

NONLINEAR PROTEIN PRODUCTION TRACKING IN A FED-BATCH CULTURE

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Abstract

In fed-batch cultures the desired metabolite production is achieved often following a predefined nutrient/inducer feeding profile. Controlling a fed-batch fermentation is not an easy task to achieve due to the nonlinearities and uncertainties present in the kinetic terms. In this paper a control strategy is designed to track a trajectory in a nonlinear fed-batch culture producing the protein luciferase through a recombinant bacterium. The proposed control scheme has three components: i) a nominal model, ii) a dynamic reduced observer, and iii) a SISO nonlinear feedback controller. A linearizing control law provides the nonlinear system with a closed loop linear behavior. Uncertain terms related with the fermentation kinetics are gathered in a function that is estimated with an observer. From the control theory point of view, the problem of estimating the uncertain kinetic terms is addressed as a combination of Model matching and Tracking problems. The proposed scheme is robust against modeling errors. The observer based controller had satisfactory performance tracking the desired trajectories.

Key words

Nonlinear control, Tracking, Model matching, Uncertain terms estimation, Fed-batch.

1 Introduction

Fed-batch systems are used to produce elevated concentrations of metabolites achieving high productivities and yields. In this culture, one or more nutrients are supplied to the bioreactor; however, nutrient overfeeding or underfeeding is detrimental to cell growth and product formation. Therefore, control strategies are necessary to satisfactorily achieve the operational objectives of the bioprocess. Unlike the continuous culture where a steady state is sought, in a fed-batch system a transient behavior is always present. Fed-batch cultures have been analyzed as a control problem where it is desired to find an optimal control trajec-

tory $u(t)$ such it takes the process from an initial state to a final state minimizing a cost function. An early approach into finding an optimal feeding profile was sought through the Pontryagin's minimum principle; however, the feeding rate was independent of the control law resulting in a singular problem failing to find a solution [Modak et al., 1986]. To solve this problem the Hamiltonian related to the feed rate is successively differentiated respect to time until an explicit relationship for the optimal flow rate is obtained for the singular arc, which can be used for control [San and Stephanopoulos, 1986]. However, these approaches were based in open loop controllers sensitive to model uncertainties deviating the process from the desired results. Later the singular control problem was transformed to nonsingular using as the controlled variable the substrate, the volume of the fermenter, or the specific growth rate.

The advances in control theory has allowed applying conventional, linear, and nonlinear controllers to fed-batch cultures, *i.e.* pH-Stat, DO-Stat, PID [Lee et al., 1999], linear [Dewasme et al., 2009] and nonlinear [Jayadeva et al., 1991; Mohsenia, et al., 2009]. Specially, geometric control has emerged as an alternative tool to deal with nonlinear systems [Isidori, 1989]. This approach is based in the nonlinear transformation of the states, and using a linearizing control input [Kravaris and Chung, 1987; Femat et al., 1999]. However, particularly in bioprocesses the lack of confident sensors has hampered the design of robust controllers [Bastin and Dochain, 1990]. Therefore, software or virtual sensors have been used in bioprocesses to estimate: *i)* unavailable sensor measurements like the biomass, the substrate and products in the culture, and *ii)* unknown kinetic terms [Bastin and Dochain, 1990]. The aim of this contribution is to design a nonlinear controller to track a signal in a biological reactor, the problem of estimating the uncertain/unknown kinetic terms is addressed as a combination of Model matching and Tracking problems. In the model matching approach a compensating control law is designed for a plant, in such way that the response of the closed-loop system

match a predefined driven model, so the closed-loop system is internally stable [Di Benedetto and Grizzle, 1994]. If the output and a reference function of a dynamical systems is given by $y(t)$, and $y_r(t)$, respectively, then the tracking problem consists in finding a control law which asymptotically decrease the tracking error $E_T = y(t) - y_r(t)$ to zero as $t \rightarrow \infty$ [Isidori, 1989]. Unknown kinetic terms are gathered in a function that is then estimated with an observer. The study case is a biological reactor operating in a fed-batch mode producing the enzyme luciferase. The fed-batch model has seven states and diverse nonlinear uncertain kinetic terms. The document has been organized as follows: after introduction, section 2 describes the luciferase fed-batch kinetic model. The model matching and tracking problem is described in section 3. Brief background in nonlinear control is given in section 4. Simulation results are given in section 5. Finally the conclusions are given.

