On-line State Estimation and Identification of a Fed-batch Bioprocess

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Abstract: - This paper deals with the problem of on-line state estimation and identification for a fed-batch bioprocess, which is in fact a lipase production process that takes place inside a Fed-batch Bioreactor. The lipase production process is highly nonlinear and, furthermore, the available on-line measurements are lack and the reaction kinetics is not perfectly known. Some on-line state estimation strategies based on extended Luenberger observer and asymptotic observer approach are proposed. The unknown kinetic parameters of the bioprocess are estimated by using a distribution based identification technique. The performance of the proposed estimation strategies is analysed using numerical simulation.

Key-Words: - Biotechnology, Nonlinear systems, Identification, Asymptotic observers

1 Introduction
The development and especially the implementation of advanced control strategies on real bioprocesses are difficult because of absence of reliable instrumentation for the biological state variables, i.e. the substrates, biomass, and product concentrations. In many cases the state variables (the concentrations), were analysed manually and as a result there is not on-line (and real-time) control. This fact together with the nonlinearity and parameter uncertainty of the bioprocesses requires an enhanced modelling effort and modern state estimation and identification strategies [1].

Several estimation strategies have been developed to provide accurate on-line estimations of state variables. Presently, two classes of state observers for bioprocesses can be found in the literature [1], [4]. The first class of observers (including classical observers like Luenberger and Kalman observers, nonlinear observers) is based on a perfect knowledge of the model structure. A disadvantage of this class is that the uncertainty in the model parameters can generate possibly large bias in estimation of unmeasured states. A second class, called asymptotic observers, is based on the idea that the uncertainty in process models lies in process kinetics models. The design of these observers is based on mass and energy balances without the knowledge of kinetics being necessary.

From the identification point of view, very important is the estimation of parameters and especially of kinetic rates inside a bioreactor (the so-called kinetics of the bioprocess) - the estimates of these rates are used for advanced control strategies. This problem can be solved using “software sensors”. A software sensor is a combination between a hardware sensor and a software estimator [1], [2], [4]. A well-known technique is the Bastin and Dochain approach based on the adaptive systems theory [1], [2]. This strategy consists in the estimation of unmeasured state with asymptotic observers, and after that, the measurements and the estimates of the state variables are used for on-line estimation of kinetic rates. This method is useful, but in some cases, when many reactions are involved, the implementation requires the calibration of too many parameters. A relative modern approach [6], [8] is the distribution based identification method. In this approach the set of nonlinear differential equations describing the state evolution is mapped into a set of linear algebraic equations respect to the model parameters.

Generally speaking, the design of stable and convergent state estimators and the design of appropriate identification method for bioprocesses is a complex task and good solutions are given only by studying each particular bioprocess.

A bioreactor is a tank in which several biological reactions occur simultaneously in a liquid medium [1]. The bioreactors can operate in three modes: the continuous mode, the fed-batch mode and the batch mode [1], [2]. For example, a Fed-Batch Bioreactor (FBB) initially contains a small amount of substrates and micro-organisms and is progressively filled with
the influent substrates. When the FBB is full the content is harvested. By contrast, in a continuous bioreactor the substrates are fed to the bioreactor continuously and an effluent stream is continuously withdrawn such that the culture volume is constant.

This paper deals with the design of some on-line state estimation and identification strategies for a lipase production bioprocess that is carried out inside a FBB. The organization of the paper is as follows. In Section 2 the lipase production process is widely analysed, and a nonlinear model of the bioprocess is presented. Section 3 deals with the design of two estimation algorithms for state variables: an extended Luenberger estimator and an asymptotic observer. In Section 4, a distribution based identification method for the unknown kinetics of the bioprocess is investigated. The performance of all estimation strategies are illustrated by using numerical simulations. Finally, concluding remarks are collected in Section 5.

