Simultaneous versus sequential optimal experiment design for the identification of multi-parameter microbial growth kinetics as a function of temperature

E. Van Derlinden, K. Bernaerts, J.F. Van Impe

PII: S0022-5193(10)00007-X
DOI: doi:10.1016/j.jtbi.2010.01.003
Reference: YJTBI5825

www.elsevier.com/locate/jtbi

To appear in: *Journal of Theoretical Biology*

Received date: 6 October 2009
Revised date: 22 December 2009
Accepted date: 5 January 2010

Cite this article as: E. Van Derlinden, K. Bernaerts and J.F. Van Impe, Simultaneous versus sequential optimal experiment design for the identification of multi-parameter microbial growth kinetics as a function of temperature, *Journal of Theoretical Biology*, doi:[10.1016/j.jtbi.2010.01.003](https://doi.org/10.1016/j.jtbi.2010.01.003)

This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting galley proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Simultaneous versus sequential optimal experiment design for the identification of multi-parameter microbial growth kinetics as a function of temperature

E. Van Derlinden^a, K. Bernaerts^a, J. F. Van Impe^a

^a CPMF² - Flemish Cluster Predictive Microbiology in Foods - www.cpmf2.be
BioTeC - Chemical and Biochemical Process Technology and Control
Department of Chemical Engineering, Katholieke Universiteit Leuven, Leuven, Belgium
(eva.vanderlinden@cit.kuleuven.be, kristel.bernaerts@cit.kuleuven.be, jan.vanimpe@cit.kuleuven.be)

Correspondence to:
Prof. J.F. Van Impe
BioTeC - Chemical and Biochemical Process Technology and Control
Department of Chemical Engineering, Katholieke Universiteit Leuven
W. de Croylaan 46, B-3001 Leuven, Belgium
jan.vanimpe@cit.kuleuven.be
Tel: +32-16-32.14.66
Fax: +32-16-32.29.91

Abstract

Optimal experiment design for parameter estimation (OED/PE) has become a popular tool for efficient and accurate estimation of kinetic model parameters. When the kinetic model under study encloses multiple parameters, different optimization strategies can be constructed. The most straightforward approach is to estimate all parameters simultaneously from one optimal experiment (*single OED/PE strategy*). However, due to the complexity of the optimization problem or the stringent limitations on the system's dynamics, the experimental information can be limited and parameter estimation convergence problems can arise. As an alternative, we propose to reduce the optimization problem to a series of two-parameter estimation problems, i.e., an optimal experiment is designed for a combination of two parameters while presuming the other parameters known. Two different approaches

30 can be followed: (i) all two-parameter optimal experiments are designed
 31 based on identical initial parameter estimates and parameters are estimated
 32 simultaneously from all resulting experimental data (*global OED/PE strat-*
 33 *egy*), and (ii) optimal experiments are calculated and implemented sequen-
 34 tially whereby the parameter values are updated intermediately (*sequential*
 35 *OED/PE strategy*).

36 This work exploits OED/PE for the identification of the Cardinal Tem-
 37 perature Model with Inflection (CTMI) [5]. This kinetic model describes the
 38 effect of temperature on the microbial growth rate and encloses four param-
 39 eters. The three OED/PE strategies are considered and the impact of the
 40 OED/PE design strategy on the accuracy of the CTMI parameter estimation
 41 is evaluated. Based on a simulation study, it is observed that the parame-
 42 ter values derived from the sequential approach deviate more from the true
 43 parameters than the single and global strategy estimates. The single and
 44 global OED/PE strategies are further compared based on experimental data
 45 obtained from design implementation in a bioreactor. Comparable estimates
 46 are obtained, but global OED/PE estimates are, in general, more accurate
 47 and reliable.

48 *Keywords:* optimal experiment design, predictive microbiology, parameter
 49 estimation, design strategy

1. Introduction

Mathematical models are the key-stone of optimal design, control and operation of bioprocesses. Next to the construction of a relevant model structure, in which the relation between the input and output variables is determined, the estimation of the model parameters is of great importance in the model building cycle. Accurate and efficient parameter estimation can be hindered by the properties of the model structure (e.g., parameter correlation and a small sensitivity of the model parameters) or by the quality and quantity of the experimental data.

In biology and biotechnology related research fields, most investigated systems are dynamic, i.e., they change as a function of time and environmental conditions. These dynamic systems are described by kinetic models which typically consist of ordinary differential equations. Accurate and efficient parameter estimation is in many research areas a bottleneck for a reliable implementation of bio-related models. Traditionally, parameters of kinetic models that describe a system's behavior as a function of changing environmental conditions are estimated from a series of static experiments, i.e., at different constant levels of the studied environmental factor. Among the many disadvantages connected to this approach, one of the most important one is its experimental load.

Different approaches can be taken to minimize the number of experiments and/or increase the quality of the experimental data. (i) The number of required experimental data can be reduced by limiting the environmental range under study. However, as a consequence, the region of validity of the constructed model is small and extrapolation outside the studied range is not guaranteed. (ii) Instead of studying the system at single levels for one environmental factor at a time, more information can be collected in a full factorial design, in which all combinations of the selected discrete levels of the different explanatory variables are considered. The number of experi-

ments can be further reduced by a well-thought selection of these levels that unravel the most information about the studied system (fractional factorial design). (iii) Presuming model validity, the mathematical technique of optimal experiment design forms an excellent starting point for the selection of a small set of highly informative, dynamic experiments, resulting in unique and accurate parameter estimates. This approach also guarantees parameter estimates which are valid under dynamic, more realistic conditions.

In the field of predictive microbiology, mathematical models are developed that can describe the microbial evolution (growth, inactivation and survival) under time-varying environmental conditions with the ultimate goal to quantify and predict microbial dynamics in food products. Important environmental conditions influencing the microbial behavior are temperature, pH, water activity and the presence of preservatives. In the past, different types of models have been constructed to quantify the effect of these conditions on microbial growth and inactivation. Acknowledging the general validity of these model structures, implementation under varying environmental conditions for different microbial strains and different food products requires accurate and reliable specific parameters.

