Optimal Startup/Shutdown Operating Policies with a Recombinant Strain Continuously Stirred Bioreactor

Letizia Luperini-Enciso, Holda Purón-Zepeda, Lorena Pedraza-Segura, and Antonio Flores-Tlacuahuac*

Departamento de Ingeniería y Ciencias Químicas, Universidad Iberoamericana Prolongación Paseo de la Reforma 880, México D.F., 01210, México

In this work dynamic optimal startup and shutdown policies were computed and validated in an experimental lab-scale continuous bioreactor. Using the theoretical optimal profile, a reduction in the startup time was achieved when compared to simple steplike changes in the manipulated variables. Therefore, the use of optimal startup policies led to raw material savings. To test the optimal startup policies, two operating points were selected. Experiments were run at these conditions to fit kinetic rate constants leading to a reliable model representation of the addressed biosystem. The practical implementation of theoretical optimization results is not a trivial task, and this is a major contribution of the present work. This is true especially for the optimal dynamic shutdown results that are not, up to our best knowledge, reported in the open literature.

1. Introduction

Bioprocess control has been highlighted as one way to improve bioprocess productivity. According to the definition provided by Alford:[1] "Bioprocess control is defined as providing a near optimal environment for microorganisms to grow, multiply, and produce a desired product. This includes providing the right concentration of nutrients to the culture, removing any toxic metabolic products, and controlling important internal cellular parameters". In fact, common advantages of bioprocess control include: (a) process variability reduction and (b) productivity improvements measured trough better use of raw materials and increase of process profit. A key component of any control strategy is the availability of set-point values of the controlled variables around which closed-loop control is enforced. Normally, for continuous operation, the set-point values refer to the values of the controlled variables they have under steady-state conditions. Sometimes an optimization approach may be used to realize optimal set-point values. For discontinuous operation, the set-point values can be changed either smoothly (e.g., by step, ramp changes, etc.) or a single overall set-point change can be imposed on the operation. However, traditionally in bioprocess control, set points for the controlled variables are determined by trial and error. Moreover, because batch reactors are common equipment in the bioprocess industry, some of those set points are time-varying making their determination a complex task. Moreover, there is the perception that the determination of set-point values for dynamic bioprocesses (e.g., batch reactors, startup/shutdown operations, state transitions, etc.) is very time-consuming work, although it may result in better understanding of the bioprocesses as discussed by Alford.[1] From a technical point of view, the determination of dynamic set-point values by optimization techniques can be formulated as a dynamic optimization problem.[2] Part of the perception that the determination of optimal set points is a complicated and time-consuming task could be attributed to the fact that those optimization problems have been solved using the numerical procedures that emerge when Pontryagin's maximum principle is applied. In the past 10−20 years, there have been important advances[3,4] in the dynamic optimization field that make much easier, in terms of the time involved and complexity of the underlying problem, the optimization of continuous, batch, and hybrid bioprocesses. In this work, our aim is to formulate the startup/shutdwon problems emerging in bioreactors as dynamic optimization problems such that improved ways to operate bioreactors during those operating steps are determined leading to improvements in process productivity.

Kameswaran and Biegler[5] proposed the use of dynamic optimization techniques for improving the dynamic operation of processing systems. In fact, this approach has been widely used for computing optimal startup and switching control policies. For medium- to large-scale systems, two optimal control methodologies dominate the numerical solution of optimal control problems. On one hand, the resulting set of differential and algebraic equations (DAE) comprising the dynamic mathematical model of the addressed system is subject to partial discretization of the output variables and the remaining differential system is numerically integrated as shown by Allgor and Barton.[6] This approach is commonly called the sequential optimal control approach. On the other hand, in the simultaneous approach, both the sets of manipulated and controlled variables are fully discretized leading to a set of algebraic equations. Therefore, the optimal control problem is transformed into a nonlinear program.[5] Although, it has been claimed that the sequential approach is easy to use it has some disadvantages. Presently, it is unable to handle open-loop unstable systems without previous stabilization. Quite the contrary, it has been shown by Flores-Tlacuahuac et al.[7] that the simultaneous approach is able to efficiently handle unstable systems. However, the simultaneous approach demands good initialization strategies and normally state-of-the-art nonlinear solvers able to handle the large systems arising from system discretization. With the ever increasing advances in computing power and availability of large-scale nonlinear optimization solvers, we hope that the simultaneous approach will be widely used for approaching large-scale and highly nonlinear optimal control problems.

One of the aims of dynamic optimization techniques lies in computing optimal startup control policies of processing equipment. Frequently, heuristic-based methodologies are used for such a purpose. However, they normally tend to demand long startup times, leading to large amounts of off-specification

* To whom correspondence should be addressed. E-mail: antonio.flores@uia.mx. Phone/fax: +52(55)59504074. http://200.13.98.241/~antonio.

10.1021/ie901138p
Published on Web 11/24/2009

material and excessive energy consumption. Dynamic optimal startup policies can contribute to remove startup problems, leading to a profitable system featuring improved operability characteristics.

In this work, we formulate the startup problem of a continuous stirred bioreactor as an open-loop optimal control problem, aiming to compute optimal startup policies featuring minimum transition time. The optimal theoretical startup policies were experimentally implemented in a lab-scale bioreactor. Good agreement was observed between the predicted and measured biomass concentration.

2. Materials and Methods

A recombinant strain of *Saccharomyces cerevisiae W303* (pRS6:: Δ*NSITPS1*/pSAL4:: *ScTPS2*) maintained on defined medium without uracil[8] was grown at a dilution rate $D = 0.096$ h^{-1} under a carbon source limitation at 30 °C in a stirred tank bioreactor (BioFlo III, New Brunswick Scientific, NJ) with a working volume of 2.5 L. The airflow was kept at 0.4 vvm (air volume × volume × minute), and the stirred speed varied in order to maintain the dissolved oxygen concentration at 20%. The concentrations of the components in the synthetic mineral medium were calculated from elementary balancing. Cultivation was initiated with a batch operation; continuous operation was started at different times and feed concentration. Biomass concentration was determined by turbidimetry at 560 nm. Sucrose was analyzed by HPLC, using an Aminex column HPX-87H (BioRad, USA) with 0.05 N H_2SO_4 like eluant, at 30 °C with an RI detector. We would like to remark that our recombinant yeast features some advantages, in comparison with wield yeasts, such as larger biomass concentration and greater stress tolerance.

