Temperature profiles are designed for both a linear increasing and decreasing temperature. For each parameter combination, the most informative temperature profile is presented in bold.

Contrary to the case study of *E. coli* [13], linearly decreasing temperature profiles are not per se the most informative for all parameter combinations. The parameter couples (T_{max}, μ_{opt}) and (T_{max}, T_{opt}) can be estimated better from a dynamic experiment including a linear temperature increase. For all parameter combinations, differences in the det(**F**) of optimal experiments given a positive and a negative temperature change are very small. Nearly equal D-criterion values can be observed for (T_{max}, T_{opt}) and (T_{min}, T_{opt}).

Due to the slow growth rates, the optimal temperature profiles are dominated by the temperature change. The optimal $(\Delta T/\Delta t)$ always coincides with the absolute maximum temperature change, i.e., 0.5°C/h. This is similar to the case study of *E. coli* [13] and the work of Bernaerts et al. [3], where rapid temperature changes are preferred to slow temperature changes. Initial or final temperatures of the optimal experiments aiming at the estimation of (T_{max}, μ_{opt}), (T_{max}, T_{min}) and (T_{max}, T_{opt}) are always equal to the upper temperature boundary. The lower temperature constraint, however, is never attained.

Between the different parameter combinations, the values of T_1, $t_{s,1}$ and $t_{s,2}$ vary. For a specific parameter couple, the initial and final temperature of the experiments with a linear temperature decrease are (almost) identical to the final and initial temperatures of the experiments with a positive slope, respectively. A larger variation exist in the duration of the constant temperature phase. In general, experiments with positive and negative slopes are seemingly each others' mirror image.

Interpretation of optimal temperature profiles with respect to sensitivity functions. Analysis of the optimal temperature profiles with respect to the sensitivities in Figure 1 (Right) is analogous to the case study of *E. coli* [13]. (*i*) Estimation of μ_{opt} is improved by sampling at and around T_{opt}. This is reflected in the temperature profiles associated with (T_{max}, μ_{opt}) and (T_{min}, μ_{opt}), for which the initial or final temperature is equal to T_{opt}. For the combination (T_{opt}, μ_{opt}), the informative region is crossed during the linear temperature change. (*ii*) Profiles for the estimation of T_{min} start or end at temperatures around 14°C-15°C, the temperature region in which the inflection point of the CTMI model is situated. (*iii*) The informative temperature zones for T_{opt} are situated around 20°C and 33°C. Therefore, one of these temperatures is mostly selected as begin or end temperature when a parameter couple encloses T_{opt}. (*iv*) As revealed before, the estimation of T_{max} requires temperature profiles including temperatures close to the true T_{max}. This is reflected in the sensitivity of μ_{max} with respect to T_{max}, which shows a very explicit extremum at T_{max}. As such, optimal experiments for the estimation of T_{max} always start or end at the upper temperature boundary of 37°C. (*v*) The lower temperature boundary is never attained since no extrema exist around this lower boundary.

Table 3: Nominal values, CTMI parameter estimates and standard deviations derived from the six optimal experiments (Figure 2) and the six non-optimized dynamic experiments (Figure 3).

		Optimal		Non-optimized	
	Nominal values	dynamic experiments		dynamic experiments	
T_{min}	10.50	7.191	(2.386×10^{-1})	9.723	(3.588×10^{-1})
T_{opt}	28.50	29.72	(1.323×10^{-1})	29.28	(1.225×10^{-1})
T_{max}	37.50	37.13	(2.897×10^{-2})	37.03	(3.003×10^{-2})
μ_{opt}	0.2800	0.3333	(3.025×10^{-3})	0.3000	(2.680×10^{-3})
MSE_{global}		5.775×10^{-2}		1.814×10^{-2}	
(n_t, n_p)		(228, 17)		(175, 17)	

4.2 Parameter estimation from optimal experiments

As in the case study with *E. coli*, the optimal temperature profiles are simplified before practical implementation in the bioreactor. The impact of the profile simplification on the information content of the experiment, i.e., the value of the D-criterion, is negligible (data not shown). The periods of sampling are scheduled such that the informative zones (see [6] and [9]), i.e., the initial lag phase, the begin and end of the exponential phase and the stationary phase, are covered. Furthermore, sampling was preferentially placed during the temperature change and at the constant phases at the very low and high temperatures. At the latter, growth is difficult to estimate without prolonged sampling, due to the low growth rates. As can be seen in Figure 2, the periods without sampling are small such that it can be expected that only a minimum of information is lost.