In this work, optimal experiment design for parameter estimation (OED/PE) is exploited for the identification of the Cardinal Temperature Model with Inflection (CTMI) [5]. This kinetic model describes the effect of temperature on the microbial growth rate and encloses four parameters, i.e., T_{min} , T_{opt} , T_{max} (the minimum, optimum and maximum growth temperature, respectively) and μ_{opt} (the maximum specific growth rate at T_{opt}). As this kinetic model encloses multiple parameters, different OED/PE design strategies can be constructed. In our previous work, the four CTMI parameters for *Escherichia coli* K12 were accurately estimated using a *global OED/PE strategy* [7]. In this approach, the optimization problem was reduced to a series of two-by-two optimization problems. 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 known. Next, the six optimal experiments were performed in a computer controlled bioreactor and CTMI parameters were *simultaneously* estimated from the experimental data. Realistic CTMI parameters were obtained, and it was concluded that OED/PE implementation can improve the statistical quality of the kinetic model parameters.

In this paper, the global OED/PE optimization approach is compared to two other optimization strategies, i.e., (i) a single approach in which the four parameters are estimated *simultaneously* from one optimal experiment, and (ii) a *sequential* approach where in sequential order, six optimal experiments are implemented which focus on two parameters, and (nominal) parameter estimates are updated intermediately. The impact of the optimal experiment design strategy on the accuracy of the identification of the microbial growth kinetics is evaluated.

In a first step, the three design strategies are evaluated based on a simulation study. Next, optimal experiments are implemented in a computer controlled bioreactor and CTMI model parameters are estimated from the resulting experimental data.

2. Materials and methods

2.1. Mathematical models

Cell density as a function of time is described by the growth model of Baranyi and Roberts [2]:

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

129 with $n(t)$ [$\ln(\text{CFU/ml})$] the natural logarithm of the cell density at time t ,
 130 $n(0)$ the initial and n_{max} the maximum value for $n(t)$, $Q(t)$ [-] the physio-
 131 logical state of the cells, and μ_{max} [1/h] the maximum specific growth rate.
 132 As the duration of the microbial lag phase, modeled via the parameter $Q(t)$,
 133 is determined by the prior and actual experimental conditions, it can not be
 134 predicted accurately. Therefore, a reduced form of the model of Baranyi and
 135 Roberts, in which the variable $Q(t)$ is omitted, was used to design the opti-
 136 mal experiments. As such, Equation 1 reduces to the logistic growth model
 137 that describes exponential growth followed by a stationary phase.

The temperature dependency of μ_{max} is incorporated in Equation (1) by the Cardinal Temperature Model with Inflection (CTMI) [5]

$$\mu_{max} = \mu_{opt} \cdot \gamma(T) \quad (2)$$

with

$$\gamma(T) = \frac{(T - T_{min})^2 \cdot (T - T_{max})}{(T_{opt} - T_{min}) \cdot ((T_{opt} - T_{min}) \cdot (T - T_{opt}) - (T_{opt} - T_{max}) \cdot (T_{opt} + T_{min} - 2T))}$$

138
 139 This model encloses four parameters: the cardinal temperatures T_{min} , T_{opt} and
 140 T_{max} [$^{\circ}\text{C}$] (the minimum, optimum and maximum temperature for growth,
 141 respectively) and μ_{opt} [1/h] (the maximum specific growth rate at T_{opt}). At
 142 temperatures below T_{min} or above T_{max} , the maximum specific growth rate
 143 (μ_{max}) is set equal to zero.

144 2.2. Optimal experiment design for parameter estimation (OED/PE)

The unknown parameters to be estimated are the four parameters of the CTMI model: $\mathbf{p} = [T_{min} \ T_{opt} \ T_{max} \ \mu_{opt}]^T$. The OED/PE technique is implemented to maximize the information embedded in the output of the dynamic growth experiments, i.e., cell density as a function of time, by optimizing the dynamic temperature input. The information content of a dynamic experiment with continuous measurement of $n(t)$ and duration t_f can be quantified

by the Fisher information matrix (see e.g., [8]):

$$\mathbf{F} = \int_0^{t_f} \left(\frac{\partial n(t)}{\partial \mathbf{p}} \Big|_{\mathbf{p}=\mathbf{p}^\circ} \right)^T \mathbf{Q} \left(\frac{\partial n(t)}{\partial \mathbf{p}} \Big|_{\mathbf{p}=\mathbf{p}^\circ} \right) dt \quad (3)$$

with $\partial n(t)/\partial \mathbf{p}$ the sensitivity matrix, and $\mathbf{Q}$ the errors on the output measurements. Here, $\mathbf{Q}$ is taken equal to the inverse of the measurement error variance, i.e., 3.27×10^{-2} . The sensitivity functions are calculated by integrating (if $n(t)$ sufficiently smooth)

$$\begin{aligned} \frac{d}{dt} \left(\frac{\partial n(t)}{\partial p_i} \right) &= \frac{\partial}{\partial p_i} \left(\frac{dn(t)}{dt} \right) \\ &= \frac{\partial \mu_{max}}{\partial p_i} \cdot (1 - e^{(n(t)-n_{max})}) - \mu_{max} \cdot e^{(n(t)-n_{max})} \cdot \frac{\partial n(t)}{\partial p_i} \end{aligned} \quad (4)$$

with $\partial \mu_{max}/\partial \mathbf{p}$ equal to

$$\begin{aligned} \frac{\partial \mu_{max}}{\partial T_{min}} &= \mu_{opt} \cdot \frac{2(T - T_{opt})^2(2T_{opt} - T_{min} - T_{max})}{(T - T_{max})(T - T_{min})^3} \cdot \gamma(T)^2 \\ \frac{\partial \mu_{max}}{\partial T_{opt}} &= \mu_{opt} \cdot \frac{2(T - T_{opt})(T_{max} + 2T_{min} - 3T_{opt})}{(T - T_{max})(T - T_{min})^2} \cdot \gamma(T)^2 \\ \frac{\partial \mu_{max}}{\partial T_{max}} &= \mu_{opt} \cdot \frac{2(T - T_{opt})^2(T_{opt} - T_{min})}{(T - T_{max})^2(T - T_{min})^2} \cdot \gamma(T)^2 \\ \frac{\partial \mu_{max}}{\partial \mu_{opt}} &= \gamma(T) \end{aligned} \quad (5)$$

145 For nonlinear models, these sensitivity functions are determined by the un-
 146 known true parameters $\mathbf{p}$ and $\mathbf{F}$ is computed using nominal values $\mathbf{p}^\circ$, an
 147 initial guess for the unknown model parameters obtained from literature or
 148 preliminary experiments. As a consequence, the optimality of the design is
 149 only locally valid, i.e., for parameters close to $\mathbf{p}^\circ$. When the nominal values
 150 differ significantly from the true parameters, convergence to the latter values
 151 is not guaranteed. OED/PE is therefore an iterative procedure in which op-
 152 timal experiment design and parameter estimation are alternated to improve
 153 the nominal values until convergence to the true parameters is obtained.