2 The dynamical model of the lipase production process

The lipase is an enzyme able to split fats and to synthesise glycerides, so the large scale production of lipase is very useful in many applications. The lipase production process usually takes place inside FBB; this process is widely discussed in [3] and a nonlinear model is designed:

\[
\begin{align*}
\dot{S}_1 &= -\eta S_1 X + F \\
\dot{S}_2 &= \eta S_1 - \mu S_2 (Y + S_2) \\
\dot{X} &= \mu S_2 X \\
\dot{L}_{in} &= v_p (S_1, X, \mu) - v_{ex} (L_{in}) - \mu S_2 L_{in} \\
\dot{L}_{ex} &= v_{ex} (L_{in}) X \\
\text{Cer} &= (a \mu S_2 + b) X
\end{align*}
\]

In these equations, \( S_1 (g/l), S_2 (g/g), X (g/l), L_{in} (u/mg) \) and \( L_{ex} (u/ml) \) are the concentrations of external (extra-cellular) substrate, internal (intra-cellular) substrate, biomass, internal (intra-cellular) and external (extra-cellular) lipase, respectively and Cer is the carbon dioxide excretion rate \( (a, b \text{ are excretion parameters}) \) [3], [7]. In (1), \( F \) is the feeding rate of substrate, i.e. the input process variable (when \( F = 0 \), the operating mode is batch) and \( Y \) is the biomass/substrate yield coefficient. The rate \( \eta \) is the absorption rate of the external substrate \( S_1 \), \( \mu \) is the specific growth rate of biomass \( X \), \( v_p \) is the production rate of the internal lipase \( L_{in} \) and \( v_{ex} \) is the excretion rate of external lipase \( L_{ex} \). The form of these rates is of Monod type for \( \eta, \mu, v_{ex}, \) but very complex for \( v_p \): Haldane law plus influence of the specific growth rate of biomass.

\[
\eta(S_1) = \frac{\eta^* S_1}{K_{M1} + S_1}; \quad \mu(S_2) = \frac{\mu^* S_2}{K_{M2} + S_2}
\]

\[
v_p (S_1, X, \mu) = \frac{v_p^* (S_1/X)}{K_p + (S_1/X) + K_i (S_1/X)^2} \cdot \mu(S_2)
\]

\[
v_{ex} (L_{in}) = \frac{v_{ex}^* L_{in}}{K_{ex} + L_{in}}
\]

All coefficients in (2), (3) and (4) are strictly positive. \( K_{M1}, K_{M2}, K_p, K_i \) are Michaelis - Menten constants. \( \eta^*, \mu^*, v_p^*, v_{ex}^* \) are maximum specific rates and \( K_i \) is an inhibition constant. The above model is valid for biomass and substrate concentrations between 0 g/l and 8 g/l [3]. The strongly nonlinear character of this model is given by the reaction kinetics. In many situations, the yield coefficients, the structure and the parameters of the reaction rates are partially known or unknown.

The main purpose of FBB control is to maximise the final lipase product quantity. This goal can be achieved through an optimal control of the bioreactor, i.e. the calculation of the optimal profile for the feeding rate, which is a common solution for FBB control [1]. This optimal control is unsatisfactory when the kinetics is imprecisely known or unknown (the nonlinear expressions of the kinetic parameters (2)-(4) are not totally realistic; in fact, these kinetic parameters are imprecisely known). Two possible suboptimal alternatives are the adaptive control and the SMC [7]. The analysis of this bioprocess [3] leads to the following main result: the regulation of the concentration of the external substrate \( S_1 \) can achieve the maximisation of the production. The dynamics of concentrations of external substrate, internal substrate and of the biomass (first three equations of (1)) are significant for the evolution of the internal and external lipase concentrations (given by the equations four and fifth of (1)) [3], [7]. Therefore, the equations describing the dynamics of \( S_1, S_2 \) and \( X \) are used for the control design. Then we consider the first three equations, compactly written as:

\[
\begin{align*}
\dot{\xi}_1 (t) &= -\eta \xi_1 \xi_2 + u, \\
\dot{\xi}_2 (t) &= \eta \xi_1 - \mu \xi_2 (Y + \xi_2), \\
\dot{\xi}_3 (t) &= \mu \xi_2 \xi_3
\end{align*}
\]

where \( \xi = [\xi_1, \xi_2, \xi_3]^T = [S_1, S_2, X]^T \) is the state vector and \( u = F \) (feeding rate is the control input).

In order to implement useful control strategies, it is necessary to design state observers and identification methods for the unknown kinetics.


3 On-line state estimation strategies

3.1 An extended Luenberger observer

In the lipase production process, often the only state variable that is on-line available is the biomass concentration, and it is necessary to reconstitute the external and especially the internal substrate concentrations. Next, for the on-line estimation of unmeasured state variables \(S_1\) and \(S_2\) an extended Luenberger observer will be designed. Firstly, we deduce that the model equations (5) constitute an exponential observable system (for more details, see [1], [4]). The equations (5) can be written:

\[
\dot{\hat{x}}(t) = f(\hat{x})
\]

where \(f(\hat{x}) = [-\eta \cdot X + F \cdot \eta - \mu \cdot (Y + S_2) \cdot \mu \cdot X]^T\).

