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On the trade-off between experimental effort and information content in optimal experimental design for calibrating a predictive microbiology model

Titre: Sur le compromis entre l'effort expérimental et le contenu des informations dans les plans d'expériences optimaux pour la calibration d'un modèle de la microbiologie prédictive

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Abstract: In predictive microbiology, dynamic mathematical models are developed to describe microbial evolution under time-varying environmental conditions. Next to an acceptable model structure, reliable parameter values are necessary to obtain valid model predictions. To obtain these accurate estimates of the model parameters, labor-and cost-intensive experiments have to be performed. Optimal experimental design techniques for parameter estimation are beneficial to limit the experimental burden. An important issue in optimal experimental design, included in this work, is the sampling scheme. Recent work illustrates that identifying sampling decisions results in bang-bang control of the weighting function in the Fisher information matrix. A second point addressed in this work is the trade-off between the amount of time an experimenter has available for measurements on the one hand, and information content on the other hand. Recently, multi-objective optimization is applied to several different optimal experimental design criteria, whereas in this paper the workload expressed as when to sample, is considered. The procedure is illustrated through simulations with a case study for the Cardinal Temperature Model with Inflection. The viability of the obtained experiments is assessed by calculating the confidence regions with two different methods: the Fisher information matrix approach and the Monte-Carlo method approach.

Résumé : Aujourd'hui, dans le domaine de la microbiologie prédictive, la croissance et la décroissance des populations bactériennes sont décrites par des modèles mathématiques dynamiques, dits primaires. Les paramètres de ces modèles primaires sont eux-mêmes liés à des facteurs environnementaux par des modèles, dits secondaires, comprenant plusieurs paramètres secondaires (typiquement des températures et des pH minimaux, maximaux et optimaux). A cette complexité hiérarchique des modèles s'ajoutent inéluctablement de nombreuses sources d'erreurs de mesure compte tenu de la nature biologique des objets expérimentaux, erreurs qui affectent les observations issues des expériences de microbiologie. A l'évidence, il est donc particulièrement crucial de disposer de protocoles expérimentaux performants, qui prennent en compte à la fois ces structures de modèles et les erreurs, pour conduire in fine à des estimations de qualité (faible biais, faible variance, ...) des paramètres secondaires, et aussi à des prédictions fiables avec les modèles estimés. Même s'il existe plusieurs critères d'optimalité pour construire de tels protocoles performants, le coût de leur mise en oeuvre reste toutefois souvent prohibitif de par le nombre d'essais expérimentaux à réaliser. Dans cet article, deux nouvelles approches sont proposées dans un cadre de critères d'optimalité de plans d'expériences bien connus : a) un schéma d'échantillonnage temporel formalisé par une fonction de pondération de type "tout ou rien" ajoutée dans la forme dynamique de la fonction d'information de Fisher ; cette fonction va permettre à l'expérimentateur de décider si on doit échantillonner ou non à un temps donné optimisé (effort expérimental), et b) l'élaboration d'un compromis entre l'effort expérimental à fournir, commandé par le protocole optimal, et le contenu de l'information obtenue grâce à ce protocole ; cette élaboration est basée sur l'utilisation de techniques d'optimisation multi-objectifs. Usuellement, quand ces techniques sont utilisées pour déterminer un protocole optimal, l'effort expérimental n'est pas pris en compte. Enfin, à partir d'un modèle bien connu des microbiologistes, des simulations ont été menées à la fois pour évaluer la faisabilité des protocoles calculés et déterminer des intervalles de confiance pour les estimations des

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paramètres.

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Mots-clés : plan d'expériences optimal, optimisation multi-objectif, l'estimation paramétrique, méthode de Monte-Carlo

1. Introduction

Dynamic models that predict microbial behavior in food products play an important role in food quality and safety. In these models, the effect of temperature, pH, water activity and preservatives are important elements. When a suitable model structure is chosen, reliable parameter estimates have to be obtained for different microbial strains. However, performing experiments is cost- and labor-intensive. The experimenter must take samples and, subsequently, perform an extensive analysis procedure. To reduce this experimental effort, optimal experimental design (OED) can be applied. In optimal experimental design for parameter estimation, some scalar function of the Fisher information matrix (Pukelsheim, 1993; Walter and Pronzato, 1997) is employed as objective function in the resulting dynamic optimization problem. Design of an optimal experimental can focus on different aspects.

The main contribution is a practical procedure for systematically evaluating trade-offs between information content and experimental effort in the design of dynamic experiments for calibrating predictive microbiology models. Advanced multi-objective dynamic optimization approaches are exploited with parameter accuracy and total time reserved for measuring as specific (conflicting) objectives. With respect to the latter objective, the determination of optimal measurement/sampling schemes specifying when to measure, is added as a degree of freedom.

Recent work discussing when to sample (Sager, 2012) indicates that the inclusion of the weighting function in the Fisher information matrix results in a bang-bang type control (Sager, 2012; Pontryagin et al., 1962). Bang-bang control is a type of control which arises in optimal control when the optimal value for the control action is either at its minimum or maximum value. The necessary conditions for the existence/occurrence of this type of control can be found in, e.g., Sonneborn and Van Vleck (1965). The maximal amount of measurement time is not a priori fixed and not necessarily equal to the experiment duration, but is added as an additional objective to be minimized.

To enable an overview of the trade-offs, multi-objective optimization is exploited to generate the set of Pareto optimal solutions, which contains different optimal alternatives. This availability of the Pareto set allows the experimenter to make a sound decision based on his/her preferences. As such, the presented procedure is an extension to the work of Telen et al. (2012) as it integrates strategies from Sager (2012).

The accuracy of the designed experiments with reduced workload is assessed in a simulation study for the Cardinal Temperature Model with Inflection (CTMI), which describes the effect of temperature on the microbial growth rate. Based on generated data, a parameter estimation procedure is carried out and confidence intervals are calculated. A first approach exploits the

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Fisher information matrix. However, one known drawback of this matrix is that, for models non-linear in the parameters (Balsa-Canto et al., 2007; Heine et al., 2008; Schenkendorf et al., 2009), it can result in inaccurate estimates of the confidence intervals. Hence, to check the validity of the Fisher information matrix approach for the CTMI model, a Monte-Carlo simulation (Walter and Pronzato, 1997) is also performed.