154 The (initial) nominal parameters ($\mathbf{p}^\circ$) for *Escherichia coli* were extracted
 155 from [3]: $T_{min}^\circ = 11.33^\circ\text{C}$, $T_{opt}^\circ = 40.85^\circ\text{C}$, $T_{max}^\circ = 46.54^\circ\text{C}$, and $\mu_{opt}^\circ =$
 156 2.397 1/h . These values are assumed realistic as they are derived from a large
 157 set of static experiments. The initial and maximum cell density are taken
 158 equal to 7.000 and $22.55 \ln(\text{CFU/mL})$, respectively.

159 D-optimal experiments are designed by minimization of the joint confi-
 160 dence region on $\mathbf{p}$ via the maximization of the determinant of $\mathbf{F}$. The time-
 161 varying temperature input $T_{input}(t)$ is parameterized using control vector
 162 parameterization approach. Four degrees of freedom characterize the tem-
 163 perature profile: T_1 [$^\circ\text{C}$] the initial temperature, t_s [h] the time at which the
 164 increase or decrease in temperature starts, $\Delta T/\Delta t$ [$^\circ\text{C/h}$] the rate of temper-
 165 ature change and Δt [h] the duration of the temperature change (Figure 1).
 166 These four parameters define the final temperature T_2 . To guarantee prac-
 167 tical implementation of the dynamic experiments and model validity, two
 168 constraints are imposed on the temperature input (i) the absolute maximum
 169 rate of temperature change was set at 5°C/h , and (ii) the dynamic tem-
 170 perature profiles are confined to $[12^\circ\text{C}, 45^\circ\text{C}]$. The duration of the designed
 171 experiments is fixed at 38h.

172 2.3. OED/PE design strategies: single, sequential and global parameter esti- 173 mation

174 For the estimation of the four CTMI parameters, three different OED/PE
 175 design strategies are constructed.

176 (i) In the *single design strategy* (SiOED/PE), one optimal experiment is
 177 designed from which all model parameters are estimated *simultaneously* (with
 178 $\mathbf{F}$ a (4×4) -matrix). It is, however, questionable that a large set of parameters
 179 can be estimated accurately from a single simple experiment. To tackle
 180 this problem, the optimization problem can be reduced to a combination of
 181 optimization problems of lower complexity. For this simplified design, two

different strategies are suggested.

(ii) In the *sequential design strategy* (SeOED/PE), the problem is reformulated as a series of two-parameter estimation problems. Taking the CTMI model with four parameters, the approach works as follows. A first design round focuses on the estimation of parameters p_1 and p_2 . An optimal experiment is determined while considering parameters p_3 and p_4 perfectly known (with $\mathbf{F}$ reduced to a (2×2) -matrix). After implementation of this optimal experiment, parameters p_1 and p_2 are estimated. The nominal parameter values are then updated and a new optimal design round is applied, e.g., aimed at the accurate estimation of parameters p_2 and p_3 . Other subsequent designs focus on the estimation of parameter combinations (p_1, p_3) , (p_1, p_4) , (p_2, p_4) and (p_3, p_4) . At the end of the cycle six experiments are performed and each parameter is considered three times.

(iii) Instead of performing experiment design and parameter estimation for each parameter couple sequentially, an optimal experiment can be designed for each parameter combination, based on common nominal values. Next, all six optimal experiments are implemented and new parameter values are *simultaneously* estimated from all experimental data sets. In contrary to the sequential strategy, nominal values are not intermediately updated. This approach is here referred to as the *global design strategy* (GLOED/PE).

In the single and global design procedure, the four CTMI parameters are simultaneously estimated from all experimental data, i.e., in a single run. This is in contrast to the sequential approach where experimental implementation and parameter estimation are altered, i.e., CTMI parameters are updated intermediately.

2.4. *In silico study*

A first comparison of the optimization strategies and the selection of the most appropriate optimization strategy is based on simulated experiments.

210 This simulation study consists of three steps.

211 (i) D-optimal experiments are designed for the temperature profile as
212 shown in Figure 1.

213 (ii) The corresponding growth curves are simulated using the simpli-
214 fied growth model (i.e., without parameter $Q(0)$), combined with the CTMI
215 model (Equation (2)), based on the parameters selected as the true param-
216 eters, i.e., $\mathbf{p}^*$. After integration and data sampling (every 30 min), an error
217 is added to each data point mimicking the uncertainty and biological vari-
218 ability on experimental data. Hereto, a random value, taken from a normal
219 distribution with zero mean and variance ($s_n^2 = 3.27 \times 10^{-2}$), is added to the
220 simulated cell density data.

221 (iii) Parameters of the growth model and the CTMI model are estimated
222 from the experimental data via the minimization of the sum of squared errors.

223 2.5. Experimental validation

224 In a second step, the modes of OED/PE implementation are compared based
225 on experimental data.

226 2.5.1. Microorganism

227 *Escherichia coli* K12 MG1655 (CGSC#6300), obtained from the *E. coli* Ge-
228 netic Stock Center from Yale University, was stored at -80°C in Brain Heart
229 Infusion broth (BHI) (Oxoid), supplemented with 25% glycerol (Acros Or-
230 ganics).

231 Inoculum cultures were prepared as follows: $5\mu\text{L}$ of the stock culture
232 was transferred into 20mL BHI and incubated on a rotary shaker at 175
233 rpm (Unimax 2010, Heidolph) placed in a temperature controlled incubator
234 (model KBP6151, Termaks) at 37°C , and after 9h, $20\mu\text{L}$ was transferred into
235 20mL fresh BHI and again placed on the rotary shaker in the incubator (15h).

2.5.2. Bioreactor experiments

Optimal experiments were performed in a bench top bioreactor (BioFlo 3000, New Brunswick Scientific Inc.) controlled by the AFS-Biocommand software (New Brunswick Scientific Inc.). The reactor vessel, filled with 4.5L BHI was aerated at 2L/min and the stirrer speed was set at 400rpm. Foaming was prevented by the addition of 1mL of antifoam agent (Sigma). Temperatures below room temperature were obtained via a circulation cooler (CFT-33, Neslab Instruments Inc.). During the experiment, pH was kept constant at 7.55 by the addition of base (1N KOH) (Acros Organics) or acid (1N H₂SO₄) (Acros Organics). Dissolved oxygen, stirring speed, pH, addition of base and acid are monitored and the time-temperature profiles were implemented via the AFS-Biocommand software.