The simplified optimal experiments are implemented in a computer controlled bioreactor. The growth curves given the dynamic temperature profiles are shown in Figure 2. (Letter codes refer to the optimal experiments as given in Table 2.) The experimental protocol was designed such that the microbial cells are in the exponential growth phase when sampling started. However, to take into account the possible presence of a (short) initial lag phase, the growth curves are fitted using the full model of Baranyi and Roberts, i.e., with Q(t), as shown in Equation (6). Growth model parameters and CTMI parameters are identified by fitting this full model of Baranyi and Roberts, combined with the CTMI, on the six optimal experiments. The resulting CTMI parameter values and standard deviations are listed in Table 3.

The trends in the growth curves are followed accurately, except for experiments (B) and (C). For both experiments, microbial cells grow faster than described by the models. As the model simulation underestimates the growth in experiments (B) and (C), this can not be due to the induction of an intermediate adaptation phase as a response to the temperature change. In experiment (C), the growth in the initial phase at approximately 33°C is characterized by two growth phases. It seems unlikely that an initial lag phase is induced as the difference between the inoculation temperature (30°C) and the temperature in the bioreactor is small (less than 5°C). Furthermore, microorganisms are adapted to the new temperature prior to the implementation of the optimal temperature profile. An explanation for the lack of fit in experiments (B) and (C) is thus not found.

The new CTMI parameter estimates are compared to the nominal parameters, selected based on a very small set of static experiments and values published in [7]. These researchers estimated T_{min}, T_{opt} and T_{max} roughly at 6.5°C, 30°C and 37°C, respectively, for a medium with 10% (w/w) glucose. Values for T_{opt}, T_{max} and μ_{opt} are very similar to the nominal values, and T_{opt} and T_{max} correspond to temperatures presented in [7]. However, the minimum temperature for growth is estimated significantly lower than its nominal value. Most probably, the nominal T_{min} is an overestimation of the lower growth boundary and the new T_{min} estimate and value given in [7] are closer to the real minimum temperature for growth. All parameter estimates are characterized by a small estimation error, with the largest estimation uncertainty associated with T_{min}. This higher uncertainty can be explained by the rather low sensitivity of the kinetic model with respect to the parameter T_{min}. As can be seen in Figure 1 (Left), the CTMI is significantly less sensitive for T_{min} as for the three other model parameters. As a result, small differences in the T_{min} value only have a small influence on the models' descriptive quality, and accurate and reliable estimation of T_{min} is not evident. Moreover, the nominal value for T_{min} is chosen significantly higher than the true minimum temperature for growth. As a result, limited information is available in the temperature region the most informative for the estimation of T_{min}. However, compared to literature, the obtained T_{min} estimate is realistic. It seems, however, very reasonable that a second iteration of OED/PE with a revision of the nominal T_{min} will improve the estimation of the minimum temperature for growth.

4.3 Evaluation and validation

Evaluation of the OED/PE strategy. To verify the advantage of OED/PE, the parameter estimates and corresponding standard deviations are compared to CTMI parameters estimated from a series of non-optimized dynamic bioreactor experiments. For the design of these dynamic experiments, the temperature region was subdivided in three parts, namely [12°C, 25°C], [25°C, 34°C] and [27.5°C, 37°C]. In each zone, a linearly increasing and de-