2 The Fed-batch dynamic model

Definition: A fed-batch culture is a process operating for a finite interval of time, $\tau = t \in \mathbb{R} | 0 \leq t \leq t_{max}$, and only can be fed during this operation time τ .

The dynamic fed-batch model used as a case of study was propounded by [Lee and Ramirez, 1992]. In this culture it is desired to grow an *E. coli* cloned microorganism and induce the production of the luciferase protein by Isopropylthiogalactoside (IPTG). The mass balance equations describing the fed-batch model are given by:

$$\begin{aligned} \dot{x}_1 &= u_1 + u_2 \\ \dot{x}_2 &= \mu(x_3, x_5, x_6, x_7)x_2 - T_1x_2 \\ \dot{x}_3 &= T_2C_n^f - T_1x_3 - Y^{-1}\mu(x_3, x_5, x_6, x_7)x_2 \\ \dot{x}_4 &= R_{fp}(x_3, x_5)x_2 - T_1x_4 \\ \dot{x}_5 &= T_3C_i^f - T_1x_5 \\ \dot{x}_6 &= -k_1(x_5)x_6 \\ \dot{x}_7 &= -k_2(x_5)(1 - x_7) \end{aligned} \quad (1)$$

where $x \in \mathbb{R}^7$ is the state vector, x_1 describes the bioreactor volume (L), x_2 is the cell density (g/L), x_3 stands for the substrate concentration (g/L), x_4 is the luciferase protein concentration (g/L), x_5 is the protein inducer IPTG in (g/L), x_6 and x_7 are the shock factor on cell growth rate and the inducer recovery factor on cell growth rate, respectively. $T_1 = (u_1 + u_2)/x_1$, $T_2 = u_1/x_1$ and $T_3 = u_2/x_1$, where u_1 and u_2 are the substrate and inducer feeding rates (L/h). The terms C_n^f and C_i^f are the nutrient and inducer concentrations in the feed. Y^{-1} is the relationship between the cellular mass produced and the nutrient consumed. The specific growth rate is given by

$$\mu = \frac{\mu_{max}x_3}{K_{CN} + x_3}(x_6 + x_7R_R) \quad (2)$$

where μ_{max} and K_{CN} are Monod constant parameters and R_R is the ratio of μ at an inducer concentration to μ_{max} .

$$R_{fp} = \frac{f_{max}x_3}{K_{CN} + x_3 + \frac{x_3^2}{K_s}} \frac{f_{I0} + x_5}{K_I + x_5} \quad (3)$$

More details of the kinetic model can be found in [Lee and Ramirez, 1992].

3 Problem description

The problem of designing a controller for tracking the protein luciferase (1) is solved as a combination of the Tracking and Model matching problem. In order to make clear the problem statement, the following systems are defined, which we called respectively the real model (RM) and nominal model (NM), see Figure 1. The RM is given by the dynamical system described by (1). The NM is a system containing all the dynamic information of the RM (*i.e.*, their dynamics are equivalent), since it is the result of a nonlinear transformation of the RM based in Lie derivatives. It is assumed that systems NM and RM have different initial conditions. In addition the NM unknowns the kinetic functions (*i.e.*, uncertain kinetics). In this way, from the Tracking point of view it is desired that the tracking error between the NM outputs (z_1, z_2) and the RM available measurements states (x_3, x_4) asymptotically decays to zero as $t \rightarrow \infty$. This, in spite of either the NM uncertainties respect to RM or the differences in they initial conditions. In order to solve the tracking problem, a SISO control scheme with dynamic estimation based in geometric control is proposed. This control scheme consisted of two parts: i) a feedback linearizing control law and ii) Luenberger type dynamic estimator, which has as objective compensate the differences of NM with respect to RM. On the other hand, from the Model matching point of view, once the tracking is achieved, the vector fields of NM are modified in such way that at closed-loop the dynamical behavior of NM equals RM. In this way, we can say that the observer problem consist in a control scheme based in geometric control and a nominal model.

