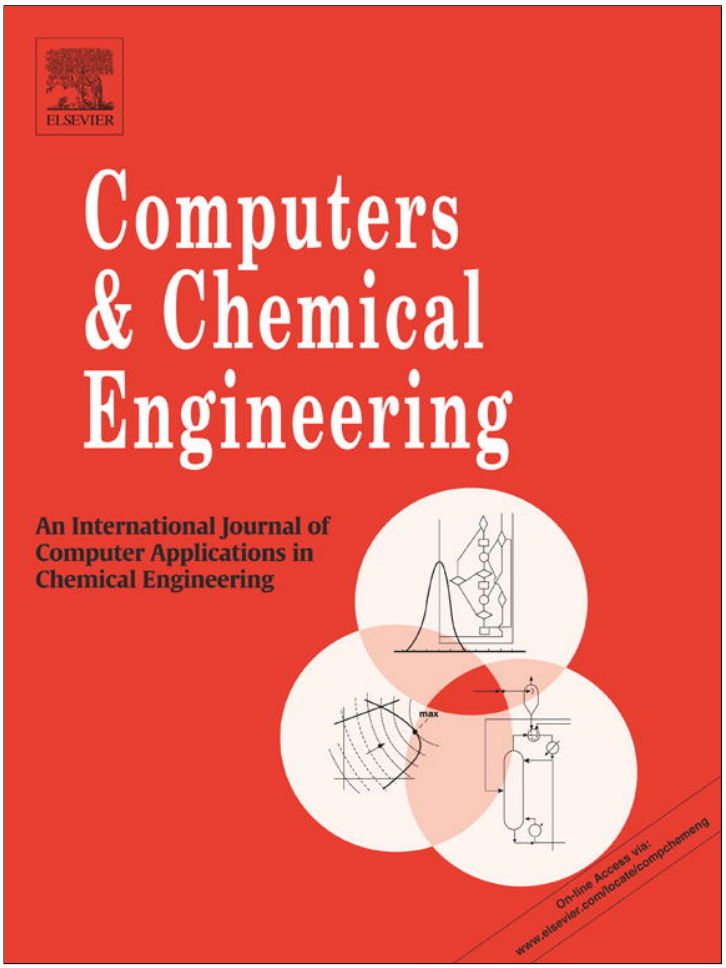

(This is a sample cover image for this issue. The actual cover is not yet available at this time.)

Computers and Chemical Engineering 49 (2013) 37–49

Contents lists available at SciVerse ScienceDirect

Computers and Chemical Engineering

journal homepage: www.elsevier.com/locate/compchemeng

Dynamic optimization of bioreactors using probabilistic tendency models and Bayesian active learning

Ernesto C. Martínez [b,*], Mariano D. Cristaldi [b], Ricardo J. Grau [a]

[a] INTEC (CONICET-UNL), Güemes 3450, Santa Fe 3000, Argentina
[b] INGAR (CONICET-UTN), Avellaneda 3657, Santa Fe S3002 GJC, Argentina

ARTICLE INFO

Article history:
Received 24 August 2011
Received in revised form 17 August 2012
Accepted 20 September 2012
Available online xxx

Keywords:
Bayesian inference
Experimental design
Fed-batch fermentation
Modeling for optimization
Run-to-run optimization
Sensitivity analysis

ABSTRACT

Due to the complexity of metabolic regulation, first-principles models of bioreactor dynamics typically have built-in errors (structural and parametric uncertainty) which give rise to the need for obtaining relevant data through experimental design in *modeling for optimization*. A run-to-run optimization strategy which integrates imperfect models with Bayesian active learning is proposed. Parameter distributions in a probabilistic model of bioreactor performance are re-estimated using data from experiments designed for maximizing information and performance. The proposed Bayesian decision-theoretic approach resorts to probabilistic tendency models that explicitly characterize their levels of confidence. Bootstrapping of parameter distributions is used to represent parametric uncertainty as histograms. The Bajpai & Reuss bioreactor model for penicillin production validated with industrial data is used as a representative case study. Run-to-run convergence to an improved policy is fast despite significant modeling errors as long as data are used to revise iteratively posterior distributions of the most influencing model parameters.

1. Introduction

Most bioprocess optimization techniques are model-based (De Tremblay, Perrier, Chavarie, & Archambault, 1993; Frahm, Lane, Märk, & Pörtner, 2003; Guthke & Knorre, 1981; Lim, Tayeb, Modak, & Bonte, 1986; Riascos & Pinto, 2004), and since accurate models are rarely available, experimental optimization of the operating policy is a difficult problem to be addressed for a successful scale-up. The best use of an imperfect first-principles model through proper handling of its inherent uncertainty is a challenging issue for fast productivity improvement of innovative fed-batch fermentations using data sampled from a small number of production runs. Bioreactors are engineered systems in which the activity of living cells is harnessed to produce an antibiotic, antibody, protein, a tissue or a host of other products of considerable impact on human life (Anesiadis, Cluett, & Mahadevan, 2008; Jain & Kumar, 2008; Ramkrishna, 2003). For maximum productivity, cells in a bioreactor must be maintained in an appropriate state of metabolic activity by tightly controlling conditions in the abiotic phase. The main problem in bioreactor modeling for optimization is that biological activity occurs in metabolic pathways which are controlled by switches through built-in regulatory networks (Geng & Yuan, 2010). Due to the complexity of metabolic regulation and limited measurements, first-principles models of bioreactor dynamics can only capture the qualitative tendency of sampled state variables such as biomass, substrate and product concentrations (Martínez, Cristaldi, & Grau, 2009; Tsobanakis, 1994). Hence, without biasing data gathering by increasingly improving the operating policy, bioreactor performance predictions are too uncertain and unreliable in quantitative terms to be useful for productivity optimization (Bonvin, 1998; Martínez & Wilson, 2003; Schenker & Agarwal, 1995). As a result, migration from laboratory conditions to production runs is often made with high levels of uncertainty about the degree of optimality of an operating policy (Terwiesch, 1995; Terwiesch & Agarwal, 1995). Consequently, a very conservative and sub-optimal operating policy is repeatedly applied to industrial bioreactors seeking reproducibility rather than improvement (Martínez & Wilson, 2003).

Run-to-run optimization of the operating policy for a fed-batch bioreactor using data gathered in production runs can be approached using two alternatives: (i) a systematic model-based iteration strategy, or (ii) a heuristic procedure using somehow past operating experience for modifying the policy directly. The heuristic optimization approach based on intuitively tweaking input profiles is very inefficient, often leads to sub-optimal solutions, and it cannot guarantee neither systematic performance improvement nor convergence to a near optimal policy. An interesting step in this direction has been proposed in Smets, Claes, November, Bastin, and Van Impe (2004) by starting from a model-derived operating policy and optimal profiles of the key state variables. Then, the optimal

* Corresponding author. Tel.: +54 342 4534451; fax: +54 342 4553439.
E-mail address: ecmarti@santafe-conicet.gov.ar (E.C. Martínez).

0098-1354/$ – see front matter © 2012 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.compchemeng.2012.09.010

Nomenclature

F_{in}	Inlet flow rate ($L\,h^{-1}$)
F_{evap}	outlet flow rate due evaporation ($L\,h^{-1}$)
J	performance index
$\boldsymbol{m}(t)$	time-dependent control variables.
$p(\theta_i)$	*prior* distribution of the *i*th model parameter.
$p(\boldsymbol{\theta}\|\boldsymbol{x})$	*posterior* probability distribution for parameters.
P	penicillin concentration (as potassium salt) (g PenGK L^{-1})
Q	global sensitivity matrix
S	substrate concentration ($g\,L^{-1}$)
t	time (h)
t_f	final time of an experimental run (h)
$\mathbf{t}^{sp}$	vector of sampling times in an evaluation experiment
V	culture broth volume (L)
$\boldsymbol{x}(t)$	vector of state variables
$\boldsymbol{x}(t_i)$	bioreactor sampled state vector at a given time.
X	biomass concentration (g-DW L^{-1})
X_d	death biomass concentration (g-DW L^{-1})
X_v	viable biomass concentration (g-DW L^{-1})
u	utility function
$\boldsymbol{w}$	time-invariant control variables

Parameters

A	feeding profile parameter ($L\,h^{-2}$)
B	feeding profile parameter (h^{-1})
C	feeding profile parameter (h^{-2})
T_{feed}	initial time for fed-batch operation (h)

Greek symbols

Θ	feasible set of model parameters
$\boldsymbol{\beta}$	set of parameters describing time-varying inputs
$\boldsymbol{\varphi}$	vector of operating policy parameters
μ	specific biomass growth rate (h^{-1})

solution is implemented in the form of a model-independent sub-optimal strategy by using a modified (semi-empirical) control function, which includes reduced terms based on heuristic observations. More effective, though, is designing dynamic experiments to extract useful information from policy evaluation runs. In this way, the operating policy is improved by introducing relevant data for optimization in an imperfect model. This approach does not rely on expert knowledge, but requires to model available data carefully. For model-based policy optimization to be successful it is mandatory to re-estimate selectively the more sensitive model parameters using optimal experimental design techniques in data gathering (Martínez et al., 2009).

An approach for model-based heuristic optimization of operating policies has been proposed in Maria (2004, 2007) and successfully applied to D-glucose oxidation. This author argues that, by using reduced order (low complexity) bioreactor models and through semi-empirical optimal control functions, it is possible to lower computational costs and experimental efforts necessary to identify and verify all model parameters and reaction steps under a wide range of operating conditions and at different time scales. The reduced order model is based on a simplified enzymatic kinetics, requires a small number of on-line measurements for model update and a few parameters are used to adjust the control function. The solution found is implemented in the form of a model-independent sub-optimal strategy based on a control function selected from a library. However, the heuristic optimization approach is highly problem-dependent (*e.g.*, enzyme oxidation) since it mostly relies on an intricate understanding of the characteristics of the bioprocess behavior and human judgment for defining an improved policy while addressing the dilemma of knowledge exploitation *versus* exploring untried operating conditions. This dilemma is at the very heart of modeling for optimization with imperfect models. When a reduced order model is used for policy improvement you cannot improve its parametric precision comprehensively. Thus, the model is only a means to find better policies at the cost of biasing data gathering in the most profitable region of operating conditions. Lacking a conceptual framework for policy optimization, generalization and incorporation of uncertainty into the decision-making process, the heuristic optimization approach is costly in terms of both time and money. Expert knowledge can be difficult to obtain, expensive, or is simply not available. Moreover, no systematic reduction of model uncertainty is made as more experimental data is available which prevents guaranteeing steady policy improvement and convergence toward a near-optimal solution.

In the attempt to compensate for a significant process-model mismatch, optimal operation under uncertainty requires using measurements from carefully designed experiments to improve on a run-to-run basis from a cautious (sub-optimal) policy. This model-based policy optimization approach consists of iteratively using new measurements to increasingly reduce parametric uncertainty in a tendency (imperfect) model and later resorting to the updated model for policy improvement (Martínez et al., 2009). A "tendency model" is a low order, nonlinear, dynamic model that approximates the stoichiometry and kinetic relationships of a bioprocess using the available plant data along with fundamental knowledge of the process characteristics (Bonvin & Rippin, 1990; Filippi, Bordet, Villermaux, Marchal-Brassey, & Georgakis, 1989; Fotopoulos, Georgakis, & Stenger, 1998; Georgakis, 1995; Uhlemann, Cabassud, LeLann, Borredon, & Cassamatta, 1994). Operating policies based on over-confident first-principles models often fail to yield productivity improvement due to a lack of parametric precision and structural errors.