2.5.3. Cell count method

At regular time intervals, a sample was taken aseptically to determine the cell density via plate counting. Samples were serially diluted and the appropriate dilutions were plated on BHI agar (i.e., BHI supplemented with 6g/L technical agar nr 3., Oxoid) using a spiral plater (Eddy Jet, IUL Instruments s.a.). Plates were counted 18h after incubation at 37°C. For each sample, two or three dilutions were plated and each cell count value shown is an average of all countable plates.

2.6. Mathematical implementation

Optimal experiments were calculated using a hybrid optimization algorithm, programmed in Fortran using standard routines from the NAG Library (Numerical Algorithms Group). Herein, the stochastic algorithm, i.e., the Integrated Controlled Random Search algorithm [1] was combined with a deterministic algorithm, i.e., the *E04UCF* from the NAG library. In ICRS, random values were generated from a normal distribution with the pseudo-random generator *G05FAF*, combined with *G05CCF* to set the seed to a

264 non-repeatable initial value. The *F03ABF* routine was used to calculate the
265 determinant of $\mathbf{F}$. Ordinary differential equations were integrated with the
266 *D02EJF* routine.

267 Model parameter estimates and standard deviations were obtained via the
268 minimization of the global sum of squared errors (SSE), using the *lsqnonlin*
269 routine of the Optimization Toolbox of Matlab version 6.5 (The Mathworks
270 Inc.). The Matlab routine *ode23s* was used for the integration of ordinary
271 differential equations. For more information with respect to data processing,
272 see [7].

3. Results and discussion

3.1. Comparison based on *in silico* data

In a first step, the three OED/PE strategies were compared in a simulation study. This *in silico* procedure was repeated for three different sets of true parameters ($\mathbf{p}^*$).

The three OED/PE design strategies are summarized as follows: (i) all four CTMI parameters are estimated from a single optimal experiment (*single OED/PE strategy*), (ii) the optimization problem is reduced to a series of two-parameter estimation problems and all two-parameter optimal experiments are designed based on identical initial parameter estimates and parameters are estimated simultaneously from all resulting experimental data (*global OED/PE strategy*), and (iii) two-parameter optimal experiments are calculated and implemented sequentially whereby the parameter values are updated intermediately (*sequential OED/PE strategy*). The advantage of the sequential and global OED/PE strategies is that the error in one (or more) nominal values is not spread over all designs, i.e., if an initial parameter 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.

For the two simultaneous approaches (SiOED/PE and GloED/PE), optimal experiments were designed based on the same nominal values, i.e., $\mathbf{p}^\circ = [11.33 \ 40.85 \ 46.54 \ 2.397]$. All optimal experiments are given in Table 1. Next, fictitious growth curves were generated using an arbitrarily chosen set of true kinetic parameters ($\mathbf{p}^*$) and models parameters were estimated simultaneously (Table 3).

For the SeOED/PE approach, the same nominal values were used to optimize the temperature profile for the first parameter combination. Next, this first growth curve was simulated using the selected true parameter values

($\mathbf{p}^*$) and model parameters were subsequently estimated. These new model parameter estimates were used to update the nominal parameter vector as shown in Table 2, i.e., the two parameters estimated in the previous optimal experiments were adapted prior to optimization of the next dynamic experiment. This intermediate adaptation of the nominal values affects the subsequent optimal experiments, i.e., values of T_1 , t_s , $(\Delta T/\Delta t)$ and Δt differ. For SeOED/PE, the optimal experiments are given in Table 2 with the updated nominal values preceding the optimal experiment. For the three repetitions, the order of parameter couples optimized was chosen different.

The different course of the dynamic temperature profiles can be explained by the sensitivity functions $(\partial n(t)/\partial p_i)$ which determine the Fisher information matrix and hence the information content of the experiments. These sensitivity functions depend on the sensitivities of the maximum specific growth rate with respect to the CTMI parameters $(\partial \mu_{max}/\partial p_i)$ which are shown in Figure 2 with respect to the nominal values for SiOED/PE and GIOED/PE, and the three subsequent sets of nominal values for the second SeOED/PE iteration (see Table 2). Around the extrema of these functions, small variations in parameter values strongly influence the CTMI model output, i.e., μ_{max} , and thus $n(t)$. The selection of the temperatures in the optimal temperature profiles affects the information content.

(i) For T_{min} , the most informative temperature region is located around the inflection point of the CTMI model. With respect to the (initial) nominal values, this region is situated between 20°C and 25°C. For most optimal experiments, this region is attained during the linear temperature change. (ii) The estimation of T_{opt} can be improved by including temperatures between 35°C and 40°C, and close to T_{max} . These temperatures are either selected as initial temperature or crossed during temperature decrease. (iii) Accurate estimation of T_{max} requires optimal temperature profiles which include temperatures close to the expected T_{max} , as an extreme maximum can be

observed at T_{max} . As a consequence, most experiments start at the upper temperature boundary. Previous experimental results identified the nominal T_{max} value and/or the upper temperature boundary as a bottleneck in the OED/PE procedure. When T_{max}° and/or T_{high} are chosen too low, accurate estimation of T_{max} is not guaranteed. (iv) The sensitivity of μ_{max} with respect to μ_{opt} shows a clear extremum at T_{opt} . Therefore, accurate estimation of μ_{opt} highly depends on sampling at and around the optimal growth temperature. Except for the parameter combination (T_{min}, T_{opt}) , the optimal growth temperature is always enclosed in the linear temperature phase.

For the subsequent designs in the SeOED/PE strategy, the largest changes in the sensitivity functions can be observed for T_{min} , and to a lesser extent for T_{opt} and T_{max} . In the SeOED/PE iteration, the minimum temperature and thus also the informative region shift to lower values. As a consequence, most optimal temperature profiles attain the lower boundary, in contrast to the SiOED/PE and GLOED/PE optimal experiments. Although T_{max}° values generally decrease during the SeOED/PE course, the initial temperature of most optimal experiments is equal to 45°C as an identical T_{high} value is used for all calculations. The closer the T_{max}° comes to the assumed true T_{max} , the longer the initial constant phase becomes. Due to the expected lower growth rate at 45°C, more time/sampling is needed to obtain an accurate $\mu_{max}(45^\circ\text{C})$ estimate.