4 Controller design

The input-output linearization method is a control law based in state feedback which provides the nonlinear system with a closed loop linear dynamic [Isidori, 1989]. This method does not require linearization of the whole nonlinear system; however, it only can be applied to minimum phase systems [Kravaris and Chung, 1987]. Consider the following class of nonlinear system

$$\begin{aligned} \dot{x}(t) &= f(x(t)) + g(x(t))u(t) \\ y(t) &= h(x(t)) \end{aligned} \quad (4)$$

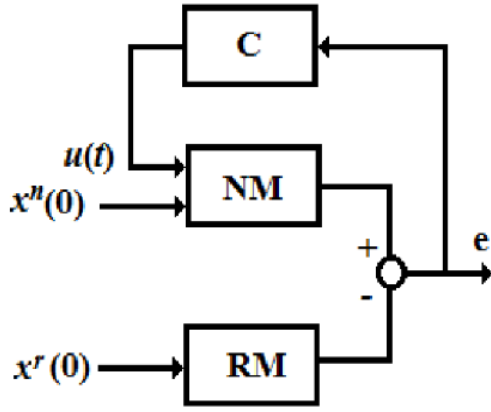


Figure 1. Diagram representing the model matching and tracking problem. NM, RM, C, and e stands for nominal model, real model, controller and error, respectively.

where $x(t) \in \mathbb{R}^n$ is the state vector, $u \in \mathbb{R}$ is a scalar input, $y \in \mathbb{R}$ is the system output $h(x(t))$, which is a smooth function. For convenience the time dependency is omitted from now on. Differentiating the output y with respect to time it is obtained that $\dot{y} = L_f h(x) + L_g h(x)u$, where $L_f h(x) = \sum_{i=1}^n f_i(x) \partial h(x) / \partial x_i$ is the Lie derivative of $h(x)$ through $f(x)$. If $L_g h(x) = 0$ for any $x(t) \in \mathbb{R}^n$ it is necessary to differentiate again to obtain $\ddot{y} = L_f^2 h(x) + L_g L_f h(x)u$. Generally, if r is the smallest positive integer, such that: (i) $L_g L_f^i h(x) = 0$ for $i = 1, 2, \dots, r-2$, and (ii) $L_g L_f^{r-1} h(x) \neq 0$, to $x = x_0$, then the system has relative degree r at the point x_0 . Therefore, it is possible to write the first r output derivatives y with respect to time as $y^{(i)} = L_f^i h(x)$, for $i = 1, 2, \dots, r-2$ and $y^{(r)} = L_f^r h(x) + L_g L_f^{r-1} h(x)u$, where $L_f^i h(x) = L_f(L_f^{i-1} h(x))$ and $L_g L_f^i h(x) = L_g(L_f^i h(x))$. The relative degree is defined as the times that is necessary to differentiate the output y before the input u explicit appears [Isidori, 1989]. Once defined the relative degree r , it is possible through a change of coordinates given by $Z_{i+1} = L_f^i h(x)$, $1 \leq i \leq r$, rewrite (4) in its canonical form proposed by [Isidori and Byrnes, 1990]; which is also known as the normal form, and may be expressed as:

$$\begin{aligned} \dot{z}_i &= z_{i+1}, \\ \dot{z}_r &= \alpha(z, \nu) + \gamma(z, \nu)u \\ \dot{\nu} &= \zeta(z, \nu) \\ y &= z_1 \end{aligned} \quad (5)$$

where $y = z_1$ is the system output, $\alpha(z, \nu) = L_f^r h(x)$ and $\gamma(z, \nu) = L_g L_f^{r-1} h(x)$ are the nonlinear part of the observable z -states of the system (5), while the ν -states represents the non-observable states. From \dot{z}_r in (5) it can be seen that if $L_g L_f^{r-1} h(x)$ remains bounded away from zero, it is possible to