For Bayesian optimization with tendency models, not only a bioreactor model for policy improvement is required, but it is also important that the model faithfully describes its own accuracy to treat uncertainties in a principled way. Humans do something similar: as it is argued in (Körding & Wolpert, 2004, 2006), whenever humans have only little experience, they employ an internal forward model for predictions and average over the uncertainty when extrapolating and making decisions. The essential characteristic of Bayesian methods is their explicit use of probability theory for quantifying uncertainty in inferences based on statistical data analysis. Without any notion of uncertainty, the model-optimized policy would be too confident and claims exact knowledge, which it actually does not have. Representation and incorporation of model uncertainty in run-to-run optimization is particularly important in the early stages of bioprocess scale-up when the available data set is very sparse and has been obtained for a wide range of operating conditions. For Bayesian optimization of bioreactors, the novel concept of a *probabilistic tendency model* that integrates first-principles and constitutive laws with probability distributions for describing parametric uncertainty is proposed.

In this work, a general and fully Bayesian decision-theoretic framework for policy optimization in innovative bioprocesses is presented. In the case of only few production runs with a full-scale bioreactor, the problem of dealing with fairly limited experience to improve the policy is successfully addressed using Bayesian active learning. In Bayesian inference, scarce experimental data are used to learn a probabilistic model of a bioreactor dynamics by updating parameter distributions. Probabilistic tendency models are able to represent and to quantify their own uncertainty for safe generalization of available experience to untried operating conditions. Thus, uncertainty is explicitly accounted for in run-to-run

optimization with imperfect models in a natural way. Model-based Bayesian optimization does not rely on expert knowledge and can readily be applied to innovative bioprocesses by simply assuming initially wide intervals of uncertainty in model parameters. In a probabilistic tendency model this uncertainty gives rise to probability distributions over plausible performance values for a given operating policy and sample predictions are thus based on averaging performance using these distributions. Posterior distributions are selectively revised upon data using bootstrapping and a utility function that trades off exploration and exploitation in biasing data gathering toward the more profitable region of operating conditions. The role of Bayesian optimal design of dynamic experiments in modeling for optimization is highlighted in order to obtain the most informative data for significant productivity improvement using a minimum of policy evaluation runs.

2. Bayesian decision-theoretic framework

Experimental design for run-to-run optimization using an imperfect model must be based on active learning with sampled data. Bayesian active learning can be seen as a strategy for optimal data gathering so as to make run-to-run optimization more efficient. Active learning is a concept very similar to sequential experimental design (Blot & Meeter, 1973). In the latter, there is available a set of experiments which may be conducted. After each observation, a decision is made as to which experiment to conduct in the next stage in order to obtain relevant data. Since some sequences of experiments may be more "informative" than others, there is a potential saving in sequential optimization of data gathering strategies for model building. In the Bayesian framework, sampling times are selected using as a guideline a utility function that pursues a balance between performance improvement and information gain from an experiment. Before running an actual experiment, these gains are uncertain. Hence, for active learning, at each policy evaluation run, the expected objective function is obtained by averaging over possible outcomes using global sensitivity analysis (GSA). Solely maximizing the expected information gain tends to select sampling times in an evaluation run which are far away from the ones used in previous experiments. Information content alone is unduly sub-optimal and often risky, hence a trade-off between exploitation of what is already known and exploration of unknown operating conditions is proposed here to deal with uncertainty.

In run-to-run optimization, it is assumed that the bioreactor dynamics is modeled using a number of state variables $\boldsymbol{x}(t)$ whereas the value of a performance index $J(t)$ can be sampled at selected times; for the sake of simplicity the dependence of $J(t)$ on other variables is implicitly assumed. Also, it is assumed that the tendency model is made up of a set of differential–algebraic equations with uncertain parameters $\boldsymbol{\theta} \in \Theta$ of the form

$$f(\dot{\boldsymbol{x}}, \boldsymbol{x}, \boldsymbol{m}(t), \boldsymbol{w}, \boldsymbol{\theta}, t) = \mathbf{0} \tag{1}$$

with given initial conditions $\boldsymbol{x}(t_0) = \boldsymbol{x}_0$, whereas $\boldsymbol{m}(t)$ and $\boldsymbol{w}$ are, respectively, the time-dependent and time-invariant control variables (manipulated inputs), Θ is the feasible space of model parameters with given *prior* distributions $p(\theta_i)$, $i = 1, \ldots, k$, and t is time. Hereafter, it is also implicitly assumed that, at least locally, the tendency model with a probability density function $p(\boldsymbol{\theta}) = \prod_i p(\theta_i)$ is able to approximate the observed bioreactor performance in the most profitable region of operating conditions by shrinking parametric uncertainty toward a given set of model parameters as more policy evaluation runs are made.

The productivity index to be maximized is estimated at *certain* times over a run based on sampled data $\boldsymbol{x}(t_i)$ for bioreactor states at specific times t_i, $i = 1, \ldots, n$, when a given policy is evaluated

$$\boldsymbol{J} = [J(t_1), \ldots, J(t_n)]^{\boldsymbol{T}} \tag{2}$$

where the formula for calculating each entry $J(t_i)$ in $\boldsymbol{J}$ mainly accounts for end-product value and downstream processing costs, and implicitly includes measurement errors of states in data sampled in an experiment. The observable performance $\boldsymbol{J}(\boldsymbol{x})$ depends on sampled data $\boldsymbol{x}$ over a run which in turn are the result of manipulated inputs to the bioreactor and sampling times. Control vector parameterization techniques are used to describe control input profiles $\boldsymbol{m}(t)$. These control profiles can be piecewise constant, piecewise linear, polynomials or splines over intervals and are parametrized accordingly. Thus, it is assumed that $\boldsymbol{m}(t) = \xi(t; \boldsymbol{\beta})$ is an m-dimensional vector of input variables whose time profiles are defined by the set of parameters $\boldsymbol{\beta}$.

Model-based optimization aims at improving on a run-to-run basis the productivity index $J(t_f)$ by acting on the following design vector for the operating policy defined as follows:

$$\boldsymbol{\varphi} = (\boldsymbol{\chi}_0, \boldsymbol{\beta}, \boldsymbol{w}, \mathbf{t}^{\mathrm{sp}}, t_f) \tag{3}$$

where $\boldsymbol{\chi}_0$ is the set of initial conditions of the bioreactor and t_f is the duration of a production run. The set of time instants at which the output variables are sampled is a design variable itself, and is expressed through the vector $\mathbf{t}^{\mathrm{sp}} = [t_1, \ldots, t_n]^T$ of n sampling times. The number n of samples taken in a run is fixed and defined as the minimum number of samples required to make the tendency model identifiable. It is worth noting that the vector of performance observations over a run, $\boldsymbol{J}$, is dependent on the chosen $\mathbf{t}^{\mathrm{sp}}$. Thus, in order to improve the policy parameters in $\boldsymbol{\varphi}$, sampling times must be carefully selected so that the performance prediction mismatch can be reduced on a run-to-run basis. To this aim, an appropriate time schedule for sampling must be defined based on the sensitivity of performance predictions as model-optimized policies are implemented (evaluated).

The basic ingredients of the Bayesian decision framework (Chaloner & Verdinelli, 1995; Lindley, 1972; Verdinelli & Kadane, 1992) are the experiment design space $\boldsymbol{\varphi} \in \Phi$, a probabilistic model $p_\varphi(\boldsymbol{\theta}, \boldsymbol{x})$ for all relevant random variables, including parameters $\boldsymbol{\theta} \in \Theta$, and the data set $\boldsymbol{x}$ sampled from an experimental output space Ω, and a posterior decision space $d \in D$ for using sampled data to revise the posterior density for model parameters. Also, the *utility* function $u(\boldsymbol{\varphi}, \boldsymbol{\theta}, \boldsymbol{x}, d)$ is used to quantify preferences for alternative experiment outcomes and assumed parameter values $\boldsymbol{\theta}$ under alternative experimental designs $\boldsymbol{\varphi}$ and alternative posterior decisions d. The probabilistic model can be factored into a prior distribution for tendency model parameters $p(\boldsymbol{\theta})$ and a sampling model $p_\varphi(\boldsymbol{x}|\boldsymbol{\theta})$ for state predictions at sampling times in $\mathbf{t}^{\mathrm{sp}}$. It is worth noting that this probabilistic model is made dependent on the chosen experimental design $\boldsymbol{\varphi}$ to highlight the decisive influence of sampled data on the optimality of the operating policy. Since the design decision $\boldsymbol{\varphi}$ has to be made before the actual experiment has produced any new data $\boldsymbol{x}$ for the posterior distribution of $\boldsymbol{\theta}$, the expectation of $u(\cdot)$ with respect to $(\boldsymbol{\theta}, \boldsymbol{x})$ should be maximized in two steps.

While the logical procedure for experimental design followed by inference/decision making proceeds in time order, utility function optimization is easier to solve in reverse time order. The posterior stage decision involves finding the best update of the probabilistic model given the observed data $\boldsymbol{x}$ that maximizes the *posterior* expected utility for the chosen experimental design

$$U(\boldsymbol{\varphi}, \boldsymbol{x}) = \max_d \int_\Theta u(\boldsymbol{\varphi}, \boldsymbol{\theta}, \boldsymbol{x}, d) p_\varphi(\boldsymbol{\theta}|\boldsymbol{x}) d\boldsymbol{\theta} \tag{4}$$

where the expectation or averaging over Θ accounts for the uncertainty regarding the unknown $\boldsymbol{\theta}$. The expectation is taken with respect to the posterior distribution of $\boldsymbol{\theta}$, which properly reflects the remaining parametric uncertainty for each alternative decision d on how data gathered in the experiment $\boldsymbol{\varphi}$ is used to modify the prior distribution $p(\boldsymbol{\theta})$ in the probabilistic model.

As the operating policy $\boldsymbol{\varphi}$ must be specified before any new data is observed, the second stage in policy optimization involves finding the best experiment $\boldsymbol{\varphi}^*$ that maximizes the *a priori* expected utility which is obtained by integrating the result in Eq. (4) over possible experiment outcomes $\boldsymbol{x}$ in the sample space $\boldsymbol{\Omega}$ of a dynamic experiment

$$U(\varphi) = \int_{\Omega} U(\boldsymbol{\varphi}, \boldsymbol{x}) p(\boldsymbol{x}|\boldsymbol{\varphi}) d\boldsymbol{x} = \int_{\Theta} \int_{\Omega} U(\boldsymbol{\varphi}, \boldsymbol{x}) p_{\varphi}(\boldsymbol{x}|\boldsymbol{\theta}) p(\boldsymbol{\theta}) d\boldsymbol{\theta}\, d\boldsymbol{x} \quad (5)$$

The integral in Eq. (5) is defined with respect to $p(\boldsymbol{x}|\boldsymbol{\varphi})$, the marginal distribution of data sampled in the experiment $\boldsymbol{\varphi}$, which is obtained by integrating $p_{\varphi}(\boldsymbol{x}|\boldsymbol{\theta})$ over possible prior values for $\boldsymbol{\theta}$, described by the prior distribution $p(\boldsymbol{\theta})$.