3. Mathematical Model

The mathematical model of a perfectly stirred nonconstant volume continuous bioreactor is given as follows:

$$\frac{dx}{dt} = -D_2 x + \frac{D_{max} s}{(k_s + s)} x - \frac{x}{V}(Q_1 - Q_2) \quad (1)$$

$$\frac{ds}{dt} = D_1 s_f - D_2 s - \frac{D_{max} s}{Y_{x/s}(k_s + s)} x - \frac{s}{V}(Q_1 - Q_2) \quad (2)$$

$$\frac{dV}{dt} = Q_1 - Q_2 \quad (3)$$

where x [g cell/L] stands for the biomass concentration, s [g substrate/L] is the substrate composition, D_1 [1/h] is the input dilution rate, D_2 [1/h] is the output dilution rate, Q_1 is the feed stream volumetric flow rate, Q_2 is the output volumetric flow rate, s_f [g substrate/L] is the substrate concentration in the feed stream. D_{max} [1/h], k_s [g substrate/L], and $Y_{x/s}$ [g cell/g substrate] are kinetic constants. It should be noted that the above model is a perfectly mixed continuous bioreactor model. We have assumed that, to optimally start up the reactor, from certain initial conditions (to be optimized as well), we manipulate the feed stream substrate concentration (s_f) and control the absorbance (A) of the product mixture. The absorbance is an indirect measure of biomass composition since $A = x/1.1$.

According to Henson,[9] mathematical models used for dynamic simulation and control of bioreactors can be classified at least in three categories, depending on the level of detail to describe a single cell: (1) unstructured and lumped models, where internal cell phenomena (such as modeling individual enzyme-catalyzed reactions) are ignored and the model equations represent the behavior of an "average" cell, (2) structured and lumped models, where mechanistic descriptions of cellular metabolism are used and are based on the modeling of individual enzyme-catalyzed reactions, (3) distributed models, which take into account population heterogeneities where cells, yeast, etc. are modeled as discrete components. The resulting model is cast in terms of population balances equations that lead to integral−differential equations, that could be hard to numerically solve. Some modeling and control results using this approach have been reported in ref 10.

In our work the model used corresponds to an unstructured and lumped model. Although the mathematical model structure looks simply, a mathematical model should not probably be judged only on its apparent complexity (i.e., the number of states to be modeled, complex nonlinear patterns, etc.), but rather on its ability to mimic the addressed phenomenon. We found that the structure of the model used in our work was enough to represent the behavior we intended to control. Moreover, the matching between experimental and theoretical results supports this statement. According to Henson,[9] this kind of model has found wide application in control studies. However, it remains as a research topic to base bioreactor modeling and control studies on distributed models.

4. Parameter Fitting

To improve model predictions, two sets of experimental runs were done. The parameters fitted were D_{max}, k_s, and $Y_{x/s}$. The first set of experimental runs were done far away from the expected operating conditions to test model prediction capabilities. Using the experimental information, a standard nonlinear parameter procedure was used to get the kinetic value constants that best fit the experimental data. Figure 1 displays the comparison between experimental vs fitted model behavior for the two sets of experimental information. Overall, as shown, the fitted model predictions are satisfactory. For the first set of experimental measurements, Figure 1a, the fitted parameter values are $D_{max} = 0.457463$, $k_s = 0.291433$, and $Y_{x/s} = 1.14612$, whereas for the second set of experimental measurements, Figure 1b, the fitted parameter values are $D_{max} = 0.39461$, $k_s = 0.0924537$, and $Y_{x/s} = 0.894432$. Even when no optimal global solutions were searched, the nonlinear parameter problem was solved from different guessed parameter values to ensure the best possible optimal solution. It should be noted that fitted $Y_{x/s}$ values are larger than the reported values. However, large $Y_{x/s}$ values could be explained noticing that most of the ethanol is quickly consumed, suggesting diauxic growth or multiple carbon substrate.

We would like to highlight the reasons why we report different parameter sets for the same organism under two different operating conditions. When operating a reactor in the biochemical batch or semibatch regime, wide variations in dynamic behavior are normally expected. It would be difficult for a single model, with fixed parameters, to represent the system behavior along the dynamic trajectory, and under all initial and steady-state conditions. There is nothing wrong in using the same mathematical model structure with different sets of parameters. The value of the fitted parameters may depend not only on the type of organism, but also on the operation region. Such a model simply reflects the fact that a single mathematical model with fixed parameters may be unable to completely describe the system dynamics, under

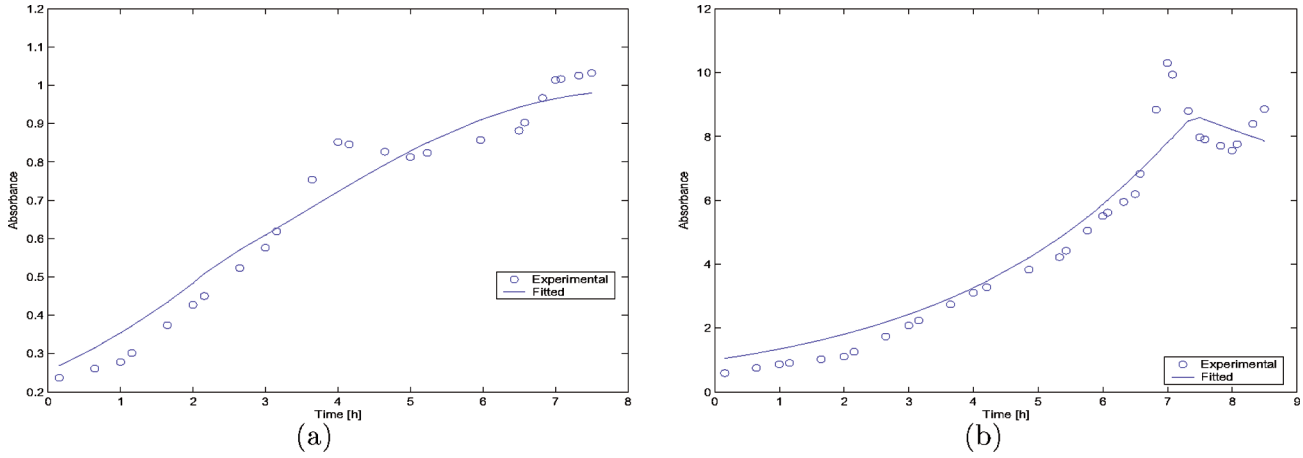

Figure 1. Comparison between measured and fitted model absorbance response for each on of the two experimental systems.

