AIChE

Novel Anticorrelation Criteria for Design of Experiments: Algorithm and Application

Gaia Franceschini and Sandro Macchietto
Dept. of Chemical Engineering, Imperial College London, South Kensington Campus, London SW7 2AZ, U.K.

DOI 10.1002/aic.11607
Published online November 4, 2008 in Wiley InterScience (www.interscience.wiley.com).

When building mathematical models to represent chemical phenomena, the identification of the system kinetics can be one of the most difficult and time-consuming processes. The high-parameter correlations, which are typical of the most common reaction networks (parallel, consecutive reactions, etc.), often make the identification and proper estimation of the model parameters extremely difficult. For this reason, a novel approach to model-based experiment design able to yield optimally informative experiments while simultaneously reducing the correlations between the model parameters was proposed in a previous publication. (Franceschini and Macchietto, AIChE J. 2008;54:109–1024) This method was demonstrated very effective when applied to a simple, illustrative case. This article investigates this novel approach in more details and presents an algorithm and a structured set of recommendations to guide a user in the formulation of the design sequence most appropriate to the features of the problem under investigation. The application to a more complex fermentation example is used to demonstrate the usefulness of the proposed algorithm, the effectiveness of the novel criteria in yielding precise parameter estimates as less correlated as possible, and the suitability of this method to various design procedures (sequential, parallel, etc.).
 AIChE J, 54: 3221–3238, 2008
Keywords: kinetics, model-based experiment design, nonlinear dynamics, parameter correlation, parameter identification

Introduction

The importance of dynamic mechanistic modeling in process engineering is undeniable: when limitations of time and money prevent the exploration of all potentially feasible solutions for a certain process, the engineer turns to the use of mathematical models to overcome this drawback. These models may be empirical or based on mechanistic knowledge (physical, chemical, or biological laws). These mechanistic models are more powerful because they allow extrapolation of the design space to conditions beyond that encountered during laboratory experimentation. However, to perform this extrapolation in safety, these mathematical models must be validated: all the unknown parameters included in the model must be estimated from experimental data and be statistically satisfactory. Collecting the data which are required to build and validate a model can therefore be costly, both in terms of time and resources. Therefore, there is a need to develop such models in a systematic way to maximize the information obtained from each experiment and to minimize the number of analyses, the cost of materials, and the time required. From this point of view, the modern technique of *Model-Based Experiment Design* is an important link between the experimental and the modeling world as it aims at obtaining the maximum information from the experimental apparatus being modeled by devising experiments that will yield the most informative data, in a statistical sense, for use in parameter estimation and model validation.

Correspondence concerning this article should be addressed to G. Franceschini at g.franceschi03@imperial.ac.uk.

In what follows, a general deterministic model described by a set of (possibly mixed) differential and algebraic equations (DAEs) is considered:

$$\begin{cases} \mathbf{f}(\dot{\mathbf{x}}(t), \mathbf{x}(t), \mathbf{u}(t), \mathbf{w}, \boldsymbol{\theta}, t) = 0 \\ \mathbf{y}(t) = \mathbf{h}(\mathbf{x}(t)) \end{cases} \tag{1}$$

where $\mathbf{f}$ is a n_{eq}-dimensional set of DAEs and is assumed to have continuous first partial derivatives with respect to its arguments. $\mathbf{x}(t)$ is a n_s-dimensional vector of time dependent state variables, $\dot{\mathbf{x}}(t)$ is an n_{diff}-dimensional vector of differential variables, $\mathbf{u}(t)$ is a n_u-dimensional vector of time-varying controls or inputs to the process, $\mathbf{w}$ is a n_w-dimensional vector of constant controls, $\boldsymbol{\theta}$ is a p-dimensional vector of model parameters to be determined, and $\mathbf{y}(t)$ is an n_{resp}-dimensional vector of measured response variables that are functions of the state variables, $\mathbf{x}(t)$. In most cases, $\mathbf{h}(\mathbf{x}(t))$ will simply be a "selector'' function, selecting those state variables that are in fact measured.

In mathematical terms, given an initial model and current values of its parameters, the aim of model-based experiment design is to minimize the expected confidence region of the new parameters, i.e., to make the elements of the parameters variance-covariance matrix small. An experiment design calculation thus involves minimizing some metrics of this matrix or of its inverse, the information matrix, by choosing a set of experiment decision variables (length, time-varying and time invariant controls, initial conditions, sampling times, etc.), which are collected in the so-called design vector ϕ, subject to equality or inequality constraints defining the allowable experimental conditions and (expected) responses.[1] The design vector therefore contains all the control variables which can be manipulated by the experimenter and optimized during the design and, of course, $\boldsymbol{\varphi}$ must be constrained to lie within the experiment design space Φ to obtain an experiment realizable in practice.

For a dynamic experiment, the information matrix can be calculated according to the following definition, first derived by Zullo[2]:

$$\mathbf{M}(\hat{\boldsymbol{\theta}}, \boldsymbol{\varphi}) = \sum_{r=1}^{n_{resp}} \sum_{s=1}^{n_{resp}} \sigma_{rs} \mathbf{Q}_r^T \mathbf{Q}_s \tag{2}$$

where n_{resp} is the number of model responses, $\hat{\boldsymbol{\theta}}$ is the vector of the best available estimates of the model parameters, ϕ is the design vector, and σ_{rs} is the element (r, s) of the inverse of the variance–covariance matrix of the experimental measurements. $\mathbf{Q}_r$ is defined as:

$$\mathbf{Q}_r = \left[\frac{\partial y_{ri}}{\partial \theta_j} \right] \quad i = 1, ..., n_{sp}; j = 1, ..., p \tag{3}$$

and is the matrix of the first-order sensitivity coefficients of the rth model response, computed at each of the n_{sp} sampling times. For a detailed description of the experiment design method, reference is made to Franceschini and Macchietto.[1]

Various real-valued functions have been suggested as suitable metrics for the "size" of the parameter variance–covariance matrix or of the information matrix.[1] The most common criteria are the A-, D-, and E-optimal, which maximize, respectively, the trace, the determinant, and the smallest eigenvalue of the information matrix. For a detailed discussion of these criteria, reference is made to Walter and Pronzato.[3]

As the information available at the time of model building is often scarce, incomplete or imprecise (the experimental data can be affected by high errors and/or the values of the parameters may be unknown or very uncertain), the approach required to validate the model statistically (experiment design for parameter precision) must be an iterative procedure (see Asprey and Macchietto[4] or Sidoli et al.[5] for a discussion on the complete systematic model-building procedure). There are three main approaches to an iterative design of experiments: sequential (the most used method[6–11]), parallel (see the very significant and recent contribution from Galvanin et al.[12] on this topic), and parallel/sequential (see Figure 1). In a sequential approach, the basic idea consists in improving the initial choice of the parameter values by alternating experiment design and identification of the parameters. Each new experiment is designed using the estimates obtained from the previous estimation step as nominal values for the parameters (Figure 1a), until the estimation can be considered statistically satisfactory (see Asprey and Naka[13] for a discussion on statistical and model adequacy tests). In a parallel approach (Figure 1b), several experiments are designed simultaneously (all adopting the same nominal values for the parameters) and then performed in the laboratory. Using the data collected from all the parallel optimal experiments, the parameters are estimated and their adequacy assessed. If the information obtained from the n_{exp}^p experiments designed in parallel is shown to be insufficient after the parameter estimation, the procedure can be repeated (parallel/sequential approach). Therefore, in a sequential and parallel/sequential approach, three consecutive steps are needed to determine and improve the accuracy of the model parameters:

1) The design of one optimal experiment (sequential approach) or n_p optimal experiments (parallel/sequential approach) based on the current knowledge of the system (model structure and the best available parameter values);

2) The execution of the designed experiment/s and collection of the new data;

3) The estimation of the model parameters and their statistical assessment.

The sequential iteration of Steps 1, 2, and 3 leads to a progressive reduction in the uncertainty region of the model parameters, thanks to the new information obtained from the experimental data. In a parallel approach, on the other hand, Steps 1, 2, and 3 are repeated only once: all the required experiments are designed simultaneously and the parameters are estimated only after all the optimal data have been collected from the laboratory experimentation. As mentioned in Galvanin et al.,[12] there are a number of research and industrial applications where a parallel approach is more suitable than a sequential one. For example, miniaturization and nanotechnology allow the definition of array of modules, such as microreactors for chemical or biochemical reactions, in which several experiments can be simultaneously performed in different conditions.

Several experiment design techniques have been developed in the past and applied successfully to a wide range of

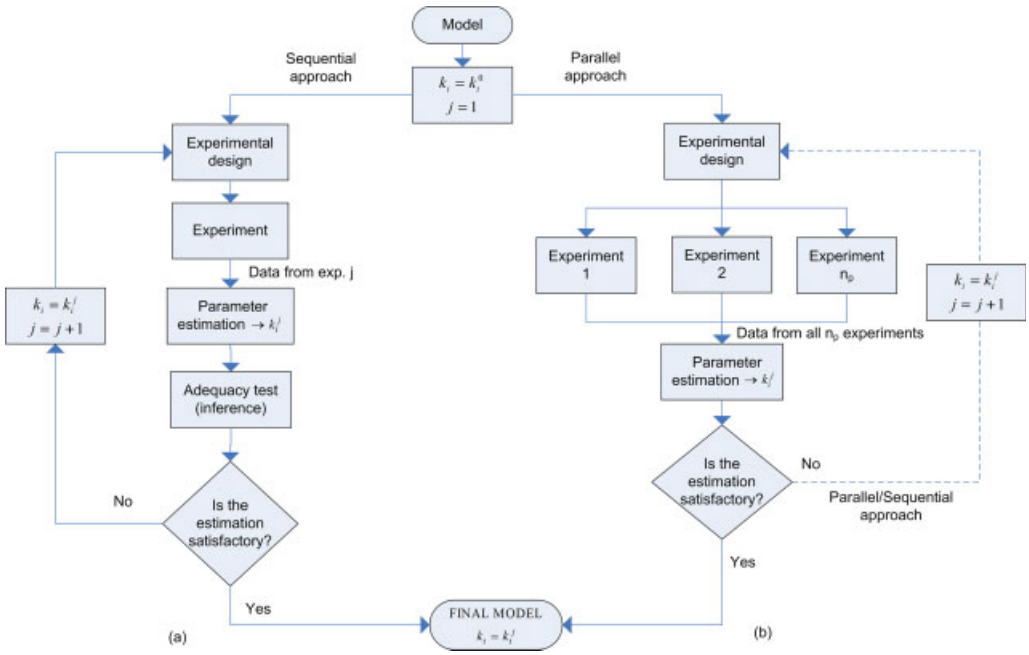

Figure 1. Sequential, parallel and parallel sequential approaches to model-based design of experiments.

The symbols used in the figure have the following meaning: k_i are the model parameters, k_i^f are the final validated model parameters, k_i^0 are the initial values of the parameters (the best available estimates), k_i^j are the model parameters obtained after the j-th estimation and j is a counter which indicates the current iteration (for sequential and parallel/sequential only). [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

systems.[14–23] One of the areas where an effective experiment design can be most useful is the identification of the kinetic parameters of a model. As highlighted in a previous publication,[24] this problem can be very much complicated by the large number of parameters often involved in kinetic mechanisms and by their significant interactions. Reaction networks constituted of parallel, sequential, competitive, or consecutive steps are all examples of kinetic models characterized by highly correlated parameters, which often prevent successful experiment design and/or parameter identification. In the view of the above issue, a novel approach to the experiment design of complex kinetic models was strongly advocated and was recently developed.[25] A previous article[26] presented this innovative experiment design method, which is capable of reducing parameter correlations while maintaining the same aims of traditional designs (improving the precision of the parameters). Four new optimal design criteria (PAC, ACE, E-AC, and AC-V) were proposed that include explicit measures of correlation as objective function or as constraints and are able to target the experiments to the improvement of specific parameter/s. The formulations of these novel criteria were presented in the above cited publication[26] and their effectiveness was tested by means of an illustrative three-parameter epoxidation example. The results of the tests proved the novel approach very successful, highly flexible, and more effective than the standard experiment design criteria in reducing the uncertainty regions of the parameters and improving the reliability of the estimates. This article deals with some of the issues which were risen in the previous publication during the presentation of the novel approach without receiving a complete and thorough discussion (such as, the choice of the most appropriate bounds and criteria, the potential rotation of the ellipsoid during the design, the preliminary analyses required for a reasoned application of the criteria). In addition, an algorithm and a structured set of recommendations will be proposed to guide a user in the formulation of the design sequence most appropriate to the features of the problem under investigation.