Final CTMI parameter estimates are compared in Table 3. Generally, parameter values derived via the sequential approach deviate more from the true parameters than the simultaneous strategy estimates. Both the single and global OED/PE approach render values very close to the chosen set of true parameters $\mathbf{p}^*$, and differentiating between the two methods is difficult. As single OED/PE only requires the implementation of one experiment, this method seems most suitable. However, SiOED/PE estimates are characterized by a significant larger standard deviations. Furthermore, estimating the

359 four parameters from one experiment makes the procedure rather sensitive
360 for experimental errors.

361 3.2. Comparison based on experimental data

362 In the second step, the two simultaneous OED/PE design strategies (SiOED/PE
363 and GIOED/PE) were compared based on experimental data. Hereto, the op-
364 timal temperature profiles were implemented in a computer controlled biore-
365 actor. (For more details on the experimental procedure, see Van Derlinden
366 et al. [7]).

367 3.2.1. Global OED/PE

368 Experimental data, parameter estimates and standard deviations for the
369 GIOED/PE design strategy are copied from [7]. The global identification
370 yields a good description of the six optimal experiments. The CTMI param-
371 eter estimates and corresponding standard deviations are repeated in Table 4.
372 T_{min} , T_{opt} and T_{max} estimates are characterized by a small estimation error,
373 i.e., less than 3%. Values for T_{opt} and T_{max} correspond to values found in lit-
374 erature and obtained from prior static and dynamic experiments. Compared
375 to literature, the minimum growth temperature seems slightly overestimated
376 (see e.g., [4, 6]). For figures and more information, see [7].

377 Optimal experiments were calculated based on (initial) nominal values
378 assumed close to the true parameters as they were derived from a large set
379 of static experiments. Although in reality nominal values tend to differ from
380 the true model parameters, this approach has a major advantage. By taking
381 nominal values approximately equal to the true values ($\mathbf{p}^{\circ}=\mathbf{p}^*$), the results
382 of this *in silico* study are not or very little affected by the (incorrectness of
383 the) nominal parameters $\mathbf{p}^{\circ}$. Moreover, a preceding *in silico* study showed
384 that, even when starting from less realistic nominal values, the GIOED/PE
385 design approach is robust and parameter estimates will easily converge to the

true values. Only when the maximum temperature is initially chosen lower than its true value, T_{max} is significantly underestimated.

Effect of reducing the number of experiments in the GLOED/PE approach

Following the definition of the global OED/PE design, the implementation included all six parameter combinations. This way, all parameters and their informative temperature regions were considered three times. It is shown in [7] that this procedure results in reliable estimates with low uncertainties. Next, the effect of reducing the number of experiments on the parameter identification outcome was studied. When the amount of experiments is brought back to four and two experiments, each parameter is regarded twice and once, respectively. Given this precondition, three combinations of parameter couples could be selected for both cases. Subsequently derived CTMI parameters are listed in Table 4 for the combination of four and two optimal experiments.

CTMI parameters estimated from the three combinations of four optimal experiments are similar to the parameter values derived from all six GLOED/PE optimal experiments. Uncertainty errors are slightly larger. The largest variability can be observed in the values of T_{min} and T_{max} . So, irrespective of the combination of parameter couples, the CTMI parameters can be identified accurately from four GLOED/PE optimal experiments.

When the number of experiments is reduced to two, a larger difference can be observed between the CTMI parameter estimates. Two parameter couple combinations, i.e., the first and third combination, yield parameter values similar to the values derived from all six GLOED/PE optimal experiments. The remaining combination, however, generates very unrealistic CTMI parameters. As such, accurate identification of the CTMI from two optimal experiments is not guaranteed.

3.2.2. Single OED/PE

Analogously, CTMI parameters were estimated from one optimal experiment, designed for the simultaneous estimation of the four CTMI parameters (see Figure 3). The resulting parameter values and standard deviations are presented in Table 4. Estimates of T_{opt} , T_{max} and μ_{opt} and associated standard deviations are very similar to the values extracted from the six GLOED/PE experiments. The estimated value for T_{min} , however, is slightly higher. This possible overestimation of T_{min} can be due to the limited information in the temperature zone beneath 30°C. Information can only be obtained during the final part of the temperature change and the final constant phase. When estimating T_{min} from the six optimal experiments, growth rates at moderate temperatures can be estimated from the six temperature changes and six constant phases at different temperature levels. Based on this abundant information, the inflection point of the CTMI can be determined more accurately.

The SiOED/PE experiment is similar to the optimal experiments (T_{max} , μ_{opt}), (T_{max} , T_{min}), (T_{max} , T_{opt}) and (T_{opt} , μ_{opt}) from the GLOED/PE design (see Table 4) with the largest difference in the final temperature (T_2) and the rate of temperature change ($\Delta T/\Delta t$). The final temperature of the GLOED/PE experiments (T_{max} , T_{min}), (T_{max} , T_{opt}) and (T_{opt} , μ_{opt}) is significantly higher than T_2 of the SiOED/PE experiment. The information embedded in the four GLOED/PE experiments with respect to the simultaneous estimation of the CTMI parameters is reflected in the value of the D-criterion (Table 5). The different ($\Delta T/\Delta t$) and T_2 values reduce the determinant of the Fisher information matrix with approximately 30%.

A large variation exists between the T_{min} , T_{opt} , T_{max} and μ_{opt} estimates and most values differ significantly from the GLOED/PE values in Table 4. The large uncertainty on the estimates is reflected in the large estimation errors. For parameter identifications from one experiment, T_{opt} and μ_{opt} are es-

442 timated from measurements during the temperature change. Possibly, growth
 443 rates are determined more accurately from the SiOED/PE experiment due
 444 to the slower growth rate. Most T_{min} estimates deviate from the expected
 445 T_{min} due to the limited knowledge of the temperature effect on the growth
 446 rate around ambient temperatures as explained above. The more accurate
 447 estimate of T_{max} from the SiOED/PE optimal experiment can be explained
 448 as follows. While the constant phase at 45°C in the GLOED/PE experiments
 449 is characterized by an adaptation phase (reflected in a small $Q_i(0)$ value),
 450 *E. coli* grows exponentially in the initial phase at 45°C in the SiOED/PE
 451 experiment. Possibly, the more accurate estimate of T_{max} pushes the other
 452 three CTMI parameter values towards more realistic values.