The paper is structured as follows. In Section 2, the employed mathematical techniques and the mathematical model are discussed. Section 3 presents the obtained results. Afterwards, in Section 4, the conclusions are formulated.

2. Methods and Model

Optimal experimental design for dynamic models gives rise to a specific subclass of dynamic optimization problems. The general concept of dynamic optimization and the techniques of optimal experimental design are discussed in the first part of this section. Because sampling schemes are considered in this paper, recent work on sampling decisions is discussed in the second part. As no single optimal criterion for optimal experimental design exists, conflicting criteria can be considered by employing multi-objective optimization (Telen et al., 2012). Hence, the specific methods of multi-objective optimization are described in the third part. The fourth part discusses the validation process of the computation of the confidence intervals. In the last subsection, the predictive microbiological case study is presented.

2.1. Optimal experimental design

An adequately general formulation of a dynamic optimization problem for the purpose of this paper, reads as follows:

$$\min_{\mathbf{x}(t),\mathbf{u}(t)} J(\mathbf{x}(t),\mathbf{u}(t),\mathbf{p},t) \tag{1}$$

subject to:

$$\begin{aligned} \frac{d\mathbf{x}}{dt} &= \mathbf{f}(\mathbf{x}(t),\mathbf{u}(t),\mathbf{p},t) && (2)\\ 0 &= \mathbf{b}_c(\mathbf{x}(0)) && (3)\\ 0 &\geq \mathbf{c}_p(\mathbf{x}(t),\mathbf{u}(t),\mathbf{p},t) && (4)\\ 0 &\geq \mathbf{c}_t(\mathbf{x}(t_f),\mathbf{u}(t_f),\mathbf{p},t_f) && (5) \end{aligned}$$

where t is the time in the interval $[0, t_f]$, $\mathbf{x}$ are the state variables, $\mathbf{u}$ are the control variables and $\mathbf{p}$ the parameters. The dynamic system equations are represented by $\mathbf{f}$. The vector $\mathbf{b}_c$ indicates the initial conditions, the vector $\mathbf{c}_p$ the path inequality constraints and the vector $\mathbf{c}_t$ the terminal inequality constraints on controls and states. Furthermore, the vector $\mathbf{y}$ containing the measured outputs, is introduced. These are usually a subset of the state variables $\mathbf{x}$. The degrees of freedoms of the optimal experimental design are grouped in a vector of trajectories $\mathbf{z} = [\mathbf{x}(t)^T, \mathbf{u}(t)^T]^T$. All vectors $\mathbf{z}$ that satisfy equations (2) to (5) form the set of feasible solutions S.

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This optimization problem is infinite-dimensional. These problems are solved within this work by converting them to a finite Non-Linear Programming (NLP) problem by means of discretization. There are two different approaches. A *sequential direct method* as *single shooting* (e.g., Sargent and Sullivan 1978; Vassiliadis et al. 1994a,b) discretizes only the controls $\mathbf{u}$ using piecewise polynomials. The differential equations are subsequently solved using standard integrators and the objective function is evaluated. The parametrized controls are updated using a standard optimization algorithm. Alternatively, *simultaneous direct methods* discretize both states and controls. The simulation and optimization are performed simultaneously which results in larger NLPs than in the sequential approach. Two different approaches exist within the class of simultaneous direct methods: *multiple shooting* (e.g., Bock and Plitt 1984; Leineweber et al. 2003) and *orthogonal collocation* (e.g., Biegler 1984, 2007). In multiple shooting, the integration range is split in a finite number of intervals while in orthogonal collocation the states are fully discretized based on orthogonal polynomials. These large NLPs require tailored algorithms that exploit the problems' structure and sparsity. In view of brevity, the interested reader is referred to Biegler (2010) for a general overview and discussion.

In optimal experimental design for parameter estimation, a scalar function $\Phi(.)$ of the Fisher information matrix is used as the objective function. This matrix is defined as:

$$\mathbf{F}(\mathbf{p}) = \int_0^{t_f} \left(\frac{\partial \mathbf{y}(\mathbf{p},t)}{\partial \mathbf{p}}\right)^T \Bigg|_{\mathbf{p}=\mathbf{p}^*} \mathbf{Q} \left(\frac{\partial \mathbf{y}(\mathbf{p},t)}{\partial \mathbf{p}}\right) \Bigg|_{\mathbf{p}=\mathbf{p}^*} dt \tag{6}$$

The true values $\mathbf{p}^*$ are unknown so the Fisher matrix depends on their current best estimate. Two parts constitute the Fisher information matrix: the matrix $\mathbf{Q}$, being the inverse of the measurement error variance-covariance matrix and $\frac{\partial \mathbf{y}(\mathbf{p},t)}{\partial \mathbf{p}}$, the sensitivities of the model output with respect to small variations in the model parameters. The latter can be found as the solution to:

$$\frac{d}{dt}\frac{\partial \mathbf{y}(\mathbf{p},t)}{\partial \mathbf{p}} = \frac{\partial \mathbf{f}}{\partial \mathbf{y}}\frac{\partial \mathbf{y}(\mathbf{p},t)}{\partial \mathbf{p}} + \frac{\partial \mathbf{f}}{\partial \mathbf{p}} \tag{7}$$

An interesting property of the Fisher information matrix is that under the assumption of unbiased estimators, the inverse of $\mathbf{F}$ is the lower bound of the parameter estimation variance-covariance matrix, i.e., the Cramér-Rao lower bound (Ljung, 1999).

The matrix $\mathbf{F}$ contains the information of a specific experiment. To optimize this amount of information a scalar function of $\mathbf{F}$ has to be selected as the optimization problem's objective function. Mathematically this is expressed as:

$$J = \Phi(\mathbf{F}(\mathbf{x}(t),\mathbf{u}(t),\mathbf{p},t)) \tag{8}$$

where $\Phi(.)$ is one of the criteria further described. The most widely used scalar functions are listed below (Pukelsheim, 1993; Walter and Pronzato, 1997; Franceschini and Macchietto, 2008).

- **A-criterion: min[trace($\mathbf{F}^{-1}$)]** A-optimal designs minimize the mean of the asymptotic variances of individual parameter estimates. The geometrical interpretation is the minimization of the enclosing frame around the joint confidence region.