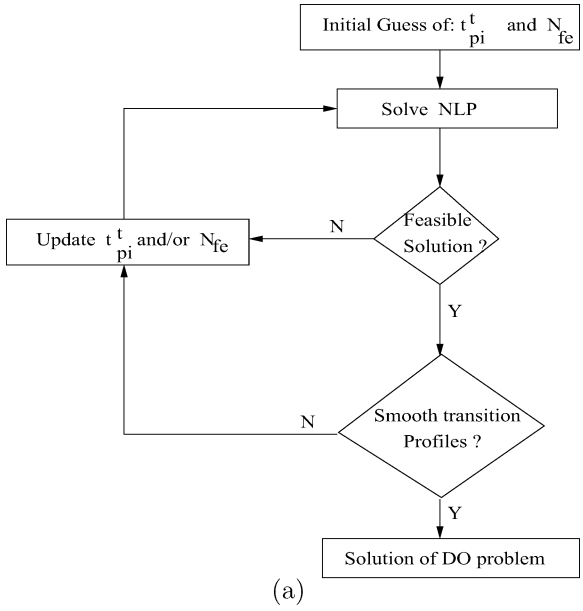

Figure 2. Iterative algorithm for the solution of the dynamic optimization (DO) problem. t^t_{pi} stands for the startup or shutdown time values, and N_{fe} is the number of finite elements.

Table 1. Information Regarding the Final Desired Steady-State Operating Conditions (x^d, s_f^d) for Each Operating Scenario[a]

	case 1	case 2
x^d [g cell/L]	10	10
s_f^d [g subsrate/L]	8.8	11.2
x_i [g cell/L]	1	1
s_i [g substrate/L]	17.806	10
N_{fe}	10	10
N_{col}	3	3
CPU time [s]	0.141	0.093

[a] x_i and s_i stand for the optimal initial biomass and substrate concentration, respectively.

all the expected operating conditions. Moreover, even when two different sets of fitted parameters are provided in our work, only the parameters fitted around the dynamic trajectory, leading to the expected steady-state conditions (see Figure 1b), were actually used for the optimal startup and shutdown calculations. As mentioned above, the parameters fitted for reproducing the results shown in Figure 1a are only provided to demonstrate that the model can reproduce experimental results, under operating conditions far away from the expected steady-state processing conditions.

5. Optimization Formulation

As described in the work of Flores-Tlacuahuac et al.,[11] simultaneous dynamic optimization (SDO) provides a way to compute optimal dynamic policies, even in the presence of challenging nonlinear behavior. These include transitions to unstable points, optimization with chaotic systems,[12] and systems with limit cycles and bifurcations. In SDO, computation of optimal transitions policies reduces to the solution of a nonlinear problem (NLP) as shown in the work of Biegler et al.[13] and provides values of the decision variables (i.e., the manipulated variables) that drive the system toward minimum transition time or off-spec product.

Startup Period. A common requirement during the startup of bioreactors is that startup features minimum transition time, waste material, or utility consumption. The minimum startup time policy requires setting the following optimization problem (where we have assumed that the manipulated variable is feed stream substrate concentration s_f and the tracking variable is the absorbance A):

$$\min_{x,s,s_f,A,s_i,x_i} \int_0^{t_f} \{\alpha_a(A - A^d)^2 + \alpha_s(s_f - s_f^d)^2\}\, dt \tag{4}$$

s.t. Mathematical model

$$\frac{dx}{dt} = -Dx + \left(\frac{D_{max}s}{k_s + s}x\right) \tag{5}$$

$$\frac{ds}{dt} = D(s_f - s) - \left(\frac{D_{max}s}{Y_{x/s}(k_s + s)}x\right) \tag{6}$$

$$A = 0.9091x \tag{7}$$

Initial conditions

$$x(0) = x_i \tag{8}$$

$$s(0) = s_i \tag{9}$$

Bounds

$$\begin{gathered} x \geq x^u \\ s \geq s^u \\ s_f^l \leq s_f \leq s_f^u \\ x_i^l \leq x_i \leq x_i^u \\ s_i^l \leq s_i \leq s_i^u \end{gathered} \tag{10}$$

where t_f is the transition horizon. The objective function represents the deviation of the absorbance and feed stream substrate concentration from their desired target values. Notice that both A^d and s_f^d represent the values of the absorbance and

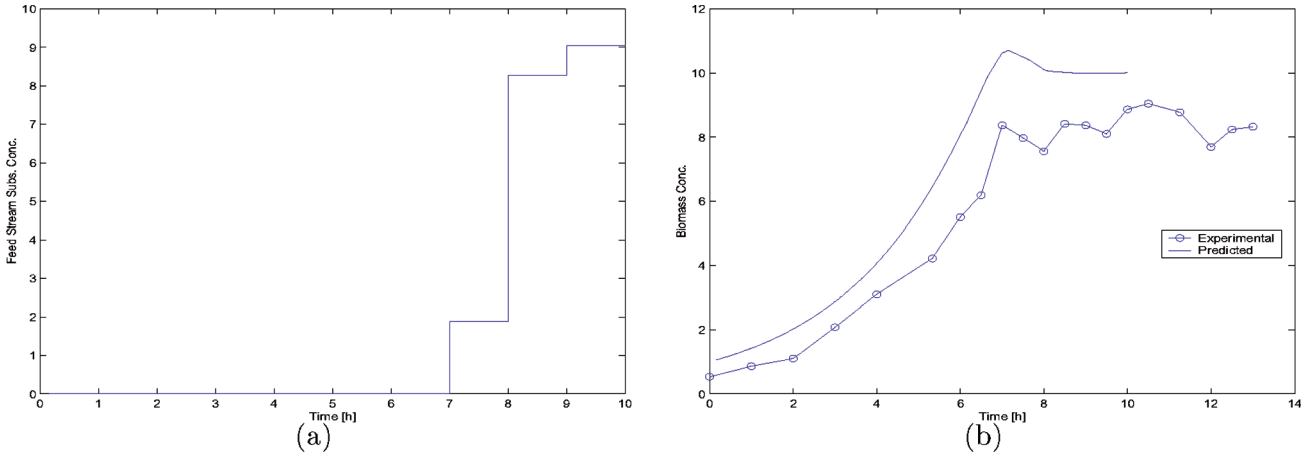

Figure 3. Results for the first operating point. (a) Optimal profile of the manipulated variable (S_f). (b) Comparison between predicted and lab-scale bioreactor responses.

