STOCHASTIC ESTIMATION TECHNIQUES FOR BIOTECHNOLOGICAL PROCESSES

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Abstract: *This paper deals with stochastic state and parameter estimation for the biotechnological processes. The lipase producing process using Candida rugosa yeast is taking into consideration. As is well known from the literature the state variables and the parameters that characterize the proper biotechnological process are not accessible to the direct measurement and they are affected by noise. In the paper the authors advance the use of two stochastic estimators (the Extended Kalman and H∞ Filters) to estimate state variables and parameters of the biotechnological processes.*

Keywords: *biotechnological process, lipase, state and parameters estimation, stochastic estimator, robust estimator.*

1. INTRODUCTION

The complexity of the bioprocesses makes their control problem very difficult. The usual modelling procedures, based on kinetic enzymatic schemes, lead to nonlinear state models, with a large number of parameters [6]. Moreover, the state variables that characterize the proper biotechnological process are not accessible to the direct measurement. In the basic configuration, the bioreactor has a number of control loops only for the physical and chemical variables of the culture environment (temperature, stirring, aeration, pH etc.). Other important variables like biomass concentration, enzyme concentration etc. are measured taking a number of samples and analyzing in the laboratory. Many times the numbers of samples is limited, the analysis is done with difficulty or the culture environment from bioreactor could contaminate [1].

In general the biotechnological processes are strongly affected by noise. There are many factors, such as the substrate composition, the primary operations performed before the bioreactor process (bioreactor washing, substrate preparation, bioreactor stirilizing and the inoculum preparation) that induce a high level of uncertainty in process modelling [3]. All these factors cannot be mathematically modelled and they contribute to the unknowing of the process initial state and to the difficulty of the process parameter identification with bad results in the process simulation.

These facts have determined the development of some specific techniques to estimate the parameters and provide the state observers synthesis [4]. State estimation theory was developed for linear systems and many solutions can be envisaged, according to the nature of the system. When nonlinear systems are considered, the estimation problem is more complicated. Traditionally used approaches are either an approximation or an extension of linear algorithms or specific nonlinear algorithms. The problem with the first method is that convergence is not guaranteed and the second only applies to one class of systems, which can be mathematically complicated [7].

The structure of the paper is as follows: the second section deals with the process model (the lipase producing process using *Candida rugosa* yeast); the third section deals with the implementation of the Extended Kalman Filter for state and parameter estimation, the fourth section presents the Extended H∞ state and parameter estimator, section 5 presents a study regarding the robustness of the solutions proposed in the paper and the last section is dedicated to the conclusions.

2. THE MODEL OF THE LIPASE PRODUCING PROCESS

Lipase is an enzyme which is produced in batch or fed-batch bioreactors [15]. The lipase is obtained using *Candida rugosa* yeast. The yeast growth takes place in a proper environment where the substrate consumed by the biomass is the oleic acid. The biosynthesis process of the lipase is very complex and strongly nonlinear. It contains four phases which do appear simultaneously: the liquid phase, the organic phase, the cellular phase and the gaseous phase.

The process is given by the following equations:

$$\frac{dS_1}{dt} = -\eta(S_1)X + F \tag{1}$$

$$\frac{dS_2}{dt} = \eta(S_1) - \mu(S_2)\cdot(Y + S_2) \tag{2}$$

$$\frac{dX}{dt} = \mu(S_2)X \tag{3}$$

$$\frac{dL_{in}}{dt} = \nu_p(S_1, X, \mu) - \nu_{ex}(L_{in}) - \mu(S_2)L_{in} \tag{4}$$

$$\frac{dL_{ex}}{dt} = \nu_{ex}(L_{in})X \tag{5}$$

$$C_{er} = (a\mu(S_2) + b)X \tag{6}$$

where S_1 is the substrate consumed for the biomass growth, S_2 is the intracellular substrate, X represents the biomass, L_{in} is the intracellular enzyme and L_{ex} is the extracellular enzyme. The last equation is algebraic and expresses the specific outflow rate of CO_2, which depends on biomass. The parameter Y is the production coefficient "biomass/substrate". The reaction rates are given by the equations (7) – (10):