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– **D-criterion: max[det(F)]** D-optimal designs minimize the geometric mean of the eigenvalues of the approximate variance-covariance matrix. Geometrically, this is minimizing the volume of the joint confidence region. Note that when only a subset of the parameters are considered, this is the D_s-criterion (Walter and Pronzato, 1997).
– **E-criterion: max[$\lambda_{min}(\mathbf{F})$]** E-optimal designs aim at minimizing the largest asymptotic variance of individual parameter estimates. This corresponds to minimizing the length of the largest uncertainty axis of the joint confidence region.

2.2. *Determination of an optimal sampling scheme*

In Sager (2012), an additional control function $v(t) \in [0,1]$ is introduced in the Fisher information matrix, which is then defined as:

$$\mathbf{F}(\mathbf{p}) = \int_0^{t_f} v(t) \left(\frac{\partial \mathbf{y}(\mathbf{p},t)}{\partial \mathbf{p}} \right)^T \Bigg|_{\mathbf{p}=\mathbf{p}^*} \mathbf{Q} \left(\frac{\partial \mathbf{y}(\mathbf{p},t)}{\partial \mathbf{p}} \right) \Bigg|_{\mathbf{p}=\mathbf{p}^*} dt \tag{9}$$

This function $v(t)$ is a binary function indicating whether or not measurements are required at time t. Mathematically this is expressed as $v(t) \in \{0,1\}\ \forall t \in [0,t_f]$. Note that in the current paper measuring several times at the same time instance is excluded. However, to provide a tractable optimal control problem, the problem is relaxed to $v(t) \in [0,1]$ and the strategy described in Sager (2012) is followed. Note that there is a subtle difference between the *experiment time* and the *measurement time*. The experiment time is assumed to be a predetermined number of hours. The measurement time is the amount of hours the experimenter is willing to spend to take measurements. This value is assumed not to be set a priori. The measurement time can of course never exceed the total experiment time. When the measurement time is not constrained to a certain value, the maximum information content is found by measuring continuously during the experiment time. As performing experiments is cost-and labor-intensive, the following additional objective is taken into consideration:

$$\min_{v(t)} W \tag{10}$$

in which:

$$\frac{dW}{dt} = v(t) \tag{11}$$

$$W(0) = 0 \tag{12}$$

This objective represents the experimental/measurement effort in the sense that it represents the total amount of hours spent measuring during the experiment. This objective can also be formulated as a constraint $W(t_f) \leq \alpha \leq t_f$ in a single objective optimization problem. This problem formulation closely resembles the work of Fedorov (1989). In the latter work, a numerical procedure for optimal design is presented based on the idea to delete less informative sets and to include more informative ones at every step of the procedure. Furthermore, the idea that the total amount of measurements is constrained to an a priori specified value is also present and equivalent to the approach in the current paper. However, the difference is that the current paper

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does not require the model to be linear in the parameters, which is one of the assumptions in Fedorov (1989). This means that the Fisher information matrix depends on the best estimates of the parameters and no guarantee on global optimality of the design can be provided. Note that, in the current work, the experimental effort is computed as the time integral of the measurement time. This approach can be generalized by taking the integral of a different function (e.g., computing the financial cost) by taking into account the cost to take samples at different points.

2.3. *Multi-objective optimization*

The formulation of a dynamic optimization problem involving multiple objectives J_1 to J_m is described as:

$$\min_{\mathbf{z}} \{J_1(\mathbf{z}), \ldots, J_m(\mathbf{z})\} \tag{13}$$

subject to equations (2) to (5).

In multi-objective optimization, *Pareto optimality* is used (see, e.g., Miettinen 1999). A solution is called Pareto optimal if there exists no other feasible solution that improves at least one objective function without worsening another. The decision variables $\mathbf{z}$ belong to the feasible set S, defined previously, and the vector of all individual cost functions is defined as $\mathbf{J} = [J_1(\mathbf{z}), \ldots, J_m(\mathbf{z})]^{\mathrm{T}}$.

The (convex) weighted sum (WS) is in practice the most widely-used technique for combining different objectives. By consistently varying the weights assigned to each cost J_i an approximation of the Pareto set is obtained. However, despite its simplicity, the weighted sum has several intrinsic drawbacks (Das and Dennis, 1997): a uniform distribution of the weights does not necessarily result in an even spread on the Pareto front, and points in non-convex parts of the Pareto set cannot be obtained.

Two recent scalarization-based multi-objective optimization techniques are the normal boundary intersection (NBI) (Das and Dennis, 1998) and the (enhanced) normalized normal constraint ((E)NNC) (Messac et al., 2003). Both techniques mitigate the discussed disadvantages of the WS and allow the use of fast gradient-based solvers. For a more detailed description of the NBI and (E)NNC, the interested reader is referred to Logist et al. (2010); Logist and Van Impe (2012). In recent, work a connection (Logist and Van Impe, 2012) between the WS, NBI and (E)NNC is proven.

In Sager (2012), a different approach is presented to make the trade-off between experimental effort and information gain. The author suggests adding a linear penalty of the control $v(t)$ to the objective. In this penalty term, there is a factor ε which is shown to be a minimum bound on the information gain. As this ε is set a priori this approach leads to a single optimal solution in a similar manner as fixing the total amount of measurement time which is equivalent to the ε-constraint method (Haimes et al., 1971). The authors of the current paper prefer to solve this trade-off in a multi-objective framework to ensure the experimenter can make an informed decision.

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2.4. Validation of the confidence regions

To assess the viability of the obtained experiments, simulations with pseudo-measurements are performed for different workload settings. To this extent, random white noise is added to the expected state evolution. This noise is drawn from a normal distribution with zero mean and a variance equal to the state measurement variance, σ_n^2.

To estimate the confidence intervals, the approximate variance-covariance matrix $\mathbf{C}$ is calculated parameter estimation. The individual confidence intervals of the parameters are given as:

$$p_i \pm t_{\alpha/2}^{n_t-n_p}\sqrt{C_{ii}} \tag{14}$$

where $t_{\alpha/2}^{n_t-n_p}$ is the upper critical value of the Student's t-distribution with tolerance level α and $n_t - n_p$ degrees of freedom, n_t is the number of measurements, n_p is the number of parameters and α is the $(1-\alpha)100\%$ confidence level employed by the user.

The model under study is non-linear in the parameters. As a result, the parameter estimation may yield an inaccurate estimation of the real confidence region (Balsa-Canto et al., 2007; Heine et al., 2008; Schenkendorf et al., 2009). In order to assess the viability of the obtained confidence regions, a Monte-Carlo-analysis is performed (Walter and Pronzato, 1997). In this work, 500 pseudo-measurement sets are generated. For each set, the model parameters are identified. Next, the 95% empirical confidence bounds on the parameters p_i are computed.