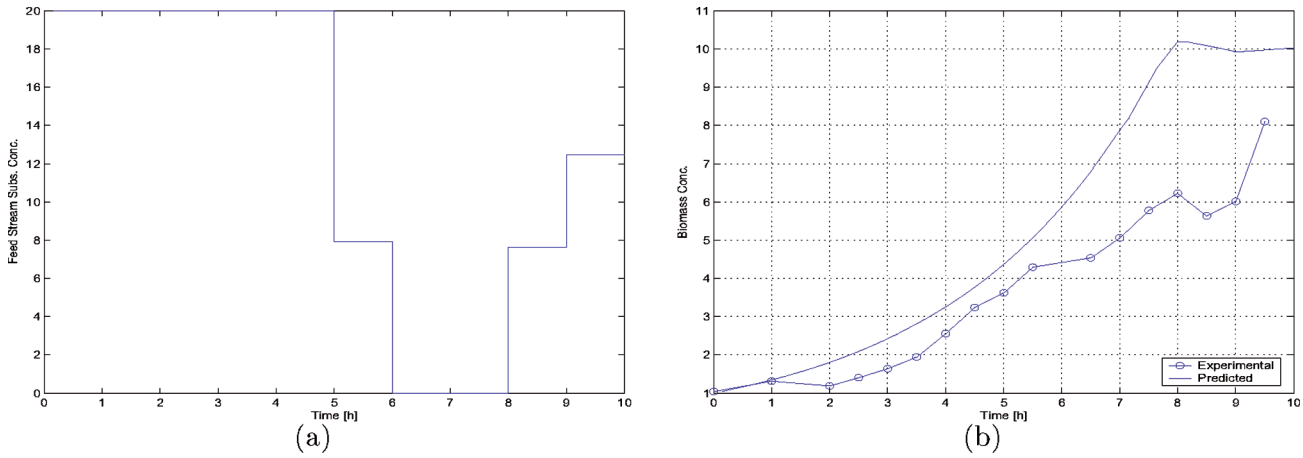

Figure 4. Results for the second operating point. (a) Optimal profile of the manipulated variable (S_f). (b) Comparison between predicted and lab-scale bioreactor responses.

feed stream substrate concentration, at the end of the desired startup period. Such values are normally available from steady-state calculations since, in order to compute a startup transition, we need to know the values of the controlled and manipulated variables at the initial and final operating points. Moreover, by trial and error, fast dynamic reactor responses were obtained using weights values of $\alpha_a = 10^8$ and $\alpha_s = 1$ in the objective function. A large α_a value was needed to get closer tracking of the absorbance A. When computing control actions, we normally would like to get "smooth" control actions, meaning control signals that do not exhibit abrupt or strong variations, because they could be difficult to implement. We found that a value of $\alpha_s = 1$ allowed us to get such smooth control actions. It should be noted that the initial concentration of both biomass and substrate (x^i, s^i) were also decision variables, because we found that minimum time optimal startup policies were highly sensitive to the initial concentrations of the reactants. The upper bounds vector is given as follows: $[x^u\ s^u\ s_f^u\ x_i^u\ s_i^u]^T = [0\ 0\ 20\ 1\ 10]$, whereas the lower bounds vector reads as follows: $[s_f^l\ x_i^l\ s_i^l]^T = [0\ 0\ 0]$.

Regarding the selection of the manipulated variable during the optimal startup period, we would like to mention that our aim was to propose an optimization formulation able to startup the reactor, from a given set of initial conditions to the desired steady-state operation region, featuring minimum startup time. We tested several optimization formulations, including of course the manipulation of Q_1^f and Q_2^f, and we came to the conclusion that the best theoretical results (i.e., minimum startup time) were obtained by filling the reactor up to the desired final volume and then taking the substrate feed stream concentration (s^f) as the single manipulated variable. By initially filling the reactor up to its final steady-state value, we did not need to manipulate Q_1^f and/or Q_2^f during the startup of the reactor. Of course, after reaching the desired steady-state processing conditions, both Q_1^f and Q_2^f were manipulated to keep continuous operation of the bioreactor, but this operating regime was not addressed in the present work.

Shutdown Period. In the research literature, shutdown operations of bioreactors have not received the same amount of attention than startup strategies. This is partially due to the fact that, during startup periods, the process has to reach the desired operating conditions in the most efficient way, since the process will remain operating under this region for a certain time period. However, during the shutdown phase, there is the feeling that the process operation should cease without paying attention to the product that invariably remains inside the process. Our shutdown approach recognizes that the product inside the process should be recovered, because it represents process profit. Therefore, our aim is to propose a shutdown optimization formulation that will take the reactor from an initial steady-state operating point and will drive the process operation until desired shutdown conditions are reached, minimizing the amount of off-specification material. The proposed shutdown optimization formulation reads as follows.

$$\min_{S^f,Q_1,Q_2,D_1,D_2,x,s,V,\text{abs}} \text{(shutdown time)} \tag{11}$$

s.t. Mathematical model

$$\frac{dx}{dt} = -D_2 x + \left(\frac{D_{max} s}{k_s + s}\right)x - \frac{x}{V}(Q_1 - Q_2) \tag{12}$$

$$\frac{ds}{dt} = D_1 s^f - D_2 s - \left(\frac{D_{max} s}{Y_{x/s}(k_s + s)}\right)x - \frac{s}{V}(Q_1 - Q_2) \tag{13}$$

$$\frac{dV}{dt} = Q_1 - Q_2 \tag{14}$$

$$Q_1 = D_1 V \tag{15}$$

$$Q_2 = D_2 V \tag{16}$$

$$A = 0.9091x \tag{17}$$

Initial conditions

$$x(0) = x^0 \tag{18}$$

$$s(0) = s^0 \tag{19}$$

$$V(0) = V^0 \tag{20}$$

End conditions

$$Q_1(t^f) = Q_1^f \tag{21}$$

$$Q_2(t^f) = Q_2^f \tag{22}$$

$$V(t^f) = V^f \tag{23}$$

Bounds

$$\begin{aligned} x^l &\le x \le x^u \\ s^l &\le s \le s^u \\ V^l &\le V \le V^u \\ s^{f,l} &\le s^f \le s^{f,u} \\ Q_1^l &\le Q_1 \le Q_1^u \\ Q_2^l &\le Q_2 \le Q_2^u \\ D_1^l &\le D_1 \le D_1^u \\ D_2^l &\le D_2 \le D_2^u \\ 0.95A^d &\le A \le 1.05A^d \end{aligned} \tag{24}$$

The objective function features minimization of the shutdown time using the input and output flow rates (Q_1^f, Q_2^f) as the manipulated variables, for driving the process until shutdown conditions are reached. We have found that optimal shutdown conditions are easier to compute, when allowing the feed stream substrate concentration (s^f) to become also a decision variable. The initial operating conditions are represented by eqs 18−20, whereas eqs 21−23 state the final desired shutdown conditions. The set of eqs 24 simply represent lower and upper bounds on the decision variables.