$$\eta = \frac{\eta^* S_1}{K_{M1} + S_1} \tag{7}$$

$$\mu = \frac{\mu^* S_2}{K_{M2} + S_2} \tag{8}$$

$$\nu_{ex} = \frac{\nu_{ex}^* L_{in}}{K_{ex} + L_{in}} \tag{9}$$

$$\nu_p = \frac{\nu_p^*(S_1/X)}{K_p + (S_1/X) + K_i(S_1/X)^2}\cdot\mu(S_2) \tag{10}$$

In industrial conditions the lipase production can be analyzed considering a part of the external lipase (the liquid lipase – L_a).

$$L_a = L_{ex}\left(1 + \frac{K_{a1}\cdot S_1}{K_{a2} + S_1}\right) \tag{11}$$

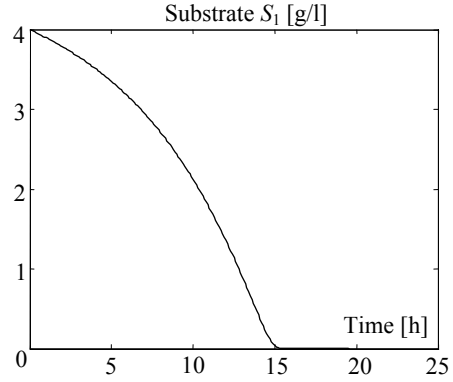

Fig. 1. The substrate (S_1) concentration

The model simulations have been made using the following values of the parameters and the results are presented in figures 1-5:

η^*=0.21h^{-1}, K_{M1}=0.11g/l, μ^*=0.25h^{-1}, F=0(g/l)h^{-1}, K_{M2}=0.25g/l, ν_p^*=123u/mg, K_P=0.26g/l, K_i=22.2g/g, ν_{ex}^*=4.09h^{-1}, K_{ex}=19.5u/mg,

Y=1.16g/g, a=0.018mol/g, b=0.0002(mol/g)h^{-1}, K_{a1}=0.5 and K_{a2}=0.19g/l [15].