Regarding the structure of this article, the next section briefly recalls the main characteristics of the novel anticorrelation (AC) approach and the formulations of the four criteria. Then, a comprehensive algorithm is described to summarize all the preliminary steps and choices required to perform an experiment design using one of the novel AC criteria. A thorough discussion of some issues strongly related to the application of the novel approach to more complex systems (structural/non-structural correlations, ellipsoid rotation, and choice of the criteria) is also presented. Finally, the effectiveness of the criteria and the helpfulness of the proposed algorithm are demonstrated through the application to a more complex and realistic case study than the epoxidation example previously used[26]: a four-parameter model describing a fermentation process. Although simple, this case study is quite substantial and has been used several times in the past to illustrate other novel experiment design approaches developed by our research group.[4,12,16,27] For this reason, the bioreactor example has been chosen here to demonstrate the application of the proposed AC algorithm. Both an ideal case with good initial values for the parameters and reliable measurements and a poor situation are tested. The second instance (imprecise data and wrong initial guesses for the parameters) is particularly suitable for investigating both a parallel and a parallel/sequential experiment design approach. The chosen example is quite simple and not particularly large but is very

suitable for a step-by-step illustration of the use of the novel algorithm. An application of our innovative AC approach to a real case of industrial interest involving the actual, as opposed to simulated, execution of laboratory experiments (a biodiesel production process) has been already published elsewhere.[28]

Novel Anticorrelation Experiment Design Criteria

In the last 20 years, several studies have highlighted the problems which can affect an estimation procedure when parameters are highly correlated[29–32] but only a few of them has tried to propose and implement a solution based on model-reparameterization and/or variable separation,[33–36] well-designed experiments[31,37] or independent estimation of as many parameters as possible.[38] The rest of these works simply noted the problem and concluded with a recommendation of trying to avoid correlated parameters as much as possible.[39] Few papers in the literature have identified a suitable experiment design procedure as a possible way of overcoming the correlation problem and the most valid criterion proposed before our study[25] to reduce parameter correlations when designing optimal experiments is Pritchard's method.[31] This criterion employs the so-called *overall correlation level* (OCL), which is the root square of individual correlations between pair of parameters, as objective function (p is the number of model parameters):

$$\mathrm{OCL} = \left\{ \sum_{\substack{i,j \\ i \neq j}} \frac{c_{ij}^2}{(p^2 - p)} \right\}^{1/2} . \tag{4}$$

As the works of Agarwal and Brisk[40] and Issanchou et al.[41] have revealed, Pritchard's criterion which aims only at reducing parameter correlations is deemed to produce large confidence regions for the parameters since the information content of the experiment is not included in the design. For this reason, most of our novel AC criteria[25] aim at finding the best possible trade-off between reduction in the parameter correlation and increase in the information content of the experiment. In addition, these criteria allow designed experiments to be specifically targeted at improving a selected parameter or group of parameters of interest and this has been proved particularly useful for sequential experiment design strategies.[26] The AC-V design achieves this experiment targeting by directly minimizing the variance of the desired parameter/s while reducing the correlations. The ACE and E-AC criteria exploit some concepts of principal component analysis to reach the goal of targeting the experiment: to improve the estimation of a certain parameter, its specific eigenvalue is maximized (see the section on ellipsoid rotation).

Anticorrelation Criteria Formulation

In this section, the formulations of the four novel criteria discussed in Franceschini and Macchietto[26] are briefly recalled.

The correlation matrix (**C**) is calculated, by definition, from the elements of the variance–covariance matrix (**V**):

$$C_{ij} = \frac{V_{ij}}{\sqrt{V_{ii}} \cdot \sqrt{V_{jj}}} \quad i,j = 1, ..., p \tag{5}$$

As **V** is the inverse of the information matrix, the correlation coefficients required in the formulation of the novel criteria can be easily computed at each iteration of the experiment design calculations by inversion of the information matrix, **M**, of Eq. 2.

PAC design

The objective function involves the sum of the two largest correlation coefficients (not more than two otherwise, based on our experience, the minimization of the function can become too difficult due to the presence of several minima) while the other elements of the correlation matrix **C** are subject to the constraints

$$\begin{aligned} &\min_{\boldsymbol{\varphi} \in \boldsymbol{\Phi}} \left(\mathrm{C}_{ij}^2(\hat{\boldsymbol{\theta}}, \boldsymbol{\varphi}) + \mathrm{C}_{kl}^2(\hat{\boldsymbol{\theta}}, \boldsymbol{\varphi}) \right) \text{with} \\ &\qquad \mathrm{C}_{ij}, \mathrm{C}_{kl} = \max \mathbf{C}|_{\text{basepoint}} \text{ and } i,j,k,l \in \{1, ..., p\} \\ &\text{s.t. } \mathrm{C}_{\mathrm{mn}}^2(\hat{\boldsymbol{\theta}}, \boldsymbol{\varphi})|_{\substack{m \neq n \\ mn \neq ij \neq kl}} < \varepsilon_{mn}^C \quad m = 1, ..., p-1; \\ &\qquad\qquad\qquad\qquad\qquad\qquad n = 2, ..., p \end{aligned} \tag{6}$$

with bounds ε_{mn}^C set differently for each constraint. p is the number of model parameters, ϕ the design vector (which includes all the design variables), Φ the design space (identified by the variability range of all the design variables contained in the vector ϕ), and $\hat{\boldsymbol{\theta}}$ the vector of the best currently available parameter estimates.

ACE design

The criterion adopts the formulation expressed by Eq. 6 with the following additional constraints on one or more eigenvalues λ of the information matrix

$$\lambda_i - \lambda_i^0 > \varepsilon_i^\lambda \quad \text{with } i \in \{1, p\} \tag{7}$$

with ε_i^λ a specified bound.

E-AC design

The objective function maximizes one of the eigenvalues of the information matrix while all the correlation coefficients are bounded in the constraints

$$\begin{aligned} &\max_{\boldsymbol{\varphi} \in \boldsymbol{\Phi}} f(\mathbf{M}(\hat{\boldsymbol{\theta}}, \boldsymbol{\varphi})) = \max_{\boldsymbol{\varphi} \in \boldsymbol{\Phi}} \lambda_i(\hat{\boldsymbol{\theta}}, \boldsymbol{\varphi}) \quad i \in \{1, p\} \\ &\text{s.t. } \mathrm{C}_{ij}^2(\hat{\boldsymbol{\theta}}, \boldsymbol{\varphi})|_{i \neq j} < \varepsilon_{ij}^C \quad i = 1, ..., p-1; \quad j = 2, ..., p \end{aligned} \tag{8}$$

with ε_{ij}^C specified bounds.

AC-V design

The criterion uses the formulation expressed by Eq. 6 with the following additional constraints on one or more parameter variances:

$$V_{ii} < \varepsilon_i^v \quad i \in \{1, p\} \tag{9}$$

with ε_i^V a specified bound and V_{ii} the variance of parameter i. As an alternative, the formulation of the E-AC design can be adopted resulting in the V-AC criterion; the objective function minimizes the variance of a certain parameter/s while all the correlation coefficients are bounded in the constraints:

$$\begin{aligned} &\min_{\boldsymbol{\varphi}\in\boldsymbol{\Phi}} V_{ii}(\hat{\boldsymbol{\theta}},\boldsymbol{\varphi}) \text{ with } V_{ii}\in \mathbf{V}(\hat{\boldsymbol{\theta}},\boldsymbol{\varphi}) \\ &\text{s.t. } C_{ij}^2(\hat{\boldsymbol{\theta}},\boldsymbol{\varphi})|_{i\neq j} < \varepsilon_{ij}^C \quad i=1,...,n_P-1; \quad j=2,...,n_P \end{aligned} \tag{10}$$

Anticorrelation Criteria: Algorithm and Critical Issues

As highlighted in a previous article,[26] the PAC criterion is not a recommended choice for the design of experiments as the information content of the new data is not considered. The optimal experiments designed with this criterion were demonstrated to be very successful in eliminating the correlations but poorly informative. Therefore, although the reduction in the parameter correlations tends to improve the estimates, the loss in information content causes this criterion to be less effective than the standard E-optimal design (larger confidence regions). However, the PAC criterion can be very useful at the preliminary stage to assist in the choice of the most appropriate combination of objective function and constraints. Before starting the design, it is very important to know the features of the system in terms of correlations and relationships between parameters and principal directions; this last information can be easily obtained by an eigenvalue–eigenvector decomposition of the information matrix at the base point. However, this knowledge alone is not sufficient, in particular, for complex systems. For example, it is essential to understand whether the correlations between the parameters are caused by a bad design (the base point is often an unplanned experiment) and so can be eliminated, or are strongly dependent on the structure of the model. In addition, the higher the number of parameters, the more likely is that the reduction in some of the correlation coefficients will cause an increase in some of the others; this information must be known a priori to choose appropriate bounds in the constraints and avoid convergence problems. Some preliminary calculations of optimal experiments using a standard and the PAC design are a very suitable way of developing this knowledge of the system.

The following algorithm is presented to summarize all the preliminary steps and choices required to perform an experiment design using one of the novel AC criteria (after the introduction of the algorithm some comments are added in order to clarify certain recommendations). The relative importance which is used in several algorithm steps is calculated according to Perger et al.[42] as the square of each eigenvector component ($imp_{ij} = e_{ij}^2$ where $\mathbf{E}$ is the eigenvector matrix) and indicates how significant the parameters are in each of the principal directions.

Given a design vector ϕ (base point for the design), a design space $\boldsymbol{\Phi}$, and a value for the parameters $\hat{\boldsymbol{\theta}}$:

1. Calculate the information matrix ($\mathbf{M_{bp}}$), the parameter variance–covariance matrix ($\mathbf{V_{bp}}$), and the parameter correlation matrix ($\mathbf{C_{bp}}$) at the base point;
2. Analyze $\mathbf{C_{bp}}$ to identify "problematic" coefficients, i.e., any coefficient that passes the "dangerous" levels of 80 and 90% of correlation (for use in Step 5);
3. Perform an eigenvalue-eigenvector decomposition of $\mathbf{M_{bp}}$ to obtain $\lambda_{\mathbf{bp}}$ and $\overline{\mathbf{E}}_{\mathbf{bp}}$; calculate the relative importance $\text{imp}_{ij}|_{bp}$ of the various parameters ($i = 1,\ldots,p$) in all the principal directions ($j = 1,\ldots,p$) and for each direction identify its dominating parameter (the one with the largest relative importance) for use in Step 10;
4. Carry out a standard design (D- or E-optimal) and analyze the predicted correlation matrix $\mathbf{C_{sd}}$ obtained to see whether the parameters are more or less correlated than at the base point;
5. Based on the results obtained in Steps 2 and 4, choose the correlation coefficients to be minimized in the objective function for the PAC design required in Step 8 (see the comments at the end of the algorithm for a recommended criterion for this decision);
6. Carry out an eigenvalue-eigenvector decomposition of the predicted optimal information matrix obtained with the standard design ($\mathbf{M_{sd}}$) to calculate $\lambda_{\mathbf{sd}}$. Identify the upper bounds for the constraints on the eigenvalues (*for the ACE design only*) as:

$$\varepsilon_i^{\lambda} \leq (\lambda_i - \lambda_i^0)|_{\text{sd}} \quad i\in\{1,p\}; \tag{11}$$