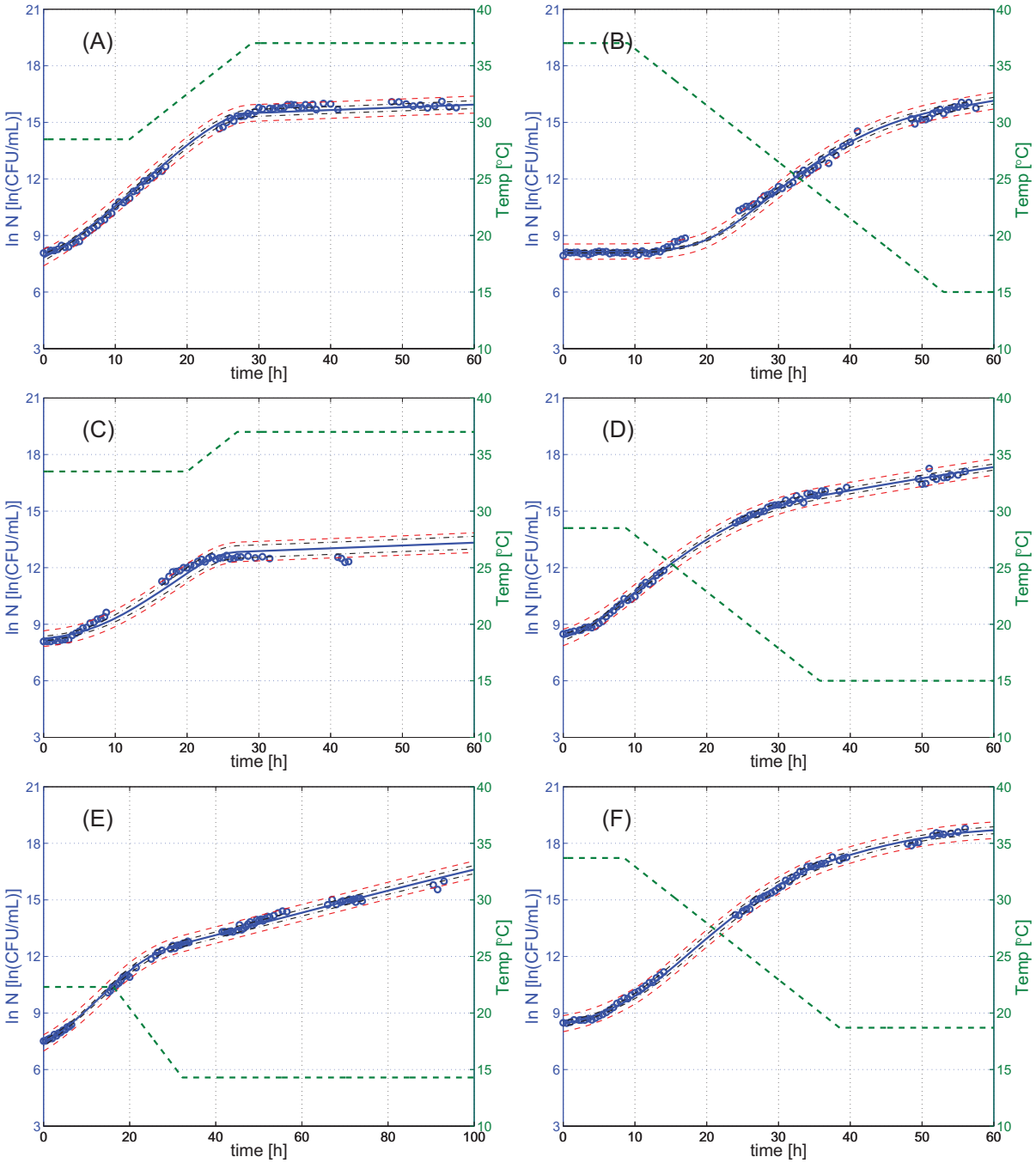

Figure 2: Global identification of the CTMI model on the six optimal experiments designed within [12°C, 37°C]: experimental data (○), global identification curves (-) and temperature profiles (- -).The confidence and prediction intervals are represented by (- ·) and (- -), respectively. The corresponding parameter estimates and standard deviations are listed in Table 3.

creasing temperature profile is designed. Profiles are selected such that temperature changes during exponential growth. Moderate temperature changes (0.2°C/h - 0.35°C/h) are selected as rapid changes can induce an intermediate adaptation phase that violates the model structure (see, e.g., [12]). When the temperature is at its final setpoint, this temperature level is maintained until the stationary phase is reached. To ensure exponential growth during temperature variation, temperature change was only initiated 5h after inoculation.