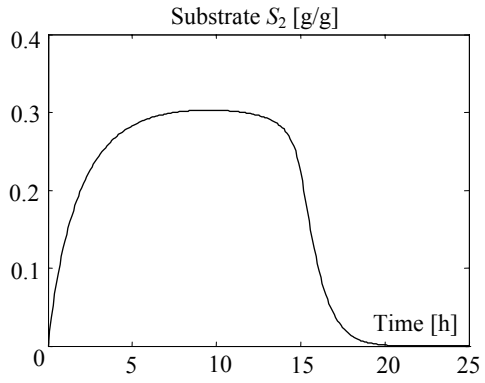

Fig. 2. The substrate (S_2) concentration

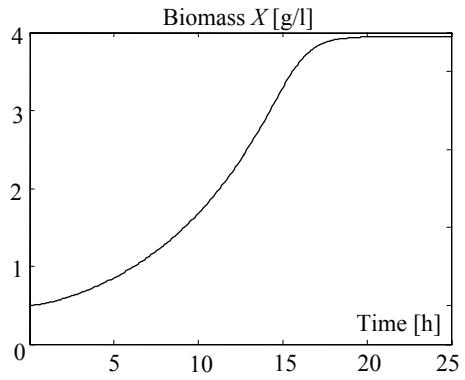

Fig. 3. The biomass (X) concentration

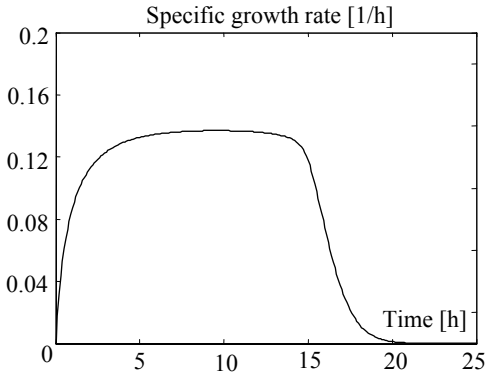

Fig. 4. The specific growth rate (μ) evolution

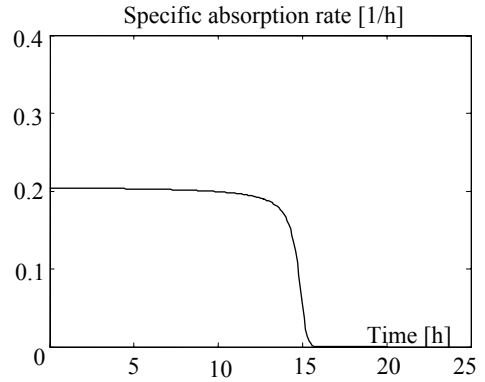

Fig. 5. The specific absorption rate (η) evolution

3. THE KALMAN STATE AND PARAMETER ESTIMATOR

3.1. *The Kalman Filter*

Let us consider the linear stochastic system described by the equations:

$$\dot{x}(t) = Ax(t) + Bu(t) + D_1 w(t) \tag{12}$$

$$y(t) = C_2 x(t) + D_2 v(t) \tag{13}$$

$$z(t) = C_1 x(t) \tag{14}$$

where $x \in R^n$ is the state vector, $y \in R^m$ is the measurements vector and $z \in R^p$ is the estimated signals vector; $w(t)$ and $v(t)$ are vectors of process and measurement noise. The process noise and the measurement noise are assumed to be white, uncorrelated and with normal probability distribution [8].

The estimator equation is:

$$\dot{\hat{x}}(t) = A\hat{x}(t) + Bu(t) + K\left[y(t) - C_2\hat{x}(t)\right] \tag{15}$$

The design of the Kalman filter consists in the calculus of the gain matrix K which minimizes the mean square of the estimation error:

$$E = \int_0^t \left\| x - \hat{x} \right\|^2 d\tau = \int_0^t \left\| e(\tau) \right\|^2 d\tau \tag{16}$$

The solution is

$$K = PC_2^{\mathrm{T}} R^{-1} \tag{17}$$

where the symmetric square matrix P is generated by the Riccati equation:

$$\dot{P} = PA^{\mathrm{T}} + AP - PC_2^{\mathrm{T}} R^{-1} C_2 P + D_1 Q \tag{18}$$

In equation (18) Q represents the process noise covariance and R is the measurement noise covariance.

3.2. *The Extended Kalman Filter*

Let us consider the nonlinear system described by the equations:

$$\dot{x}(t) = f(x(t), u(t)) + D_1 w(t) \tag{19}$$

$$y(t) = h(x(t)) + D_2 v(t) \tag{20}$$

In the case of the nonlinear systems the Extended Kalman Filter is used. It uses the equations of the linear filter, where the matrices A, B and C_2 are obtained by linearizing the

nonlinear system around each functioning point [12]:

$$A(\hat{x}) = \frac{\partial f(\hat{x},u)}{\partial \hat{x}};\ B(\hat{x}) = \frac{\partial f(\hat{x},u)}{\partial u};$$
$$C_2(\hat{x}) = \frac{\partial h(\hat{x})}{\partial \hat{x}}$$

The estimator equation is:

$$\dot{\hat{x}}(t) = f(\hat{x}(t),u(t)) + K\left[y(t) - h(\hat{x}(t))\right] \quad (21)$$

3.3. *The Extended Kalman Filter for the state estimation of the biotechnological processes*

Let us consider the general state space model of a biotechnological process [4]:

$$\dot{\xi}(t) = K\varphi(\xi) - D\xi + F - Q + D_1 w(t) \quad (22)$$

$$\xi_1 = C_2\xi + D_2 v(t) \quad (23)$$

where ξ represents the state vector, D – the dilution rate, K – the matrix of the production coefficients, φ - the reaction rates, F – the input flow, Q - the output gaseous flow and ξ_1 represents the vector of the measured states.