2.5. Predictive microbial growth model for E. coli

In this case study, optimal experiments for identifying the parameters of the Cardinal Temperature Model with Inflection (CTMI) (Rosso et al., 1993) are designed. This is a secondary model to the model of (Baranyi and Roberts, 1994). This latter model describes the cell density as a function of time while the former incorporates the dependency on temperature. The model equations of the Baranyi and Roberts model are:

$$\frac{dn(t)}{dt} = \frac{Q(t)}{Q(t)+1}\mu_{max}(T(t))[1-\exp(n(t)-n_{max})] \tag{15}$$

$$\frac{dQ(t)}{dt} = \mu_{max}(T(t))Q(t) \tag{16}$$

with the states $\mathbf{x}(t) = [n(t)\ Q(t)]^T$. The state $n(t)$ [ln(CFU/ml)] is the natural logarithm of the cell density and $Q(t)$ [-] the physiological state of the cells. The observable state is $\mathbf{y}(t) = n(t)$. The control input to this system is the temperature profile $\mathbf{u}(t) = T(t)$. The temperature dependency of the CTMI is given by:

$$\mu_{max} = \mu_{opt}\gamma(T(t)) \tag{17}$$

with:

$$\gamma(T(t)) = \frac{(T(t)-T_{min})^2(T(t)-T_{max})}{(T_{opt}-T_{min})[(T_{opt}-T_{min})(T(t)-T_{opt})-(T_{opt}-T_{max})(T_{opt}+T_{min}-2T(t))]} \tag{18}$$

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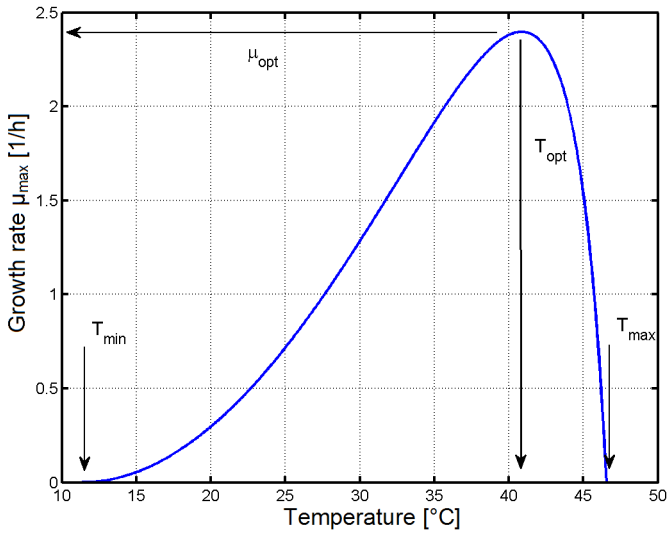

FIGURE 1. *Cardinal Temperature Model with Inflection with illustration of the meaning of the four parameters.*

The values of the parameters $\mathbf{p} = [\mu_{max}\ T_{min}\ T_{opt}\ T_{max}]^T$ for the model (Van Derlinden et al., 2010) are shown in Table 1. The Cardinal Temperature Model with Inflection is illustrated in Figure 1. T_{min} is the minimal temperature needed for growth, T_{max} is the maximal temperature where growth can occur. In case of temperatures lower than T_{min} or higher than T_{max}, no growth is assumed. T_{opt} is the temperature at which the growth rate μ is optimal. When designing experiments, the end time is fixed to 38 h (Van Derlinden et al., 2010). For model validity reasons the dynamic temperature profiles are constrained to:

$$15^\circ\mathrm{C} \leq \quad T(t) \quad \leq 45^\circ\mathrm{C} \tag{19}$$

$$-5^\circ\mathrm{C/h} \leq \quad dT(t)/dt \quad \leq 5^\circ\mathrm{C/h} \tag{20}$$

The temperature profile is designed as a combination of piecewise linear parts (Van Derlinden et al., 2010) to ensure practical implementation. The simplest approach is to estimate the four parameters from one single experiment. In previous work, the following practical strategy was proposed and validated experimentally. The four parameters are divided in six 2-parameter combinations $((T_{max}, \mu_{opt})$, (T_{max}, T_{min}), (T_{max}, T_{opt}), (T_{min}, μ_{opt}), (T_{min}, T_{opt}) and $(T_{opt}, \mu_{opt}))$ and for each combination an optimal experiment was designed. By only considering two parameters, the D_s-criterion is actually used instead of the D-criterion (Walter and Pronzato, 1997). Although the four parameters are unknown, only two are of interest in the design while the others are considered nuisance. Note that D_s-designs can be singular. By performing these six experiments, each individual parameter is considered three times (with each of the other parameters). This approach increases the experimental load, but was chosen as an acceptable trade-off with respect to parameter accuracy (Van Derlinden, 2009; Van Derlinden et al., 2010). In Van Derlinden et al. (2010) it was illustrated on simulation level that four 2-parameter combinations lead to an

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acceptable level of parameter accuracy provided each individual parameter is considered twice in two combinations. Note that three such possible combinations exist. To enable a fair comparison, the strategy of dividing into six 2-parameter combinations is also followed in this work.

TABLE 1. *Parameter values used for the design of the optimal experiments for the predictive growth model.*

T_{min}	11.33 °C	T_{opt}	40.85 °C
T_{max}	46.54 °C	μ_{opt}	2.397 1/h
n_{max}	22.55 ln(CFU/mL)	σ_n^2	3.27×10^{-2} ln(CFU/mL)^2

3. Results

In the first subsection, the individual results for the different parameter combinations are discussed. The trade-off between the experimental burden and the information content is discussed in the second subsection. The third subsection presents the discussion of the parameter accuracy.

