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EXPERIMENTAL VERIFICATION OF OPTIMAL START-UP POLICIES IN A CONTINUOUS STIRRED BIOREACTOR

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Abstract: In this work dynamic optimal start-up policies were computed and validate in an experimental lab scale continuous bioreactor. Using the theoretical optimal profile a reduction in the start-up time was achieved when compared to simple step-like changes in the manipulated variables. Therefore, the use of optimal start-up policies led to raw material savings. To test the optimal start-up 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. Our aim was to demonstrate that dynamic optimization is a valuable tool when approaching the dynamic operation of bioreactors assuming that a good model representation of the system is available.

1. INTRODUCTION

Dynamic optimization has been proposed for improving the dynamic operation of processing system (Kameswaran and Biegler, 2006). In fact, this approach has been widely used for computing optimal start-up and switching control policies. For medium to large scale systems, two optimal control methodologies seem to 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 (Allgor and Barton, 1999). This approach is commonly called the sequential optimal control approach. On the other hand, in the simultaneous approach both the set 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 (Kameswaran and Biegler, 2006). Although it has been claimed that the sequential approach is easy to use it has some disadvantages. Presently, it seems to be unable to handle open-loop unstable systems without previous stabilization. Quite the contrary, it has been shown that the simultaneous approach is able to efficiently handle unstable systems (A. Flores-Tlacuahuac, 2005). However, the simultaneous approach demand 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 the

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availability of large scale nonlinear optimization solvers, it seems 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 start-up control policies of processing equipment. Frequently, heuristic based methodologies are used for such purpose. However, they normally tend to demand long start-up times leading to large amounts of off-specification material and excessive energy consumption. Dynamic optimal start-up policies can contribute to remove start-up problems leading to a profitable system featuring improved operability characteristics.

In this work we formulate the start-up problem of a continuous stirred bioreactor as an open-loop optimal control problem aiming to compute optimal start-up policies featuring minimum transition time. The optimal theoretical start-up 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 (Pedraza-Segura, 2005), was grown at D=0.096 h^{-1} under carbon source limitation at 30^{o}C in a stirred tank bioreactor (BioFlo III, New Brunswick Scientific, N.J., USA) with a working volume of 2.5 L. The airflow was kept at 0.4 vvm 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 were 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^{o}C with 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 the bioreactor is given as follows:

$$\frac{dx}{dt} = -Dx + \frac{\mu_{max} s}{(k_s + s)} x \quad (1)$$

$$\frac{ds}{dt} = D(s_f - s) - \frac{\mu_{max} s}{Y_{x|s}(k_s + s)} x \quad (2)$$

where x [gr cell/l] stands for the biomass concentration, s [gr substrate/l] is the substrate composition, D [h^{-1}] is the dilution rate, s_f [gr substrate/l] is the substrate concentration in the feed stream. μ_{max} [h^{-1}], k_s [gr substrate/l] and $Y_{x|s}$ [gr cell/gr substrate] are kinetic constants. It should noticed that the above model is a perfectly mixed continuous bioreactor model. We have assumed that, to optimally starting-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 $A = x/1.1$.

4. PARAMETER FITTING

To improve model predictions two sets of experimental runs were done. The parameters fitted were μ_{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, Fig. 1(a), the fitted parameter values are $\mu_{max} = 0.457463, k_s = 0.291433, Y_{x|s} = 1.14612$, while for the second set of experimental measurements, Fig. 1(b), the fitted parameter values are $\mu_{max} = 0.39461, k_s = 0.0924537, Y_{x|s} = 0.894432$. 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 noticed that fitted $Y_{x|s}$ values are beyond reported values. However, large $Y_{x|s}$ values could be explained noticing that most of the ethanol is quickly consumed.

5. OPTIMAL START-UP FORMULATION

As described in (Flores-Tlacuahuac *et al.*, 2005), 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