7. Choose the bounds $\varepsilon_{ij}^C|_{\text{PAC}}$ for the PAC design required in the next step within the range:

$$\varepsilon_{ij}^C\Big|_{\text{PAC}} \in [0, C_{ij}^2|_{bp}] \quad i,j=1,...,p \text{ and } i\neq j \tag{12}$$

8. Carry out a PAC design with objective function from Step 5 and bounds for the constraints from (12) to understand to which extent the correlations can be eliminated and identify the potentially problematic coefficients (the ones which cannot be significantly reduced without a great worsening of the others);
9. Perform an eigenvalue–eigenvector analysis of the predicted optimal information matrix obtained with the PAC design ($\mathbf{M_{PAC}}$) to calculate $\lambda_{\mathbf{PAC}}$ and $\overline{\mathbf{E}}_{\mathbf{PAC}}$; compute the relative importance $\text{imp}_{ij}|_{\text{PAC}}$ of the various parameters ($i = 1,\ldots,p$) in all the principal directions ($j = 1,\ldots,p$) and for each direction identify its dominating parameter (the one with the largest relative importance);
10. Compare the relative importance of the previous step with that at the base point (Step 3) for all the parameters in order to recognize possible ellipsoid rotations and identify the parameter-eigenvalue connection required for the *ACE and E-AC criteria*:
    - if the principal directions are dominated by the same parameters in both cases, no rotation has occurred and the required parameter-eigenvalue association is identified,
    - otherwise, use the connection between parameters and eigenvalues obtained in Step 9;
11. Choose the bounds for the constraints on the correlation coefficients within the range identified by the bounds used for the PAC design in Step 8, $\varepsilon_{ij}^C|_{\text{PAC}}$ (lower bound) and the base point correlation coefficients $C_{ij}|_{bp}$ (upper bound):

$$\varepsilon_{ij}^{C}|_{\mathrm{PAC}} \le \varepsilon_{ij}^{C} \le C_{ij}^{2}|_{bp} \quad i,j = 1,...,p \text{ and } i \neq j \qquad (13)$$

12. If the AC-V (or the V-AC) criterion is adopted:
    - select the parameter/s for which the experiment is targeted (the parameter dominating the most uncertain principal direction is the best choice for the first experiment);
    - decide the desired predicted t-value for the selected parameter/s (approximately around or higher than the one/s obtained with the standard design) and then calculate the bounds for the constraints on each parameter variance from the following equation:

$$t_i = \frac{\hat{\theta}_i}{X_i(0.95)} \text{ with } X(\alpha) = t\left(\frac{1+\alpha}{2}, n-p\right) \cdot \sqrt{V_{ii}} \qquad (14)$$

    $\hat{\theta}_i$ is the current best estimate of the i-th parameter, α the probability level (typically $\alpha = 95\%$), n the number of measurement data available, p the number of parameters, and t the statistical Student's t-distribution;
13. If the ACE (or the E-AC) criterion is used:
    - choose the parameter/s for which the experiment is targeted according to the parameter-eigenvalue connection identified in Step 10;
    - for the ACE design, decide the bounds for the constraints on the eigenvalues within the range identified by the eigenvalues obtained with the PAC (lower bound) and the standard design (upper bound):

$$(\lambda_i - \lambda_i^0)|_{\mathrm{PAC}} \le \varepsilon_i^{\lambda} \le (\lambda_i - \lambda_i^0)|_{\mathrm{sd}} \quad i \in \{i,p\} \qquad (15)$$

14. Perform the experiment design and assess the results obtained using the predicted statistics, i.e., verify by means of the predicted t-values whether or not the expected precision of the parameters is sufficient for a satisfactory estimation.

Step 5 of the algorithm involves the choice of the correlation coefficients to be minimized in the objective function. Based on our experience, the best results in terms of convergence are obtained when the "problematic" correlation coefficients (the ones which can be reduced only with a significant worsening of the others) are minimized in the objective function and not bounded in the constraints. Therefore the recommended criterion requires the minimization in the objective function of those of the "problematic coefficients" identified in Step 2 which are not reduced in the standard design without causing a significant worsening of the others.

Step 7 of the algorithm addresses the choice of the bounds to be used in the PAC design; in case of complex models with a large number of correlation coefficients, the recommended criterion is to identify two–three levels of acceptable correlation (a medium and a low one at least) based on the correlation matrix at the base point. The medium bound ($\sim$ 50–60%) can be used to constraint the largest correlation coefficients not employed in the objective function and the low level ($\sim$ 10–20%) can be employed for the smallest correlations. It is worth noting however that in order to reduce the largest correlations to medium levels, it is very often necessary to adopt a less strict bound for the smallest coefficients. Allowing these coefficients to rise if necessary can make a reduction of the largest correlations easier and very often feasible. The upper bound indicated in (12) can therefore be exceeded sometimes for the smallest coefficients to ease convergence. The same observation is valid for point (11) of the algorithm.

Finally, the PAC design performed in Step 8 of the algorithm is also useful to verify whether the choice of the correlation coefficients to include in the objective function and in the constraints (based on the results of the E-optimal criterion, see Step 4) is valid for use in the calculations of the optimal experiments. If the criterion proves to be effective and successful in reducing the correlations below the desired levels, the choice made in Step 5 can be deemed suitable for the other AC designs.

The application to the bioreactor case study presented in the results section will show the importance of these preliminary analyses and the effectiveness of the proposed recommendations by discussing the main points of the algorithm in details. Before this illustrative part, the next sections will deal with some critical issues which can rise from the application of the novel AC approach to complex systems.

Structural/Nonstructural Correlations

The novel AC criteria aim at identifying regions of the design space where the parameters are as little correlated as possible. These methods therefore can reduce/eliminate the correlations caused by poor designs. However, if the parameter correlations are structural (i.e., due to the model equations), the novel criteria are unable to decrease them. As pointed out by several studies,[13,31,43] in these cases the correlations can be reduced only by using a reparameterization of the model. A typical example of such a situation is a system involving an Arrhenius equation: very often the correlations between pre-exponential factors and activation energies can be reduced only if the temperature is allowed to span a wide range, which may be unrealistically large for the vast majority of chemical reactions. The Arrhenius equation is a clear example of a structural correlation but, when complex kinetic networks or a large number of parameters are involved in a model, it is very likely that not all the correlations can be eliminated. Some of the correlation coefficients can be "problematic," that is they cannot be significantly reduced without causing a worsening in other coefficients. This situation too can be considered a structural problem since, due to the model structure, only a partial reduction of the "problematic" coefficients must be accepted and sought in the design. Only in this way, optimal experiments showing an improvement in all the parameter correlations can be obtained.

An easy way to look for potential parameter correlations in models is to calculate the information matrix and from it the correlation matrix (5) at different base points. Of course, this method is not comprehensive but can quickly provide an idea whether parameter correlations may represent a problem for the model under investigation. If this preliminary analysis shows parameter correlations to be significant, the second step is to identify whether these correlations are structural or not. The PAC designs performed during the preliminary analysis (as required by the proposed algorithm) allow structural correlations to be assumed when no convergence is reached

even with very loose constraints. However, a more structured approach than this trial and error procedure is highly desirable and will be part of our future research on this topic.

Ellipsoid Rotation

As mentioned earlier, the novel AC criteria exploit some concepts of principal component analysis[44–46] to target the optimal experiments to desired parameters. This aspect was explained and discussed in a previous article[26]; some basic principles are recalled here for the sake of clarity. From the inverse of the information matrix (the parameter variance–covariance matrix), a hyper-ellipsoid can be obtained in the parameter space which represents the true joint confidence region of the parameters. An eigenvalue–eigenvector decomposition of the information matrix allows the orientation of this ellipsoid with respect to the parameter axes to be determined (each eigenvector identifies a principal direction) and the relative lengths of the axes of the ellipsoid are given by the eigenvalues of **M**. If there are no correlations between the parameters, the confidence ellipsoid is parallel to the axes.[47] In such a situation, each of the principal directions of the information matrix (or of the variance–covariance matrix) is dominated by a specific parameter. This derives directly from the condition of parallelism: only one eigenvector component for each principal direction is significantly different from zero if the ellipsoid and the parameter axes are parallel. Therefore, if there are no correlations between the parameters, the principal component analysis of the information matrix can easily relate the parameters with the corresponding eigenvalues. In this way, maximizing an eigenvalue is equivalent to increase the information content of the corresponding parameter.

When the model structure prevents a complete elimination of the parameter correlations, at the end of the design the ellipsoid is not anymore parallel to the axes. This can influence the outcomes of the novel ACE and E-AC designs which, as shown in the previous section, are based on a parameter-eigenvalue association. Two main possibilities can be encountered as follows:

1. Each principal direction is dominated by a specific parameter (and this is the most likely case based on our experience because even the information matrix at the base point often reveals an evident parameter-eigenvalue connection);
2. Some principal directions can be controlled by two or more parameters approximately in an equal measure.

In the first case, the criteria can be formulated as mentioned in the introduction; in the second case, the design must work on the smallest eigenvalue of the principal directions which are controlled by the desired parameter. As the variance–covariance matrix is the inverse of the information matrix, the variance of a parameter is mainly due to the most uncertain principal direction of those controlled by the parameter.

A second aspect worth discussing is the choice of the eigenvalue/s to use in the ACE and E-AC criteria when the ellipsoid tends to rotate during the design (as in the bioreactor example considered here—see the results section). If the ellipsoid rotates, the connection between parameters and eigenvalues changes and so improving a certain principal direction can have a different result from the expected one. In such a case, the analysis of the information matrix at the base point is no longer sufficient for the eigenvalue choice. To identify if the model under investigation is subject to these ellipsoid rotations during the design, a comparison (in terms of eigenvalue–eigenvector analysis) between the optimal information matrix obtained after the PAC design and the same matrix at the base point is performed. If the PAC design causes an ellipsoid rotation, this may also happen when the other AC criteria are used because it is probably required to reduce the correlations. In such a case, the eigenvalue choice can be based on the optimal information matrix obtained after the PAC design.

In general, a completely random ellipsoid rotation between the various designs can also occur. This cannot be detected a priori but only after the design has been performed; in this case, three solutions may be adopted:

- To redesign the experiment using a different eigenvalue according to the new parameter-eigenvalue connection identified;
- To impose a constraint to prevent an ellipsoid rotation from changing the parameter-eigenvalue association shown at the base point. If the aim is to have parameter i dominating the principal direction j, it is sufficient to ensure that its relative importance in that direction is greater than 50%; the following constraint formulation can therefore be used: $imp_{ij} > \varepsilon_{ij}^{\text{imp}}$ with $\varepsilon_{ij}^{\text{imp}} \in [55,100]$. The closer the bound $\varepsilon_{ij}^{\text{imp}}$ is to 100, the more the principal direction j is dominated by the desired parameter but the more difficult convergence can be;
- To use a different criterion (AC-V design), which is completely independent of any eigenvalue.