3.1. Maximizing information content

In order to estimate the four model parameters, six possible two-parameter combinations can be used. For each combination, the corresponding cost function is optimized using the D_s-criterion (Van Derlinden et al., 2010). The six criteria as used in Van Derlinden et al. (2010) are: $J_1 = D_{s T_{max},\mu_{opt}}$, $J_2 = D_{s T_{max},T_{min}}$, $J_3 = D_{s T_{max},T_{opt}}$, $J_4 = D_{s T_{min},\mu_{opt}}$, $J_5 = D_{s T_{min},T_{opt}}$, $J_6 = D_{s T_{opt},\mu_{opt}}$. The individual maxima are summarized in Table 2. These values are obtained using a multiple shooting (Bock and Plitt, 1984) approach using the ACADO-toolkit (Houska et al., 2011) which allows for a fast computation of the optimal profiles. The integrator tolerance of the RungeKutta78 and the KKT-tolerance of the resulting SQP-problem are set to 10^{-6}. In this case, the assumption is that the measurements are performed continuously during the 38 hours of the experiment. These results are used as initialization in the next section as they represent the upper bound of available information content. The results in Table 2 are similar to those in Van Derlinden et al. (2010). It should be noted that the employed non-linear programming does not guarantee global optimality, only local optimality is ensured. The effect of increasing the number of discretization intervals is clearly visible in Table 2. The third column depicts the results for a different control action every fifteen minutes whereas the second column requires a control action every two hours. By a finer control discretization, slightly better results are obtained. However, nineteen control intervals will be chosen in the subsequent simulations to limit computational time. Compared with the approach in Van Derlinden et al. (2010), there are more degrees of freedom in this paper. In Van Derlinden et al. (2010) the temperature profile is constrained to two constant phases and a connecting linear section. The degrees of freedom in Van Derlinden et al. (2010) are: the initial temperature profile, the switching time to start the linear phase, the slope of the linear phase and the switching point to the second constant phase.

3.2. The trade-off between information content and measurement burden

In order to study the trade-off between information content and the measurement burden, a multi-objective optimization approach is employed, where the additional objective is the minimization of

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TABLE 2. *Values for the* D_s*-criterion for all the possible two-parameter combinations for 19 and 152 discretization intervals.*

Combination	19 intervals	152 intervals
$T_{max} - \mu_{opt}$	8.41×10^5	8.45×10^5
$T_{max} - T_{min}$	9.38×10^4	1.12×10^5
$T_{max} - T_{opt}$	7.40×10^4	8.49×10^4
$T_{min} - \mu_{opt}$	1.80×10^6	1.81×10^6
$T_{min} - T_{opt}$	7.76×10^4	7.76×10^4
$T_{opt} - \mu_{opt}$	1.58×10^6	1.62×10^6

the experimental effort. The extra decision variable introduced in this case is $v(t)$, which indicates whether to measure or not. Each of the six criteria described in Section 3.1 are combined with the criterion which aims at minimizing the measurement time. Note that nineteen control intervals are again used in order to limit the computational burden. These trade-off experiments are designed using NNC, as possible non-convex parts are expected. As software tool, the multi-objective extension of the ACADO-toolkit (Logist et al., 2010, 2012, 2013) is employed. For each of the combinations, the number of Pareto points is set to 21. The maximal observed CPU time for 1 of the 6 combinations is 167 seconds, the minimal time observed is 73 seconds. The maximum number of SQP iterations is 256, the minimum number is 118. The 6 combinations are computed in 1002 seconds. The resulting Pareto fronts for each of the parameter combinations are displayed in Figure 2. Note that the values for the information content are normalized. The obtained Pareto fronts clearly indicate non-convex feasible regions. Hence, the choice for NNC is justified. These Pareto fronts allow the experimenter to select a specific experiment with a given amount of work. Based on the Pareto front he or she then knows the expected information amount of the experiment.

The decision when to measure influences the designed experiment. This is illustrated in Figures 3 and 4. In this paragraph w, is used to indicate which multi-objective point of the utopia plane is considered. The utopia plane is the line/plane connecting the two individual objectives. The weights, w indicate how this line is discretized in the NNC. The case with $w = [1\ 0]^T$ corresponds to measuring at each of the multiple shooting nodes during the experiment, so every two hours and a maximum total of 20 samples per experiment. Other weight combinations have a reduced workload. The lowest workload combination taken into consideration relates to weight combination $w = [0.25\ 0.75]$. In the cases with a reduced workload, the experimenter is expected to sample continuously in the time horizons indicated by the rectangles in the temperature profiles of Figures 3 and 4. The total amount of hours denoted by the integral over the intervals indicated by the rectangles constitutes the measurement time. These coincide again with the 2-hourly multiple shooting nodes and a possible change in control action.

For the three combinations where T_{max} is involved, the temperature profile starts at the maximal allowed temperature of 45°C. For these experiments, the decreasing part of the temperature profile is an interesting part for optimizing the information content. So, if only a few samples are taken, it is advisable to do it in this part of the experiment. Note that the temperature profile changes in view of when samples are taken. This is shown for the parameter combination $T_{max} - T_{opt}$.

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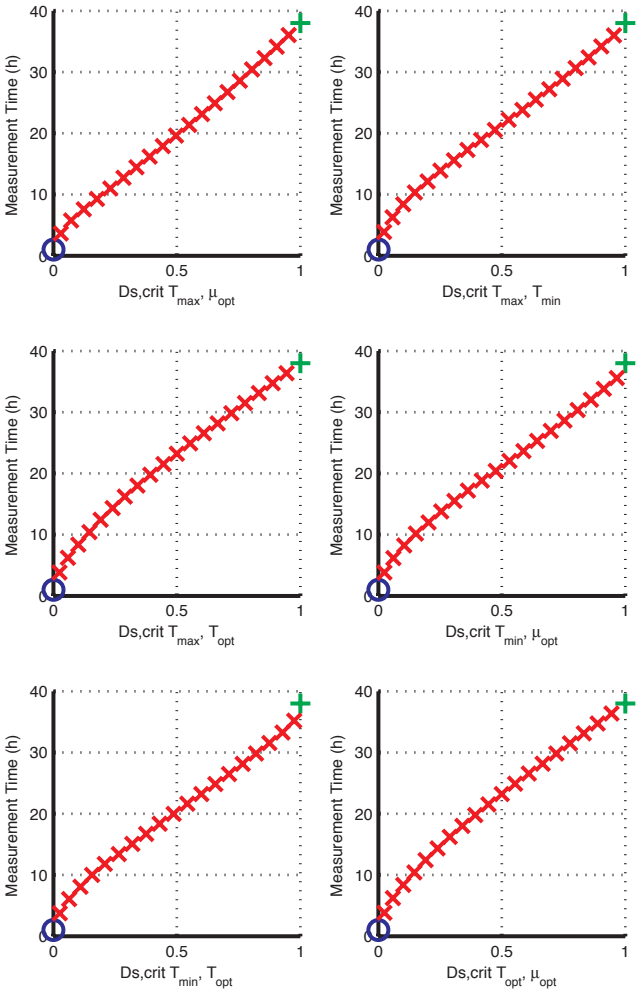

FIGURE 2. *Pareto fronts for the six different criteria in relation to measurement time. The plus and circle indicate the individual optima of the two considered objectives, respectively.*

When T_{min} is combined with T_{opt} or μ_{opt}, the temperature profile does not start at the maximal value. There also seems to be a more gentle decrease in the lower temperature region of the experiment compared with the profiles where T_{max} is present.