propound a control law $u = [-L_f^r h(x) + x_R^{(r)} - V(x)] / L_g L_f^{r-1} h(x)$, which induce the linear dynamic $V(x, t) = \sum_{i=1}^r K_{i-1} (L_f^{i-1} h(x) - x_R^{i-1})$ on the subsystem (5); such is able to track a desired trajectory $X_R(t)$. This control law is known as linearization by static state feedback (LSSF). On the other hand, if the coefficients K_i ($i = 1, 2, \dots, r$) of $V(x, t) = K_r (L_f^{r-1} h(x)) + \dots + K_1(y)$, are such that the polynomial $P_r(s) = s^r = K_r s^{r-1} + \dots + K_2 s + K = 0$ is Hurwitz, then the LSSF allows the asymptotic stabilization of system (5). In addition, the LSSF can be expressed as: $u(z, \nu) = (1/\gamma(z, \nu))[\alpha(z, \nu) + \sum_{i=1}^r K_{i-1} (z^{(i-1)} - x_R^{(i-1)})]$ where $x_R^{(i-1)}$ represents the vector of the derivatives used as reference.

Consequently the closed loop dynamic of system (5) can be rewritten as $\dot{z} = Az$. $\dot{\nu} = \zeta(z, \nu)$ where A is a matrix r -dimensional composed by the K_i coefficients of the characteristic equation given by the polynomial $P_r(s)$. Since the matrix A is Hurwitz, the closed loop system is stable if and only if the ν -states non observable (which corresponds to the zero dynamic) are bounded for every $t \geq 0$ and the dynamic of the system $\dot{\nu} = \zeta(z, \nu)$ is stable for a constant value of \dot{z} , namely, the dynamic of the ν -states is non-minimum phase.

4.1 An equivalent dynamic representation

If the z -states observable are not available for feedback, the LSSF cannot be implemented directly. Consequently, it is required to estimate these non observable states. Let $\delta(z, \nu) = \gamma(z, \nu) - \hat{\gamma}(z)$, then, it is possible to express the system (5) as:

$$\begin{aligned} \dot{z}_i &= z_{i+1}, \\ \dot{z}_r &= \eta + \hat{\gamma}(z)u \\ \dot{\eta} &= \Xi(z, \eta, \nu, u) \\ \dot{\nu} &= \zeta(z, \nu) \end{aligned} \quad (6)$$

where $\hat{\gamma}(z)$ is the estimated function of $\gamma(z, \nu)$, $\eta = \Theta(z, \eta, u)$, is the function that gathers the uncertain terms, $\Theta(z, \nu, u) = \alpha(z, \nu) + \delta(z, \nu)u$ and $\Xi = \sum_{k=1}^{r-1} z_{k+1} \partial_k \Theta(z, \nu, u, t) + [\eta + \hat{\gamma}(z)u] \partial_r \Theta(z, \nu, t) + \partial_\nu \Theta(z, \nu, t) \zeta(z, \nu)$, $\partial_k \Theta(z, \nu, u) = \partial \Theta(z, \nu, u) / \partial x_k$. To estimate the uncertain terms we propound a high gain Luenberger type observer, which provides the estimated values of the z -states. This approach has demonstrated to have satisfactory performance in estimating uncertain terms related with chemical processes [Femat et al., 1999]. The design of Luenberger filters can be carried as follows:

$$\begin{aligned} \dot{\hat{z}}_i &= \hat{z}_{i+1} + L^i \beta_i (z_1 - \hat{z}_1) \\ \dot{\hat{z}}_r &= \hat{\eta} - \hat{\gamma}(\hat{z})u + L^r \beta_r (z_1 - \hat{z}_1) \\ \dot{\hat{\eta}} &= L^{r+1} \beta_{r+1} (z_1 - \hat{z}_1) \end{aligned} \quad (7)$$

where $\hat{\eta}$ and \hat{z} stands for the estimated values of the function that gathers the uncertainty of the z -states, re-

spectively; while $\hat{\gamma}(\hat{z})$ is the estimated function of the z -states. The β_i -values are chosen in such way that the polynomial $s^{r+1} + \beta_{r+1}s^r + \dots + \beta_1 = 0$ is Hurwitz, while L is a positive parameter used for tuning. Therefore, the linearizing control law can be expressed as:

$$u(\hat{z}) = \frac{1}{\hat{\gamma}(\hat{z})} \left[-\hat{\eta} + \sum_{i=1}^r K_{i-1}(\hat{z}_i - x_R^{(i-1)}) \right] \quad (8)$$