ACE Vs. AC-V Design

When the correlations cannot be completely eliminated, the confidence ellipsoid is not anymore parallel to the parameter axes and ACE and AC-V criteria answer to different requirements. If the aim is to design an experiment specifically targeted at minimizing the uncertainty of one parameter, the AC-V design is the most appropriate tool and is much more immediate to employ than the ACE design since it requires only the preliminary analysis of the correlation coefficients (see the algorithm described earlier). On the other hand, the ACE design needs more effort initially because the association between parameters and eigenvalues must be identified and, in case of ellipsoid rotations, this can be not so direct (see previous section). This criterion is, however, very effective in designing experiments targeted at a desired parameter/s when this preliminary analysis is properly performed (see the examples reported in the results section). In addition, the ACE criterion is very suitable when all the parameters in the model need improvements. In this case, optimal experiments can be calculated (for example in a parallel approach) constraining a different eigenvalue in each of the designs, after having established how many experiments are to be performed (depending on the budget available). So, when all the parameters are to be improved, using this method instead of an AC-V criterion with a different parameter variance in each of the experiments should give better results. By improving the most uncertain principal directions, two advantages can be gained:

- The precision of the parameters which dominate those directions is enhanced and

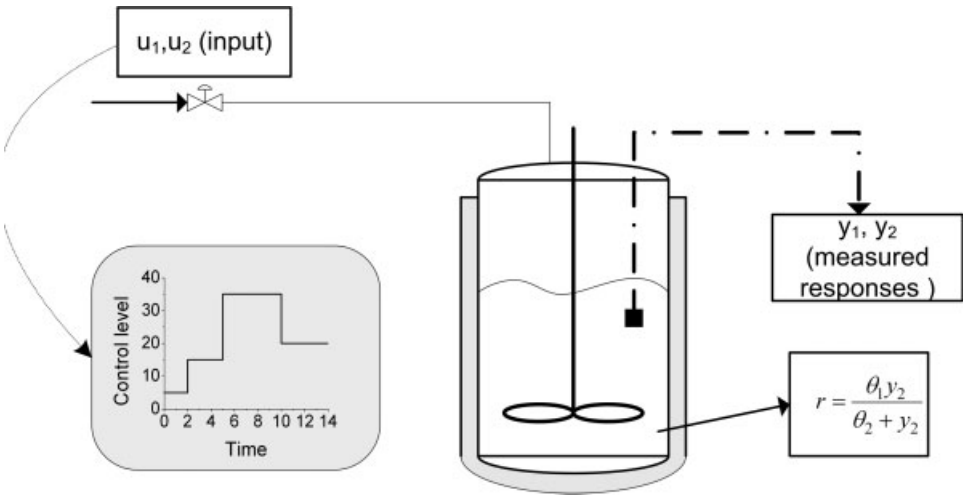

Figure 2. Scheme of the bioreactor used for the fermentation process.

• As a secondary effect, all the other parameters which influence even slightly the directions considered can achieve more reliable estimates.

To summarize, the ACE design is therefore recommended if the aim of the new set of experiments is to improve the estimation of all the parameters with a minimum number of experiments while the AC-V criterion is suitable to specifically improve the estimation of one or more specific parameters. The ACE design can also be used for the latter requirement but, in this case, a rigorous preliminary analysis of the system is required according to the steps indicated in the proposed algorithm.

Application to a Bioreactor Model

To illustrate the effectiveness and flexibility of the novel criteria when applied to a more complex model than the pedagogical, three-parameter epoxidation example employed in a previous publication,[26] a fermentation process is considered here as case study. The following procedure was adopted for the analysis of the case study: first, the optimal experiments were designed using the novel AC criteria and various possible design approaches (sequential, parallel, parallel-sequential) were tested. The required optimal experimental data were then simulated using the true values of the parameters (see later) and a normally distributed random error with a specified variance–covariance matrix (see later) was added to the simulated data to reproduce the uncertainty typical of real measurements. Finally, the parameters were estimated and their estimates statistically assessed. The results of these tests are reported in the following sections after a brief description of the example used.

Case Study

The bioreactor model used here as case study considers the fermentation stage of an intracellular process[48] as presented in Benabbas et al.[16] for the fermentation of baker's yeast. The model does not take into account the internal composition and structure of the cells (unstructured model) and assumes all the cells to be identical (unsegregated model). Only one key substrate is considered to be the limiting factor for growth and product formation; the product is an intracellular enzyme and nonviable cells are also formed. An isothermal operation of the fermenter and a feed free from the product are assumed. Figure 2 shows a schematic illustration of the bioreactor including the inlet stream, the fermenter body, and measured variables. Assuming Monod-type kinetics for biomass growth and substrate consumption, the system can be described by the following set of DAEs[48]:

$$\begin{aligned} \frac{dy_1}{dt} &= (r - u_1 - \theta_4) \cdot y_1 \\ \frac{dy_2}{dt} &= -\frac{r \cdot y_1}{\theta_3} + u_1(u_2 - y_2) \\ r &= \frac{\theta_1 y_2}{\theta_2 + y_2} \end{aligned} \tag{16}$$

where y_1 and y_2 are the biomass and substrate concentrations (g/l), u_1 the dilution factor (h^{-1}), and u_2 the substrate concentration in the feed (g/l). r is the micro-organism specific growth rate (g/(l·h)]) and θ_i are the unknown parameters of the model.

Analysis of the system

The experimental conditions that can be optimized during an experiment design are as follows:

• The initial biomass concentration (or inoculation), y_1^0, with range 1–10 g/l;

• The dilution factor, u_1, with range 0.05–0.2 h^{-1};

• The substrate concentration in the feed, u_2, with range 5–35 g/l;

• The sampling times for the two responses;

• The switching times for the two control variables.

The initial substrate concentration, y_2^0, is assumed equal to 0 g/l and cannot be manipulated for experiment design purposes. The two controls, u_1 and u_2, are modeled as piecewise-constant variables over five intervals and with different switching times.

As indicated in Figure 2, both state variables, y_1 and y_2, are measurable; the variance–covariance matrices for the experimental data are assumed to be diagonal (independent measurements) and a constant variance model is adopted:

Table 1. Initial Guess and Range for the Design Variables of the Bioreactor Case Study

Variable	Symbol	Initial Guess	Lower Bound	Upper Bound
Initial biomass concentration (g/l)	y_1^0	1	1	10
Sampling times (h)	t_{sp}	[1/7/15/20/30]	0.1	40
Switch time for first control (h)	τ_{sw1}	[1/6/10/20]	0.1	40
Switch time for second control (h)	τ_{sw2}	[1/6/10/20]	0.1	40
Dilution factor (h^{-1})	u_1	[0.05/0.05/0.05/0.05/0.05]	0.05	0.2
Feed substrate concentration (g/l)	u_2	[5/5/5/5/5]	5	35
Experiment duration* (h)	τ	30	0.1	40

*The initial guess of the experiment duration equals the initial guess of the last sampling time.

$$\Sigma_A = \begin{bmatrix} 0.01 & 0 \\ 0 & 0.05 \end{bmatrix} \text{and } \Sigma_B = \begin{bmatrix} 0.1 & 0 \\ 0 & 0.5 \end{bmatrix}. \quad (17)$$

Matrix $\Sigma_\mathbf{A}$ represents the case of a reliable and precise measurement system whereas matrix $\Sigma_\mathbf{B}$ simulates the more realistic situation of experimental data affected by high errors. In both cases, the experimental variances are assumed to be known a priori. The choice of independent measurements has no effect on the application of the algorithm: a diagonal matrix simply makes the calculation of the information matrix at each optimization step quicker.

As the number of sampling times is chosen equal to five (limited budget) and both responses are assumed to be measured at the same time, there are in total 24 design variables for this problem. The duration of the experiment which is defined by the last sampling time is chosen to be maximum 40 h. Table 1 summarizes the initial guesses and the ranges for all the variables involved in the design.

Some additional constraints are required for the sampling and switching times:

- The earliest time at which a measurement can be taken is equal to 1 h;
- The minimum time between consecutive measurements is again equal to 1 h;
- The minimum time between consecutive switching times is equal to 0.5 h.

The initial guesses of the parameters for the case of low-variance data (matrix $\Sigma_\mathbf{A}$) are taken from Galvanin et al.,[12] $\boldsymbol{\theta}_A$ = [0.357, 0.153, 0.633, 0.043], while for the case of unreliable data the following values are adopted: $\boldsymbol{\theta}_B$ = [0.62, 0.09, 1.0, 0.025]. The true values of the parameters used for simulating the optimal experimental data are assumed as in Galvanin et al.[12] equal to $\boldsymbol{\theta}_{true}$ = [0.31, 0.18, 0.55, 0.05].

Criteria assessment: test results

When the novel criteria briefly described in the introduction were applied to this case study, two different instances were tested:

1. Instance 1 corresponds to the ideal case of good initial guesses for the parameters ($\theta_\mathbf{A}$) and precise experimental data (matrix $\Sigma_\mathbf{A}$);
2. Instance 2 represents a more demanding application which is often encountered in practice. This case is characterized by poor quality data (matrix $\Sigma_\mathbf{B}$) and unreliable initial guesses for the parameters ($\theta_\mathbf{B}$). These values show a deviation from the true parameters of 100%; they were chosen all equidistant from the true values so that differences in the precision obtained after the estimation could not be ascribed to better or worse initial guesses.

This section presents the results obtained after the parameter estimation was carried out using the data from the (simulated) optimal experiments to assess the performance and effectiveness of the novel criteria in reducing correlations and selectively enhancing the information content of desired parameter/s, both in a sequential and in a parallel-sequential experiment design approach. For Instance 1, the first procedure was adopted with two experiments designed sequentially: the parameter values obtained after the first estimation were used as initial guesses for the second design iteration and, according to a standard sequential experiment design approach, the second estimation was performed using the data from both the optimal experiments. For Instance 2, a parallel-sequential procedure was employed. In the first design iteration, two experiments were planned in parallel and, after the estimation, the new parameter values were used to calculate a third and final experiment in the second design iteration (the aim was to see whether significant

Table 2. Eigenvalue–Eigenvector Analysis of the Information Matrix at the Base Point and After the PAC Design for Instance 2 (PD_1 is the First Principal Direction Which Corresponds To the Largest Eigenvalue)

		Base Point				PAC Optimal			
		PD_4 (λ_1)	PD_3 (λ_2)	PD_2 (λ_3)	PD_1 (λ_4)	PD_4 (λ_1)	PD_3 (λ_2)	PD_2 (λ_3)	PD_1 (λ_4)
λi		$0.28e^{-2}$	$0.43e^{-1}$	$0.102e^{2}$	$0.349e^{3}$	$0.117e^{2}$	$0.152e^{2}$	$0.297e^{2}$	$0.172e^{4}$
$\bar{e}_{ij}$	θ_1	−0.933	0.256	−0.252	$-0.253e^{-1}$	0.168	0.115	0.973	−0.11
	θ_2	−0.291	−0.125	0.944	$0.908e^{-1}$	0.974	−0.166	−0.143	$0.507e^{-1}$
	θ_3	0.202	0.919	0.209	−0.266	−0.136	−0.96	0.111	−0.224
	θ_4	$0.59e^{-1}$	0.274	−0.38e−1	0.959	$-0.63\ e^{-1}$	−0.2	0.143	0.967
imp_{ij}* (%)	θ_1	**87.08**	6.53	6.33	0.06	2.83	1.31	**94.66**	1.19
	θ_2	8.47	1.55	**89.15**	0.82	**94.92**	2.77	2.05	0.26
	θ_3	4.10	**84.42**	4.38	7.10	1.84	**91.91**	1.24	5.00
	θ_4	0.35	7.49	0.14	**92.02**	0.40	4.00	2.04	**93.55**

The entries in bold denote the largest relative importance in each direction.
*The subscripts i and j indicate, respectively, the parameters and the principal directions (PD).

Table 3. Objective Functions and Constraints Used in the Various Cases Tested for Instance 1

Design Iteration	Case	AC Criterion	Objective Function	Bounds on Correlation Coefficients*	Other Constraints
1	1	ACE	min $(C_{12}^2 + C_{34}^2)$	ε^C = [–, 0.01, 0.01, 0.01, 0.01, –]	$\lambda_1\lambda_1{}^0 > 750$, $\lambda_2\lambda_2{}^0 > 50{,}000$
	2	AC-V			$V_{22} < 0.00098$
	3	PAC			–
2	1	E-AC	max $\lambda_1{}^\dagger$	ε^C = [0.64, 0.115, 0.194, 0.032, 0.04, 0.722]	–‡
	2	AC-V	min $(C_{12}^2 + C_{34}^2)$	ε^C = [–, 0.35, 0.476, 0.01, 0.01, –]	$V_{22} < 0.00098$

*The bounds contained in the vector ε^C refer to the following correlation coefficients: C_{12}, C_{13}, C_{14}, C_{23}, C_{24}, and C_{34}.
†λ_1 is the smallest eigenvalue of the information matrix.
‡The symbol – indicates that no constraint on that particular correlation coefficient/eigenvalue/variance is used.

improvements in the precision of the estimates could be obtained with an additional experiment). Three experiments in total were therefore designed for each case of Instance 2.