Combining $T_{opt} - \mu_{opt}$ results again in a temperature profile that starts at the maximum allowed temperature value. Note again the change of the obtained temperature profile when reducing the amount of samples that are taken.

A general observation is that all parameter combinations except $T_{max} - \mu_{opt}$ have some measurements taken at the end of the experiment. Also the sharp decrease in temperature present in all profiles generates information. Biologically, this corresponds to the fast growing phase of the cell,

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as illustrated in the corresponding cell concentration profiles (see Figure 3 and 4). Based on these observations, one can conclude that it is important to sample at the start (i) of the experiment, (ii) during a period of decreasing temperature, and at the end (iii) of the experiment. In previous work, this was done from practical point of view (need of night rest) (Van Derlinden et al., 2010), but is now validated with these results.

3.3. Assessing parameter accuracy when reducing number of samples

The obtained parameter values and the corresponding confidence regions are presented in Tables 3, 4, 5 and 6. In each table, the results are grouped according to the number of measurements in the experiments.

Parameters are estimated using a least squares objective function. Multiple shooting is employed, where the shooting nodes coincide with the in silico measurements. The bounds on the parameters are:

$$1.5 \leq \mu_{opt} \leq 4.5 \tag{21}$$
$$6 \leq T_{min} \leq 13 \tag{22}$$
$$37 \leq T_{opt} \leq 42 \tag{23}$$
$$44 \leq T_{max} \leq 50. \tag{24}$$

The initial parameter values passed to the optimization routine are the middle values of the above intervals. Two different approaches for computing the parameter variance are compared. One of the main possible drawbacks of the Fisher information matrix is that it can lead to an underestimation of the confidence bounds (Balsa-Canto et al., 2007). This means that the confidence regions obtained by the Fisher information matrix are smaller than those obtained by other approaches (Heine et al., 2008; Schenkendorf et al., 2009). In this work, a Monte-Carlo simulation is performed to check whether this is also the case for the CTMI. In the first approach, the model parameters are identified, and their uncertainty is computed using the obtained FIM. The second approach uses 500 Monte-Carlo noise realizations, each time re-estimating the parameters. The 500 individual estimates are used to construct the empirical 95% confidence region. Their average value is taken as the final parameter estimate.

The results show that the original parameters are each time located inside the obtained confidence region. As can be expected, the parameter confidence intervals become broader when the number of measurement points are decreased (and thus the workload reduced). This holds for both the Fisher information approach and the Monte-Carlo approach. When only 45 samples are taken compared to the 120 in the nominal case, the confidence interval, on average doubles in size.

From the simulation results shown in Tables 3 to 6, one infers that the size of the confidence intervals of the FIM and MC approaches is approximately equal. In Figure 5, the evolution of the parameter estimates and the confidence bounds for both approaches are displayed. This figure illustrates that there are only three cases where the 95% confidence bound given by the asymptotic

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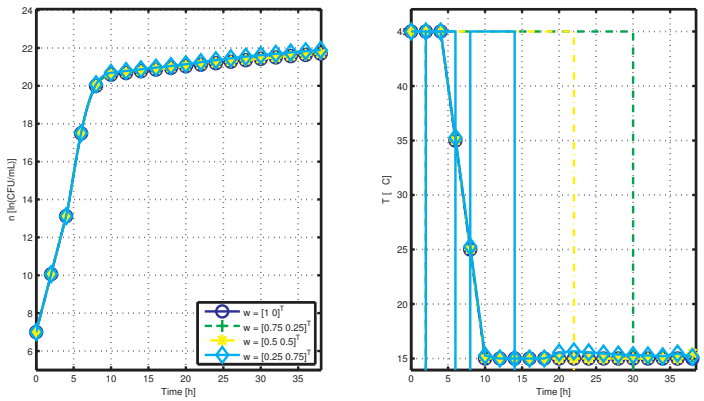
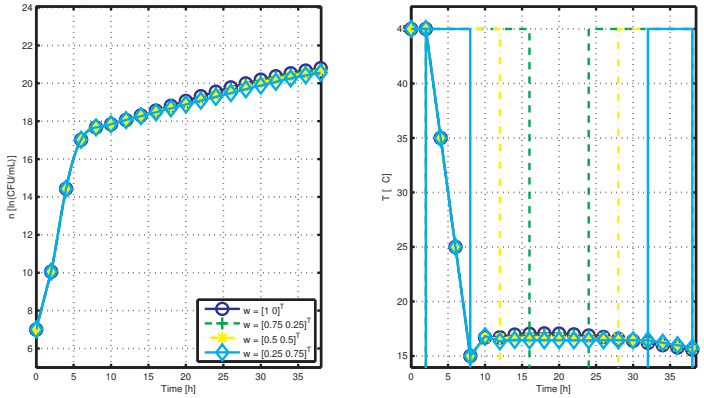
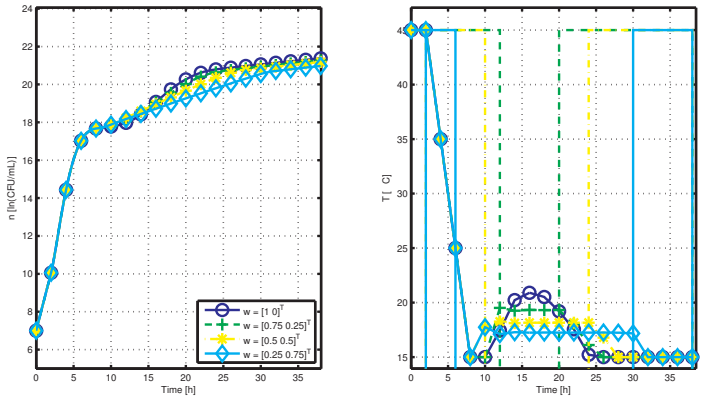


(a) State evolution $n(t)$ and temperature profile $T(t)$ for parameter combination $T_{max} - \mu_{opt}$.