A general class of observers for bioprocesses is proposed by Bastin and Dochain [1]:

\[
\frac{d\hat{\xi}}{dt} = f(\hat{\xi}) + \Omega(\hat{\xi})(\xi_1 - \hat{\xi}_1)
\]

where \(\hat{\xi}\) is the estimated state vector, \(\Omega(\hat{\xi})\) is a gain matrix and \(\xi_1\) is the vector of measurable state variables, \(\xi_1 = L \hat{\xi}\), with \(L\) a selection matrix.

The design of the observer consists in the choice of gain matrix. The dynamic of the estimation error \(e = \xi - \hat{\xi}\) is:

\[
e = f(\hat{\xi} + e) - f(\hat{\xi}) - \Omega(\hat{\xi})Le
\]

It is clear that \(e = 0\) is an equilibrium point of (8). The linear approximation around \(e = 0\) can be easily obtained:

\[
\dot{e}(t) = (A(\hat{\xi}) - \Omega(\hat{\xi})L)e, \quad A(\hat{\xi}) = \frac{\partial f(\hat{\xi})}{\partial \xi}\bigg|_{\xi = \hat{\xi}}
\]

If it is possible to impose desired values for the eigenvalues of matrix \(A(\hat{\xi}) - \Omega(\hat{\xi})L\) by choosing the gain matrix, then the system (6) is exponentially observable and the observer (7) is an exponential observer. A necessary condition of exponential observability is that the observability matrix

\[
O = [L \quad LA(\hat{\xi}) \quad LA(\hat{\xi})^2 \quad \cdots \quad LA(\hat{\xi})^{n+1}]
\]

is a full rank matrix: \(\text{rank}(O) = n\) along the state trajectories, with \(n\) the dimension of state vector.

In the case of our lipase bioprocess, \(\xi_1 = X = \hat{\xi}_3\), \(L = [0 \quad 0 \quad 1]^T\). After straightforward calculations the matrix \(A = [a_y(\hat{\xi})]_{j=1,3}\) is obtained:

\[
A = \begin{bmatrix}
\eta^* K_{M1} \hat{\xi}_3 & 0 & \eta^* \hat{\xi}_1 \\
\frac{K_{M1}}{(K_{M1} + \hat{\xi}_1)} & -\mu^* (K_{M2} + 2K_{M2} \hat{\xi}_2 + \hat{\xi}_2) & 0 \\
0 & \mu^* K_{M2} \hat{\xi}_2 & \frac{K_{M1} + \hat{\xi}_1}{(K_{M1} + \hat{\xi}_1)}
\end{bmatrix}
\]

Then the observability matrix is:

\[
O = \begin{bmatrix}
0 & a_{32}(\hat{\xi}) & a_{33}(\hat{\xi}) \\
0 & a_{32}(\hat{\xi}) a_{21}(\hat{\xi}) & a_{33}(\hat{\xi}) a_{21}(\hat{\xi}) + a_{32}(\hat{\xi}) a_{22}(\hat{\xi}) + a_{33}(\hat{\xi}) a_{22}(\hat{\xi})
\end{bmatrix}
\]

and \(\text{det}(O) = -a_{32}(\hat{\xi}) a_{21}(\hat{\xi}) \neq 0 \quad (\hat{\xi}_3 > 0)\). Therefore \(\text{rank}(O) = 3\) and the necessary condition of exponential observability is achieved.

With this condition fulfilled it is possible to try the design of an extended observer for the system (6). The design of the observer consists in the choice of gain matrix \(\Omega(\hat{\xi})\) such that the equilibrium point \(e = 0\) of (9) is asymptotically stable. Therefore, the gain matrix must to obey two conditions: (i) the matrix \(A(\hat{\xi}) - \Omega(\hat{\xi})L\) and his derivative are bounded; (ii) the real parts of eigenvalues of \(A(\hat{\xi}) - \Omega(\hat{\xi})L\) are strictly negative. The characteristic polynomial of \(A(\hat{\xi}) - \Omega(\hat{\xi})L\) is:

\[
\text{det}(\lambda I - A(\hat{\xi}) - \Omega(\hat{\xi})L) = \lambda^3 + \alpha_1 \lambda^2 + \alpha_2 \lambda + \alpha_3
\]