The Bayesian optimal experimental design using a probabilistic tendency model $p_{\varphi}(\boldsymbol{\theta}, \boldsymbol{x})$ can be formally stated as

$$U(\varphi^*) = \max_{\varphi} \int_{\Omega} \max_{d} \int_{\Theta} U(\boldsymbol{\varphi}, \boldsymbol{x}) p_{\varphi}(\boldsymbol{x}|\boldsymbol{\theta}) p(\boldsymbol{\theta}) d\boldsymbol{\theta}\, d\boldsymbol{x} \quad (6)$$

Since in modeling for optimization the information content in new data is useful as long as productivity can be improved, the expected utility function to be maximized must combine performance outcome and information gain as follows:

$$U(\varphi) = \int \int \left[\omega p(\boldsymbol{\theta}) J(t_f|\boldsymbol{\theta}) + \delta p(\boldsymbol{\theta}|\boldsymbol{x}) \ln \frac{p(\boldsymbol{\theta}|\boldsymbol{x})}{p(\boldsymbol{\theta})} \right] d\boldsymbol{\theta}\, d\boldsymbol{x} \quad (7)$$

where $p(\theta|\boldsymbol{x})$ is the *posterior* probability distribution for parameters in the probabilistic tendency model. The values of the weights ω and δ express the relative contribution of exploiting the model for improving the policy (first term in the bracket) and seeking more information to profit from (exploration), respectively. It is worth mentioning that the second term in the bracket of Eq. (7) is the *Kullback–Leibler* (KL) "distance" between the prior $p(\boldsymbol{\theta})$ and the posterior $p(\theta|\boldsymbol{x})$ distributions and measures the novelty, or *interestingness*, of the information provided by sampled data to be obtained in the next evaluation run. Since this distance between both distributions directly measures the performance prediction mismatch, data that increases the KL distance allows exploring apparently sub-optimal policies. By seeking to explore only the most profitable space of operating conditions put training inputs for the probabilistic model in the most relevant part of the state space. As more data are sampled in this subspace of improved operating conditions the prior $p(\boldsymbol{\theta})$ tends to the posterior $p(\theta|\boldsymbol{x})$ which decreases exploration and then exploitation (model-based optimization) is emphasized. However, as soon as the prior and posterior distributions become almost identical, no further performance improvements are possible and policy iteration converges. By resorting to Bayesian active learning, the model-based policy iteration strategy in the next section is a systematic approach to the design of a rather short sequence of optimally informative experiments to explore safely operating conditions while information gained provides a direction for improvement. Later on, by exploiting data gathered, convergence to an operating policy $\boldsymbol{\varphi}$ which is optimal, at least locally, is achieved.

Model-based run-to-run optimization

1: *Policy evaluation* ▷ Exploratory run.
2: *Model initialization* ▷ Define priors $p(\boldsymbol{\theta})$ for parameter distributions.
3: **Loop**
4: *Model-based policy optimization* ▷ Exploiting knowledge .
5: *Optimal sampling* ▷ Maximize information gain.
6: *Policy evaluation* ▷ Data gathering.
7: *Performance sensitivity analysis* ▷ Modeling bias.
8: *Probabilistic model update* ▷ Bootstrapping.
9: **End Loop**

Fig. 1. High-level description of the run-to-run optimization strategy.

3. Run-to-run optimization

3.1. High-level description

A high-level description of the proposed model-based strategy for run-to-run optimization is given in Fig. 1. It is important to highlight that the activity called *policy evaluation* corresponds to the actual running of a designed experimental run whereas other activities such as *experimental design*, *performance sensitivity analysis* and *model update* are entirely based on model simulations. The operating policy is first initialized by resorting to expert judgment, if any, and/or *a priori* knowledge from lab scale to avoid undesirable physiological states. Samples are taken along this experiment so as to make a rough estimation of probability distributions or histograms for parameters in the tendency model. Equipped with a probabilistic model which explicitly addressed its own uncertainty, the policy improvement loop can be entered. First, a model-optimized operating policy is obtained based on the prior distributions of model parameters. Using this policy an optimally informative experiment is designed to define sampling times along the next evaluation run. The policy is then evaluated experimentally and new data are gathered. To use data more efficiently, a sensitivity analysis is made to pinpoint which is the subset of parameters that explain most the variance of the chosen performance index. Finally, the tendency model is updated by re-estimating the corresponding distributions of most sensitive parameters, and a new iteration begins. Starting from ample initial uncertainty, data gathered on a run-to-run basis introduce the relevant data for policy optimization so that only the most relevant operating conditions are explored.

In order for the model to describe the observed bioreactor dynamics as accurately as possible, the tendency model must faithfully represent its own fidelity of how accurate it is. For example, if a bioreactor physiological state is found on a simulated trajectory about which not much knowledge has been previously acquired, the tendency model must be able to quantify this uncertainty, and not simply assume that its best guess is close to the truth. A probabilistic tendency model quantifies its lack of knowledge and can be considered as a model that captures all plausible dynamics in a distribution over fitted models. The use of probabilistic tendency models for the dynamics allows us to keep track of the uncertainties in the simulations used for policy optimization. Typically, in early iterations, parameter distributions will reflect that without properly biasing data gathering, the model-optimized policy can give rise to a significant performance–prediction mismatch. As more data are sampled, model uncertainty is increasingly reduced and the operating policy will converge to a local optimal solution. Accordingly, as the number of runs increases the probabilistic tendency model will tend to a nearly deterministic one where the output variance is mostly due to model-process mismatch. Model-based policy iteration is stopped when observed performance improvement in two successive runs is lower than

a small tolerance which is conveniently chosen in accordance to the intrinsic bioprocess variability and measurement errors.

3.2. Experimental design

As the probabilistic tendency model has a mismatch with the process, the model-optimized policy using the prior distribution $p(\boldsymbol{\theta})$ is not necessarily the policy obtained when new data $\boldsymbol{x}$ are used to revise (*a posteriori*) parameter distributions, which do not necessarily give rise to a better policy. Exploiting what is already known must be balanced with exploring untried operating conditions to find better policies. To address this dilemma between exploiting the tendency model—based on the prior—and bringing novel information to generate the required data to revise the posterior $p(\theta|\boldsymbol{x})$ so that the operating policy $\boldsymbol{\varphi}$ is actually improved, experimental design is split in two sub-problems using two sets of manipulated inputs as follows

$$\boldsymbol{\varphi_1} = (\boldsymbol{\chi}_0\boldsymbol{\beta}, \boldsymbol{w}, t_f); \quad \boldsymbol{\varphi_2} = \mathbf{t}^{\mathrm{tp}} \tag{8}$$

which makes room for the next evaluation run to be designed by optimizing $\boldsymbol{\varphi_1}$ and $\boldsymbol{\varphi_2}$, separately. Since the first term in the bracket of Eq. (7) only depends on the prior distribution of parameters, exploitation of available knowledge can be approached by solving the following optimization problem

$$\max_{\boldsymbol{\varphi_1}} \int p(\boldsymbol{\theta})\tilde{J}(t_f|\boldsymbol{\theta})d\boldsymbol{\theta} \tag{9a}$$

subject to:

$$f(\dot{\boldsymbol{x}}, \boldsymbol{x}, \boldsymbol{u}(t), \boldsymbol{w}, \boldsymbol{\theta}, t) = \mathbf{0}, \quad \text{tendency model in Eq. (1)} \tag{9b}$$

$$\boldsymbol{\varphi}_{\mathbf{1}}^{\boldsymbol{L}} \le \boldsymbol{\varphi_1} \le \boldsymbol{\varphi}_{\mathbf{1}}^{\boldsymbol{U}}, \quad \text{upper/lower constraints for design variables} \tag{9c}$$

$$\boldsymbol{x}(t) - \boldsymbol{G}(t) \le \mathbf{0}, \quad \text{path constraints for state variables} \tag{9d}$$

Solving the stochastic optimization problem in Eq. (9) is expensive computationally since it requires a Monte Carlo approach to generate a representative sample of possible realizations of the parameter vector $\boldsymbol{\theta}$ over all the parametric uncertainty modeled by $p(\boldsymbol{\theta})$. A significantly less costly alternative would be resorting to the "most probable" parameterization $\tilde{\boldsymbol{\theta}}$ for the tendency model in Eq. (9b) and then replacing the integral in Eq. (9a) with $\tilde{J}(t_f|\tilde{\boldsymbol{\theta}})$ as the objective function. However, by doing this the prediction capability of a probabilistic tendency model is not fully used as it is required in a Bayesian decision-theoretic approach.

Having obtained model-optimized policy parameters in $\boldsymbol{\varphi}_{\mathbf{1}}^{*}$, what is left for active learning (exploration) is choosing *a priori* the best sampling times, namely $\boldsymbol{\varphi_2} = \mathbf{t}^{\mathrm{sp}}$, over an evaluation run. To this aim the following optimization problem is proposed in Martínez et al. (2009)

$$[\mathbf{t}^{\mathrm{sp}}]^{*} = \max_{\mathbf{t}^{\mathrm{sp}}} \det|M(\tilde{\boldsymbol{\theta}}, \boldsymbol{\varphi}_{\mathbf{1}}^{*}, \mathbf{t}^{\mathrm{sp}})|; \quad \boldsymbol{M} = \boldsymbol{Q}^{T}\boldsymbol{Q} \tag{10a}$$

Subject to:

$$\Delta t^{L} \le t_{i+1} - t_i \le \Delta t^{U}; \quad t_i \in \mathbf{t}^{\mathrm{sp}}, \quad i = 1, \ldots, n. \tag{10b}$$

here Δt^{L} and Δt^{U} are the minimum and maximum time intervals between two successive samples, respectively, and Q is the following *global sensitivity matrix*

$$\boldsymbol{Q} = \begin{pmatrix} Si_{11} & \cdots & Si_{1k} \\ \vdots & \ddots & \vdots \\ Si_{1n} & \ldots & si_{nk} \end{pmatrix} \tag{10c}$$

Analogous to the well-known Fisher Information Matrix (FIM), each entry of the matrix $\boldsymbol{Q}$, Si_{ij}, measures the sensitivity of the performance index at the ith sampling time with respect to the jth policy parameter in $\boldsymbol{\varphi}_{\mathbf{1}}^{*}$. However, each entry in matrix $\boldsymbol{Q}$ is a global sensitivity index instead of a local one. As a result, the design criterion in Eq. (10) can be named Global FIM (GFIM); the reader is referred to the works of Rodriguez-Fernandez, Kucherenko, Pantelides, and Shah (2007) and Hamisu (2010) for further details regarding the GFIM criterion. The number of samples along each run will be defined in accordance to the budget for processing samples and bearing in mind that this number should be, at least, equal to the number of policy parameters in $\boldsymbol{\varphi}_{\mathbf{1}}^{*}$.