Some designs were also performed for both instances using the standard criteria (E- and D-optimal) so as to compare their performance with that of the novel AC approach.

Preliminary analysis

According to the algorithm previously described, the preliminary steps required for the application of the AC approach include the following: an analysis of the correlation and information matrix at the base point, a design of experiments with a standard and with the PAC criterion, and an analysis of the correlation and information matrices obtained after these designs. This section briefly illustrates the conclusions drawn from these preliminary analyses for the case study investigated. The following base point correlation matrices were obtained for the two instances considered in the tests (OCL is the overall correlation level calculated according to Pritchard's metric):

$$\mathbf{C}|_{\text{inst.1}} = \begin{bmatrix} 1 & 0.9989 & -0.2025 & -0.2623 \\ & 1 & -0.2138 & -0.275 \\ & & 1 & 0.9948 \\ & & & 1 \end{bmatrix} \quad \text{OCL} = 0.608$$

$$\mathbf{C}|_{\text{inst.2}} = \begin{bmatrix} 1 & 0.985 & -0.207 & -0.22 \\ & 1 & 0.0395 & 0.05 \\ & & 1 & 0.99 \\ & & & 1 \end{bmatrix} \quad \text{OCL} = 0.58 \tag{18}$$

It can be seen that two pairs of parameters ($\theta_1 - \theta_2$ and $\theta_3 - \theta_4$) present the maximum degree of correlation while the remaining coefficients reveal a low interaction between the other parameters for both instances (that is C_{12} and C_{34} are the "problematic" coefficients).

To see whether a significant reduction in the correlations could be achieved, both an E-optimal and a PAC design were employed. The latter criterion was formulated with the following objective function and constraints for both instances:

$$\text{OF} = \min_{\boldsymbol{\varphi} \notin \boldsymbol{\Phi}} (\text{C}_{12}^2 + \text{C}_{34}^2)$$
$$\text{s.t. } C_{ij}^2 < 0.01 \text{ for all } ij \text{ pairs and with } C_{ij} \neq C_{12} \neq C_{34} \tag{19}$$

The following optimal correlation matrices were obtained for Instance 2 after the E-optimal and the PAC design (the results for Instance 1 are not reported because very similar):

$$\mathbf{C}|_{\text{E-opt.}}^{\text{inst.2}} = \begin{bmatrix} 1 & 0.603 & 0.909 & 0.96 \\ & 1 & 0.119 & -0.015 \\ & & 1 & 0.88 \\ & & & 1 \end{bmatrix} \quad \text{OCL} = 0.695$$

$$\mathbf{C}|_{\text{PAC}}^{\text{inst.2}} = \begin{bmatrix} 1 & 0.148 & -0.11 & 0.18 \\ & 1 & -0.018 & -0.199 \\ & & 1 & 0.848 \\ & & & 1 \end{bmatrix} \quad \text{OCL} = 0.371 \tag{20}$$

Table 4. Parameter Estimation Results for the E- and D-Optimal Designs (First and Second Iteration, Instance 1)

Design/Iteration	Parameter	Estimated Value	95% CI	95% *t*-Values	Correlation Matrix	χ^2-Test
E-optimal/iteration 1	θ_1	0.312	0.1181	2.64	$\begin{bmatrix} 1 & -0.946 & 0.999 & 0.999 \\ & 1 & -0.957 & -0.96 \\ & & 1 & 0.999 \\ & & & 1 \end{bmatrix}$	4.5474 vs. 12.592
	θ_2	0.199	0.2323	0.86*		
	θ_3	0.552	0.2445	2.26		
	θ_4	0.049	0.1197	0.41*		
D-optimal/iteration 1	θ_1	0.312	0.0189	16.5	$\begin{bmatrix} 1 & 0.907 & 0.881 & 0.813 \\ & 1 & -0.243 & -0.365 \\ & & 1 & 0.975 \\ & & & 1 \end{bmatrix}$	4.4013 vs. 12.592
	θ_2	0.191	0.8725	0.22*		
	θ_3	0.556	0.0318	17.5		
	θ_4	0.051	0.0078	6.59		
E-optimal/iteration 2	θ_1	0.312	0.0092	34.11	$\begin{bmatrix} 1 & 0.852 & 0.884 & 0.862 \\ & 1 & 0.277 & 0.349 \\ & & 1 & 0.963 \\ & & & 1 \end{bmatrix}$	10.95 vs. 26.296
	θ_2	0.195	0.0627	3.10		
	θ_3	0.553	0.0195	28.35		
	θ_4	0.0501	0.0042	11.84		

*Failed *t*-test (the reference *t*-values are 1.943 and 1.746 for the first and second iteration, respectively).

Table 5. Parameter Estimation Results for Case 1 (Instance 1, ACE and E-AC Design for First and Second Iteration, Respectively)

Iteration	Parameter	Estimated Value	95% CI	95% *t*-Values*	Correlation Matrix	χ^2-Test
1	θ_1	0.312	0.0072	43.43	$\begin{bmatrix} 1 & 0.743 & 0.283 & 0.298 \\ & 1 & -0.208 & -0.237 \\ & & 1 & 0.818 \\ & & & 1 \end{bmatrix}$	2.9831 vs. 12.592
	θ_2	0.221	0.1039	2.13		
	θ_3	0.534	0.0210	25.36		
	θ_4	0.046	0.0046	10.17		
2	θ_1	0.309	0.0028	112.21	$\begin{bmatrix} 1 & 0.619 & 0.457 & 0.407 \\ & 1 & -0.174 & -0.21 \\ & & 1 & 0.687 \\ & & & 1 \end{bmatrix}$	10.13 vs. 26.296
	θ_2	0.177	0.0270	6.56		
	θ_3	0.546	0.0090	60.62		
	θ_4	0.049	0.0020	24.93		

*The reference *t*-values are 1.943 and 1.746 for the first and second iteration respectively.

It can be seen that the standard design slightly reduces the correlations between the "problematic" coefficients but causes a significant increase in the correlation between θ_1 and the other two parameters. Therefore, at the optimal point, three pairs of parameters instead of two are highly correlated (between 0.88 and 0.96) and this is reflected in the increase shown in the OCL (0.695 vs. 0.58). For this reason, the PAC design minimizes both C_{12} and C_{34} in the objective function (according to the algorithm, these correlations cannot be reduced in the standard design without causing a worsening of the other coefficients and therefore it is better to minimize them in the objective function). The PAC design reduces all correlations significantly compared to the base point (C_{34} remains quite high but well below the 0.98 level of the initial situation). The OCL is 36% lower than at the base point.

These results show that for this particular case study the structure of the model makes it impossible to eliminate all the correlations simultaneously. They can, however, be individually eliminated; some tests were performed with only one correlation coefficient involved in the design and in these cases, a final value very close to zero was always obtained for the correlation considered. However, the elimination of the correlation between $\theta_1 - \theta_2$ and $\theta_3 - \theta_4$ causes all the remaining coefficients to show very high values (around 0.9) and so the OCL worsens significantly. This means that only a partial reduction of the problematic coefficients must be accepted and sought in the designs due to the model complexity because, only in this way, optimal experiments showing an improvement in all the parameter correlations can be obtained.

Beside the correlation matrix analysis, the other main steps in the algorithm involve the study of the information matrix with the aim of establishing a connection between parameters and eigenvalues for use in the ACE and E-AC designs. Table 2 presents the results of this preliminary analysis for Instance 2 of the bioreactor example (Instance 1 showed the same ellipsoid rotation); the eigenvalue–eigenvector analysis of the information matrices at the base-point and after the PAC design is reported (the relative importance of the various parameters is also shown). As it can be seen, the ellipsoid rotates significantly after the PAC design: only the first- and third-principal directions remain dominated by the same parameter as at the base point (θ_4 and θ_3, respectively). All the cases tested (see later) for the bioreactor example showed this same rotation; in this case, therefore, the analysis of the PAC-optimal information matrix is deemed sufficient to identify the desired parameter-eigenvalue association.

Instance 1

Table 3 contains a summary of the various cases tested and described in this section for Instance 1; the AC criteria and the formulations (objective function and constraints) adopted in all the design iterations are reported. The bounds used in the constraints were all chosen within the ranges identified in the proposed algorithm.

For the first design iteration (starting from the base point described earlier), both the ACE and the novel AC-V criteria were tested and their performance compared with that of the two most commonly used standard methods (E- and D-optimal). Table 4 presents the results obtained in the first itera-

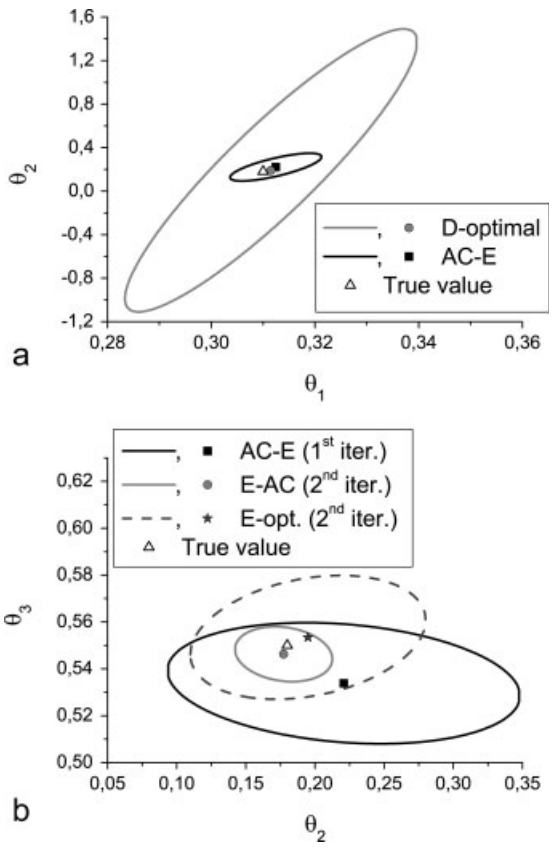

Figure 3. Confidence ellipses (Case 1, Instance 1).

(a) Comparison between D-optimal and ACE design (first iteration), ellipse $\theta_1 - \theta_2$; (b) Comparison between E-optimal (second iteration) and AC design (ACE in the first iteration and E-AC in the second one), ellipse $\theta_2 - \theta_3$.

Table 6. Parameter Estimation Results for Case 2 (Instance 1, AC-V Design for Both Iterations)

Iteration	Parameter	Estimated Value	95% CI	95% t-Values*	Correlation Matrix	χ^2-Test
1	θ_1	0.309	0.0116	26.60	$\begin{bmatrix} 1 & 0.67 & 0.041 & 0.067 \\ & 1 & -0.196 & -0.305 \\ & & 1 & 0.86 \\ & & & 1 \end{bmatrix}$	4.8051 vs. 12.592
	θ_2	0.183	0.0837	2.19		
	θ_3	0.551	0.0223	24.69		
	θ_4	0.049	0.0052	9.38		
2	θ_1	0.310	0.0028	109.20	$\begin{bmatrix} 1 & 0.417 & 0.163 & 0.285 \\ & 1 & 0.112 & -0.082 \\ & & 1 & 0.718 \\ & & & 1 \end{bmatrix}$	8.1334 vs. 26.296
	θ_2	0.179	0.0326	5.49		
	θ_3	0.551	0.0121	45.51		
	θ_4	0.050	0.0024	20.49		

*The reference t-values are 1.943 and 1.746 for the first and second iteration, respectively.

tion for these two traditional approaches. As it can be seen, the performances of the D- and E-optimal criteria differ significantly. The latter yields very poor results: the t-values obtained for all the estimates are very low and two parameters fail the test. In addition, all the correlations are very close to the maximum level (0.99) with three coefficients actually reaching this value. The predicted t-values (41, 4.3, 24, 5.3) expected all the parameters to be properly estimated but such high correlations are very likely responsible for the poor estimates actually obtained.