453 4. Conclusion

454 Optimal experiment design for parameter estimation is an efficient approach
 455 for accurate estimation of kinetic model parameters. When the model under
 456 study encloses multiple parameters, different optimization strategies can be
 457 applied. Here, three different optimization strategies are introduced. In the
 458 *single OED/PE design strategy*, all four CTMI parameters are estimated si-
 459 multaneously from a one optimal experiment. For the *sequential* and *global*
 460 design strategies, the optimization problem is reformulated as a series of two-
 461 parameter estimation problems. In the sequential approach, two-parameter
 462 optimal experiments are designed and implemented sequentially and param-
 463 eter estimates are updated intermediately. In the global OED/PE design,
 464 optimal experiments are calculated for all six two-parameter combinations
 465 and the four parameters are estimated simultaneously from these six exper-
 466 iments.

467 In a first step, the three optimization strategies were evaluated based on
 468 simulated experiments. This simulation study revealed that the sequential
 469 design results repeatedly in unrealistic CTMI parameters. CTMI parameter

estimates obtained with the simultaneous strategies, i.e., single and global OED/PE, are similar and realistic.

Next, the GLOED/PE and SiOED/PE optimal experiments were implemented in a computer controlled bioreactor. Reliable and accurate CTMI parameter estimates were derived from the six GLOED/PE optimal experiments. Equally good parameter estimates were obtained when only four GLOED/PE optimal experiment were taken into account. Further reduction to two optimal experiments could not guarantee accurate CTMI identification. Accurate estimation of the four CTMI model parameters from the single optimal experiment, designed for the simultaneous estimation of the CTMI parameters, was not guaranteed. In the GLOED/PE design approach, each CTMI parameter is considered three times and as such, accurate information for each parameter can be extracted from several experiments. Information, however, is limited to one experiment in the SiOED/PE.

Acknowledgments

This research is supported in part by projects OT/09/25 and BOF EF/05/006 (OPTEC Optimization in Engineering) of the Research Council of the KULeuven, project KP/09/005 (SCORES4CHEM) of the Industrial Research Fund, and the Belgian Program on Interuniversity Poles of Attraction, initiated by the Belgian Federal Science Policy Office. K. Bernaerts is a Postdoctoral Fellow with the Fund for Scientific Research Flanders. J. Van Impe holds the chair Safety Engineering sponsored by the Belgian chemistry and life sciences federation essenscia.

References

- [1] Banga, J. R. and Alonso, A. A. and Singh, R. P. Stochastic dynamic optimization of batch and semicontinuous bioprocesses. *Biotechnology Progress*, 13:326–335, 1997.

- 497 [2] Baranyi, J. and Roberts, T. A. A dynamic approach to predicting bac-
498 terial growth in food *International Journal of Food Microbiology*, 23:
499 277–294, 1994.
- 500 [3] Bernaerts, K. and Van Derlinden, E. and Van Impe Estimation of
501 cardinal temperature parameters from dynamic microbial growth ex-
502 periments: a comparison of different approaches *Proceeding of the 9th*
503 *European conference Food Industry and Statistics*, 2006, 201-208
- 504 [4] Nauta, M. J. and Dufrenne, J. B. Variability in growth characteristics
505 of different *E. coli* O157:H7 isolates, and its implications for predictive
506 microbiology *Quantitative Microbiology*, 1:137-155, 1999.
- 507 [5] Rosso, L. and Lobry, J. R. and Flandrois, J. P. An unexpected correla-
508 tion between cardinal temperatures of microbial growth highlighted by
509 a new model *Journal of Theoretical Biology*, 162:447–463, 1993.
- 510 [6] Shaw, M. K. and Marr, A. G. and Ingraham, J. L. Determination
511 of the minimal temperature for growth of *Escherichia coli* *Journal of*
512 *Bacteriology*, 105:683–684, 1971.
- 513 [7] Van Derlinden, E. and Bernaerts, K. and Van Impe, J. F. Accurate
514 estimation of cardinal temperatures of *Escherichia coli* from optimal
515 dynamic experiments *International Journal of Food Microbiology*, 128:
516 89–100, 2008.
- 517 [8] Walter, E. and Pronzato, L. *Identification of Parametric Models from*
518 *Experimental Data*, Springer, Masson 1997

Table 1: D-optimal temperature profiles for the GLOED/PE (top) and SiOED/PE (bottom) design strategies calculated based on the same nominal values, i.e., $\mathbf{p}^\circ = [11.33 \ 40.85 \ 46.54 \ 2.397]$. The parametrization of the temperature profile is illustrated in Figure 1. Temperature is bounded within $[15^\circ\text{C}, 45^\circ\text{C}]$. The rate of temperature change is constrained between $[-5^\circ\text{C/h}, 5^\circ\text{C/h}]$ and t_f is fixed at 38h.

	T_1 [°C]	t_s [h]	$\Delta T/\Delta t$ [°C/h]	Δt [h]	T_2 [°C]	$\det(\mathbf{F})$
(T_{max}, μ_{opt})	45.00	3.835	-5.000	6.000	15.00	8.482×10^5
(T_{max}, T_{min})	45.00	2.854	-5.000	5.812	15.94	1.117×10^5
(T_{max}, T_{opt})	45.00	2.938	-5.000	5.676	16.62	8.198×10^4
(T_{min}, μ_{opt})	40.85	1.780	-5.000	4.788	16.91	1.800×10^6
(T_{min}, T_{opt})	31.15	4.382	-2.388	5.978	16.87	7.674×10^4
(T_{opt}, μ_{opt})	45.00	2.801	-5.000	5.666	16.67	1.547×10^6
$(T_{max}, T_{min}, T_{opt}, \mu_{opt})$	45.00	2.514	-4.187	7.138	15.11	6.103×10^4

Table 2: D-optimal temperature profiles for the SeOED/PE design strategy. Three arbitrarily chosen selection schemes of parameter couples for three sets of true parameters ($\mathbf{p}^*$) are presented. For each parameter combination, the (updated) nominal values $\mathbf{p}^\circ$ are preceding the optimal experiment. The parametrization of the temperature profile is illustrated in Figure 1. Temperature is bounded within $[15^\circ\text{C}, 45^\circ\text{C}]$. The rate of temperature change is constrained between $[-5^\circ\text{C/h}, 5^\circ\text{C/h}]$ and t_f is fixed at 38h.