T_{opt} and T_{max}, estimated from the six non-optimized dynamic experiments are very similar to the estimates resulting from the optimal experiments, and correspond with the results given in [7]. A larger difference is observed for T_{min}, with a lower, more realistic value derived from the six optimal experiments. The overestimation of T_{min} from the non-optimized experiments is due to a lack of sufficient and accurate information at moderate and low temperatures. Growth rate estimates can only be obtained during the linear temperature change and at the arbitrarily chosen temperature levels (12°C and 25°C), and the constant temperature levels were often only maintained for short periods. Moreover, growth at 12°C was that slow that estimation of μ_{max} from the available information was inconclusive. In the optimal experiments, however, information can not only be collected during the temperature change but also during the initial or final constant phases. The latter temperature levels are situated between 14°C and 23°C and are different for most optimal experiments. In most cases, these constant phases are rather long ($\approx$ 20h), making the growth rate estimates more accurate.

Validation of the cardinal temperatures estimates. The overall validity and transferability of the CTMI parameter estimates is further confirmed by predicting the growth of the six non-optimized experiments. Values for $n(0)_i$, $Q(0)_i$ and n_{max} are estimated, while the CTMI parameters are fixed at the OED/PE estimates. Confidence and prediction intervals are constructed. As can be seen in Figure 3, the general trends in the dynamic experiments are well described. Except for experiment (1) where the growth at 12°C is overestimated, approximately all cell density measurements fall within the prediction interval. This observation confirms the general validity of the CTMI parameters within the investigated temperature region.

5 Conclusions

In this manuscript, *Z. bailii* is used to evaluate conclusions drawn in [13] with respect to the implementation of OED/PE for the identification of the CTMI for *E. coli* K12 MG1655.

From the results presented in [13], a major drawback of the application of OED/PE for the identification of the CTMI was formulated. Only when the nominal T_{max}, and also the upper temperature constraint (T_{high}), are close to the true T_{max}, T_{max} can be estimated accurately. Therefore, attention is paid to the selection of the nominal T_{max} and T_{high} for the case study of *Z. bailii*. The nominal maximum temperature for growth and the upper temperature boundary are chosen very close to the expected true T_{max}.

Analogous to the case study with *E. coli*, D-optimal experiments are calculated for all six combinations of two CTMI parameters. T_{min}, T_{opt} and T_{max} values estimated from these six experiments are realistic, i.e., very similar to the values obtained by Jermini et al. [7]. Associated standard deviations are very small. The largest estimation error is associated with the estimation of T_{min}. The nominal T_{min} estimate is chosen too high such that the optimal experiments lack information in the most information region. Additionally, accurate estimation of T_{min} is hardened by the lower sensitivity of the CTMI model with respect to T_{min}, and the slow growth rates at low temperatures. Estimation of T_{min} can most likely be improved in a second OED/PE design round with revised nominal values and an adapted lower temperature boundary.

The performance of OED/PE is compared to a series of non-optimized dynamic experiments. The minimum temperature for growth estimated from the optimal experiments is better than the value derived from the non-optimized dynamic experiments. In general, the main conclusion drawn from the *E. coli* case study can be confirmed: implementation of OED/PE improves the one-step identification of the CTMI compared to an arbitrarily chosen series of dynamic experiments.