A general class of state observers is given by equation:

$$\frac{d\hat{\xi}(t)}{dt} = K\varphi(\hat{\xi}) - D\hat{\xi} + F - Q + \Omega(\hat{\xi})(\xi_1 - \hat{\xi}_1) \quad (24)$$

Observation: Ω and ξ are specific notations in the field of biotechnological processes (Ω - the gain matrix and ξ - the state vector). In the same field the standard notations, K and x, have another significance: K – the matrix of the production coefficients and x – the viable biomass concentration.

The estimation error is defined as follows:

$$e = \xi - \hat{\xi} \quad (25)$$

The dynamics of the estimation error is given by the equation

$$\dot{e}(t) = K\left[\varphi(\hat{\xi} + e) - \varphi(\hat{\xi})\right] - D\cdot e - \Omega(\hat{\xi})C_2\cdot e \quad (26)$$

It can be noticed that $e = 0$ is an equilibrium point of the model (26). The linear approximation around the value $e = 0$ is given by equation:

$$\dot{e}(t) = \left[A(\hat{\xi}) - \Omega(\hat{\xi})C_2\right]\cdot e \quad (27)$$

where

$$A(\hat{\xi}) = K\left[\frac{\partial\varphi(\xi)}{\partial\xi}\right]_{\xi=\hat{\xi}} - D\cdot I_n \quad (28)$$

In the case of the lipase producing process using *Candida rugosa* yeast the measured variable is the biomass X and the variables S_1, S_2 and X are estimated. This is due to the fact that the control algorithms need the knowledge of the two substrates at every moment. The equations of the estimator are the following:

$$\frac{d\hat{S}_1}{dt} = -\eta(\hat{S}_1)\hat{X} + F + \omega_1(X - \hat{X}) \quad (29)$$

$$\frac{d\hat{S}_2}{dt} = \eta(\hat{S}_1) - \mu(\hat{S}_2)\cdot(Y + \hat{S}_2) + \omega_2(X - \hat{X}) \quad (30)$$

$$\frac{d\hat{X}}{dt} = \mu(\hat{S}_2)\hat{X} + \omega_3(X - \hat{X}) \quad (31)$$

where $\Omega = [\omega_1\ \omega_2\ \omega_3]^T$ is obtained using equations (17) and (18) around each functioning point.

The matrices used for the filter implementation are:

$$P = \begin{bmatrix} P_{11} & P_{12} & P_{13} \\ P_{12} & P_{22} & P_{23} \\ P_{13} & P_{23} & P_{33} \end{bmatrix};\ C_2 = \begin{bmatrix} 0 & 0 & 1 \end{bmatrix};$$

$$D_1 = \begin{bmatrix} 1 & 0 & 0 \\ 0 & 1 & 0 \\ 0 & 0 & 1 \end{bmatrix};$$

$$A(\hat{\xi}) = \left.\frac{\partial f(\xi)}{\partial\xi}\right|_{\xi=\hat{\xi}} = \left[a_{ij}(\hat{\xi})\right]_{i,j=\overline{1,3}}$$

where

$$a_{11}(\hat{\xi}) = -\frac{\eta^* K_{M1}\hat{X}}{(K_{M1} + \hat{S}_1)^2};\ a_{12}(\hat{\xi}) = 0;$$

$$a_{13}(\hat{\xi}) = -\frac{\eta^*\hat{X}}{K_{M1} + \hat{S}_1};\ a_{21}(\hat{\xi}) = \frac{\eta^* K_{M1}}{(K_{M1} + \hat{S}_1)^2};$$