Using the notation \(\Omega(\hat{\xi}) = [\alpha_1(\hat{\xi}) \quad \alpha_2(\hat{\xi}) \quad \alpha_3(\hat{\xi})]^T\) (in this case the gain matrix is in fact a vector), and the connection between the coefficients \(\alpha_i, i = 1,2,3\) and the eigenvalues \(\lambda_i, i = 1,2,3\), we can obtain the components of \(\Omega(\hat{\xi})\) after some direct calculations:

\[
\begin{align*}
\omega_1(\hat{\xi}) &= a_{11} + a_{22} + a_{33} - (\lambda_1 + \lambda_2 + \lambda_3) \\
\omega_2(\hat{\xi}) &= \frac{1}{a_{32}}(\lambda_1 \lambda_2 + \lambda_1 \lambda_3 + \lambda_2 \lambda_3 - a_{11} a_{22}) \\
\omega_3(\hat{\xi}) &= \frac{1}{a_{21} a_{32}}(\lambda_1 \lambda_2 \lambda_3 + a_{11} a_{22} a_{33} + a_{21} a_{32} a_{33} - a_{11} a_{22} a_{33} a_{33}) \\
\omega_4(\hat{\xi}) &= \frac{1}{a_{21} a_{32}}(\lambda_1 \lambda_2 \lambda_3 + a_{11} a_{22} a_{33} + a_{21} a_{32} a_{33} - a_{11} a_{22} a_{33} a_{33})
\end{align*}
\]

with \(a_y = a_y(\hat{\xi})\).

Finally, the state estimator for the unmeasured state variables \(\hat{\xi}_1, \hat{\xi}_2\) consists in the system (7) where the gain matrix is given by (12) with the design parameters \(\lambda_i \in \mathbb{R}^+, i = 1,2,3\).
3.2 The design of an asymptotic observer

The extended Luenberger observer is based on a perfect knowledge of the model structure, which is not a true assumption. Therefore, the main drawback of this observer is that the uncertainty in the model parameters can generate possibly large bias in the estimation of the unmeasured states. In order to overcome this disadvantage, it is possible to design an asymptotic observer, without the knowledge of the process kinetics being necessary.

The design of an asymptotic observer is based on some useful changes of coordinates, which lead to a submodel of (5) independent of the kinetics [4].

For instance, we will suppose that the internal substrate concentration is not measurable and it will be estimated. If we define an auxiliary variable as

$$z = (\xi_2 + Y)\xi_3,$$

with the dynamics

$$\frac{dz}{dt} = h(\xi_1)\xi_3,$$  \hspace{1cm} (13)

then the estimate of the internal substrate concentration is

$$\hat{\xi}_2 = \frac{z - Y}{\xi_3}$$  \hspace{1cm} (14)

From these relations it can be easily seen that the dynamics of auxiliary state variable is independent of the kinetics. The estimations of $\hat{\xi}_2$ obtained can be used in the design of control laws.

The asymptotic observer (13), (14) has good convergence and stability performance [1], [4]. The design is quite simple and natural; the observer is independent of the kinetics, the only drawback being a possible low rate of estimation convergence, which depends on the operating conditions.

3.3 Simulation results

For the lipase production process were performed some simulation experiments, where for the bioprocess parameters from equations (1)-(4) were considered the following values [3]:

$$\eta^* = 0.21\text{h}^{-1}; K_{M1} = 0.11\text{g/l}; \mu^* = 0.25\text{h}^{-1};$$

$$K_{M2} = 0.25\text{g/l}; v_p^* = 123\text{u/mg}; b = 0.000216(\text{mol/g})\text{h}^{-1};$$

$$a = 0.0185\text{mol/g}; K_p = 0.26\text{g/g}; K_i = 22.2\text{g/g};$$

$$v_{ex}^* = 4.09\text{h}^{-1}; K_{ex} = 19.5\text{u/mg}; Y = 1.16\text{g/g}.$$  

The bioprocess evolution was examined in open loop. This simulation is considered in order to analyse the lipase production and the performance of the state observers when the control law is missing. The following simulation cases are considered:

1) The extended Luenberger estimation algorithm was implemented for the design parameters $\lambda_1 = -1$, $\lambda_2 = -8$, $\lambda_3 = -0.01$. The "measured" variable $\xi_1 = X$ is vitiated with 3% additive white noise. The estimated state variables $\hat{\xi}_1 = \hat{S}_1, \hat{\xi}_2 = \hat{S}_2$ are compared with the simulated state values provided by the model equations (1)-(4). Fig. 1 presents the time evolution of the external substrate concentration and its estimate. In Fig. 2 the internal substrate concentration and its estimate are depicted. The "vitiated" measurements of the biomass concentration are presented in Fig. 3. Finally, Fig. 4 depicts the extracellular lipase concentration; from this figure it can be seen that after the consumption of external substrate, the growth of external lipase is limited. The simulation shows that the estimation algorithm is good. The measurement noise induces some noisy estimates of internal substrate concentration, but the effect on the estimates of external substrate concentration is small.

2) The asymptotic observer (13), (14) was implemented in order to reconstitute the internal substrate concentration from the measurements of biomass concentration. The bioprocess parameters were the same as in simulation case 1). Fig. 5 depicts the simulation results – the internal substrate concentration versus its estimate. It can be observed that the convergence of the asymptotic observer seems to be better than the convergence of the extended Luenberger estimator. The same problem regarding the noise sensitivity affects the estimates.

Fig. 1. Evolution of $S_1$ and its estimate

Fig. 2. Time profile of $S_2$ and its estimate
Identification results using a distribution based method

When the parameters and the kinetics of the bioprocess are partially known or unknown, it is necessary to use identification procedures. One method to identify nonlinear model parameters is the distribution based method. In this approach the set of nonlinear differential equations describing the state evolution is mapped into a set of linear algebraic equations with respect to the model parameters. Using techniques utilized in distribution approach, the measurable functions and their derivatives are represented by functionals on a fundamental space of testing functions [5], [6], [8].

If $\Phi_n$ is the fundamental space from the distribution theory, of the real functions $\varphi : \mathbb{R} \rightarrow \mathbb{R}, t \rightarrow \varphi(t)$. Let $q : \mathbb{R} \rightarrow \mathbb{R}, t \rightarrow q(t)$ be a function which admits a Riemann integral on any compact interval $T$ from $\mathbb{R}$. This function, a unique distribution

$$F_q : \Phi_n \rightarrow \mathbb{R}, \varphi \rightarrow F_q(\varphi) \in \mathbb{R}$$

(15)

can be building by the relation:

$$F_q(\varphi) = \int q(t)\varphi(t)dt, \forall \varphi \in \Phi_n$$

(16)

In distribution theory, the notion of k-order derivative is introduced. If $F_q \in \Phi_n$, then its k-order derivative is a new distribution $F_q^{(k)} \in \Phi_n$ uniquely defined by the relations:

$$F_q^{(k)}(\varphi) = (-1)^k F_q(\varphi^{(k)}), \forall \varphi \in \Phi_n$$

(17)

$$\varphi \rightarrow F_q^{(k)}(\varphi) = (-1)^k \int q(t)\varphi^{(k)}(t)dt \in R$$

(18)

where $\varphi^{(k)} : R \rightarrow R, t \rightarrow \varphi^{(k)}(t) = \frac{d^k \varphi(t)}{dt^k}$ is the k-order time derivative of the testing function.

When $q \in C^k(\mathbb{R})$, then

$$F_q^{(k)}(\varphi) = \int q^{(k)}(t)\varphi(t)dt = (-1)^k \int q(t)\varphi^{(k)}(t)dt$$

(19)

that means the k-order derivative of a distribution generated by a function $q \in C^k(\mathbb{R})$ equals to the distribution generated by the k-order time derivative of the function $q$.

The state equations used for parameters estimation are (5) and (2). Therefore the system contains rational dependencies between parameters and measured variables. To obtain linear equations in unknown parameters, the identification problem is split in several simple interlinked identification problems called identification layers. For simplicity, shall we denote the plant parameters by the vector:

$$\theta = [\theta_1, \theta_2, \theta_3, \theta_4, \theta_5]^T$$

(20)

$$\theta_1 = \eta^*, \theta_2 = K_{M1}, \theta_3 = \mu^*, \theta_4 = K_{M2}, \theta_5 = Y$$

(21)

Based on the specific structure of this system, it is possible to group the state equations, in such way to determine two interconnected identification problems. First, some state equations are utilized to obtain a set of linear equations in some parameters. The results of this first stage of identification are utilized for expressing other parameters by linear equations in the second stage.