The D-optimal criterion performs better: only one parameter fails the test and the t-values of the other estimates are well above the reference value. The correlation levels are on average still high; with the exception of C_{23} and C_{24}, all the other coefficients are above the 0.8 threshold and the pair $\theta_3 - \theta_4$ exhibits a correlation (0.975) very close to the maximum value.

When the second iteration was tried, the D-optimal design showed convergence problems and no experiment could be obtained. On the other hand, the E-optimal criterion, although so poorly effective in the first design iteration, was successful and the new experiment significantly improved the estimates achieved after the first parameter estimation (see Table 4). It can be noted that all the parameters show satisfactory t-values and only one correlation coefficient (C_{34}) remains close to the maximum level. The correlations between $\theta_2 - \theta_3$ and $\theta_2 - \theta_4$ are significantly reduced whereas the other coefficients present lower values than in the first iteration, which are, however, still high.

All these results show that there is much room for improvement in the design of optimal experiments for this bioreactor example. In the following sections, the performance of the new criteria in the first design iteration is compared only with the results of the D-optimal as the E-optimal method worked so poorly. However, for the second design iteration, the E-optimal criterion is used as means of comparison (as the D-optimal experiment did not converge).

From the preliminary tests performed to understand the behavior of the system, θ_2 emerged as the most difficult parameter to estimate because it dominates the most uncertain principal direction and its corresponding eigenvalue is two orders of magnitude lower than the following one. The second principal direction (which corresponds to λ_2) is, however, responsible for almost the entire variance of the other three parameters (results not shown here). For this reason, Case 1 employed the ACE design in the first iteration with both eigenvalues bounded in the constraints ($\lambda_1 - \lambda_1^0 > 750$ and $\lambda_2 - \lambda_2^0 > 50{,}000$). In this way, an improvement in all the parameter estimates was expected. From the values of the bounds used, it can be noted that this design aims mainly at improving the estimation of θ_1, θ_3, and θ_4 but does not neglect Parameter 2. Table 5 reports the estimation results obtained after each design iteration.

It can be seen that the criterion performs as expected: all the parameters are estimated with sufficient precision with θ_1 in particular exhibiting the largest t-value obtained in all the tests. Thanks to the constraint on λ_1, parameter θ_2 is estimated with sufficient precision (none of the standard designs was able to yield a satisfactory t-value for this parameter).

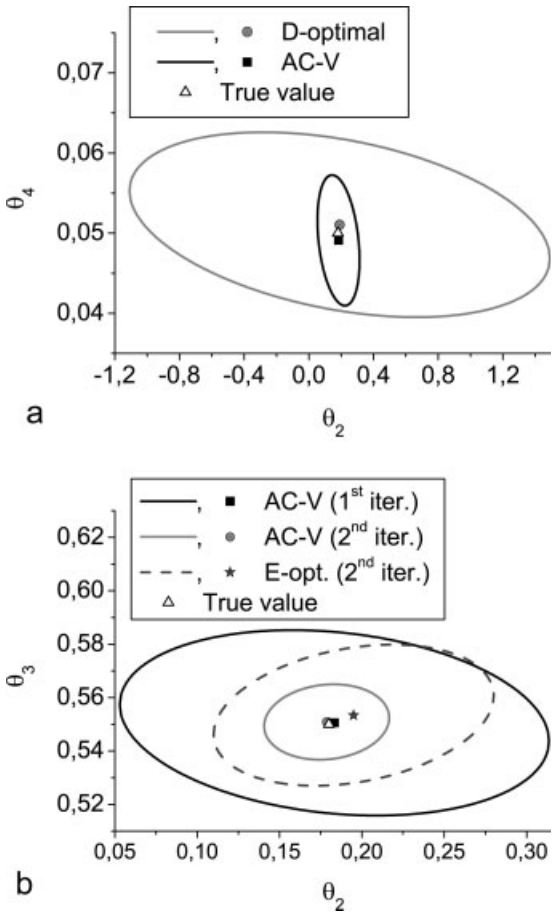

Figure 4. Confidence ellipses (Case 2, Instance 1).

(a) Comparison between D-optimal and AC-V design (first iteration), ellipse $\theta_2 - \theta_4$; (b) Comparison between E-optimal (second iteration) and AC-V design (first and second iteration), ellipse $\theta_2 - \theta_3$.

Table 7. Parameter Estimation Results for Case 3 (Instance 1, PAC Design Versus Pritchard's Criterion)

Design	Parameter	Estimated Value	95% CI	95% t-Values*	Correlation Matrix	χ^2-Test
PAC	θ_1	0.308	0.0089	34.59	$\begin{bmatrix} 1 & 0.268 & 0.509 & 0.514 \\ & 1 & -0.337 & -0.271 \\ & & 1 & 0.784 \\ & & & 1 \end{bmatrix}$	5.9905 vs. 12.592
	θ_2	0.149	0.1077	1.18*		
	θ_3	0.551	0.0239	23.09		
	θ_4	0.050	0.0085	5.93		
Pritchard	θ_1	0.280	0.0745	3.76	$\begin{bmatrix} 1 & 0.102 & 0.726 & 0.689 \\ & 1 & -0.574 & 0.642 \\ & & 1 & 0.984 \\ & & & 1 \end{bmatrix}$	4.9972 vs. 12.592
	θ_2	0.019	0.0148	0.13*		
	θ_3	0.599	0.2571	2.33		
	θ_4	0.066	0.0785	0.84*		

*Failed t-test (the reference t-values are 1.943 and 1.746 for the first and second iteration, respectively).

All the correlation coefficients present lower values than in the standard case, in particular, C_{34}, and this allows a better estimate to be obtained for all the parameters. All the confidence regions present much smaller areas compared with the D-optimal design as a result of the lower correlations and higher precisions achieved (see Figure 3a for an example of a confidence ellipse).

In the second iteration of Case 1, the E-AC criterion was adopted with the aim of maximizing the smallest eigenvalue (λ_1) as θ_2 was still the most uncertain parameter after the first estimation. As Table 5 shows, a 208% increase in the t-value of this parameter is obtained together with a very substantial improvement in the other estimates and a further reduction in the two largest correlation coefficients (C_{12} and C_{34}). Compared with the second iteration of the E-optimal design, the E-AC criterion shows a much better performance: all the parameters are less correlated and estimated with higher precision and this results in smaller confidence regions (Figure 3b).

Case 2 employed the novel AC-V criterion with the aim of improving the estimation of the most uncertain parameter θ_2 by directly reducing its variance. Besides the bounds on the correlation coefficients (see Table 3), a constraint on the variance of θ_2 is imposed requiring V_{22} to be below 0.00098 (which corresponds to a predicted t-value of 2, see Eq. 14). As it can be seen from Table 6 (Iteration 1), the criterion performs very effectively: the desired precision in the estimation of θ_2 is achieved and the correlation coefficients are significantly reduced compared to the standard case. The estimates of the other parameters are highly reliable and therefore very small confidence regions are obtained (Figure 4a). In the second iteration, the same criterion was adopted with another constraint on V_{22} as θ_2 was still the most uncertain parameter ($V_{22} < 0.00036$ which corresponds to a predicted t-value of 4). As Table 6 (Iteration 2) shows, the desired improvement in the precision of θ_2 is achieved; the correlation coefficients (C_{12} and C_{34} in particular) are further reduced and the estimation of the other parameters is enhanced thus yielding smaller confidence regions (Figure 4b).

As a final test for Instance 1, a comparison between the novel AC approach and a literature method is presented. As mentioned in the introduction, the only AC criterion for experiment design available in the literature and valid for models with more than two parameters is Pritchard's method.[31] As this criterion (Eq. 4) does not take into account the information content of the optimal experiment, its performance is compared here with that of the PAC design, which similarly aims only at reducing the correlations coefficients. Case 3 employed the PAC criterion to design an experiment starting from the base point described above and the results obtained are reported in Table 7, where they are compared with those achieved using Pritchard's metric (from the same base point).

As it can be seen, the PAC criterion performs much better than Pritchard's design. The estimates of the parameters obtained with the latter method are all very far from the true values (further than the initial guesses used for the design); this is evident in particular for the most uncertain parameter θ_2 (0.01946 instead of the true value 0.18). Two parameters fail the t-test (only one with the PAC criterion) and all the correlation coefficients except C_{12} result higher; C_{34} in particular is very close (0.98) to the maximum correlation level. The better performance of the PAC criterion can be immediately visualized by comparing the confidence ellipses (Figure 5), which all show much larger areas when Pritchard's design is used.

Instance 2

Instance 2 (characterized by unreliable initial guesses for the parameters and measurement data affected by high errors) was adopted to test the performance of the novel criteria in a parallel/sequential approach starting from the base point described above. Table 8 contains a summary of the various cases tested and described in this section; the AC criteria and the formulations (objective function and constraints)

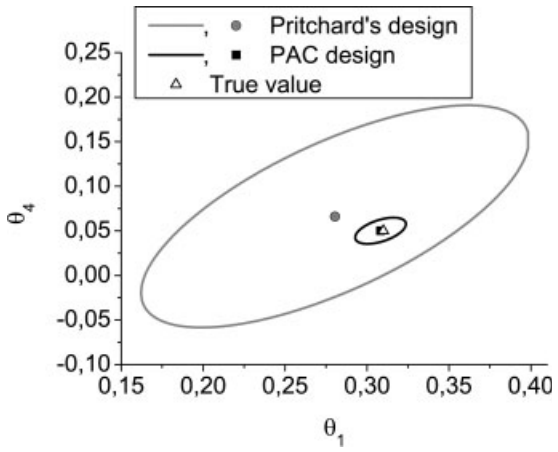

Figure 5. Confidence ellipses (Case 3, Instance 1).

Comparison between PAC design and Pritchard's criterion, ellipse $\theta_1 - \theta_4$.

Table 8. Objective Functions and Constraints Used in the Various Cases Tested for Instance 2 (Two Experiments Were Designed in Parallel in the First Design Iteration, Only One in the Second Iteration)

Design Iteration	Case	Exp.	AC Criterion	Objective Function	Bounds on Correlation Coefficients*	Other Constraints
1	4	1	ACE	min $(C_{12}^2 + C_{34}^2)$	ε^C = [–, 0.09, 0.09, 0.09, 0.09, –]	$\lambda_1\lambda_1^0 > 500$
		2	ACE			$\lambda_1\lambda_1^0 > 400$
						$\lambda_2\lambda_2^0 > 2500$
	5	1	AC-V			$V_{22} < 9.4e^{-4}$
		2	AC-V			$V_{44} < 8.5e^{-6}$
2	4		V-AC	min (V_{22})	ε^C = [0.25, 0.64, 0.49, 0.01, 0.01, 0.77]	–
	5		E-AC	max λ_2†	ε^C = [0.36, 0.42, 0.42, 0.01, 0.04, 0.49]	–‡

*The bounds contained in the vector ε^C refer to the following correlation coefficients: C_{12}, C_{13}, C_{14}, C_{23}, C_{24}, and C_{34}.
†λ_2 is the second smallest eigenvalue of the information matrix.
‡The symbol – indicates that no constraint on that particular correlation/eigenvalue/variance is used.

adopted in all the design iterations are reported. As before, the standard D- and E-optimal criteria are used as benchmarks to test the performance of the novel designs. Table 9 shows the results obtained with these traditional approaches after the first iteration (two parallel experiments). It can be noted that, in both cases, two parameters fail the *t*-test and the estimate of Parameter 2 is very far from the true value (0.18). Regarding the correlations, all the coefficients, except C_{23} and C_{24}, show very high values in the case of the E-optimal design (C_{34} is very close to the maximum level). With the D-optimal criterion, C_{23} and C_{24} worsen (but their value is still medium–low), C_{12} is significantly lower (-42%) whereas C_{34} is now equal to the maximum value of 0.99.