3.3. Performance sensitivity analysis

For effective model update in policy iteration, it is important to pinpoint the sub-set of parameters whose distributions must be re-estimated using new data in order to reduce the performance prediction mismatch. To this aim, global sensitivity analysis (GSA) is now used to assess how bioreactor performance can be apportioned to the uncertainty in different parameters of the tendency model. To understand the rationale behind GSA, it is assumed that, for a given policy $\boldsymbol{\varphi}$, the probabilistic model in Eq. (1) implicitly defines a nonlinear mapping from parameters $\boldsymbol{\theta}$ to performance $\boldsymbol{J}$ over a dynamic experiment such that the regression model must fit

$$\tilde{\boldsymbol{J}} = \mathcal{F}(\boldsymbol{\theta}) + \varepsilon \tag{11}$$

where the vector $\tilde{\boldsymbol{J}} = [J(t_1), \ldots, J(t_n)]^{\boldsymbol{T}}$ has the sampled performance data, the corresponding predictions are $\mathcal{F}(\theta) = [\mathcal{F}(t_1, \boldsymbol{\theta}), \ldots, \mathcal{F}(t_n, \boldsymbol{\theta})]^{\boldsymbol{T}}$, whereas $\boldsymbol{\varepsilon} = [\varepsilon(t_1), \ldots, \varepsilon(t_n)]^{\boldsymbol{T}}$ is the vector of measurement errors. Various sensitivity measures can be used to carry out performance sensitivity analysis bearing in mind parametric uncertainty. The variance-based method is the most commonly used since sensitivity indices are calculated based on Monte Carlo simulations. Variance-based sensitivity indices are calculated based on the prior information in the probabilistic model by a (joint) probability density function $p(\boldsymbol{\theta}) = \prod_i p_i(\theta_i)$. Performance sensitivity $S_i(t_j)$ to the parameter θ_i at a given sampling time t_j requires calculating the conditional variance (Chu & Hahn, 2010; Saltelli, Ratto, Tarantola, & Campolongo, 2006; Sobol', 1993):

$$var[E[\mathcal{F}(t_j, \boldsymbol{\theta})|\theta_i]] = E[(E[\mathcal{F}(t_j, \boldsymbol{\theta})|\theta_i)] - E[\mathcal{F}(t_j, \boldsymbol{\theta})]^2] = \int \left(\int \cdots \int \mathcal{F}(t_j, \boldsymbol{\theta}) \prod_{k \neq i} p_k(\theta_k) \prod_{k \neq i} d\theta_k - \int \cdots \int \mathcal{F}(t_j, \boldsymbol{\theta}) \prod_{k} p_k(\theta_k) \prod_{k} d\theta_k \right)^2 p_i(\theta_i) d\theta_i \tag{12}$$

The first term in the bracket of Eq. (12) is the conditional mean of the performance index at time t_j according to a particular realization of the model parameter θ_i, whereas the second term is the mean of the model output over all plausible parameter values. Accordingly, performance sensitivity for a given parameter is measured by the conditional variance divided by the total variance of model predictions for a given policy

$$S_i(t_j) = \frac{var[E[\mathcal{F}(t_j, \boldsymbol{\theta})|\theta_i]]}{var[\mathcal{F}(t_j, \boldsymbol{\theta})]} \tag{13}$$

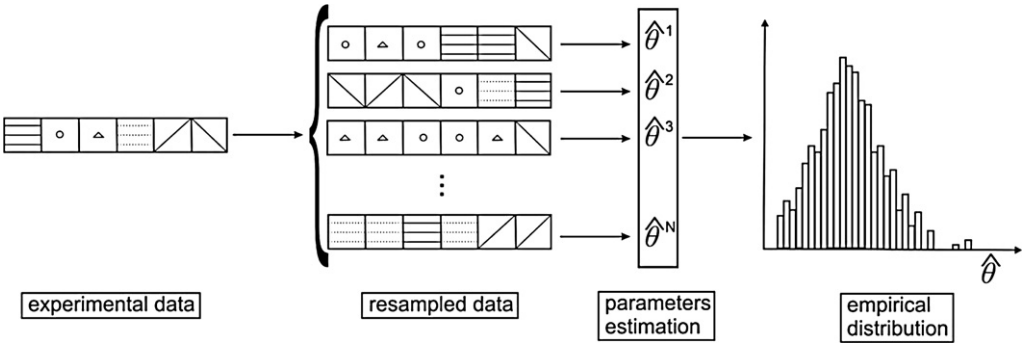

Fig. 2. Data bootstrapping for shaping parametric uncertainty.

Several approaches can be followed to compute the conditional variance including the regression method, the Sobol's method, the Fourier amplitude sensitivity test (FAST) and extensions of FAST. To study how parametric uncertainty translates into performance variability, the tendency model in Eq. (1) must be simulated for specific realizations of the entries in the vector of model parameters $\boldsymbol{\theta}$ as it has been proposed in Chu and Hahn (2010)

$$\theta_i = \alpha_i \vartheta_i(U_i) \tag{14}$$

where $\alpha_i = \theta_i^U - \theta_i^L$, $U_i \in [0, 1]$, is a pseudo-random number and the input function ϑ_i must be chosen such that it is bounded as follows

$$\vartheta_i(U_i) \in \left[\frac{\theta_i^L}{\theta_i^U - \theta_i^L}, \frac{\theta_i^U}{\theta_i^U - \theta_i^L} \right]. \tag{15}$$

Using the cumulative distribution of the parameter θ_i, $F(\theta_i)$, the input function above can be readily defined as

$$\vartheta_i(U_i) = \frac{1}{\alpha_i} F^{-1}(U_i) \tag{16}$$

Once the performance sensitivity $S_i(t_j)$ for each parameter is known, the subset of parameters to be re-estimated using data obtained in the last evaluation run is determined and their probability distributions are updated.

3.4. Model update

In tendency models (Bonvin & Rippin, 1990; Filippi et al., 1989; Fotopoulos et al., 1998; Georgakis, 1995; Uhlemann et al., 1994), it is typically assumed that bioreactor dynamics can be approximated reasonably well using a single parameter set, which is fixed, but unknown. Probabilistic tendency models are based on a completely different—the Bayesian one—approach to parameter estimation: the tendency model parameter $\boldsymbol{\theta}$ is not fixed, but described as a random variable. By choosing the prior probability distributions in $p(\boldsymbol{\theta}) = \prod_i p_i(\theta_i)$ for each parameter model uncertainty is represented in such a way prior knowledge is used to express general beliefs about plausible values for model parameters in advance. For each prior distribution $p(\boldsymbol{\theta})$ and a given policy $\boldsymbol{\varphi}$, it is assumed that a specific sampled data $\boldsymbol{x}$ will be observed with a probability density (likelihood) $p_\varphi(\boldsymbol{x}|\theta)$. Hence, parameters and sample data follow a joint probability distribution with density $p(\boldsymbol{x}, \theta) = p_{\boldsymbol{\varphi}}(\boldsymbol{x}|\theta)p(\theta)$. From this distribution, the conditional probabilities of the model parameters given the data $\boldsymbol{x}$ are computed using the "Bayes' formula"

$$p(\boldsymbol{\theta} \,|\, \boldsymbol{x}) = \frac{p_\varphi(x \,|\, \boldsymbol{\theta})p(\boldsymbol{\theta})}{p(\boldsymbol{x})} \tag{17}$$

which states that the posterior probability for a given vector $\boldsymbol{\theta}$ of model parameters is proportional to the product of the likelihood and the *prior* density and represents a compromise between them. For given data $\boldsymbol{x}$, the denominator in Eq. (17) is a fixed number which only serves the purpose of normalization constant. Typically, the posterior distribution is narrower compared to the prior one, which reflects the information gain by accounting for data sampled in an evaluation run.

Bayesian parameter estimation differs considerably from maximum likelihood estimation in the type of approximation and interpretation of the nature of parameters obtained. In maximum likelihood estimation, the regression problem is about finding a model parameterization which makes the data look probable, whereas in Bayesian estimation the idea is pinpointing which parameter set in a model appears as the most probable given the observed data. Moreover, the goal in Bayesian parameter estimation for tendency models is not to choose a single parameter set, but to characterize the entire probability distributions, *i.e.* marginal distributions of individual parameters and probabilities for performance predictions at different sampling times. Bearing in mind the small number of samples in a production run, a practical approach to Bayesian estimation of parameters in a tendency model is *bootstrapping* (Efron & Tibshirani, 1993; Joshi, Seidel-Morgenstern, & Kremling, 2006).

Due to structural mismatch and scarce data it is not possible to determine the "true" parameters in the tendency model, but only an estimate $\widehat{\boldsymbol{\theta}}(\boldsymbol{x}, \boldsymbol{\varphi})$ for the chosen operating policy. Each time the estimation is carried out with a different data set, a different estimate of model parameters is obtained. However, only a single data set is available in practice from a policy evaluation run. Therefore, only a single point estimate $\widehat{\boldsymbol{\theta}}$ is obtained with little insight or no knowledge at all about its distribution or confidence intervals. Bootstrapping provides a way to determine, at least approximately, the statistical properties of this estimator.

As it is shown in Fig. 2, bootstrapping is a simulation method for statistical inference using re-sampling with replacements (Efron & Tibshirani, 1993). A main application of the method is approximating non-parametric distributions for statistical variables in model fitting. The method has been successfully applied in quantifying confidence intervals of uncertain kinetic parameters in metabolic networks (Joshi et al., 2006). To construct a histogram for some parameters in a tendency model, bootstrapping simulates the effect of artificially excluding some data points in the data set $\boldsymbol{x}$ when parameters are estimated. We randomly sample n data points with replacement from the current data set, where the probability of each data point being selected is $1/n$. These n data points are regarded as a re-sampled training data $\boldsymbol{x}^1$. The bootstrap approach uses Monte Carlo simulation to generate a large number N of re-sampled experimental data sets $\boldsymbol{x}^1, \boldsymbol{x}^2, \ldots, \boldsymbol{x}^N$ such that the probability for a data point to be part of any of these artificial replicas for parameter fitting are all equal. Accordingly, the probability of a simulated data set having all the original sampled data $\boldsymbol{x}$ is quite

Table 1
Parametric uncertainty for the tendency model with the right structure.

Parameter	Symbol	Units	Uncertainty interval	"*In silico*" bioreactor
Maximum specific growth rate	μ_{max}	h^{-1}	0.12–0.17	0.13
Saturation parameter for biomass production	K_x	g-substrate/g-DW	0.006–0.4	0.131
Cellular death rate	K_d	h^{-1}	0.005–0.01	0.006
Cellular lysis constant	k_{lis}	h^{-1}	0.00001–0.008	0.0008
Yield factor for substrate to biomass	Y_{xs}	g-DW/g-substrate	0.40–0.58	0.52
Yield factor for substrate to penicillin	Y_{ps}	g-Penicillin/g-substrate	0.4–1	0.97
Maximum specific synthesis rate of penicillin	π_{max}	h^{-1}	0.003–0.015	0.011
Saturation parameter for penicillin production	K_p	$g\,L^{-1}$	0.00001–0.0002	0.0001
Maximum substrate uptake rate for maintenance	ζ_{max}	h^{-1}	0.014–0.029	0.02
Saturation parameter for cellular maintenance	K_s	$g\,L^{-1}$	0.00001–0.0002	0.0001
Penicillin hydrolysis rate	K_h	h^{-1}	0.002–0.01	0.002

low. Even though the number of samples in each replicated data set is the same, most of the re-sampled data sets $\boldsymbol{x}^1, \boldsymbol{x}^2, \boldsymbol{x}^3, \ldots, \boldsymbol{x}^N$ will provide a different estimation of model parameters. Histograms for each model parameter are obtained using these alternative estimators based on a given criterion, *e.g.* minimum least-square (LS) or maximum likelihood (ML).

In bootstrapping, a ML estimator is preferable over a LS estimator because the former is more flexible and can incorporate statistical tests more readily than the latter. However, point-wise estimation of the most sensitive parameters in a tendency model is an inherently nonlinear problem. That is, the surface to be minimized or maximized in parameter fitting is likely to have multiple optima and a complex topography. Estimating likelihoods when the approximated shape of parameter distributions is not known is computationally intensive to comprehensively explore this surface. Moreover, some problems may arise when bootstrap regression is made using small sample data sets. For example, in parameter estimation, a matrix whose inverse is needed to obtain the ML estimator may be rank-deficient in a bootstrap sample because some observations are missing. For ordinary LS, small samples makes mandatory resorting to nonlinear optimization techniques such as the "Levenberg–Marquardt" with multiple initializations, or better, global optimization methods such as genetic algorithms. Bayesian bootstrap predictions with *bagging* (see Fushiki, 2010, for details) of parameter histograms directly is the alternative of choice, mainly when errors in model structure are significant.