$$a_{22}(\hat{\xi}) = \frac{\mu^*(K_{M2}Y + 2K_{M2}\hat{S}_2 + \hat{S}_2^2}{(K_{M2} + \hat{S}_2)^2};$$

$$a_{23}(\hat{\xi}) = 0;\ a_{31}(\hat{\xi}) = 0;$$

$$a_{32}(\hat{\xi}) = \frac{\mu^* K_{M2}\hat{X}}{(K_{M2} + \hat{S}_2)^2};\ a_{33}(\hat{\xi}) = \frac{\mu^*\hat{S}_2}{K_{M2} + \hat{S}_2}$$

The simulation results are presented in figures (6) – (8).

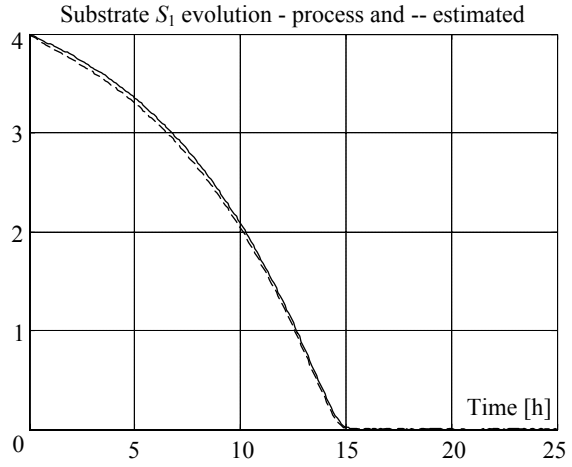

Fig. 6. The substrate S_1 concentration (model and estimation)

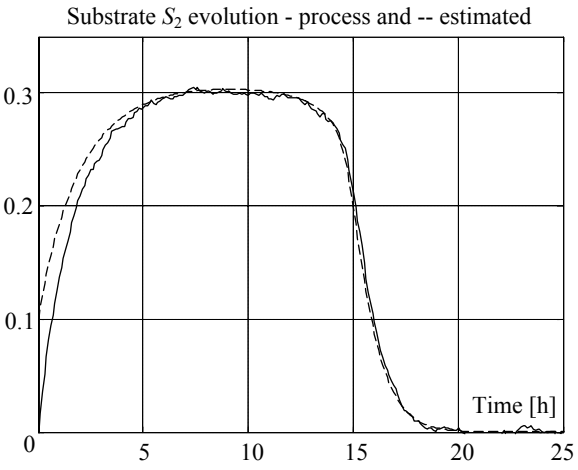

Fig. 7. The substrate S_2 concentration (model and estimation)

3.4. *The Extended Kalman Filter for the state and parameter estimation*

In the case of the process parameter estimation, they can be modeled as integrators driven by the white noise and augmented to the system states. So that the parameters need to be estimated are added to the state vector of the system [2], [11]. The new state vector is:

$$x(t) = \begin{bmatrix} x_1(t) & \theta(t) \end{bmatrix} \tag{32}$$

where $x_1(t)$ represents the process states and $\theta(t)$ the process parameters which will be estimated. The function $f(x,t)$ becomes:

$$f(x,t) = \begin{bmatrix} f(x,u,\theta) & 0 \end{bmatrix};\ D_1 = \begin{bmatrix} D_x & 0 \\ 0 & D_\theta \end{bmatrix} \tag{33}$$

Taking into account the partition of the state vector, the matrix A from the equation (32) has the following form:

$$A = \begin{bmatrix} \dfrac{\partial f(\hat{x},u,\hat{\theta})}{\partial \hat{x}_1} & \dfrac{\partial f(\hat{x},u,\hat{\theta})}{\partial \hat{\theta}} \\ 0 & 0 \end{bmatrix} \tag{34}$$