In the above formulation, the initial conditions and end point vectors are given as follows: $[x^0\ s^0\ V^0]^T = [9.99\ 0.4141\ 2.5]$, $[Q_1^f\ Q_2^f\ V^f]^T = [0\ 0\ 2.5]$, respectively. The upper and lower bound vectors are given as follows: $[x^u\ s^u\ V^u\ s^{f,u}\ Q_1^u\ Q_2^u\ D_1^u\ D_2^u]^T = [20\ 2\ 5\ 60\ 0.6\ 1\ 0.5\ 0.5]$, $[x^l\ s^l\ V^l\ s^{f,l}\ Q_1^l\ Q_2^l\ D_1^l\ D_2^l]^T = [0\ 0\ 0\ 0\ 0\ 0\ 0\ 0]$, respectively. Regarding the absorbance, it is always constrained to lie between lower and upper bounds around its initial steady-state value. In this way, we ensure that the material remaining inside the reactor during shutdown operations can always be sold as main product, because it meets product specification:

$$0.95A^d \le A \le 1.05A^d \tag{25}$$

where $A^d = 9.08$ is the desired absorbance value, around which the absorbance of the material remaining inside the reactor should be kept.

In the SDO approach, the DAE optimization problem is converted into an NLP by approximating both the state and control profiles by a family of polynomials on finite elements. Here a Runge−Kutta discretization with Radau collocation points is used, as it allows constraints to be set easily at the end of each element and to stabilize the system more efficiently if high index DAEs are present. In addition, the integral objective function is approximated with Radau quadrature with N_{fe} finite elements and N_{col} quadrature points in each element. As shown in the work of Flores-Tlacuahuac et al.,[11] substitution of this discretization into (5) and (6) or (12)−(14) applied at the collocation points leads to the following NLP.

$$\min_{x \in R^n} f(x) \tag{26}$$

s.t.

$$c(x) = 0 \tag{27}$$

$$x_L \le x \le x_U \tag{28}$$

where x represents coefficients of the piecewise polynomials that make up the control and state profiles. More details of this approach can be found in the work of Flores-Tlacuahuac et al.[11] The dynamic optimization formulation given by (26)−(28) was implemented using the AMPL mathematical programming language and solved using the IPOPT algorithm developed in the work of Wachter and Biegler[14] for large-scale nonlinear programming.

Remarks on Models Used for Optimization Purposes. The model described in eqs 1−3 represents the operation of a continuously stirred tank reactor (CSTR), where reactor volume (V) is allowed to change. Commonly, during the dynamic operation of CSTRs, reactor volume variations are not modeled because perfect holdup control is assumed. This normally means that the volumetric input flow rate (Q_1) has the same value than the volumetric output flow rate (Q_2), therefore avoiding the need to introduce eq 3. Because in our work, we are interested in the computation of dynamic optimal profiles during startup and shutdown operating scenarios, dynamic volume variations ought to be taken into account. As it can be noticed, eqs 5−6 are similar to eqs 1−3 providing $Q_1 = Q_2$ and $D_1 = D_2 = D$. The reason why we removed eq 3 in section (5) about optimal startup calculations, is because the manipulated variable (s_f) used for optimal startup calculations does not change reactor volume. Hence, eq 3 is not needed as part of the optimal startup dynamic mathematical model as given by eqs 5−6. We would like also to highlight that the situation is different for the dynamic optimal shutdown reactor calculations as represented by eqs 12−14. Here, because the manipulated variables, among other variables, are Q_1 and Q_2, the reactor holdup will not be constant, and therefore, we ought to model dynamic volume variations.

6. Theoretical and Experimental Results

We would like to highlight that our aim was to compute control actions leading to minimum startup and shutdown times. There are several ways of doing this. One of them is to explicitly minimize such startup or shutdown times. However, there is a reason why we decided not to follow this approach: it might increase the nonconvexity of the optimization formulation leading to larger CPU times for getting optimal solutions. There is an alternative approach for getting minimum transition times, that is based on minimizing the euclidian norm between system states and manipulated variables, as shown in eq 4. We have

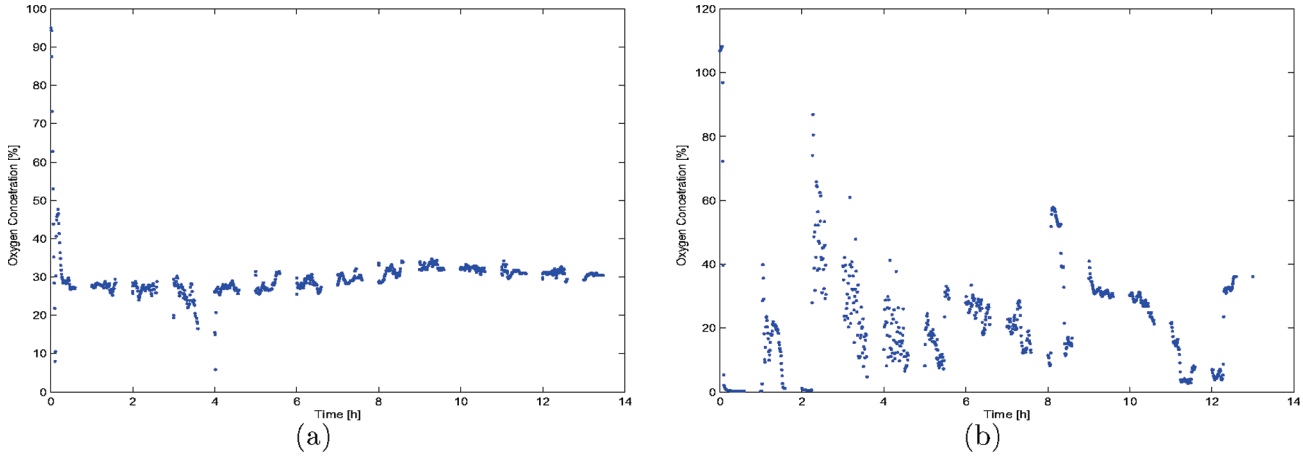

Figure 5. Measured oxygen concentration during startup of the bioreactor: (a) first and (b) second experiments.