(b) State evolution $n(t)$ and temperature profile $T(t)$ for parameter combination $T_{max} - T_{min}$.



(c) State evolution $n(t)$ and temperature profile $T(t)$ for parameter combination $T_{max} - T_{opt}$.

FIGURE 3. *Evolution of cell concentration $n(t)$ and temperature profile $T(t)$ of different parameter combinations for several weight combinations of the Pareto front obtained by NNC.*

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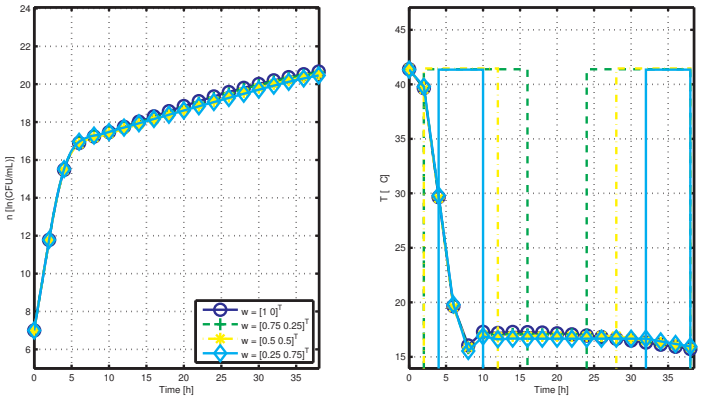
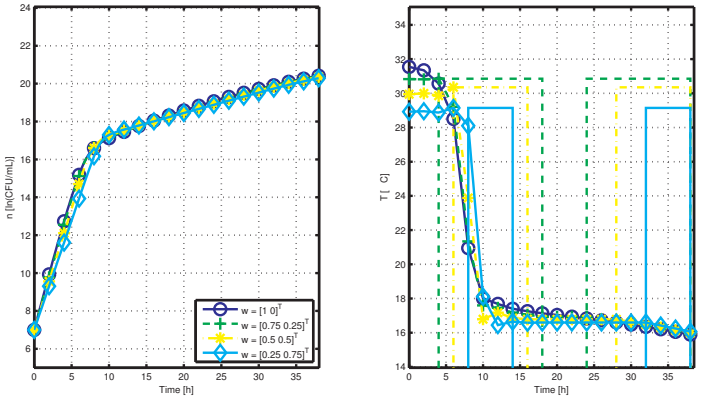
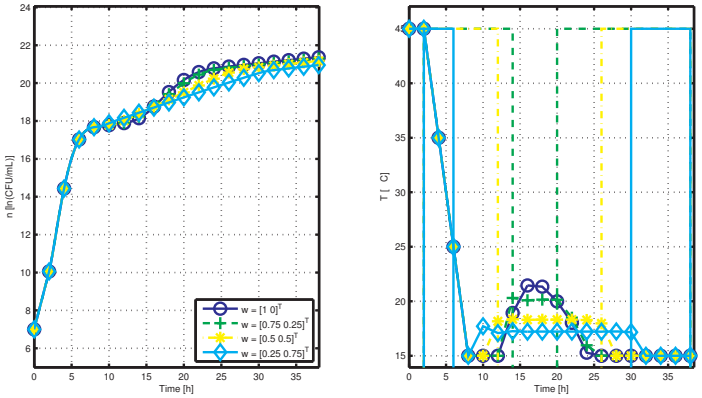


(a) State evolution $n(t)$ and temperature profile $T(t)$ for parameter combination $T_{min} - \mu_{opt}$.



(b) State evolution $n(t)$ and temperature profile $T(t)$ for parameter combination $T_{min} - T_{opt}$.



(c) State evolution $n(t)$ and temperature profile $T(t)$ for parameter combination $T_{opt} - \mu_{opt}$.

FIGURE 4. *Evolution of cell concentration $n(t)$ and temperature profile $T(t)$ of different parameter combinations for several weight combinations of the Pareto front obtained by NNC.*

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normal distribution of the least squares estimator is smaller than the Monte-Carlo estimate of the 95% bound. Note, however, that in all cases the total width of the confidence region obtained by the Fisher information is broader than the one obtained by the Monte-Carlo simulation. From these observations, it can be inferred that the Fisher information matrix can be used to get a robust estimate of the real confidence bounds of the parameter estimates for the current case study.

An additional observation is the fact that the confidence regions for T_{opt} and T_{max} exhibit a non-symmetrical confidence region in the Monte-Carlo simulations. The empirically obtained distributions have been tested for normality using the Kolmogorov-Smirnov test. The hypothesis that T_{opt} is normal, could not be rejected on a 5% discrimination level for all workload cases. For T_{max} this hypothesis can be rejected using the same test with the same discrimination level. Note, that the Fisher information matrix yields a conservative bound for T_{max}, even in view of the non-normal distribution of T_{max}. This clearly illustrates that the uncertainty estimation by the Fisher information matrix can be inaccurate and too conservative in this case study. In this case study, the uncertainty was too conservative. Therefore, a careful assessment of the uncertainty through other means than the Fisher information matrix is advisable if computationally feasible. Furthermore, if the assumption that the parameters are normal, is rejected, this means that the sample size is too small and that asymptotic normality is not reached.