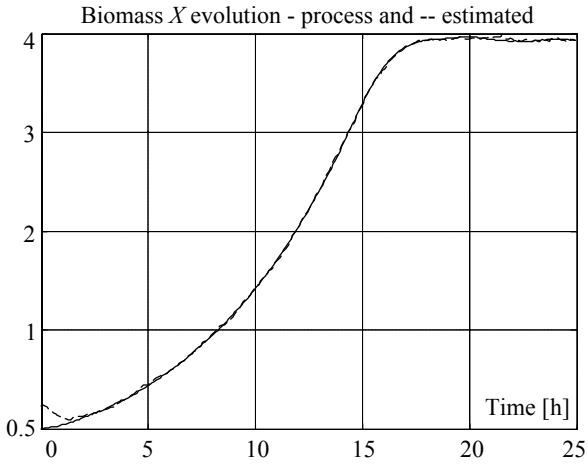

Fig. 8. The biomass X concentration (model and estimation)

3.5. *The Extended Kalman Filter for the state and parameter estimation of the biotechnological processes*

In the case of biotechnological processes we assume that the vector $\varphi(\xi)$ of reaction rates is partially unknown and is written as follows [4]:

$$\varphi(\xi) = H(\xi)\theta(\xi) \tag{35}$$

where $H(\xi)$ is a $M \times r$ matrix of known functions of the state and $\theta(\xi)$ a vector of unknown functions of ξ, with $\dim(\theta(\xi)) = r$.

With this definition, the general state space model of a biotechnological process is rewritten:

$$\dot{\xi} = KH(\xi)\theta(\xi) - D\xi + F - Q \tag{36}$$

The state observer is given by the equation:

$$\frac{d}{dt}\begin{bmatrix}\hat{\xi}\\ \hat{\theta}\end{bmatrix}=\begin{bmatrix}KH(\hat{\xi})\theta(t)-D\hat{\xi}+F-Q\\ 0\end{bmatrix}+ \begin{bmatrix}\Omega_1(\hat{\xi},\hat{\theta})\\ \Omega_2(\hat{\xi},\hat{\theta})\end{bmatrix}\left[\xi-\hat{\xi}\right] \tag{37}$$

In the case of the lipase producing process using *Candida rugosa* yeast the vector $\theta(t)$ of the parameters that must be estimated is:

$$\theta(t)=\left[\eta(t)\quad \mu(t)\right]^{\mathrm{T}} \tag{38}$$

In this case the measured variables are S_1 and X and the variables S_1, X, η and μ are estimated.

The matrices used for the filter implementation are:

$$x(t)=\left[S_1(t)\quad X(t)\quad \eta(t)\quad \mu(t)\right]^{\mathrm{T}};$$

$$A=\begin{bmatrix}0 & -\hat{\eta} & -\hat{X} & 0\\ 0 & \hat{\mu} & 0 & \hat{X}\\ 0 & 0 & 0 & 0\\ 0 & 0 & 0 & 0\end{bmatrix};\ D_1=\begin{bmatrix}1 & 0 & 0 & 0\\ 0 & 1 & 0 & 0\\ 0 & 0 & 1 & 0\\ 0 & 0 & 0 & 1\end{bmatrix}$$

$$P=\begin{bmatrix}P_{11} & P_{12} & P_{13} & P_{14}\\ P_{12} & P_{22} & P_{23} & P_{24}\\ P_{13} & P_{23} & P_{33} & P_{34}\\ P_{14} & P_{24} & P_{34} & P_{44}\end{bmatrix};$$

$$C_2=\begin{bmatrix}1 & 1 & 0 & 0\end{bmatrix}.$$

The simulation results are presented in figures (9) – (12).