When the above estimates obtained from the parallel design were used to calculate an additional optimal experiment in the second design iteration, no convergence was reached with the D-optimal criterion whereas the E-optimal yielded the results reported in Table 9. It can be noted that the improvement in the precision of the estimates is quite poor; θ_2 and θ_4 still fail the test (the *t*-value is slightly better for θ_2 but unchanged for θ_4) and, regarding the correlations, C_{34} is reduced but now C_{13} and C_{14} are very high. The estimate of Parameter 2 is, however, closer to the true-value. As no convergence was obtained with the D-optimal design, the results of the E-optimal criterion will be used to assess the second iteration of the following cases.

As for this case study θ_2 is the most difficult parameter to estimate (as highlighted both by the cases of Instance 1 and by the standard designs described earlier), Case 4 employed the ACE criterion in both the experiments of the first iteration with λ_1 ($\lambda_1 - \lambda_1^0 > 500$) constrained in one of the parallel experiments and both λ_1 and λ_2 bounded in the other ($\lambda_1 - \lambda_1^0 > 400$ and $\lambda_2 - \lambda_2^0 > 2500$). Table 10 (Iteration 1) shows the results obtained and it can be seen that, compared with the standard designs, only one parameter, θ_2, now fails the *t*-test. All the other estimates present very satisfactory *t*-values (in particular Parameters 1 and 3) and much higher precision than in the D- or E-optimal case is achieved for Parameter 3. The design is much more effective than the standard criteria and reduces all the correlation coefficients to medium or low levels; the improvement in the confidence regions is substantial compared with both the standard designs (see Figure 6a for an example).

The second iteration tried to improve the estimation of Parameter 2 (the most uncertain) by employing the novel V-AC design with the variance of θ_2 as objective function to be minimised. As reported in Table 10 (iteration 2), the *t*-value of the desired parameter doubles but still results slightly insufficient; the precision obtained here is, however, the highest of the various tests performed for Instance 2. The correlation levels remain very similar to the previous iteration and a further improvement in the confidence regions, which are much smaller than in the E-optimal case, can be noted (Figure 6b).

As a final test, the novel AC-V criterion was employed for both the parallel experiments of the first iteration (Case 5); one experiment required $V_{22}<9e^{-4}$ (predicted *t*-value of 1.1) and the other $V_{44}<8.5e^{-6}$ (predicted *t*-value of 4). As

Table 9. Parameter Estimation Results for the E- and D-Optimal Designs (First and Second Iteration, Instance 2)

Design/Iteration	Parameter	Estimated Value	95% CI	95% *t*-Values	Correlation Matrix	χ^2-Test
E-optimal/iteration 1	θ_1	0.366	0.148	2.47	$\begin{bmatrix} 1 & 0.891 & 0.668 & 0.786 \\ & 1 & -0.052 & -0.157 \\ & & 1 & 0.987 \\ & & & 1 \end{bmatrix}$	6.9101 vs. 26.296
	θ_2	0.434	0.8033	0.54*		
	θ_3	0.52	0.0881	5.90		
	θ_4	0.035	0.038	0.91*		
D-optimal/iteration 1	θ_1	0.309	0.0377	8.17	$\begin{bmatrix} 1 & 0.521 & 0.872 & 0.86 \\ & 1 & -0.352 & -0.365 \\ & & 1 & 0.992 \\ & & & 1 \end{bmatrix}$	15.709 vs. 26.296
	θ_2	0.456	0.6868	0.66*		
	θ_3	0.508	0.1683	3.01		
	θ_4	0.0401	0.02994	1.37*		
E-optimal/iteration 2	θ_1	0.301	0.0394	7.64	$\begin{bmatrix} 1 & 0.669 & 0.945 & 0.93 \\ & 1 & 0.244 & -0.166 \\ & & 1 & 0.644 \\ & & & 1 \end{bmatrix}$	10.95 vs. 38.885
	θ_2	0.24	0.1709	1.41*		
	θ_3	0.521	0.0846	6.16		
	θ_4	0.034	0.0304	0.94*		

*Failed *t*-test (the reference *t*-values are 1.746 and 1.706 for the first and second iteration, respectively).

Table 10. Parameter Estimation Results for Case 4 (Instance 2, ACE and V-AC Design for First and Second Iteration, Respectively)

Iteration	Parameter	Estimated Value	95% CI	95% *t*-Values*	Correlation Matrix	χ^2-Test
1	θ_1	0.339	0.0289	10.27	$\begin{bmatrix} 1 & 0.692 & 0.41 & -0.27 \\ & 1 & 0.18 & -0.335 \\ & & 1 & 0.652 \\ & & & 1 \end{bmatrix}$	16.937 vs. 26.296
	θ_2	0.277	0.3501	0.79*		
	θ_3	0.548	0.0392	13.99		
	θ_4	0.046	0.0117	3.93		
2	θ_1	0.326	0.0971	16.24	$\begin{bmatrix} 1 & 0.568 & 0.519 & 0.164 \\ & 1 & 0.354 & -0.143 \\ & & 1 & 0.652 \\ & & & 1 \end{bmatrix}$	20.982 vs. 38.885
	θ_2	0.160	0.0201	1.65*		
	θ_3	0.549	0.0342	16.05		
	θ_4	0.051	0.0078	6.51		

*Failed *t*-test (the reference *t*-values are 1.746 and 1.706 for the first and second iteration, respectively).

Table 11 (Iteration 1) shows, the criterion performs as expected and the results obtained are quite similar to the previous case (Parameter 2 still fails the test) except for the lower precision of Parameter 3. The correlation levels result slightly higher than in the previous example with the exception of the problematic coefficient C_{12}, which is now reduced to a very small value (0.17). The confidence regions obtained are much smaller than in both the standard cases (Figure 7a).

To highlight the flexibility of the novel approach to target experiments to desired parameters, in the second iteration of Case 5, the E-AC criterion with λ_2 as objective function was employed (θ_1, θ_3, and θ_4 were the targets). From the results reported in Table 11 (Iteration 2), it can be seen that the precision of all the estimates (except θ_2 as expected) is very high (in particular that of Parameter 1 which now presents a *t*-value of 29). Most of the correlation coefficients are significantly reduced compared with the first iteration and much smaller confidence regions are achieved (Figure 7b). The confidence ellipses calculated after this second iteration present the smallest area obtained during the tests of the bioreactor example in the conditions simulated by Instance 2.

Criteria assessment: discussion on the results

The main objective of the novel design criteria is to reduce the correlations between the parameters as much as possible. As six-correlation coefficients are involved in this case study, Pritchard's scalar measure of correlation (Eq. 4) can be used to immediately assess whether the optimal experiments reduce or increase the OCL. Table 12 reports the values of this metric calculated for all the examples discussed in the previous two sections (the results of the standard criteria are included for comparison). It can be seen that in all the cases tested the novel criteria perform very effectively: the reduction in the correlation levels is very significant compared with the values obtained with the standard design. As highlighted during the preliminary analysis of the system, the structure of the model here used as case study prevents an easy decoupling of the parameters and so makes a complete elimination of all parameter correlations impossible. This characteristic is common to the vast majority of chemical process models and so makes the particular example here used a very important benchmark to test the effectiveness of the criteria.

The comparison between the novel and the standard designs (D- and E-optimal) showed how the AC criteria are able to improve significantly the precision of the estimates (much smaller confidence regions) thanks to the reduction in the parameter correlations and the capability of targeting the experiments so as to specifically improve the information content of desired parameters. The high flexibility of choosing which eigenvalue/s or parameter variance/s to bind in the constraints is a powerful tool, very suitable for any possible design procedure: sequential, parallel, and parallel-sequential, as demonstrated by the cases here reported.

The application of the novel AC approach requires a certain degree of preliminary analysis of the system under investigation in order to be able to fully exploit the high flexibility of the method. The "problematic" correlation coefficients, possible ellipsoid rotations, and the parameter-eigenvalue connection must be identified. The proposed algorithm contains a set of recommendations on how to perform these investigations and the results of the case study reported here

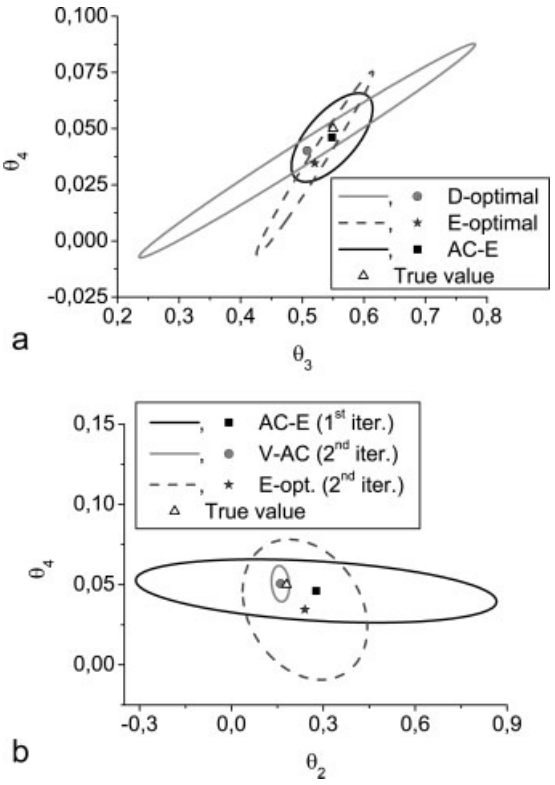

Figure 6. Confidence ellipses (Case 4, Instance 2).

(a) Comparison between D- and E-optimal and ACE design (first iteration), ellipse $\theta_3 - \theta_4$; (b) Comparison between E-optimal (second iteration) and AC design (ACE in the first iteration and V-AC in the second one), ellipse $\theta_2 - \theta_4$.

Table 11. Parameter Estimation Results for Case 5 (Instance 2, AC-V and E-AC Design for First and Second Iteration, Respectively)

Iteration	Parameter	Estimated Value	95% CI	95% *t*-Values*	Correlation Matrix	χ^2-Test
1	θ_1	0.305	0.0241	12.64	$\begin{bmatrix} 1 & 0.169 & 0.717 & 0.679 \\ & 1 & -0.0327 & -0.244 \\ & & 1 & 0.816 \\ & & & 1 \end{bmatrix}$	9.4898 vs. 26.296
	θ_2	0.227	0.2708	0.84*		
	θ_3	0.527	0.0949	5.55		
	θ_4	0.043	0.0103	4.17		
2	θ_1	0.311	0.0107	29.02	$\begin{bmatrix} 1 & 0.507 & 0.681 & 0.584 \\ & 1 & 0.466 & -0.335 \\ & & 1 & 0.687 \\ & & & 1 \end{bmatrix}$	16.015 vs. 38.885
	θ_2	0.193	0.1368	1.41*		
	θ_3	0.555	0.0282	19.67		
	θ_4	0.049	0.0064	7.70		

*Failed *t*-test (the reference *t*-values are 1.746 and 1.706 for the first and second iteration, respectively).

proved both the necessity of these preliminary steps and the usefulness of the suggested approach.