	T_1 [$^{\circ}\text{C}$]	t_s [h]	$\Delta T/\Delta t$ [$^{\circ}\text{C}/\text{h}$]	Δt [h]	T_2	$\det(\mathbf{F})$
Series 1 ($\mathbf{p}^* = [8.000 \ 40.00 \ 46.00 \ 2.200]$)						
			$\mathbf{p}_0^{\circ}=[\ 11.33 \ 40.85 \ 46.54 \ 2.397 \]$			
(T_{max}, T_{min})	45.00	2.854	-5.000	5.812	15.94	1.117×10^5
			$\mathbf{p}_1^{\circ}=[\ 7.856 \ 40.85 \ 45.47 \ 2.397 \]$			
(T_{max}, T_{opt})	45.00	5.477	-5.000	6.000	15.00	1.513×10^6
			$\mathbf{p}_2^{\circ}=[\ 7.856 \ 41.37 \ 45.46 \ 2.397 \]$			
(T_{max}, μ_{opt})	45.00	5.892	-5.000	6.000	15.00	3.642×10^7
			$\mathbf{p}_3^{\circ}=[\ 7.856 \ 41.37 \ 45.51 \ 2.326 \]$			
(T_{min}, T_{opt})	45.00	5.254	-5.000	6.000	15.00	6.665×10^4
			$\mathbf{p}_4^{\circ}=[\ 7.036 \ 41.10 \ 45.51 \ 2.326 \]$			
(T_{min}, μ_{opt})	41.10	1.722	-5.000	5.427	17.86	1.207×10^6
			$\mathbf{p}_5^{\circ}=[\ 6.163 \ 41.10 \ 45.51 \ 2.313 \]$			
(T_{opt}, μ_{opt})	45.00	5.322	-5.000	6.000	15.00	3.302×10^6
Series 2 ($\mathbf{p}^* = [6.500 \ 39.80 \ 45.70 \ 2.000]$)						
			$\mathbf{p}_0^{\circ}=[\ 11.33 \ 40.85 \ 46.54 \ 2.397 \]$			
(T_{max}, T_{min})	45.00	2.854	-5.000	5.812	15.94	1.117×10^5
			$\mathbf{p}_1^{\circ}=[\ 7.152 \ 40.85 \ 45.20 \ 2.397 \]$			
(T_{min}, μ_{opt})	40.85	1.619	-5.000	5.388	18.06	1.214×10^6
			$\mathbf{p}_2^{\circ}=[\ 6.125 \ 40.85 \ 45.20 \ 2.152 \]$			
(T_{min}, T_{opt})	44.95	8.962	-5.000	6.000	15.00	8.581×10^4
			$\mathbf{p}_3^{\circ}=[\ 4.256 \ 41.76 \ 45.20 \ 2.152 \]$			
(T_{opt}, μ_{opt})	45.00	8.854	-5.000	6.000	15.00	8.349×10^6
			$\mathbf{p}_4^{\circ}=[\ 4.256 \ 42.04 \ 45.20 \ 2.173 \]$			
(T_{max}, μ_{opt})	45.00	7.993	-5.000	6.000	15.00	3.451×10^8
			$\mathbf{p}_5^{\circ}=[\ 4.256 \ 42.04 \ 45.19 \ 2.173 \]$			
(T_{max}, T_{opt})	45.00	7.703	-5.000	6.000	15.00	1.084×10^7
Series 3 ($\mathbf{p}^* = [10.00 \ 40.50 \ 45.30 \ 2.350]$)						
			$\mathbf{p}_0^{\circ}=[\ 11.33 \ 40.85 \ 46.54 \ 2.397 \]$			
(T_{min}, T_{opt})	31.15	4.382	-2.388	5.978	15.11	7.674×10^4
			$\mathbf{p}_1^{\circ}=[\ 10.25 \ 41.00 \ 46.54 \ 2.397 \]$			
(T_{max}, μ_{opt})	45.00	3.485	-5.000	6.000	15.00	6.440×10^5
			$\mathbf{p}_2^{\circ}=[\ 10.25 \ 41.00 \ 45.17 \ 2.509 \]$			
(T_{min}, μ_{opt})	41.00	1.618	-5.000	4.950	20.25	1.451×10^6
			$\mathbf{p}_3^{\circ}=[\ 9.763 \ 41.00 \ 45.17 \ 2.413 \]$			
(T_{max}, T_{opt})	45.00	1.546	-0.1642	1.286	44.79	5.950×10^7
			$\mathbf{p}_4^{\circ}=[\ 9.763 \ 40.52 \ 45.28 \ 2.413 \]$			
(T_{opt}, μ_{opt})	45.00	11.52	-5.000	6.000	15	4.728×10^6
			$\mathbf{p}_5^{\circ}=[\ 9.763 \ 40.61 \ 45.28 \ 2.392 \]$			
(T_{max}, T_{min})	45.00	8.027	-5.000	5.934	15.33	7.815×10^6

Table 3: Parameter estimates and associated standard deviations from the simulation study for the comparison of the single, sequential and global OED/PE design strategies. Nominal values for the experiment design are 11.33°C, 40.85°C, 46.54°C and 2.397 1/h.

	$\mathbf{p}^*$	SiOED/PE	SeOED/PE	GLOED/PE
T_{min}	8.000	8.073 (4.371×10^{-1})	6.163 (1.852×10^{-1})	7.822 (1.072×10^{-1})
T_{opt}	40.00	40.10 (5.288×10^{-1})	40.84 (1.241×10^{-1})	40.12 (7.278×10^{-2})
T_{max}	46.00	46.17 (4.112×10^{-1})	45.51 (3.278×10^{-2})	45.92 (3.549×10^{-4})
μ_{opt}	2.200	2.184 (7.795×10^{-2})	2.368 (2.305×10^{-2})	2.204 (1.110×10^{-2})
T_{min}	6.500	6.464 (4.385×10^{-1})	4.256 (5.445×10^{-1})	6.491 (1.146×10^{-1})
T_{opt}	39.80	40.00 (5.601×10^{-1})	42.04 (6.403×10^{-2})	40.09 (8.706×10^{-2})
T_{max}	45.70	45.78 (2.184×10^{-1})	45.19 (1.012×10^{-4})	45.55 (2.437×10^{-5})
μ_{opt}	2.000	2.007 (5.656×10^{-2})	2.173 (1.698×10^{-2})	2.051 (1.246×10^{-2})
T_{min}	10.00	10.08 (3.683×10^{-1})	9.828 (2.779×10^{-1})	9.889 (8.118×10^{-2})
T_{opt}	40.50	40.47 (5.744×10^{-1})	40.61 (1.123×10^{-1})	40.58 (1.576×10^{-1})
T_{max}	45.30	45.40 (1.635×10^{-1})	45.27 (1.063×10^{-2})	45.29 (3.415×10^{-2})
μ_{opt}	2.350	2.318 (9.301×10^{-2})	2.392 (7.863×10^{-2})	2.348 (2.823×10^{-2})