4. Simulation results

4.1. Case study—fed-batch fermentation of penicillin G

Penicillin production is an established benchmark in fed-batch fermentation for testing new approaches in modeling, optimization and control of novel bioprocesses (Bajpai & Reuss, 1980; Cinar, Parulekar, Ündey, & Birol, 2003, chap. 2; Li, Zhao, & Zuan, 2005; Menezes, Alves, & Lemos, 1994; Riascos & Pinto, 2004). Typically, industrial cultures of *Penicillium crysogenum* are operated in two phases. Firstly, a *batch* mode of operation is used to favor mycelium growth. Later on, for penicillin production, cells in the culture are put in a condition of metabolic stress. To improve yield and productivity of antibiotic expression, a *fed-batch* fermentation mode is used to add substrates continuously to the culture alongside with a specific inductor. Penicillin and biomass are obtained at the expense of substrates (S) such as glucose, which is taken as the limiting carbon source, and organic nitrogen compounds that are generally provided in excess by using corn steep liquor. Since the concentrations of viable (v) and dead (d) biomass (X), penicillin (P) and glucose (S) are routinely measured, they are chosen as the descriptive state variables along with broth volume (V) which varies with time t in this fed-batch bioprocess. Model equations for an unstructured tendency model of a fed-batch bioreactor are given below whereas uncertainty intervals of model parameters are given in Table 1 (Menezes et al., 1994). Moreover, the tendency model takes into account a culture media evaporation rate F_{evap} which is set as constant in the present study.

$$
\begin{aligned}
&\frac{dV(t)}{dt} = F_{in} - F_{evap}\\
&\frac{dX_v}{dt} = \mu X_v - K_d X_v - \frac{(F_{in}-F_{evap})}{V} X_v; \quad \mu = \frac{\mu_{max} S}{K_x X_v + S}\\
&\frac{dX_d}{dt} = K_d X_v - k_{lis} X_d - \frac{(F_{in}-F_{evap})}{V(t)} X_d\\
&\frac{dS}{dt} = -\sigma X_v + \langle S_{in} F_{in}\rangle - \frac{(F_{in}-F_{evap})}{V(t)} S; \quad \sigma = \frac{\mu}{Y_{xs}} + \frac{\pi}{Y_{ps}} + \zeta; \quad \zeta = \frac{\zeta_{max} S}{K_s + S}\\
&\frac{dP}{dt} = \pi X_v - \frac{(F_{in}-F_{evap})}{V(t)} P - K_h P; \quad \pi = \frac{\pi_{max} S}{K_p + S}
\end{aligned}
\tag{18}
$$

The policy optimization problem is defined such that the amount of penicillin obtained at the end of a production run, $J = P(t_f)\cdot V(t_f)$), is maximized. The fed-batch bioreactor needs some discharges of culture medium in order to maintain both viability and axenity of the penicillin producing fungi strain since intermittent drain-offs reduce the possibility of mutations and productivity reduction. Such discharges must be made at some specific moments along the production run and with certain frequency. At any time t, the operating policy $\boldsymbol{\varphi_1}$ is defined by the set of parameters ($\boldsymbol{\chi}_0$, $\boldsymbol{\beta}$, $\boldsymbol{w}$, t_f) corresponding to two different degrees of freedom for process optimization. All policy parameters in $\boldsymbol{\varphi_1}$ correspond to inputs that can be modified from run-to-run but are time-invariant in a given run such as the initial bioreactor volume ($\boldsymbol{\chi}_0$) or the vector of parameters $\boldsymbol{w}$ whose entries are: t_{feed}, the substrate feed concentration, the first drain-off time, the drain-off volume and the drain-off frequency. Policy parameters also include the vector $\boldsymbol{\beta}$ corresponding to parameters which are used here to describe the profile of time-varying control variables such as the feeding rate. In the latter case, a key issue is the mathematical description to be used so as to provide ample room for different profile patterns within economic and safety constraints with a minimum number of independent parameters in $\boldsymbol{\beta}$.

To complete the experimental design of each policy evaluation run, ten optimal sampling times $\mathbf{t}^{sp}$ are obtained by solving the mathematical program in Eq. (10) using the current estimation of the optimal operating policy and global sensitivities describing the variability of the total amount of penicillin present at different sampling times. For this purpose, 45 min of CPU time are typically required when using the fmincon solver from the optimization toolbox of Matlab R2008a running on a PC equipped with an Intel i7 1.73 GHz processor and 4 GB of memory. From the computational standpoint, the optimization problem for optimal sampling in Eq. (10) is far more demanding than the one in Eq. (9) for optimization of the operating policy. The latter requires less than 5 min of CPU time using the fmincon solver and the same PC.

In previous works there have been various approaches to implement bioreactor feeding policies which can be defined as constant,

Table 2
Performance improvement in model-based policy iteration under parametric uncertainty.

Policy parameter	Exploratory run	Lower bound	Upper bound	Run #1	Run #2	Run #3	Run #4	"Real" optimum
A [L h^{-2}]	0.6882	0	4.13	1.5537	1.0357	1.0993	1.1153	1.2100
B [h^{-1}]	0.1431	0.1	0.86	0.1120	0.1	0.1000	0.1006	0.1000
C [h^{-2}]	0.0002	−0.0008	0.0012	0.0012	0.0012	0.0005	0.0006	0.0007
t_{feed} [h]	0	0	24	24	0	24	24	24
t_{final} [h]	240	200	300	243	223.22	294.3	283.0	282.7
Substrate feed concentration [g L^{-1}]	240	200	350	350	350	350	350	350
First drain-off time [h]	24	24	48	24	48	24	24	24
Drain-off volume [L]	60	30	80	80	80	80	80	80
Drain-off frequency [h]	24	24	60	24	60	24	25.9	25.13
Initial volume [L]	600	500	700	500	500	500	500	500
Penicillin G, J (Kg)	**22.54**			**47.23**	**45.90**	**50.61**	**51.88**	**51.92**
Pred. error (J) (Kg)				4.42	4.08	2.58	1.27	

piecewise constant, piecewise continuous or run-wide continuous functions of time. In this section, the feeding rate profile is varied with time using an inverse quadratic polynomial. Inverse polynomials (see Nelder, 1966, for details), resort to a small number of parameters to define time trajectories which are quite flexible for modeling a rich variety of continuous feeding patterns in bioreactor optimization. It is worth noting that the methodology proposed in Sections 2 and 3 is by no means limited to a given family of mathematical functions to describe time-varying input controls. However, bioreactor dynamics slowly unfolds cell responses to environmental changes which make smooth continuous profiles definitively more appealing to drive the physiological state along a profitable trajectory of states.

In penicillin production, the feasible range of variation for each parameter in $\boldsymbol{\beta}$ shaping the substrate feeding profile have been defined from an exhaustive analysis of alternative parameterizations of an inverse quadratic polynomial so that the feed rate is constrained to the interval [0–10] L h^{-1} at any time over a production run with length $t_f \leq 300$ h. The chosen family of inverse polynomials is used to specify the feed rate profile as:

$$F_{in} = \begin{cases} 0, & t < r_{feed} \\ \dfrac{At'}{1 + Bt' + Ct'} & t \geq t_{feed} \end{cases} \tag{19}$$

where $t' = t - t_{feed}$. Feed rate profiles defined by Eq. (19) have been chosen to eliminate problems found when applying GSA techniques to assess performance sensitivity to profile parameters $\boldsymbol{\beta} = (A, B, C)$. To this aim, it is important to guarantee that all parameters defining the feed rate profile are independent of each other which makes Sobol's main effects $S_i(t_j)$ in Eq. (13) optimally informative. For assessing the performance sensitivity to the policy parameters $\boldsymbol{\varphi_1}$ at given sampling times t_j, $j = 1, \ldots, n$, Eqs. (14)–(16) are used. It is worth remembering that the conditional variance in Eq. (13) describes the portion of the total variance that can be explained by the uncertainty regarding a given parameter θ_i. Once the performance sensitivity $S_i(t_j)$ for each parameter is known, the subset of relevant parameters to be re-estimated using data gathered in the last evaluation run is established and their probability distributions are then updated using bootstrapping.

4.2. Tendency model with the right structure

To demonstrate the effectiveness and convergence to an optimal policy of the proposed model-based run-to-run optimization methodology, the case where the tendency model has significant parametric uncertainty but the correct structure is addressed. To this aim, productivity optimization using the methodology in Fig. 1 has been made upon sampled data provided by the *in silico* model in Eq. (18) and parameters from Table 1 with 5% added random noise such that observations correspond to $y_i = h(\boldsymbol{x}_i) + \varepsilon$, $\varepsilon \sim \mathcal{N}(0, \sigma_\varepsilon^2)$ where the variance is $\sigma_\varepsilon^2 = 0.05\, h(\boldsymbol{x}_i)$. It is worth noting that the probabilistic tendency model is capable of faithfully approximating the *in silico* model as long as data gathering is increasingly biased toward the more profitable region of operating conditions. Also, all parameter values for the *in silico* model are included in the initial subspace of parametric uncertainty which provides enough room for obtaining an input policy which is near-optimal. In Table 2, the model-optimized values for the input policy parameters in four evaluation runs along with the exploratory run are shown. The feed rate profile in the exploratory run is an approximation using an inverse quadratic polynomial to the one proposed in Menezes et al. (1994). Also, the actual values obtained (including the measurement noise) for the objective function J after each policy evaluation run and its standard deviation $std(J)$ are shown. Performance prediction errors are computed using GSA to account for parametric uncertainty in the probabilistic tendency model. Finally, the "true" optimal policy parameterization has been obtained using the *in silico* model in Table 1 along with the upper/lower bound constraints for policy parameters. To this aim, a nonlinear optimization problem is solved using a deterministic tendency model with structure in Eq. (18) along with *in silico* model parameters.

As it can be seen in Table 2, the optimal penicillin production is reached after just four policy evaluation runs using the model-based policy iteration strategy in Fig. 1. Moreover, the penicillin obtained using the model-optimized input policy after the first evaluation run is more than twice the amount obtained in the exploratory run. Productivity improvement is highlighted using bold figures for the amount of penicillin obtained. It is noteworthy how run-to-run reduction of parametric uncertainty in the probabilistic tendency model can be readily seen in the levels of confidence for performance predictions. As more sampled data are available to re-estimate some kinetic parameters and their probability distributions (histograms), the policy parameters fast converge toward the parameter values of the optimal policy. In particular, both the substrate feeding concentration and the initial culture volume as well as the discharge frequency fast converge to their optimal values just after the first evaluation run. In Fig. 3, the evolution of feed rate profiles in model-based policy iteration is shown. Model-based optimized profiles alternate between above and under the optimal feed rate as more data are introduced in the tendency model. Moreover, despite added measurement errors in sampled data, feed rates in run #3, #4 and the optimal profile are not statistically different from the viewpoint of penicillin production.