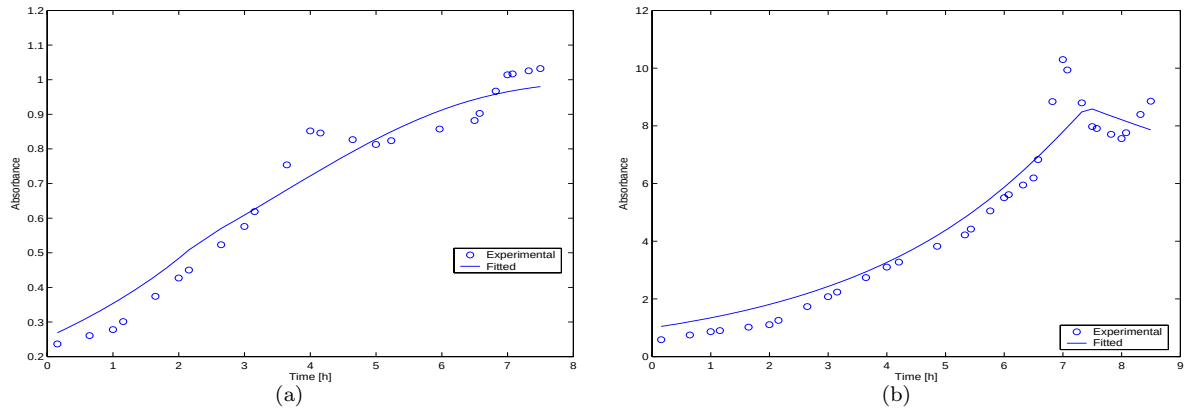

Fig. 1. Comparison between measured and fitted model absorbance response for each on of the two experimental systems.

(Schloeder-H and Bock Kallratch, 1993) and systems with limit cycles and bifurcations. In SDO, computation of optimal transitions policies reduces to the solution of an NLP (Biegler *et al.*, 2002) and provides values of the decision variables (i.e. the manipulated variables) that drive the system toward minimum transition time or off-spec product.

A common requirement during the start-up of bioreactors is that start-up feature minimum transition time, waste material or utility consumption. The minimum start-up 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):

$$\min_{\mathbf{z}_1,\mathbf{z}^o} \int_0^{t_f} \{\|A(t) - \hat{A}\|^2 + \|s_f(t) - \hat{s}_f\|^2\} dt \quad (3)$$

s.t. Semi-explicit DAE model:

$$\frac{d\mathbf{z}(t)}{dt} = \mathbf{F}\left(\mathbf{z}(t), \mathbf{y}(t), \mathbf{u}(t), t, \mathbf{p}\right) \quad (4)$$

$$0 = \mathbf{G}\left(\mathbf{z}(t), \mathbf{y}(t), \mathbf{u}(t), t, \mathbf{p}\right) \quad (5)$$

Initial conditions:

$$\mathbf{z}(0) = \mathbf{z}^0 \quad (6)$$

Bounds:

$$\begin{array}{rcccl} \mathbf{z}^L & \leq & \mathbf{z}(t) & \leq & \mathbf{z}^U \\ \mathbf{y}^L & \leq & \mathbf{y}(t) & \leq & \mathbf{y}^U \\ \mathbf{u}^L & \leq & \mathbf{u}(t) & \leq & \mathbf{u}^U \\ \mathbf{z}^{o,L} & \leq & \mathbf{z}^o & \leq & \mathbf{z}^{o,U} \\ \mathbf{p}^L & \leq & \mathbf{p} & \leq & \mathbf{p}^U \end{array} \quad (7)$$

where $\mathbf{F}$ is the vector of right hand sides of differential equations in the DAE model, $\mathbf{G}$ is the vector of algebraic equations, assumed to be index one, $\mathbf{z}$ is the differential state vector, $\mathbf{z}^0$ are the initial values of $\mathbf{z}$, $\hat{\mathbf{z}}$ is the new desired transition state, $\mathbf{y}$ is the algebraic state vector, $\mathbf{u} = s_f$ is the control profile vector, $\hat{\mathbf{u}} = \hat{s}_f$ is the reference control vector, $\mathbf{p}$ is a time-independent parameter vector, and t_f is the transition horizon. Notice that $\hat{s}_f$ represents the value of the decision variable at the end of the desired start-up period. Such values are normally available from steady-state calculations since, in order to compute a start-up transition, we need to know the values of the controlled and manipulated variables at the initial and final operating points. In the above formulation, $\mathbf{z_1}$ stands for a vector which contains those states that are part of the objective function, while $\hat{\mathbf{z}}_\mathbf{1}$ means the desired final values of the same states. In our case $\mathbf{z_1} = 1.1x$. So, $\mathbf{z}_1$ stands for the value of the states that we desire to track. The objective function represents the deviation of the states and the inputs from the desired product. It should be noticed that the initial concentration of both biomass and substrate, $\mathbf{z}^o$, were also decision variables since we found that minimum time optimal start-up policies were highly sensitive to the initial concentrations of the reactants. The upper bound on the initial conditions, $\mathbf{z}^{o,U}$, reflected the maximum physically allowed initial biomass and substrate concentrations, while the lower value, $\mathbf{z}^{o,L}$, was always set to zero.

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 (Flores-Tlacuahuac *et al.*, 2005), substitution of this discretization into (3)-(7) applied at the collocation points leads to the following NLP.

$$\min_{x \in \mathcal{R}^n} \; f(x) \tag{8}$$

$$s.t. \; c(x) = 0 \tag{9}$$

$$x_L \leq x \leq x_U \tag{10}$$

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 (Flores-Tlacuahuac *et al.*, 2005). The dynamic optimization formulation given by (8)-(10) was implemented using the AMPL mathematical programming language and solved using the IPOPT algorithm (Wachter and L.T., 2004) for large-scale nonlinear programming.