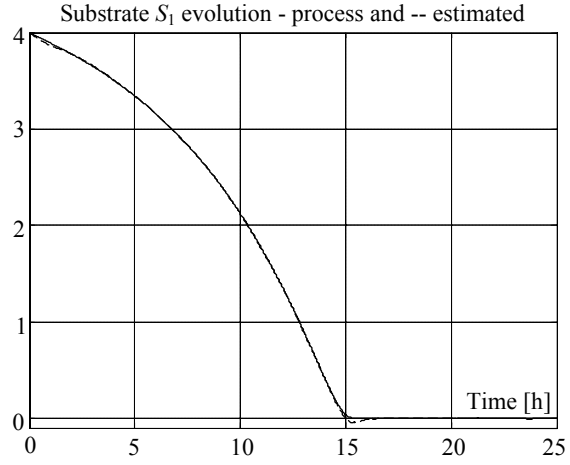

Fig. 9. The substrate S_1 concentration (model and estimation)

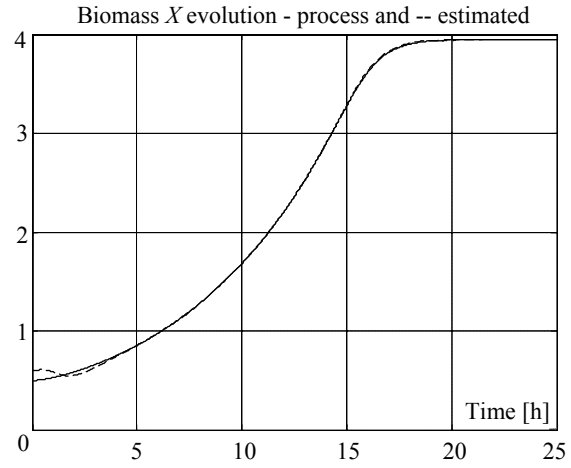

Fig. 10. The biomass X evolution (model and estimation)

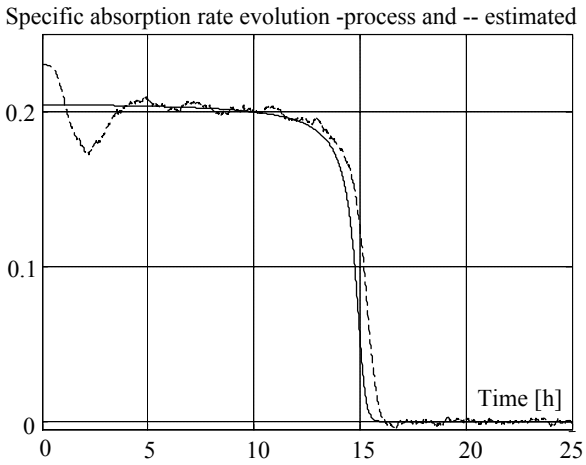

Fig. 11. The specific absorption rate (model and estimation)

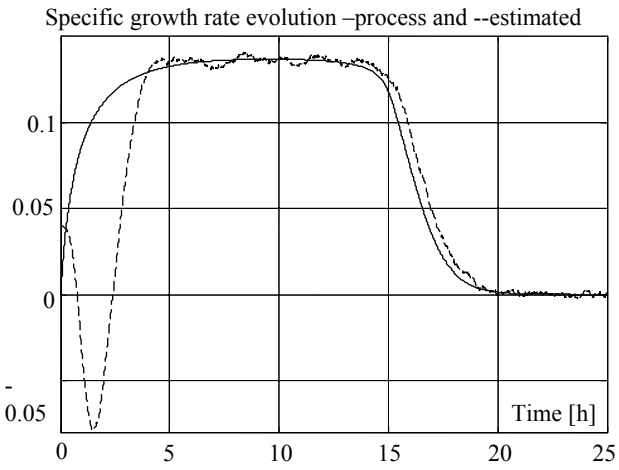

Fig. 12. The specific growth rate (model and estimation)

4. THE H∞ STATE AND PARAMETER ESTIMATOR

4.1. *The H∞ Filter*

Considering the linear stochastic system described by the equations (12)-(14), the filtering problem is to determine an estimation $\hat{z}(t)$ of $z(t)$ using the measures of variable y at the moment t. The H∞ Filter must minimize the cost function [13]:

$$J = \sup_{w \in L_2[0,\infty)} \frac{\|\tilde{z}\|_2^2}{\|w\|_2^2} < \gamma^2, x(0) = 0 \tag{39}$$

for a known value of $\gamma > 0$ and $\tilde{z}(t) = z(t) - \hat{z}(t)$.