Data gathered in each dynamic experiment done with the *in silico* model are used to (re)estimate selectively model parameter distributions in accordance with their first order (main effects) sensitivity indices (Si). These indices have been computed using empirical probability density function (pdf) or histograms estimated based on bootstrapping data in the exploratory run. In Table 3, performance sensitivity indices after the exploratory run

Table 3
Sensitivity indices for the tendency model with the right structure.

Model parameter	*Si* (exploratory run)	*Si* (run #1)	*Si* (run #2)	*Si* (run #3)
μ_{max}	0.1818	0.0187	0.0731	0.0840
K_x	0.0282	0.0194	0	0.0134
K_d	0.0891	0	0.1051	0.1166
k_{lis}	0.0327	0.0193	0.0563	0.0845
Y_{xs}	0.1641	0.1635	0.0535	0.1024
Y_{ps}	0.0906	0.0684	0.1308	0.1325
π_{max}	0.0408	0.2306	0.0450	0.0122
K_p	0.0342	0.0195	0.0708	0.0849
ζ_{max}	0	0.1659	0.1344	0.0705
K_s	0.0327	0.0192	0.1124	0.0847
K_h	0.3031	0.0947	0.1738	0

and three evaluation runs are shown. Initially, most of the uncertainty of interest in the probabilistic tendency model is located in just three parameters: to μ_{max}, Y_{xs} and K_h. The corresponding histograms for these parameters are shown in Fig. 4. The parameter K_h alone is able to explain 30% of performance uncertainty regarding the model-optimized policy obtained. As more policy evaluation runs are carried out, performance variance is spread among an increasing number of parameters. Sobol's main effects *Si* in Table 3 highlight that a further reduction in the uncertainty of performance predictions for the model-optimized policy can be achieved by using data from the second experimental run to re-estimate π_{max}, Y_{xs}, and K_h. However, uncertainty reduction for model parameters K_x, k_{lis} and K_p is never a relevant issue to better explain performance variance of a model-optimized operating policy in an evaluation experiment.

As can be seen, a selective decrease in parametric uncertainty is obtained when data from a handful of designed experiments are used to compute bootstrapping histograms for parameters that best explain the variance in performance predictions. After just four runs, the initial model uncertainty has been reduced to a level of variability that renders model-optimized policies statistically identical. Thus, performance predictions cannot be further improved using new data. In Fig. 5, run-to-run evolution of the corresponding empirical *pdf* (histogram) for Y_{xs} is shown to highlight fast shrinking of parametric uncertainty when the model has the correct structure. As more data are introduced into the probabilistic tendency model, the parameter value **0.52** used for the *in silico* model (see Table 1) becomes definitively the most probable within a much smaller confidence interval. Also, as the policy is improved using data from evaluation runs, the parameter distribution is clearly not Gaussian, but left-skewed. Fast convergence of the model-optimized policy is the direct result of using data from

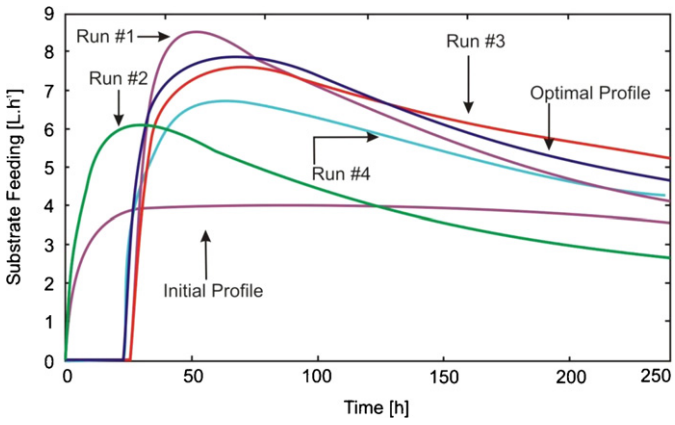

Fig. 3. Run-to-run improvement of the substrate feed rate profile using a tendency model with the right structure.

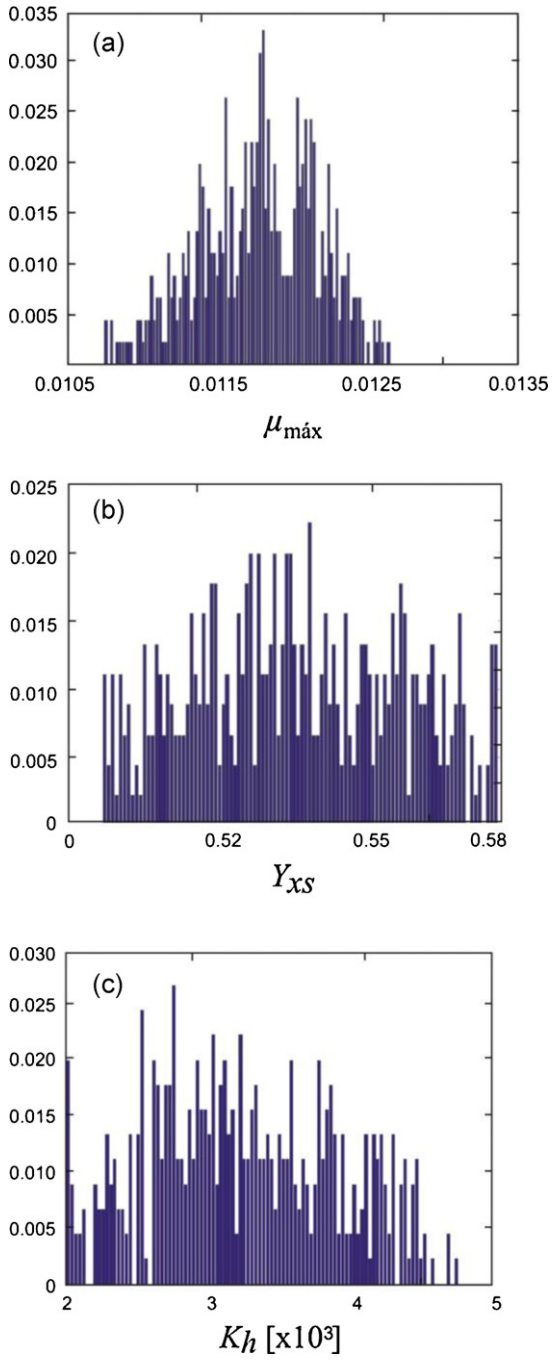

Fig. 4. Histograms for the most influencing model parameters based on bootstrapping data obtained in the exploratory run. (a) μ_{max}; (b) Y_{xs}; (c) K_h.

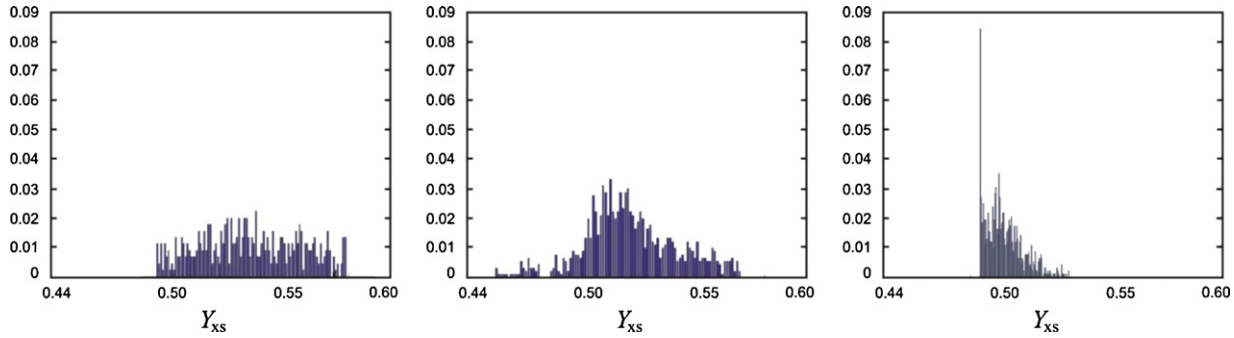

Fig. 5. Run-to-run evolution of histograms for Y_{xs} highlighting uncertainty reduction when only parametric uncertainty must be reduced. (a) Exploratory run; (b) run #1; (c) run #3.

designed experiments to revise selectively posterior distributions of the most influencing model parameters.

4.3. Process-model structure mismatch

To assess the effect of a structure mismatch between the bioreactor behavior and the tendency model used for run-to-run optimization, the model proposed in Riascos and Pinto (2004) has been implemented as the *in silico* bioreactor. The Riasco and Pinto's model structure is described by the following set of differential equations

$$\begin{aligned}
\frac{dV}{dt} &= F_{in} - F_{evap} \\
\frac{dX}{dt} &= \mu X - K_d X - \frac{(F_{in} - F_{evap})}{V} X; \quad \mu = \frac{\mu_{max} S}{K_x X + S} \\
\frac{dS}{dt} &= -\sigma X + \left\langle S_{in} F_{in} \right\rangle - \frac{(F_{in} - F_{evap})}{V} S; \quad \sigma = \frac{\mu}{Y_{xs}} + \frac{\pi}{Y_{ps}} + \zeta; \quad \zeta = \frac{\zeta_{max} S}{K_s + S} \\
\frac{dP}{dt} &= \pi X - \frac{(F_{in} - F_{evap})}{V} P - K_h P; \quad \pi = \frac{\pi_{max} S}{K_p + S(1 + (S/K_{in}))}
\end{aligned} \tag{20}$$

Table 4 provides a convenient parameterization for the *in silico* model so that the tendency model proposed in Menezes et al. (1994) equipped with a probabilistic representation of its parameters can be used as a rough guideline for optimizing the operating policy. Results obtained in run-to-run optimization of the operating policy for three iterations following the exploratory run are summarized in Table 5 and Fig. 6. It is worth noting that an improvement of roughly 300% is obtained from the exploratory run in just three evaluation experiments. However, policy convergence is achieved with a 9% productivity loss. Comparing results obtained using the probabilistic tendency model with the optimal productivity for the *in silico* bioreactor is quite clear that due to structural errors the productivity cannot be improved further. For run #4, modeling errors are so significant that actually a lowering of productivity is observed when the model-optimized policy is evaluated in run #4. This fact can be understood as the necessary result of over-fitting parameter distributions.

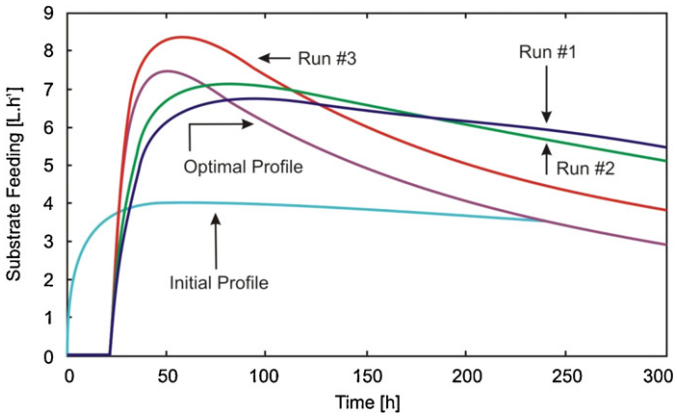

Fig. 6. Run-to-run improvement of the substrate feed rate profile when the tendency model has a process-model structural error.

Histograms for model parameters have been obtained using bootstrapping with sampled data. In Fig. 7, distributions based on data gathered in the exploratory run are shown for two parameters: Y_{ps} and π_{max},. It was found that for some parameters the most probable values coincide with either the upper or lower bounds in their uncertainty intervals. A plausible explanation of such bias is that a significant structural process-model mismatch in bootstrapping gives rise to a very low frequency of inner parameter values which cannot fit data properly.