5 Luciferase protein tracking approach

In this section the system described by (1) will be analyzed with the tools described in the former section. We made the following assumptions:

- 1.- The system is SISO, and there is only one input given by u_1 .
- 2.- The state x_4^r is known and available for feedback.
- 3.- The nominal model NM does not know the kinetic terms defined in the real model RM related with μ and R_{fp} .

Lets rewrite NM in the form given by (4) where the state vector $x = (x_1, \dots, x_7)$ belongs to a open and compact set $U \in \mathbb{R}^7$, while the output and the input are given by the scalars $x_4, u_1 \in \mathbb{R}^1$. Once rewritten NM, a change in coordinates is made in order to reach the system to the form of (5). Since $L_f^0 h(x) = x_4$ and $L_g L_f^0 h(x) = -x_4/x_1$, the relative degree of system (1) is one and it is well defined for every set U , since $x_1 > 0$ for all x_1 in $U \subset \mathbb{R}^7$ and $t > 0$. Consequently, the trajectories of the states of NM in the normal form will evolve in the space $W \subset \mathbb{R}^1$ for all $z \in W$. Since the relative degree ($r = 1$) resulted to be less than the system order ($n = 7$), then it is necessary to propound $n - r = 6$ complementary functions to generate a diffeomorphism that maps $z = \Phi(x)$. The complementary functions must fullfil with the condition given by $L_g \nu_i(x) = 0$. Then the complementary functions are $\nu_1 = x_6, \nu_2 = x_7, \nu_3 = x_1 x_2, \nu_4 = x_1 x_4, \nu_5 = x_1 x_5$ and $\nu_6 = x_1 x_3 - x_1 C_n^f$. Once completed the diffeomorphic mapping $z = \Phi(x)$, NM can be written as in (5) as follows:

$$\dot{z}_1 = R_{fp}(z_1, \nu_4, \nu_5, \nu_6)(z_1 \nu_3 / \nu_4) - (u_1 + u_2) z_1^2 / \nu_4 \quad (9)$$

$$\begin{aligned} \dot{\nu}_1 &= -k_1(z_1, \nu_4, \nu_5) \nu_1 \\ \dot{\nu}_2 &= k_2(z_1, \nu_4, \nu_5)(1 - \nu_2) \\ \dot{\nu}_3 &= \mu(z_1, \nu_1, \nu_4, \nu_5, \nu_6) \nu_3 \\ \dot{\nu}_4 &= R_{fp}(z_1, \nu_4, \nu_5, \nu_6) \nu_3 \\ \dot{\nu}_5 &= C_n^f u_2 \\ \dot{\nu}_6 &= -Y^{-1} \mu(z_1, \nu_1 \nu_4, \nu_5, \nu_6) - C_n^f u_2 \end{aligned} \quad (10)$$

where the systems (9) and (10) represent the observable and non observable states of system (1) in the normal form. In systems where $r < n$, it is necessary

that the zero dynamics described by the complementary functions be of minimum phase. Assumption 3 limits NM from the knowledge of the kinetic terms μ and R_{fp} . Defining $\eta = R_{fp}(x_3^r, x_5^r) x_2^r - (x_4^r/x_1^r) u_2^r$, it is possible rewrite the subsystem (1) as:

$$\begin{aligned} \dot{z}_1 &= \dot{x}_4^n = \eta - \frac{x_4^n}{x_1^n} u_4^n \\ \dot{\eta} &= \Gamma(\eta, t) \end{aligned} \quad (11)$$

where $\Gamma(\eta, t) = \sum_{i=1}^7 [\partial R_{fp}(x_3^r, x_5^r) \dot{x}_1 / \partial x_1] x_2^r + R_{fp}(x_3^r, x_5^r) \dot{x}_2^r - (x_1^r, \dot{x}_4^r - x_4^r, \dot{x}_1^r) u_2^r / x_3^{r2} + \dot{u}_2^r x_4^r / x_1^r$.