6. NUMERICAL RESULTS

The dynamic optimization problem represented by Eqs 3-7 was solved for the two identified models described earlier. Minimum time optimal start-up policies were sought. Even when the start-up time was not directly a decision variable, we used an iterative approach (see Fig. 2) to determine minimum start-up times. We have found that this approach works well and avoids potential non-convexities introduced when the start-up time is taken as a decision variable. Moreover, we have used, in other type 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 $\hat{x}$ was 10, while the final value of the manipulated variable $\hat{s}_f$ is different because they represent two different operating scenarios. The decision variables were bounded as follows: $x \geq 0, s \geq 0, 0 \leq s_f \leq 20, 0 \leq s^o \leq s^{o,U}, 0 \leq x^o \leq 1$, where $s^{o,U}$, the upper limit on the initial substrate concentration, was set to 20 and 10 for the first and second cases, respectively. As noticed from Table 1, in both cases only 10 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. As noticed from Table 1, the CPU time was quite low since, by today 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 h^{-1}.

The dynamic optimal theoretical results are displayed in Figs. 3 and 4. The continuous lines represent 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 step-like behavior.

Table 1. Information regarding the final desired steady-state operating conditions $(\hat{x}, \hat{s}_f)$ for each operating scenario. x^o and s^o stand for the optimal initial biomass and substrate concentration, respectively.

	Case 1	Case 2
$\hat{x}$ [gr cell/l]	10	10
$\hat{s}_f$ [gr subsrate/l]	8.8	11.2
x^o [gr cell/l]	1	1
s^o [gr substrate/l]	17.806	10
N_{fe}	10	10
N_{col}	3	3
CPU Time [s]	0.141	0.093

7. EXPERIMENTAL RESULTS

As displayed in Fig. 3, the experimental results, obtained by applying the optimal control profile, closely track the theoretical profile. Initially, the yeast consumes the initial amount of substrate since no substrate is fed during the first 7 operating hours. When most of the substrate has been consumed, the optimal solution decides to 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 5(a).

Fig. 4 displays the results of using the dynamic optimal control profiles for the second example. In this case, the feed stream substrate concentration hit 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 5 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 semi-batch reactor. Finally, 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 5(b), good oxygen concentration characteristics were maintained.

In continuous operation good aeration and yeast characteristics lead to acceptable oxygen concentration as displayed in Figure 5. Maintain-

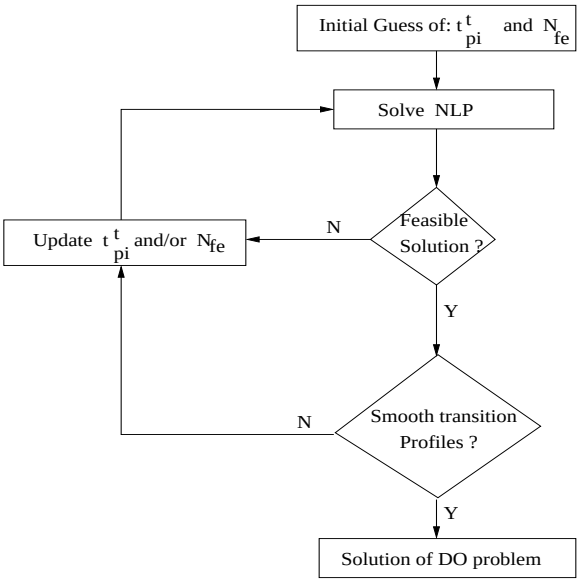

Fig. 2. Iterative algorithm for the solution of the dynamic optimization (DO) problem.