The structural mismatch between the *in silico* model and the tendency model is vividly shown in Fig. 8 using sampled data for penicillin in the exploratory run and the third optimization run when evaluating operating policies in model-based policy iteration. For the exploratory run (see Fig. 8(a)), the level of parametric uncertainty is considerably high which makes structural errors less evident. As more data have been introduced both the parameter distributions and the operating policy are changed significantly to reflect structure errors in the tendency model. The model-process

Table 4
The *in silico* model parameters used to assess the effect of structural modeling errors.

Parameter	Notation	Units	Value
Maximum specific growth rate	μ_{max}	h^{-1}	0.15
Saturation parameter for biomass production	K_x	g-substrate/g-DW	0.06
Yield factor for substrate to biomass	Y_{xs}	g-DW/g-substrate	0.45
Yield factor for substrate to penicillin	Y_{ps}	g-Penicillin/g-substrate	0.9
Maximum specific synthesis rate of penicillin	π_{max}	h^{-1}	0.012
Saturation parameter for penicillin production	K_p	$g\,L^{-1}$	0.0001
Maximum substrate uptake rate for maintenance	ζ_{max}	h^{-1}	0.025
Saturation parameter for cellular maintenance	K_s	$g\,L^{-1}$	0.0001
Inhibition parameter for penicillin production rate	K_{in}	$g\,L^{-1}$	0.1
Penicillin hydrolysis rate	K_h	h^{-1}	0.003

Table 5
Performance improvement in model-based policy iteration under structural modeling errors.

Policy parameter	Exploratory run	Lower bound	Upper bound	Run #1	Run #2	Run #3	Run #4	"Real" optimum
A [L h^{-2}]	0.6882	0	4.13	0.8707	0.9697	1.3494	1.3036	1.2755
B [h^{-1}]	0.1431	0.1	0.86	0.1	0.1022	0.1015	0.1000	0.1018
C [h^{-2}]	0.0002	−0.0008	0.0012	2e−4	3e−4	9e−4	8e−4	0.0012
t_{feed} [h]	0	0	24	24	23.6	24	24	22.37
t_{final} [h]	240	200	300	300	300	294.8	300	300
Substrate feed concentration [g L^{-1}]	240	200	500	500	500	500	500	500
First drain-off time [h]	24	24	48	24	24.17	24.02	24	24
Drain-off volume [L]	60	30	80	80	80	80	80	79.57
Drain-off frequency [h]	24	24	60	24	24	24.62	24	35.28
Initial volume [L]	600	500	700	500	500	500	500	500
Penicillin G, J (kg)	**16.12**			**35.47**	**40.49**	**57.51**	**53.6**	**63.24**
Pred. error (J) (kg)				3.1	2.1	2.5	1.9	

structure mismatch is clearly revealed in Fig. 8(b) through penicillin concentrations that are outside the prediction ranges for the probabilistic tendency model.

In Table 5, sensitivity indices *Si* (main effects) for the performance index, namely the amount of penicillin obtained, on a run-to-run basis are shown. Figures in bold highlight productivity improvement based on model-based policy iteration. These indices highlight the sensitivity of productivity predictions due parametric uncertainty and model-process mismatch. At the beginning of policy optimization main sources of performance uncertainty are related to only two parameters, Y_{ps} and π_{max}, which explain almost the same amount of variability. Despite at evaluation run #2 and run #3 the amount of variance explained by principal effects is reduced, it is interesting to observe that variability appears mainly due to a few uncertainty sources and is not spread evenly as in previous example of Section 4.2. In Figs. 9 and 10, histograms that highlight run-to-run uncertainty reduction for parameters Y_{xs} and π_{max} are shown. It is noteworthy that uncertainty reduction for these parameters drastically changes the median of the histograms. These noticeable changes in the shape of parameter histograms are rather expected whenever a model with a significant

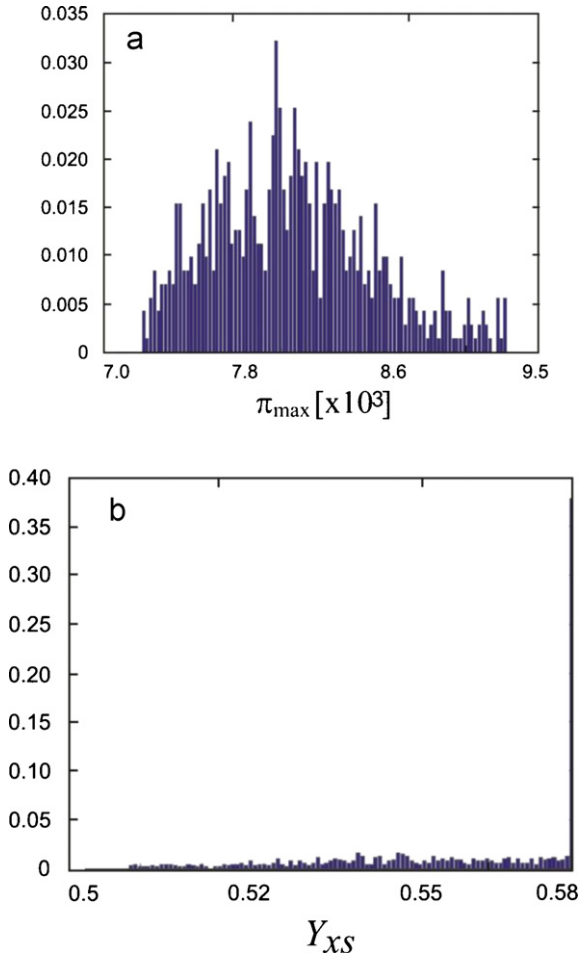

Fig. 7. Histograms for the most influencing model parameters based on bootstrapping data obtained in the exploratory run. (a) π_{max}; (b) Y_{ps}.

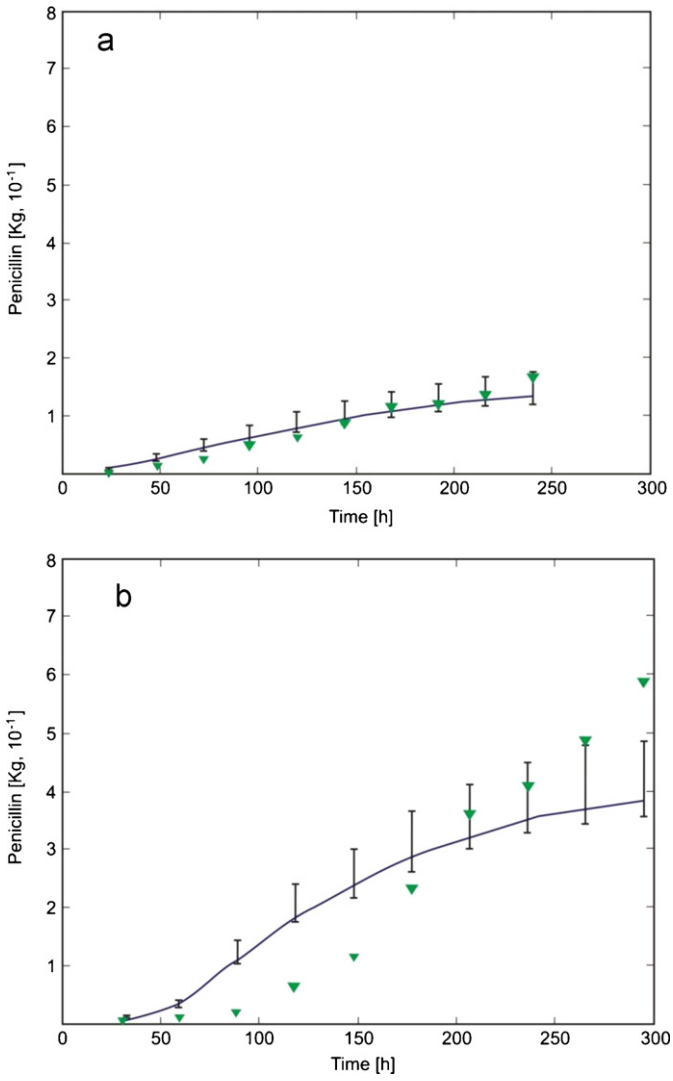

Fig. 8. Penicillin prediction degradation as more data bias are introduced into the tendency model with structural errors. (a) After the exploratory run; (b) after run #3.

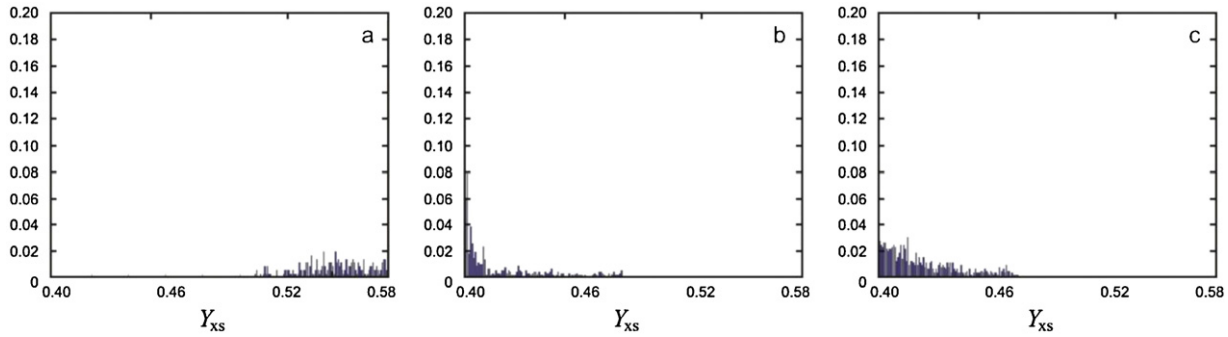

Fig. 9. Run-to-run evolution of histograms for Y_{xs} highlighting uncertainty reduction under significant model-process structure mismatch. (a) Exploratory run; (b) run #1; (c) run #3.

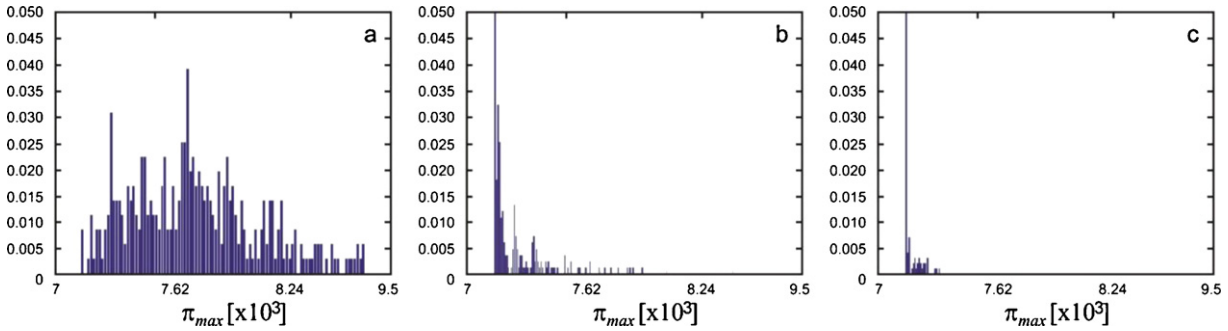

Fig. 10. Run-to-run evolution of histograms for π_{max} highlighting uncertainty reduction under significant model-process structure mismatch. (a) Exploratory run; (b) run #1; (c) run #3.