Conclusions

Because of the increasing importance of mathematical modeling in process engineering, there is a growing need of developing effective tools to help the engineer in the rapid building and especially validation of these models. One of the problems often encountered during this phase of model validation is the high correlation between the model parameters (especially between kinetic ones), which can make parameter identification extremely difficult (and sometimes impossible). This issue has been well known in the literature but very few articles have proposed a suitable experiment design procedure as a means of overcoming this problem. Based on our knowledge, none of the solutions proposed up to now is comparable with our novel AC approach, which has been introduced in a previous publication. This innovative method has been demonstrated capable of significantly reducing the nonstructural parameter correlations while maintaining the same aims of traditional design criteria (i.e., improved information content). This article has dealt with some issues which can rise from the application of this approach to complex models and has proposed an algorithm based on our experience to help a user in successfully exploiting the flexibility and effectiveness of the method. Unlike the standard experiment design criteria, the AC approach requires a certain degree of preliminary analysis and knowledge of the system structure. However, once these steps have been performed (according to the recommendations proposed), the available tool is much more powerful. The application of the novel approach to a fermentation model (often employed in the literature) proved all criteria very successful in reducing correlations and improving parameter precision and more effective than the standard experiment design methods (all confidence regions resulted much smaller). The comparison with Pritchard's method (the most valid of the experiment design criteria proposed in the literature for correlation reduction) highlighted the greater advantages and effectiveness of the proposed approach thanks to its high flexibility in the choice of objective functions and constraints. Moreover, the results shown in this article demonstrated how the AC criteria are very suitable for any experiment design procedure (sequential, parallel, etc.),

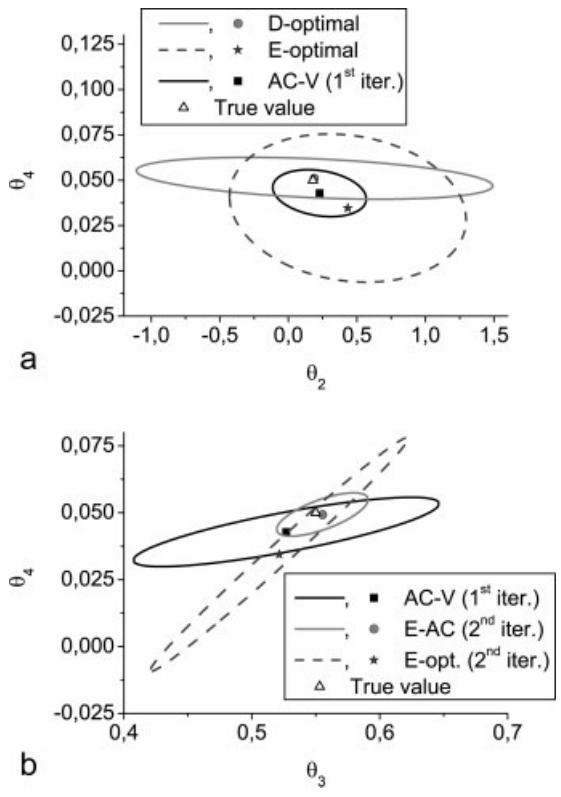

Figure 7. Confidence ellipses (Case 5, Instance 2).

(a) Comparison between D- and E-optimal and AC-V design (first iteration), ellipse θ_2 – θ_4; (b) Comparison between E-optimal (second iteration) and AC design (AC-V in the first iteration and E-AC in the second one), ellipse $\theta_3 - \theta_4$.

Table 12. Overall Correlation Level for the Examples Reported in the Previous Two Sections (NC Means That No Convergence Was Reached, While the Symbol-Indicates A Case Not Reported in This article)

Instance 1			Instance 2		
Case	Iteration 1	Iteration 2	Case	Iteration 1	Iteration 2
E-optimal	0.977	0.75	E-optimal	0.69	0.672
D-optimal	0.753	NC	D-optimal	0.708	NC
1	0.498	0.466	4	0.45	0.445
2	0.472	0.37	5	0.537	0.524
3	0.482	–			

thanks to their capability of targeting the optimal experiments and so enhancing in a selective and progressive way the precision of the parameters.

As demonstrated by the case study, many choices required for the experiment design are left to the modeler/experimenter, such as for example the number of samples to collect, the number of experiments to perform, the design procedure (parallel, sequential, etc.) or the number of responses to measure. Most of these decisions are dictated by considerations of economical nature and strongly influence the outcome of the design because of a trade-off between precision of the estimates and laboratory load.[24] An incorporation of some measures of cost or effort for the experimental and analytical work (both in terms of time and resources) could represent a significant improvement for the experiment design methodology and a first step towards an automatic procedure for saturating the degrees of freedom of the design. As illustrated by the algorithm, the novel AC approach requires a significant amount of preliminary analyses of the system. A set of recommendations have been proposed to help in this stage and this is another area where an automatic and rigorous procedure for choosing in particular the bounds for the constraints could be very beneficial in making the approach more user friendly. These topics will be addressed in our future research.

Literature Cited

1. Franceschini G, Macchietto S. Model-based design of experiments for parameter precision: state of the art. *Chem Eng Sci*. 2007. In press, DOI: 10.1016/j.ces.2007.11.034.
2. Zullo L. *Computer Aided Design of Experiments. An Engineering Approach, PhD Thesis.* UK: University of London, 1991.
3. Walter E, Pronzato L. Qualitative and quantitative experiment design for phenomenological models—a survey. *Automatica*. 1990;26:195–213.
4. Asprey SP, Macchietto S. Statistical tools for optimal dynamic model building. *Comput Chem Eng*. 2000;24:1261–1267.
5. Sidoli FR, Mantalaris A, Asprey SP. Modelling of mammalian cells and cell culture processes. *Cytotechnology*. 2004;44:27–46.
6. Bernaerts K, Servaes RD, Kooyman S, Versyck KJ, Van Impe JF. Optimal temperature input design for estimation of the square root model parameters: parameter accuracy and model validity restrictions. *Int J Food Microbiol*. 2002;73:145–157.
7. DiStefano JJ. Optimized blood sampling protocols and sequential design of kinetic experiments. *Am J Physiol*. 1981;9:259–265.
8. Ford I, Titterington DM, Wu CF. Inference and sequential design. *Biometrika*. 1985;72:545–551.
9. Hosten LH, Emig G. Sequential experimental design for precise parameter estimation in ordinary differential equations. *Chem Eng Sci*. 1975;30:1357–1364.
10. Walter E, Pronzato L. *Identification Of Parametric Models From Experimental Data*. Berlin: Springer, 1997.
11. Wu CF. Asymptotic inference from sequential design in a nonlinear situation. *Biometrika*. 1985;72:553–558.
12. Galvanin F, Macchietto S, Bezzo F. Model-based design of parallel experiments. *Ind Eng Chem Res*. 2007;46:871–882.
13. Asprey SP, Naka Y. Mathematical problems in fitting kinetic models—some new perspectives. *J Chem Eng Jpn*. 1999;32:328–337.
14. Bardow A, Marquardt W, Göke V, Koss HJ, Lucas K. Model-based measurement of diffusion using Raman spectroscopy. *AIChE J*. 2003;49:323–334.
15. Bauer I, Bock G, Korkel S, Schloder JP. Numerical methods for optimum experimental design in DAE systems. *J Comput Appl Math*. 2000;120:1–25.
16. Benabbas L, Asprey SP, Macchietto S. Curvature-based methods for designing optimally informative experiments in multiresponse nonlinear dynamic situations. *Ind Eng Chem Res*. 2005;44:7120–7131.
17. Bernaerts K, Van Impe JF. Optimal dynamic experiment design for estimation of microbial growth kinetics at sub-optimal temperatures: modes of implementation. *Simulat Model Pract Theory*. 2005;13:129–138.
18. Korkel S, Kostina E, Bock HG, Schloder JP. Numerical methods for optimal control problems in design of robust optimal experiments for nonlinear dynamic processes. *Optim Methods Softw*. 2004;19:327–338.
19. Lindner PFO, Hitzmann B. Experimental design for optimal parameter estimation of an enzyme kinetic process based on the analysis of the Fisher information matrix. *J Theor Biol*. 2006;238:111–123.
20. Nahor HB, Scheerlinck N, Van Impe JF, Nicolaï BM. Optimization of the temperature sensor position in a hot wire probe set up for estimation of the thermal properties of foods using optimal experimental design. *J Food Eng*. 2003;57:103–110.
21. Versyck KJ, Claes JE, Van Impe JF. Practical identification of unstructured growth kinetics by application of optimal experimental design. *Biotechnol Prog*. 1997;13:524–531.
22. Versyck KJ, Claes JE, Van Impe JF. Optimal experimental design for practical identification of unstructured growth models. *Math Comput Simulat*. 1998;46:621–629.
23. Versyck KJ, Van Impe JF. Trade-offs in design of fed-batch experiments for optimal estimation of biokinetic parameters. *Proceedings of the 1998 IEEE International Conference on Control Applications (Cat. No 98CH36104), Trieste, Italy*. 1998; 1: 51–55.
24. Franceschini G, Macchietto S. Validation of a model for biodiesel production through model-based experiment design. *Ind Eng Chem Res*. 2007;46:220–232.
25. Franceschini G. *New Formulations for Model-Based Experiment Design and Application to a Biodiesel Production Process, PhD Thesis*. UK: University of London, 2007.
26. Franceschini G, Macchietto S. Novel anti-correlation criteria for model-based experiment design: theory and formulations. *AIChE J*. 2008;54:109–1024.
27. Asprey SP, Macchietto S. Designing robust optimal dynamic experiments. *J Process Control*. 2002;12:545–556.
28. Franceschini G, Macchietto S. Anti-correlation approach to model-based experiment design: application to a biodiesel production process. *Ind Eng Chem Res*. 2008;47:2331–2348.
29. Cheng ZM, Yuan WK. Simultaneous estimation of kinetic and heat transfer parameters of a wall-cooled fixed-bed reactor. *Chem Eng Sci*. 1996;51:4791–4800.
30. Pritchard D, Bacon DW. Statistical assessment of chemical kinetic models. *Chem Eng Sci*. 1975;30:567–574.
31. Pritchard D, Bacon DW. Prospects for reducing correlations among parameter estimates in kinetic models. *Chem Eng Sci*. 1978;33:1539–1543.
32. Rippin DWT. Statistical methods for experimental planning in chemical engineering. *Comput Chem Eng*. 1988;12:109–116.
33. Agarwal A, Brisk ML. Sequential experiment design for precise parameter estimation, Part 1: Use of reparameterization. *Ind Eng Chem Process Des Dev*. 1985;24:203–207.
34. Dovi VG. Optimal experiment planning for environmental kinetic models subject to data heteroscedasticity. *Environmetrics*. 1997;8:303–311.
35. Dovi VG, Reverberi AP, Acevedo-Duarte L. New procedure for optimal design of sequential experiments in kinetic models. *Ind Eng Chem Res*. 1994;33:62–68.
36. Espie DM, Macchietto S. Nonlinear transformations for parameter-estimation. *Ind Eng Chem Res*. 1988;27:2175–2179.
37. Bernaerts K, Van Impe JF. Data-driven approaches to the modelling of bioprocesses. *Trans Inst Meas Control*. 2004;26:349–372.
38. Paterson W, Carberry JJ. Fixed bed-catalytic reactor modelling: the heat transfer problem. *Chem Eng Sci*. 1983;38:175–180.
39. Grijspeerdt K, Vanrolleghem P. Estimating the parameters of the Baranyi model for bacterial growth. *Food Microbiol*. 1999;16:593–605.
40. Agarwal A, Brisk ML. Sequential experiment design for precise parameter estimation, Part 2: Design criteria. *Ind Eng Chem Process Des Dev*. 1985;24:207–210.
41. Issanchou S, Cognet P, Cabassud M. Precise parameter estimation for chemical batch reactions in heterogeneous medium. *Chem Eng Sci*. 2003;58:1805–1813.

42. Perger T, Kovács T, Turányi T, Treviño C. Determination of adsorption and desorption parameters from ignition temperature measurements in catalytic combustion systems. *J Phys Chem B*. 2003;107:2262–2274.
43. Box GEP. Fitting empirical data. *Ann N Y Acad Sci*. 1960;86:792–816.
44. Saltelli E, Chan K, Scott EM. *Sensitivity Analysis, Hardcover edition*. New York: Wiley, 2000.
45. Turányi T. Sensitivity analysis of complex kinetic systems. Tools and applications. *J Math Chem*. 1990;5:203–248.
46. Vajda S, Valko P, Turányi T. Principal component analysis of kinetic models. *Int J Chem Kinet*. 1985;17:55–81.
47. Draper NR, Smith H. *Applied Regression Analysis*, 1st ed. New York: Wiley, 1966.
48. Nihtilä M, Virkkunen J. Practical identifiability of growth and substrate consumption models. *Biotechnol Bioeng*. 1977;19:1831–1850.

Manuscript received Aug. 2, 2007, and revision received July 11, 2008.