Table 4: CTMI parameter estimates and corresponding standard deviations. For combinations of 4 optimal experiments (OEs), the following combinations are considered: (A) $[(T_{max}, \mu_{opt}), (T_{max}, T_{min}), (T_{min}, T_{opt}), (T_{opt}, \mu_{opt})]$, (B) $[(T_{max}, \mu_{opt}), (T_{max}, T_{opt}), (T_{min}, T_{opt}), (T_{min}, \mu_{opt})]$, and (C) $[(T_{max}, T_{min}), (T_{max}, T_{opt}), (T_{min}, \mu_{opt}), (T_{opt}, \mu_{opt})]$. For combinations of 2 optimal experiments (OEs), the following combinations are considered: (A) $[(T_{max}, \mu_{opt}), (T_{min}, T_{opt})]$, (B) $[(T_{max}, T_{min}), (T_{opt}, \mu_{opt})]$, and (C) $[(T_{max}, T_{opt}), (T_{min}, \mu_{opt})]$.

	SiOED/PE		GIOED/PE			
	1 OE		6 OEs			
T_{min}	9.801	(2.847×10^{-1})	9.144	(2.251×10^{-1})		
T_{opt}	39.73	(2.460×10^{-1})	39.58	(2.793×10^{-1})		
T_{max}	46.70	(3.749×10^{-1})	46.71	(2.693×10^{-1})		
μ_{opt}	2.142	(4.660×10^{-2})	2.094	(4.011×10^{-2})		
MSE_{global}	1.318×10^{-2}		7.082×10^{-2}			
(n_t, n_p)	(56, 7)		(245, 17)			
	GIOED/PE		GIOED/PE		GIOED/PE	
4OEs	(A)		(B)		(C)	
T_{min}	9.110	(2.483×10^{-1})	8.834	(2.434×10^{-1})	9.868	(3.507×10^{-1})
T_{opt}	39.40	(2.881×10^{-1})	39.93	(3.423×10^{-1})	39.00	(4.063×10^{-1})
T_{max}	47.32	(3.715×10^{-1})	46.08	(3.077×10^{-1})	47.05	(4.795×10^{-1})
μ_{opt}	2.000	(4.752×10^{-2})	2.158	(5.698×10^{-2})	2.091	(4.928×10^{-2})
MSE_{global}	6.427×10^{-2}		8.193×10^{-2}		6.223×10^{-2}	
(n_t, n_p)	(184,13)		(163,13)		(177, 13)	
	GIOED/PE		GIOED/PE		GIOED/PE	
2 OEs	(A)		(B)		(C)	
T_{min}	8.819	(2.631×10^{-1})	11.49	(6.313×10^{-1})	9.422	(5.013×10^{-1})
T_{opt}	39.43	(3.827×10^{-1})	37.59	(4.634×10^{-1})	39.70	(6.805×10^{-1})
T_{max}	47.21	(2.830×10^{-5})	51.06	(1.981×10^0)	46.04	(1.482×10^0)
μ_{opt}	1.952	(5.001×10^{-2})	1.826	(6.206×10^{-2})	2.218	(8.649×10^{-2})
MSE_{global}	7.908×10^{-2}		4.250×10^{-2}		7.550×10^{-2}	
(n_t, n_p)	(85,9)		(99,9)		(78,9)	

Table 5: CTMI parameter estimates and corresponding standard deviations derived from single GIOED/PE optimal experiments (see Table 1). The presented $\det(\mathbf{F})$ -values are calculated using a (4×4) Fisher information matrix (as defined in Equation 3) with values for T_1 , t_s , $\Delta T/\Delta t$ and Δt copied from Table 1.

	Experiment (T_{max} , μ_{opt})		Experiment (T_{max} , T_{min})	
T_{min}	10.03	(1.079×10^0)	9.294	(5.836×10^{-1})
T_{opt}	37.19	(9.490×10^{-1})	39.21	(4.690×10^{-1})
T_{max}	49.65	(2.864×10^0)	48.03	(1.005×10^0)
μ_{opt}	1.729	(1.246×10^{-1})	2.039	(7.152×10^{-2})
$\det(\mathbf{F})$	4.334×10^4		4.926×10^4	
	Experiment (T_{max} , T_{opt})		Experiment (T_{opt} , μ_{opt})	
T_{min}	10.24	(8.397×10^{-1})	12.83	(8.884×10^{-1})
T_{opt}	37.02	(1.005×10^0)	35.11	(7.549×10^{-1})
T_{max}	46.55	(6.708×10^{-1})	50.00	(1.329×10^0)
μ_{opt}	1.738	(7.752×10^{-2})	1.694	(7.571×10^{-2})
$\det(\mathbf{F})$	4.130×10^4		4.110×10^4

Figure 1: Representation of the parameterized temperature profile, which is characterized by four degrees of freedom: T_1 the initial temperature, t_s the time at which the increase or decrease in temperature starts, $(\Delta T / \Delta t)$ the rate of temperature change, and Δt the duration of the temperature change. These four parameters define the final temperature T_2 . Temperature is bounded between T_{low} and T_{high} .

Figure 2: Sensitivities of the CTMI with respect to its parameters. In (-), the sensitivities for the nominal values $\mathbf{p}^o = [11.33 \ 40.85 \ 46.54 \ 2.97]$ are given. (-), (- · ·) and (· · ·) correspond to the sensitivities for parameter combinations (T_{min}, μ_{opt}) , (T_{min}, T_{opt}) and (T_{opt}, μ_{opt}) from the second series of SeOED/PE optimal experiments as listed in Table 2.

Figure 3: SiOED/PE: global identification of the CTMI (experimental data ($\circ$), global identification curve (-) and temperature profile (- -)). The confidence and prediction intervals are represented by (- ·) and (- -), respectively. Parameter estimates and standard deviations are listed in Table 4.