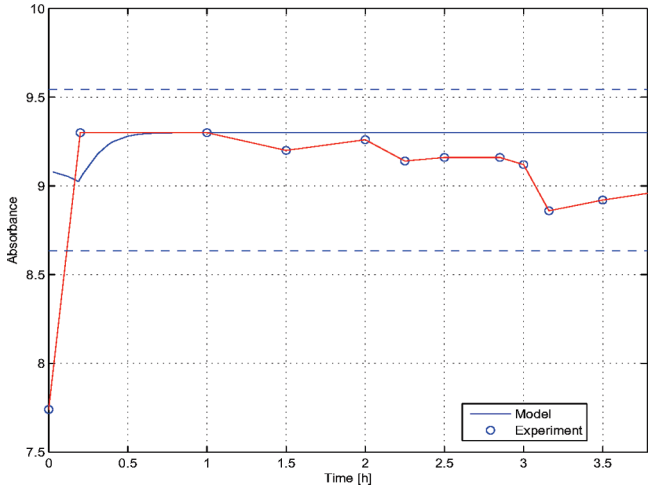

Figure 6. Comparison between predicted vs measured experimental absorbance during the shutdown period. The two dashed lines specify the upper and lower bounds on product purity.

found that, using the optimization formulation given by eqs 4−10, leads to practically the same optimal startup time, as compared to the case when the startup time was used in the objective function. Moreover, because in this optimization formulation the transition time is not a decision variable, the nonconvexity of the problem is not unnecessarily increased leading to an optimization problem easier to solve.

Startup Period. The dynamic optimization problem represented by eqs 4−10 was solved for the two aforementioned fitted models. Minimum time optimal startup policies were sought. Even when the startup time was not directly a decision variable, we used an iterative approach (see Figure 2) to determine minimum startup times. We have found that this approach works well and avoids potential nonconvexities introduced when the startup time is taken as a decision variable. Moreover, we have used, in other types of dynamic systems, the time as decision variable and found that, after a small number of iterations, the results from the iterative and direct approaches are similar.

Table 1 shows the computed results for each case. As shown, in all cases, the desired biomass concentration x^d was 10, while the final value of the manipulated variable s_f^d is different, because they represent two different operating scenarios. As noticed from Table 1, in both cases, only ten finite elements N_{fe} and three collocation points N_{col} were required to represent system dynamics. In both cases, the number of decision variables and equality constraints was 172 and 160, respectively. The CPU time was low since, by today's standards, this a small-scale problem. Finally, it should be stressed that the dilution rate D was not used as a manipulated variable due to difficulties for handling small dilution rate values in our experimental facilities. Instead, the dilution rate was always set to 0.096 1/h.

The dynamic optimal theoretical results are displayed in Figures 3 and 4. The continuous line in Figure 3a represents the predicted dynamic optimal control profiles, that should be used to drive the output system response (biomass concentration x) to the desired steady-state. Because the optimal control policies will be manually implemented in a lab-scale bioreactor, all the optimal control policies were enforced to take steplike behavior. As displayed in Figure 3, the experimental results, obtained by applying the optimal control profile, closely track the theoretical profile. At the beginning, the yeast consumes the initial amount of substrate, because no substrate is fed during the first seven operating hours. When most of the substrate has been consumed, the optimal solution decides to feed substrate for the first time. Initially, a small substrate shot is used to drive the system close to the desired steady state. Finally, a larger substrate increase is used near the end of the operating period. We would like to stress that, using the optimal operation profiles, enough oxygen concentration was always maintained as displayed in Figure 5a.

Figure 4 displays the results of using the dynamic optimal control profiles for the second example. In this case, the feed stream substrate concentration hits the upper limit because, as shown in Table 1, the initial amount of substrate seems to be small. High feed stream concentration is kept for the first five operating hours. After enough substrate has been accumulated within the system, the optimizer decides to stop feeding substrate. From this point on, the system behaves as a semibatch reactor. The optimizer uses large control actions for reaching the desired steady-state. The tracking of the theoretical signal was not as good as for the past example, because it takes more control action to drive the system to the desired steady state. Similarly, as shown in Figure 5b, good oxygen concentration characteristics were maintained.

In continuous operation, good aeration and yeast characteristics lead to acceptable oxygen concentration as displayed in Figure 5. Maintaining high oxygen concentration levels (beyond the oxygen critical concentration) promotes better biomass growth. In comparison, we have observed that in pure batch operation, oxygen is quickly exhausted. Therefore, during batch operation, changes in the metabolic network could occur leading to undesired products.

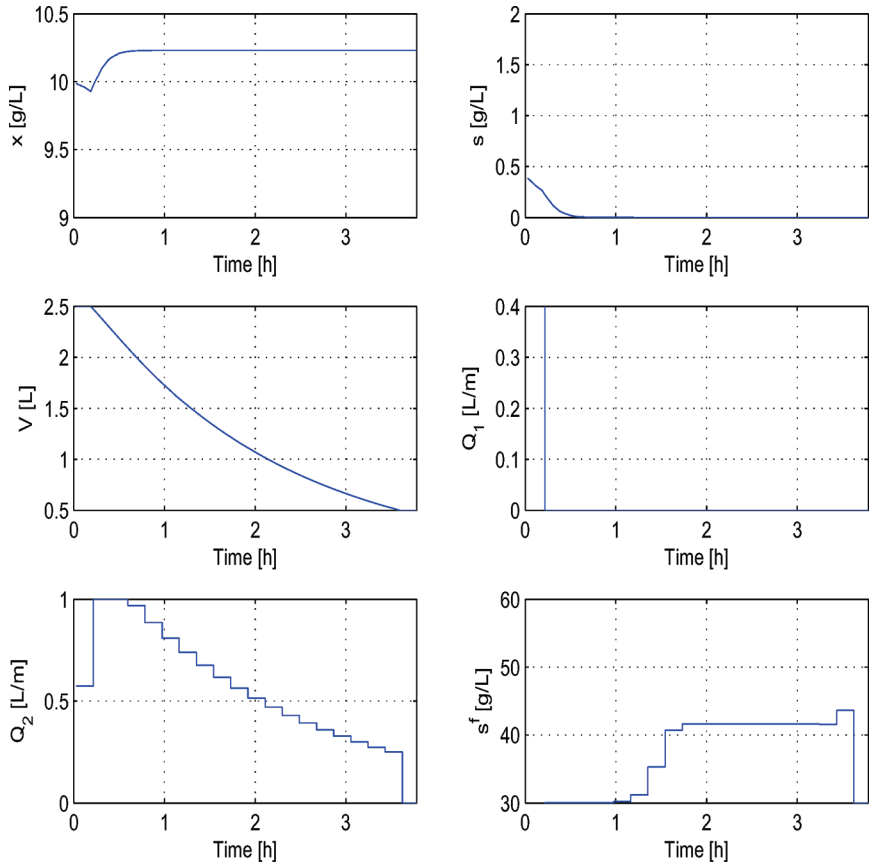

Figure 7. Dynamic optimal response of the bioreactor system during the shutdown procedure. The terms x and s are the biomass and substrate concentration, respectively; V is the reactor volume. Q_1^f and Q_2^f are the input and output flow rates, respectively, and s^f is the feed stream substrate concentration which are also the manipulated variables driving the process during the optimal shutdown transition.