TABLE 3. *Parameter values and confidence regions for 120 samples in the six experiments.*

Parameter	FIM approach (95% CI)	Monte-Carlo approach (95% CI)
μ_{opt}	2.357 ($\pm$0.1299)	2.399 ($-$0.1042 $+$0.1030)
T_{min}	11.44 ($\pm$0.4571)	11.32 ($-$0.3436 $+$0.3876)
T_{opt}	40.68 ($\pm$0.5203)	40.86 ($-$0.4108 $+$0.4679)
T_{max}	46.94 ($\pm$0.8874)	46.55 ($-$0.5018 $+$0.5995)

TABLE 4. *Parameter values and confidence regions for 95 samples in the six experiments.*

Parameter	FIM approach (95% CI)	Monte-Carlo approach (95% CI)
μ_{opt}	2.329 ($\pm$0.2380)	2.400 ($-$0.1651 $+$0.1685)
T_{min}	11.54 ($\pm$0.7664)	11.34 ($-$0.5012 $+$0.4803)
T_{opt}	40.65 ($\pm$1.012)	40.86 ($-$0.7078 $+$0.7837)
T_{max}	47.02 ($\pm$1.867)	46.60 ($-$0.8269 $+$1.1326)

TABLE 5. *Parameter values and confidence regions for 71 samples in the six experiments.*

Parameter	FIM approach (95% CI)	Monte-Carlo approach (95% CI)
μ_{opt}	2.298 ($\pm$0.2191)	2.399 ($-$0.1624 $+$0.1837)
T_{min}	11.34 ($\pm$0.7546)	11.35 ($-$0.5803 $+$0.6183)
T_{opt}	40.60 ($\pm$1.0550)	40.87 ($-$0.9038 $+$0.8960)
T_{max}	47.07 ($\pm$1.960)	46.64 ($-$0.9326 $+$1.3696)

TABLE 6. *Parameter values and confidence regions for 45 samples in the six experiments.*

Parameter	FIM approach (95% CI)	Monte-Carlo approach (95% CI)
μ_{opt}	2.415 ($\pm$0.2748)	2.4059 ($-$0.1936 $+$0.1973)
T_{min}	11.52 ($\pm$0.9985)	11.33 ($-$0.6406 $+$0.6547)
T_{opt}	40.68 ($\pm$1.6010)	40.91 ($-$0.9943 $+$1.0931)
T_{max}	46.75 ($\pm$2.0020)	46.59 ($-$0.9596 $+$1.4530)

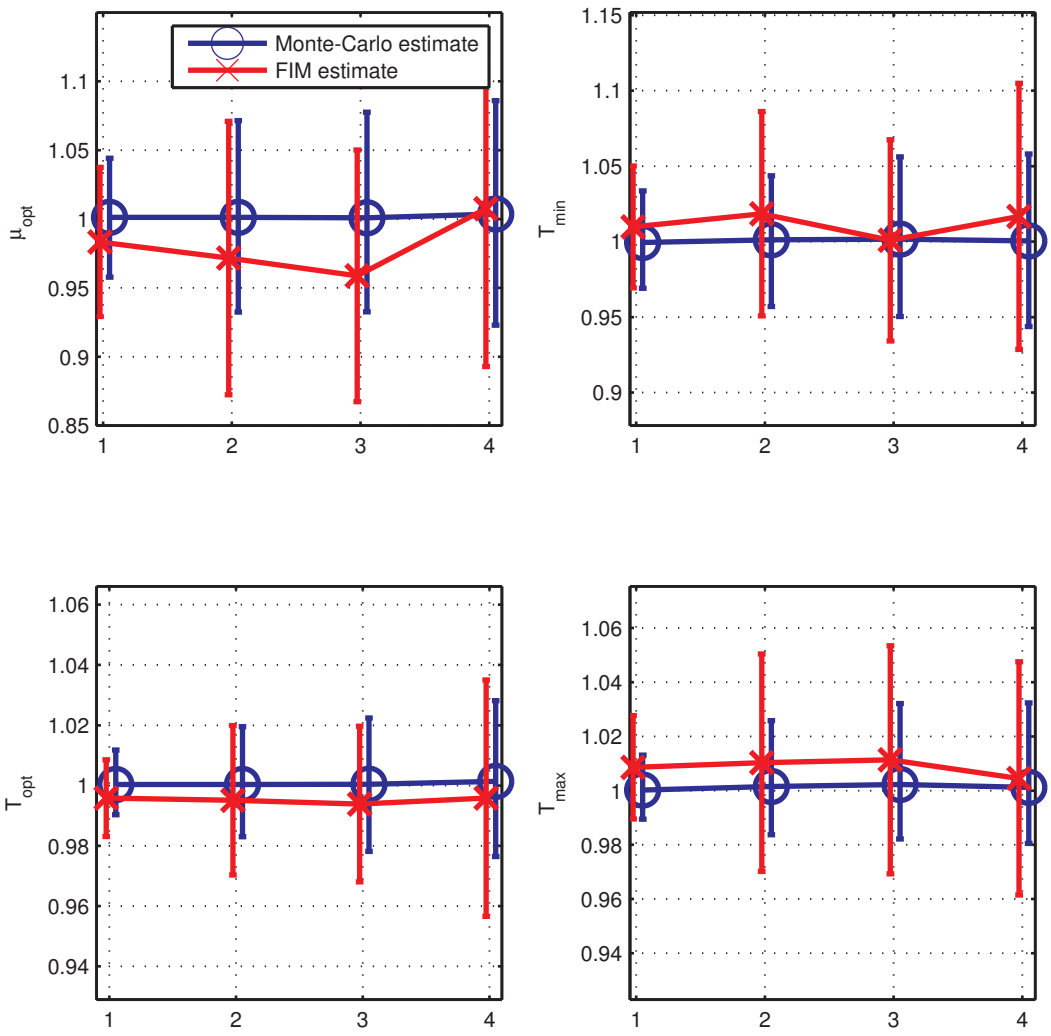

FIGURE 5. *Evolution of the parameter estimates (relative to their nominal values) obtained by the Fisher information approach and the Monte-Carlo approach and the corresponding 95% confidence regions for a decreasing work load scheme (with* $1 = [1\ 0]^T$, $2 = [0.75\ 0.25]^T$, $3 = [0.5\ 0.5]^T$, $4 = [0.25\ 0.75]^T$*).*

4. Conclusion

In this work, a practical procedure is proposed to systematically evaluate trade-offs between information content and experimental effort in the design of dynamic experiments for calibrating the Cardinal Temperature Model with Inflection. In the employed procedure, advanced multi-objective dynamic optimization approaches are exploited. The considered objectives were parameter accuracy on the one hand, and the total time reserved for measuring on the other. With respect to the latter objective, the determination of optimal measurement/sampling schemes specifying when to measure is added as a degree of freedom. The use of advanced multi-objective optimization allowed the experimenter to generate the set of Pareto optimal solutions. This set enables the

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experimenter to obtain an overview of all the possible trade-offs. The accuracy of the designed experiments with reduced workload is assessed in a simulation study, because one known drawback of the Fisher information matrix approach is that, for models non-linear in the parameters, it can give a possibly inaccurate estimate of the confidence intervals. Hence, the validity of the Fisher information matrix approach is checked by performing a Monte-Carlo simulation.

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