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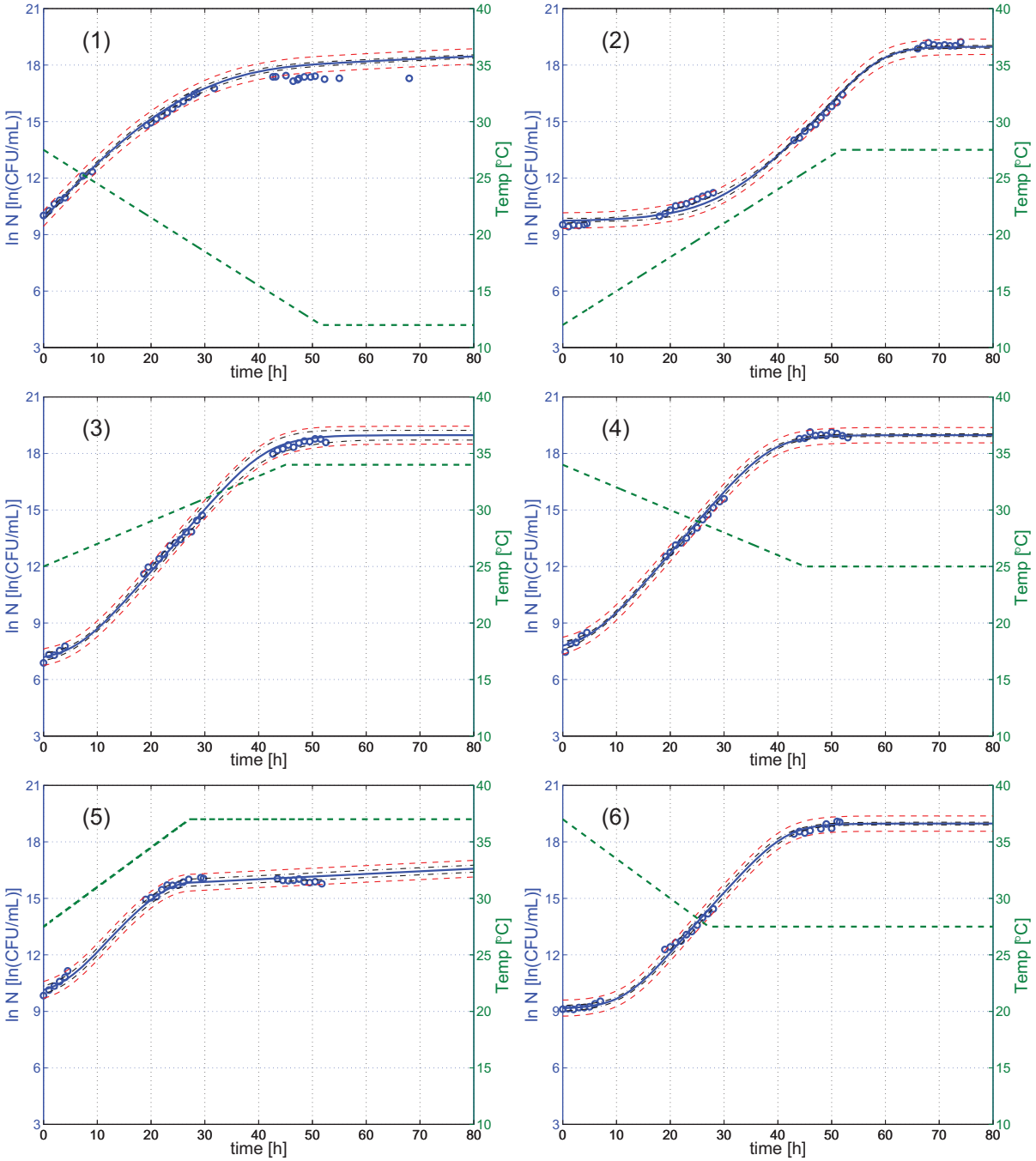

Figure 3: (Validation) Simulation of the six dynamic experiments within [12°C, 37°C] with global cardinal estimates from the six optimal experiments within [12°C, 37°C] and corresponding confidence and prediction regions. Estimates for $n(0)_i$, $Q(0)_i$ and n_{max} are obtained by globally fitting these six experimental data sets with a combination of the Baranyi model and the CTMI, while fixing the CTMI parameters at the best estimates from the optimal experiments. (experimental data (○), simulation curve (-), temperature profile (- -), confidence interval (- ·) and prediction interval (- -)).

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