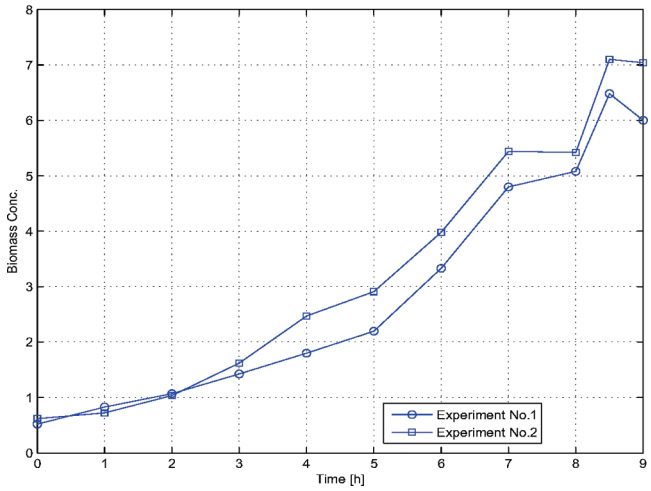

Figure 8. Experimental results of two runs in batch operation mode.

Shutdown Period. For solving the shutdown dynamic optimization formulation represented by eqs 11−24, the time solution space was discretized using 20 finite elements with 3 internal collocation points. This formulation gives rise to 758 decision variables and 701 equality constraints. The optimization formulation was solved in 1.688 s CPU time using a laptop pc with 4 M RAM. The results of the shutdown optimization formulation are depicted in Figures 6 and 7. As clearly seen in Figure 6, the shutdown procedure is carried out without violating the purity constraints as measured by the absorbance. Hence, during the shutdown procedure, the product inside the reactor satisfies the purity constraints. This means that the material remaining in the reactor when the shutdown procedure starts is a profitable product and not a waste or off-specification product, as can occur when using nonoptimal shutdown procedures. Using the design information provided in section 5, the optimal shutdown conditions were achieved in around 4 h. In Figure 7, the values of both the states and manipulated variables optimal dynamic profiles are shown. Because the final reactor volume ought to be smaller than the initial one, the optimizer finds that $Q_2^f > Q_1^f$ for most of the dynamic transition, except at the end of the transition. The changes in Q_1^f and Q_2^f are small leading to a smooth dynamic response in the reactor volume. We demand that, at the end of the transition, the reactor volume stays at 0.5 L such that a new startup procedure could be initiated using the reactor content. We have observed that optimal shutdown conditions that satisfy the purity constraints, must also manipulate the feed stream substrate concentration (s^f). As a matter of fact, the dynamic optimization formulation shown in eqs 12−24 cannot determine an optimal solution if s^f is maintained fixed. Therefore, by allowing s^f to become a decision variable permits us to meet the product constraints. From Figures 6 and 7, it can be noticed that the proposed shutdown optimization formulation is able to find an optimal shutdown strategy, minimizing the amount of off-specification material normally formed during shutdown operations.

Remarks on the Implementation of Control Actions. During the startup phase, we found that the manipulation of the substrate feed stream concentration (s^f) allowed us to quickly reach steady-state conditions. We also found that for optimal shutdown of the bioreactor from steady-state operating conditions, the simultaneous manipulation of s^f and the input/output volumetric flow rates (Q_1 and Q_2, respectively) resulted in

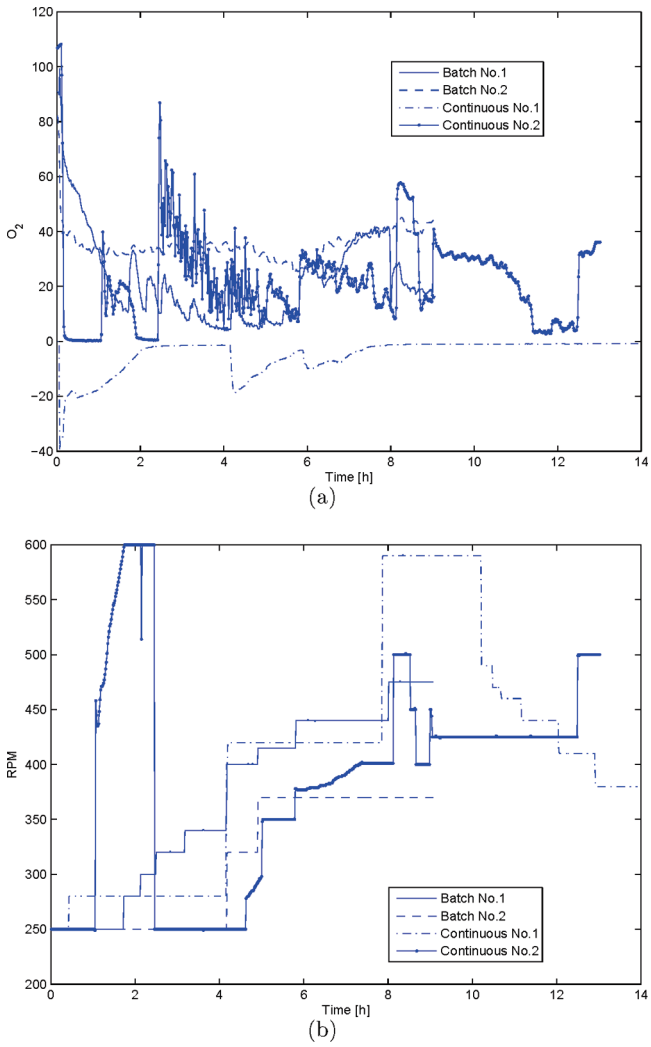

Figure 9. Comparison of oxygen consumption between batch and continuous processes.

minimum shutdown time. For the online manipulation of s^f, several samples were prepared before starting up the reactor. This is because all the optimal operating policies were calculated off-line, meaning that the optimal operating policies were ready available for their experimental implementation. For instance, from Figure 3a, we can see that the optimal startup operating policy indicates that after 7 h of operation the s^f value should be changed from 0 to a value denoted by s_1^f. Then after 8 h, the s^f value changes from s_1^f to an s_2^f value, and finally after 9 h, the value changes from s_2^f to an s_3^f value. Because we already knew the values of s_1^f (2 g/L), s_2^f (8.2 g/L) and s_3^f (9 g/L), three substrate samples with these requested substrate concentrations were prepared, before starting up the experimental bioreactor. For the online manipulation of s^f, we carried out the following procedure. Around 5 min before changing each one of the three s^f values, a 10 mL sample was taken out from the bioreactor to analyze product composition. Then, we injected also 10 mL of the requested substrate feed stream concentration (s_1^f, s_2^f, or s_3^f depending upon the desired value of the control action). Because the amount of the sample that was taken out was similar to the amount of injected substrate, the reactor volume did not change. Moreover, the amount of product sample was small compared to the reactor volume (2 L). Exactly the same approach was used for the implementation of the s^f control actions during the optimal shutdown procedure.