Table 6
Sensitivity indices for the tendency model with process-model structure mismatch.

Model parameter	*Si* (exploratory run)	*Si* (run #1)	*Si* (run #2)	*Si* (run #3)
μ_{max}	0	0	0.0329	0
K_x	0.0236	0.0012	**0.5910**	0.0029
K_d	0.0005	0.0013	0.0015	0.0056
k_{lis}	0.0003	0.0014	0.0384	0
Y_{xs}	0.0341	**0.1289**	0	0
Y_{ps}	**0.3838**	0.0012	0.0062	0.0112
π_{max}	**0.3888**	0.0426	0.0191	**0.2501**
K_p	0.0004	0.0013	0.0015	0
ζ_{max}	0.0875	**0.5224**	0.0364	0.0001
K_s	0.0004	0.0014	0.0015	0
K_h	0.0114	**0.2627**	0.0072	0

model-process structural mismatch is used for performance predictions in an ample range of operating conditions (Table 6). In Table 6, sensitivity indices in bold correspond to the most sensitive model parameters at each iteration.

5. Concluding remarks

A Bayesian decision-theoretic approach for run-to-run productivity optimization of bioreactors under uncertainty has been proposed. An important contribution of the presented work is integrating probabilistic tendency models with Bayesian active learning for experimental design in modeling for optimization. Global sensitivity analysis has been used to formulate the optimal sampling strategy in each dynamic experiment as an optimization problem whose solution provides the optimal sampling times at which the performance objective is most sensitive to changes in the policy parameters. Results obtained for the penicillin G case study highlight that, even when there may exist significant errors in a process model structure, a significant increase in bioreactor productivity can be achieved using model-based policy iteration.

For run-to-run optimization of the operating policy, it was proposed that parameter distributions in a probabilistic model of bioreactor performance must be selectively re-estimated on a run-to-run basis based on bootstrapping. Accordingly, a probabilistic tendency model is instrumental for designing optimally informative experiments in experimental optimization and reducing parametric uncertainty using a *learning-from-last-samples* type of approach. Solely based on gathered data in a few policy evaluation runs, model-based policy iteration is able to explore only the most profitable region of operating conditions guided by a utility function which combines information gain and performance improvement to trade off exploitation with exploration. First-principles and constitutive laws in a probabilistic tendency model constitute soft constraints for policy optimization to avoid physiological states in the bioreactor which are undesirable from the productivity viewpoint. Sample data, in turn, allow introducing the needed bias in gathering the most informative data to selectively explore the subspace of policy parameters.

Current research efforts attempt to extend the proposed approach to take advantage of several tendency models in policy

iteration. To this aim, model selection and multi-model inference in modeling for optimization is being addressed using the Kullback–Leibler divergence—or relative entropy—between prior and posterior distributions for each model before and after the data produced by the new experiment have been used for parameter re-estimation. Accordingly, models are ranked in order to maximize the "interestingness" of new data from the viewpoint of productivity improvement. A promising alternative in this regard is the *anticipatory* approach to optimal experimental design recently proposed by Donckels, De Pauw, De Baets, Maertens, and Vanrolleghem (2009). Currently, the presented Bayesian framework is being extended so as to rely on a library of tendency models with different structures for safely exploring operating conditions while collecting new information to discover better policies with less time and money at stake.

Acknowledgments

The authors wish to express their gratitude to Agencia Nacional de Promoción Científica y Tecnológica (ANPCYT), Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Universidad Tecnólogica Nacional (UTN) and Universidad Nacional del Litoral (UNL) of Argentina, for the financial support granted to this contribution.

References

Anesiadis, N., Cluett, W. R., & Mahadevan, R. (2008). Dynamic metabolic engineering for increasing bioprocess productivity. *Metabolic Engineering, 10*, 255–266.

Bajpai, R. K., & Reuss, M. (1980). A mechanistic model for penicillin production. *Journal of Chemical Technology and Biotechnology*, *30*, 332–344.

Blot, W. J., & Meeter, D. A. (1973). Sequential experimental design procedures. *Journal of the American Statistical Association*, *68*, 586–593.

Bonvin, D. (1998). Optimal operation of batch reactors: A personal viewpoint. *Journal of Process Control*, *8*, 355–368.

Bonvin, D., & Rippin, D. (1990). Target factor analysis for the identification of stoichiometric models. *Chemical Engineering Science*, *45*, 3417–3426.

Chaloner, K., & Verdinelli, I. (1995). Bayesian experimental design: A review. *Statistical Sciences*, *10*, 273–304.

Chu, Y., & Hahn, J. (2010). Quantitative optimal experimental design using global sensitivity analysis via quasi-linearization. *Industrial and Engineering Chemistry Research*, *49*, 7782–7794.

Cinar, A., Parulekar, S. J., Ündey, C., & Birol, G. (2003). *Batch fermentation—Modeling, monitoring and control*. New York: Marcel Dekker.

De Tremblay, M., Perrier, M., Chavarie, C., & Archambault, J. (1993). Fed-batch culture of hybridoma cells: Comparison of optimal control approach and closed loop strategies. *Bioprocess Engineering, 9*, 13–21.

Donckels, B., De Pauw, D., De Baets, B., Maertens, J., & Vanrolleghem, P. A. (2009). An anticipatory approach to optimal experimental design for model discrimination. *Chemometrics and Intelligent Laboratory Systems, 95*, 53–63.

Efron, B., & Tibshirani, R. J. (1993). *An introduction to the bootstrap*. London: Chapman & Hall.

Filippi, C., Bordet, J., Villermaux, J., Marchal-Brassey, S., & Georgakis, C. (1989). Batch reactor optimisation by use of tendency models. *Computers and Chemical Engineering*, *13*, 35–47.

Fotopoulos, J., Georgakis, C., & Stenger, H. G., Jr. (1998). Use of tendency models and their uncertainty in the design of state estimators for batch reactors. *Chemical Engineering and Processing*, *37*, 545–558.

Frahm, B., Lane, P., Märk, H., & Pörtner, R. (2003). Improvement of a mammalian cell culture process by adaptive, model-based dialysis fed-batch cultivation and suppression of apoptosis. *Bioprocess and Biosystems Engineering, 26*, 1–10.

Fushiki, T. (2010). Bayesian bootstrap prediction. *Journal of Statistical Planning and Inference*, *140*, 65–74.

Geng, J., & Yuan, J. (2010). Cybernetic modeling based on pathway analysis for *Penicillium chrysogenum* fed-batch fermentation. *Bioprocess and Biosystems Engineering, 33*, 665–674.

Georgakis, G. (1995). Modern tools of process control: The case of black, gray and white models. *Entropie*, *194*, 34–48.

Guthke, R., & Knorre, W. A. (1981). Optimal substrate profile for antibiotic fermentations. *Biotechnology and Bioengineering*, *23*, 2771–2777.

Hamisu, A. (2010). *Applications of global sensitivity analysis for design of experiments*. Master of Science thesis. Department of Chemical Engineering and Chemical Technology, Imperial College, London.

Jain, E., & Kumar, A. (2008). Upstream processes in antibody production: Evaluation of critical parameters. *Biotechnology Advances*, *26*, 46–72.

Joshi, M., Seidel-Morgenstern, A., & Kremling, A. (2006). Exploiting the bootstrap method for quantifying parameter confidence intervals in dynamical systems. *Metabolic Engineering*, *8*, 447–455.

Körding, K. P., & Wolpert, D. (2004). Bayesian integration in sensorimotor learning. *Nature*, *427*, 244–247.

Körding, K. P., & Wolpert, D. (2006). Bayesian decision theory in sensorimotor control. *Trends in Cognitive Sciences*, *10*, 320–326.

Li, X. B., Zhao, G. R., & Zuan, Y. J. (2005). A strategy of phosphorus feeding for repeated fed-batch fermentation of penicillin G. *Biochemical Engineering Journal*, *27*, 53–58.

Lim, H. C., Tayeb, Y. J., Modak, J. M., & Bonte, P. (1986). Computational algorithms for optimal feed rates for a class of fed-batch fermentation. *Biotechnology and Bioengineering*, *28*, 1408–1420.

Lindley, D. V. (1972). *Bayesian statistics – A review*. Philadelphia: SIAM.

Maria, G. (2004). A review of algorithms and trends in kinetic model identification for chemical and biochemical systems. *Chemical and Biochemical Engineering Quarterly*, *18*, 195–222.

Maria, G. (2007). Model-based heuristic optimized operating policies for D-glucose oxidation in a batch reactor with pulsate addition of enzyme. *Computers and Chemical Engineering*, *31*, 1231–1241.

Martínez, E. C., & Wilson, J. A. (2003). Evolutionary optimization of batch process systems using imperfect models. *Proceedings of the Indian Academy of Sciences – Chemical Sciences*, *69*, 403–428.

Martínez, E. C., Cristaldi, M., & Grau, R. (2009). Design of dynamic experiments in modeling for optimization of batch processes. *Industrial and Engineering Chemistry Research*, *48*, 3453–3465.

Menezes, J. C., Alves, S. S., & Lemos, J. M. (1994). Mathematical modelling of industrial pilot-plant penicillin-G fed-batch fermentations. *Journal of Chemical Technology and Biotechnology*, *61*, 123–138.

Nelder, J. A. (1966). Inverse polynomials, a useful group of multi-factor response functions. *Biometrics*, *22*(1), 128–141.

Ramkrishna, D. (2003). On modeling of bioreactors for control. *Journal of Process Control, 13*, 581–589.

Riascos, C., & Pinto, J. M. (2004). Optimal control of bioreactors: A simultaneous approach for complex systems. *Chemical Engineering Journal*, *99*, 23–34.

Rodriguez-Fernandez, M., Kucherenko, S., Pantelides, C., & Shah, N. (2007). Optimal experimental design based on global sensitivity analysis. *Computer Aided Chemical Engineering*, *24*, 63–68.

Saltelli, A., Ratto, M., Tarantola, S., & Campolongo, F. (2006). Sensitivity analysis practices. Strategies for model-based inference. *Reliability Engineering & System Safety*, *91*, 1109–1125.

Schenker, B., & Agarwal, M. (1995). Prediction of infrequently measurable quantities in poorly modeled processes. *Journal of Process Control*, *5*, 329–339.

Smets, I. Y., Claes, J. E., November, E. J., Bastin, G. P., & Van Impe, J. F. (2004). Optimal adaptive control of (bio)chemical reactors: Past, present and future. *Journal of Process Control*, *14*, 795–805.

Sobol', I. M. (1993). Sensitivity analysis for non-linear mathematical models. *Mathematical Modelling & Computational Experiment*, *1*, 407–414.

Terwiesch, P. (1995). Cautious on-line correction of batch process operation. *AIChE Journal*, *41*, 1337–1340.

Terwiesch, P., & Agarwal, M. (1995). Robust input policies for batch reactors under parametric uncertainty. *Chemical Engineering Communications*, *131*, 33–52.

Tsobanakis, P. (1994). *Tendency modelling and optimisation of fed-batch fermentations*. Ph.D. thesis. Lehigh University.

Uhlemann, J., Cabassud, M., LeLann, M., Borredon, E., & Cassamatta, G. (1994). Semi-batch reactor optimization and control for the production of furfural. *Chemical Engineering Science*, *49*, 3169–3191.

Verdinelli, I., & Kadane, J. B. (1992). Bayesian designs for maximizing information and outcome. *Journal of the American Statistical Association*, *87*, 510–515.