Step 1: From the first state equation of (5), using (21) one obtains the following differential equation:
that is a linear in parameters $\theta_1$ and $\theta_2$.

Multiplying both sides of relation (22) with two sinusoidal type test functions $\varphi_i(t), i = 1, 2$ and integrating over $\mathbb{R}$ on get a system of linear equations in respect with parameters $\theta_1$ and $\theta_2$:

$$\begin{cases} w_1\theta_1 + w_2\theta_2 = v_1 \\ w_3\theta_1 + w_4\theta_2 = v_2 \end{cases}$$

(23)

$$v_1 = \int_{\mathbb{R}} -\xi_1(t)\xi_2(t)\varphi_1(t)dt , \quad v_2 = \int_{\mathbb{R}} -\xi_1(t)\varphi_1^{(1)}(t)dt ,$$

and

$$w_1 = \int_{\mathbb{R}} \xi_1(t)\xi_2(t)\varphi_1(t)dt , \quad w_2 = \int_{\mathbb{R}} -\xi_1(t)\varphi_1^{(1)}(t)dt .$$

Solving the system (23) one obtain the parameters $\theta_1$ and $\theta_2$. Using these estimates of the parameters we can now estimate the rate $\dot{\hat{n}}(t)$ that we use in the second step.

**Step 2:** From the second state equation of (5), using (21) one gets the next differential equation:

$$\frac{1}{2} \frac{d\xi_2^2(t)}{dt^2} - \hat{\eta}(t)\xi_2 =$$

$$= \theta_4 \left( \frac{d\xi_2^2(t)}{dt} + \hat{\eta}(t) \right) - \theta_1\theta_5\xi_2(t) - \theta_3\xi_2^2(t)$$

(24)

If one denote $\theta' = \theta_1\theta_5$ one obtain a linear equation in parameters $\theta_3$, $\theta_4$ and $\theta'$.

In the same way to step 1, a third order system of linear equations in respect with parameters $\theta_3$, $\theta_4$ and $\theta'$ is obtained, and by solving it one get the parameters $\theta_3$, $\theta_4$ and $\theta'$. Then, the systems of equation for all the five unknown parameters are:

$$\begin{bmatrix} 0.3193 & 5.7975 & \theta_1 \\ 0.6070 & 8.4127 & \theta_2 \end{bmatrix} = \begin{bmatrix} 1.2566 \\ 1.8403 \end{bmatrix} \Rightarrow \begin{cases} \hat{\theta}_1 = 0.2103 \\ \hat{\theta}_2 = 0.1172 \end{cases}$$

$$\begin{bmatrix} 0.6227 & -0.5424 & -0.1219 & \theta_3 \\ 0.5138 & -0.7473 & -0.2234 & \theta_4 \\ 0.4497 & -0.7344 & -0.2159 & \theta_5 \end{bmatrix} \Rightarrow \begin{cases} \hat{\theta}_3 = 0.2613 \\ \hat{\theta}_4 = 0.3012 \\ \theta' = -0.1327 \end{cases}$$

$$\Rightarrow \begin{cases} \hat{\theta}_3 = 0.2613 \\ \hat{\theta}_4 = 0.3012 \\ \hat{\theta}_5 = 0.2754. \end{cases}$$

Real (see subsection 3.3) and estimated values of the parameters are presented in the next table.

<table>
<thead>
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<th>$\theta_1 = \eta^*$</th>
<th>$\theta_2 = K_{M_1}$</th>
<th>$\theta_3 = \mu^*$</th>
<th>$\theta_4 = K_{M_2}$</th>
<th>$\theta_5 = Y^*$</th>
</tr>
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<td>0.11</td>
<td>0.25</td>
<td>0.25</td>
</tr>
<tr>
<td>Estimate</td>
<td>0.2103</td>
<td>0.1172</td>
<td>0.2754</td>
<td>0.2613</td>
</tr>
</tbody>
</table>

5 Conclusion

In this paper, some estimation strategies for a lipase production process have been presented. This bioprocess takes place inside a FBB, is strongly nonlinear and the kinetics is imprecisely known. A state estimation strategy based on extended Luenberger observer was designed and implemented. In order to deal with the parameter uncertainty of the bioprocess, an asymptotic observer was developed. The simulation results show a quite good behaviour of the proposed observers. The unknown kinetic parameters of the process were estimated with encouraging results by using a distribution based identification technique.

The proposed estimation techniques can be used for the design of nonlinear control strategies.

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