7. Comparison against Batch Operation Mode

In this part we compare the performance of the continuous vs the batch operating mode. The first continuous reactor is started with 17.8 g/L, whereas the biomass initial concentration was 1 g/L. As it can be noticed from Figure 9a, most of the oxygen is quickly consumed leading to an increase in stirrer speedup and rising oxygen concentration. This is so because oxygen concentration is controlled by the impeller speed. Hence, the feed stream profile allowed meeting the oxygen demand. Regarding the second continuous reactor, the feed stream was started right from the beginning, and because oxygen was consumed by the initial biomass, there was a sudden decrease in oxygen concentration, giving rise to a large increase in impeller speed aiming to recover the normal oxygen set point. Later, there is a time period where oxygen concentration oscillates, because the biomass is not growing further and there is small oxygen consumption. Only when the biomass has adapted does the growing stage starts and agitation speed increase as well. However, it can be noticed that the oxygen concentration in the second continuous experiment changes abruptly, with respect to the first continuous experiment. This behavior could be associated with the feed stream policy. On the other hand, in batch experiments (see Figure 8), neither stirrer speed nor oxygen concentration change markedly until the yeast starts the growing stage leading to an oxygen demand increase.

8. Conclusions

In this work, we have addressed the practical implementation of dynamic optimal control policies for the startup and shutdown of a continuous stirred bioreactor. Optimal control policies were open-loop implemented, although closed-loop implementation is highly desirable for industrial-scale bioreactors. We have used a new kind of recombinant strain capable of greater alcohol production and maintaining good oxygenation characteristics.

When addressing optimal control calculations, the same values of the fitted parameters were maintained along the whole startup procedure. There is a good chance that both the structure of the model and the parameters embedded change with respect to the operating conditions. Using different models and parameter values for approaching system behavior at different operating regions is hardly a new idea, but it has been reported as a critical point in some bioengineering systems. To address optimal control calculation for systems featuring different model structures and parameter values, a hybrid optimal control problem ought to be solved as discussed in the work of Doyle et al.[15] This is a relatively new research area, mostly for nonlinear systems, since some work has been reported for linear ones.[16] Other research areas that deserve attention are related to the modeling and online optimal control of bioreaction systems described in terms of partial and integral equations, like the one described in the work of Henson,[10] and to the modeling and control of bioreaction tubular systems. We have started to address this last problem for the continuous production of ethanol using cellulosic raw materials.

Literature Cited

(1) Alford, J. S. Bioprocess control: Advantages and challenges. *Comput. Chem. Eng.* **2006**, *30*, 1464–1475.

(2) Bryson, A. E.; Ho, Y. C. *Applied Optimal Control*; Taylor and Francis: London, 1981.

(3) Cervantes, A.; Biegler, L. T. Large-Scale DAE Optimization using a simultaneous NLP formulation. *AIChE J.* **1998**, *44*, 1038–1041.

(4) Biegler, L. T. An overview of simultaneous strategies for dynamic optimization. *Chem. Eng. Process.* **2007**, *46* (11), 1043–1053.

(5) Kameswaran, S.; Biegler, L. T. Simultaneous Dynamic Optimization Strategies: Recent Advances and Challenges. *Comput. Chem. Eng.* **2006**, *30* (10−12), 1560–1575.

(6) Allgor, R. J.; Barton, P. Mixed-Integer Dynamic Optimization I: Problem Formulation. *Comput. Chem. Eng.* **1999**, *23* (4−5), 567–584.

(7) Flores-Tlacuahuac, A.; Biegler, L. T.; Saldívar-Guerra, E. Optimization of HIPS Open-Loop Unstable Polymerization Reactors. *Ind. Eng. Chem. Res.* **2005**, *44*, 2659–2674.

(8) Pedraza-Segura, L. Efecto de la sobrexpresión del gen ScTPS2 en el metabolismo decarbono de Saccharomyces cerevisiae. M.Phil. thesis, Universidad Autónoma del Estadode Morelos, 2005.

(9) Henson, M. A. Biochemical Reactor Modeling and Control. *IEEE Control Syst. Mag.* **2006**, 54–62.

(10) Henson, M. A. Dynamic Modeling and Control of Yeast Cell Populations in Continuous Biochemical Reactors. *Comput. Chem. Eng.* **2007**, *27*, 1185–1199.

(11) Flores-Tlacuahuac, A.; Saldívar-Guerra, E.; Ramírez-Manzanares, G. Grade Transition Dynamic Simulation of HIPS Polymerization Reactors. *Comput. Chem. Eng.* **2005**, *30*, 357–375.

(12) Kallrath, J.; Schloeder, J. P.; Bock, H. G. Least squares parameter estimation in chaotic diferential equations. *Celestial Mechan. Dyn. Astron.* **1993**, *56*, 353–371.

(13) Biegler, L. T.; Cervantes, A. M.; Watcher, A. Advances in Simultaneous Strategies for Dynamic Process Optimization. *Chem. Eng. Sci.* **2002**, *57*, 575–593.

(14) Wachter, A.; Biegler, L. T. On the implementation of an interior point filter line search algorithm for large-scale nonlinear programming. *Math. Prog.* **2006**, *106* (1), 25–57.

(15) Doyle, F. J., III.; Harrison, C. A.; Crowley, T. J. Hybrid model-based approach to batch-to-batch control of particle size distribution in emulsion polymerization. *Comput. Chem. Eng.* **2003**, 1153–1163.

(16) Bemporad, A.; Morari, M. Control of systems integrating logic, dynamics, and constraints. *Automatica* **1999**, *35* (3), 407–427.

Received for review July 15, 2009
Revised manuscript received October 13, 2009
Accepted October 20, 2009

IE901138P