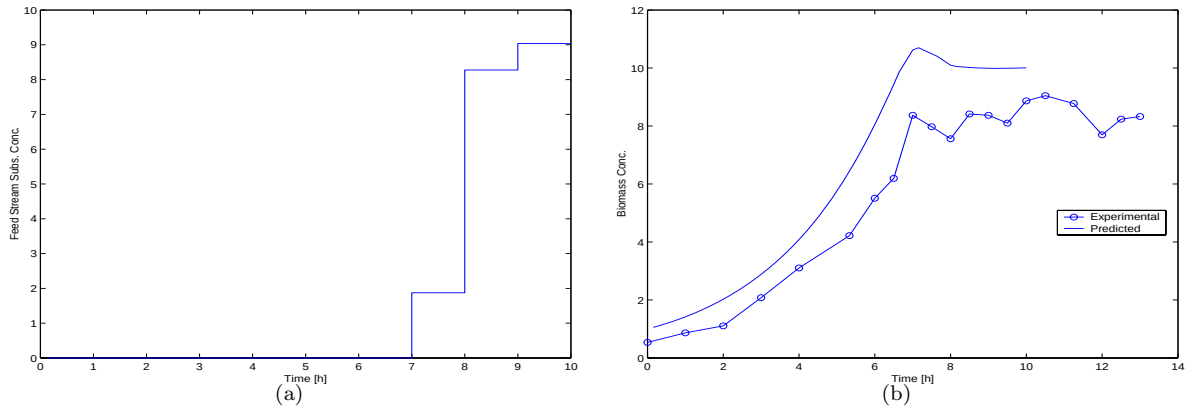

Fig. 3. Results for the first operating point. (a) Optimal profile of the manipulated variable (S_f), (b) Comparison between predicted and lab scale bioreactor responses.

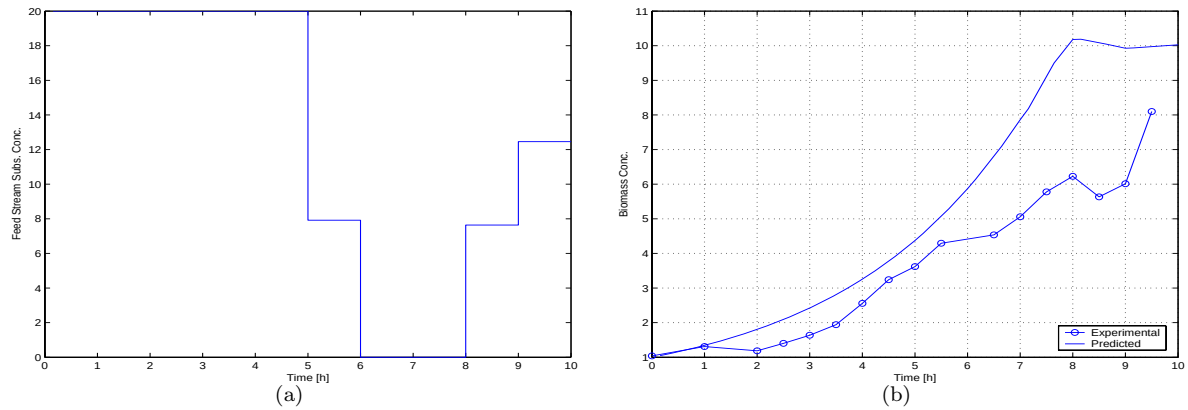

Fig. 4. Results for the second operating point. (a) Optimal profile of the manipulated variable (S_f), (b) Comparison between predicted and lab scale bioreactor responses.

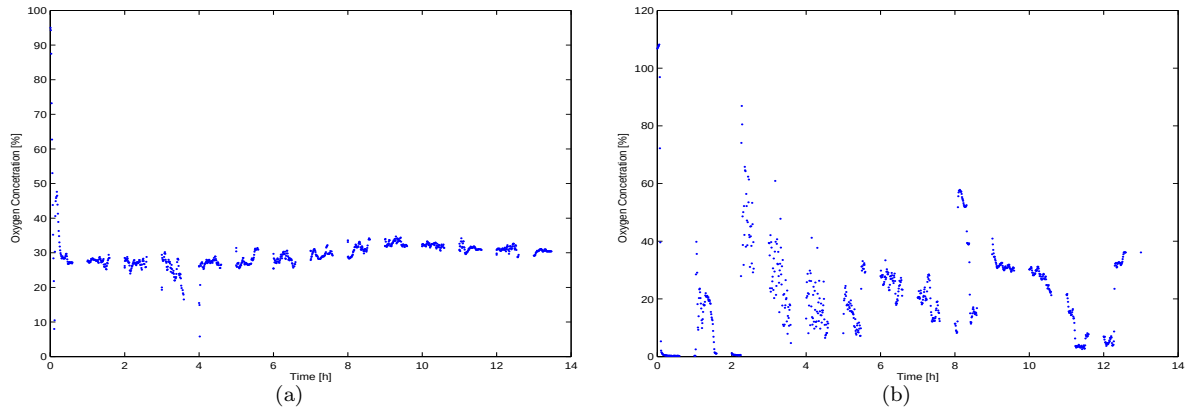

Fig. 5. Measured oxygen concentration during start-up of the bioreactor. (a) First and (b) Second experiments.

ing 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.

8. CONCLUSIONS

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

When addressing optimal control calculations, the same values of the fitted parameters were maintained along the whole start-up 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 (Doyle *et al.*, 2003). This is a relatively new research area, mostly for nonlinear systems since some work has been reported for linear ones (Bemporad and Morari, 1999).

Due to the small scale dimension of the mathematical, real time control seems to be a feasible option for the start-up and dynamic operation of bioreactors. We will address these points in future work.

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