Designing a Luenberger filter for system (6) it is obtained that

$$\begin{aligned} \dot{\hat{z}}_1 &= \hat{\eta} - \frac{x_4}{x_1} u_1 + L \beta_1 (z_1 - \hat{z}_1) \\ \dot{\hat{\eta}} &= L^2 \beta_2 (z_1 - \hat{z}_1) \end{aligned} \quad (12)$$

where it can be seen that it is possible design a control law with a similar effect to the LSSF, that can be expressed as:

$$u_1^n = [-\hat{\eta} + K_c(x_4^n - x_4^r)] \left(-\frac{x_1^n}{x_4^n} \right) \quad (13)$$

5.1 Numeric simulation results

In order to evaluate the performance of the feedback control law (12) and (13), numerical simulations were carried out over the NM. The following numerical values were considered for the designed controller $L = 5.0, K_c = -5.0$, obtained from tuning the controller, while $\beta_1 = 2.0$ and $\beta_2 = 1.0$ were chosen searching that the eigenvalues of the characteristic polynomial of the matrix of coefficients $A(\beta)$ were located in -1 . The initial condition used were $x^r(0) = [1.0, 0.1, 40.0, 0.001, 0.0, 1.0, 0.0]$ and $x^n(0) = [0.5, 0.2, 20.0, 0.01, 0.01, 0.9, 0.01]$. As can be seen in the upper part of Figure 2, the feedback given by (12) and (13), achieved the control goal, since the state x_4^n (dashed line) tracked closely the trajectory described by the state x_4^r (continuous line), in spite of the differences of NM regarding RM, even under different initial conditions. The feedback law given by (12) and (13), performed similar to the LSFF, this can be seen in the lower part of Figure 2 where the values of $\hat{\eta}$ (dashed line) estimated by the Luenberger filter tracked satisfactory the real value of $\hat{\eta}$ (continuous line), which is the function that gathers the uncertain kinetic terms.

6 Conclusion

The main contributions of the propounded control scheme is the robustness in spite of the possible errors in the mathematical model, external disturbances,

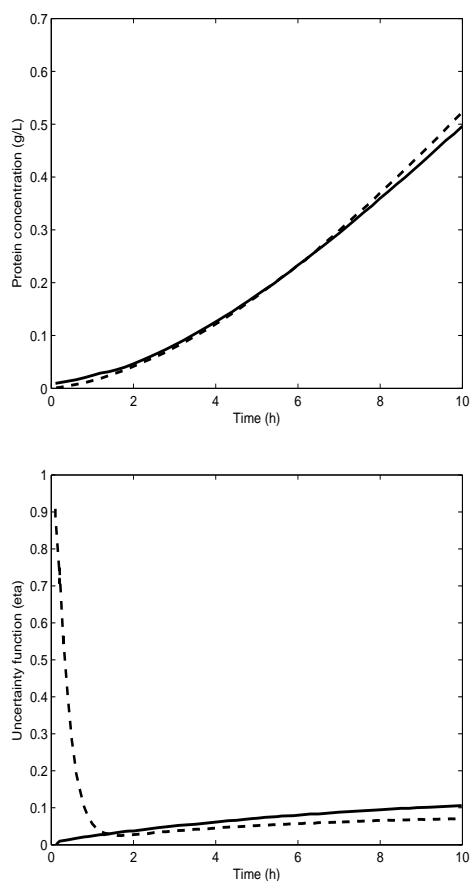


Figure 2. Upper figure: Controller performance (dashed line) during the tracking of the protein luciferase (continuous line). Lower figure: Behavior of the η state (dashed line) during the estimation of the unknown kinetic terms.

and the total or partially unknown kinetic terms. This was achieved creating a new state gathering the unknown terms, which is estimated by an observer. Simulations results show that the control system had satisfactory performance tracking the protein production x_4^r . Therefore experimental implementation of the observer-controller is promissory.

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