The gain of H∞ Filter is similar to the one of Kalman Filter, when γ has a big value. As long as γ decreases, the filter converges to the optimal H∞ Filter [9].

The estimator equation is:

$$\dot{\hat{x}}(t) = A\hat{x}(t) + Bu(t) + K\left[y(t) - C_2\hat{x}(t)\right] \tag{40}$$

where K is the filter gain:

$$K = P \cdot C_2^{\mathrm{T}} \cdot R^{-1} \tag{41}$$

In equation (41) P is the solution of H∞ Riccati equation:

$$\dot{P} = P \cdot A^{\mathrm{T}} + A \cdot P + P \cdot \left(\gamma^{-2} \cdot C_1 \cdot C_1^{\mathrm{T}} - C_2 \cdot R^{-1} \cdot C_2^{\mathrm{T}}\right) \cdot P + D_1 \cdot Q \tag{42}$$

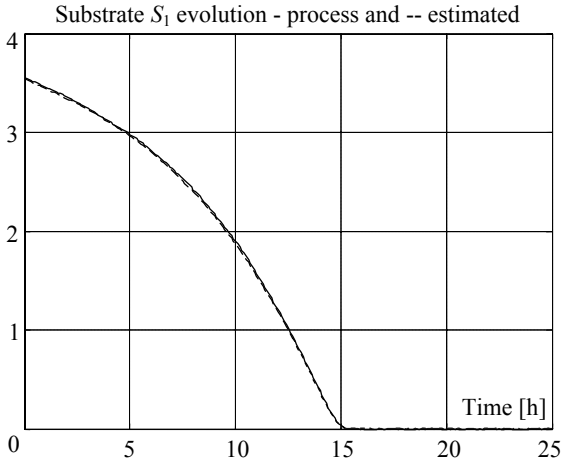

Fig. 13. The substrate S_1 concentration (model and estimation)

4.2. *The Extended H∞ Filter for the state estimation of the biotechnological processes*

In this case the measured variable is the biomass X and the variables S_1, S_2 and X are estimated. For the filter implementation the matrices A, P, C_2 and D_1, presented in section 3.3, together with the matrix C_1 and the variable γ, are used:

$$C_1 = \begin{bmatrix} 1 & 1 & 1 \end{bmatrix};\ \gamma = 0.01$$

The simulation results are presented in figures 13 – 15.

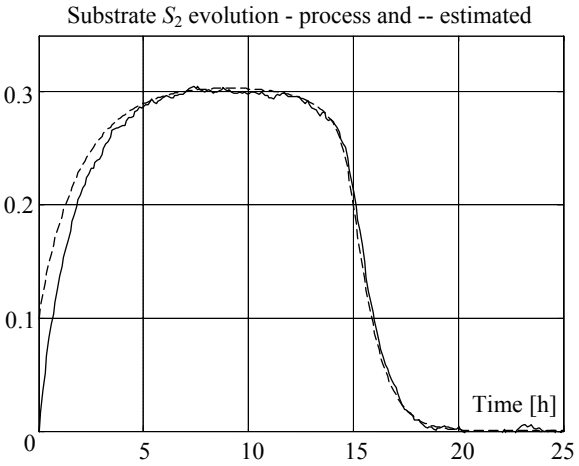

Fig. 14. The substrate S_2 concentration (model and estimation)

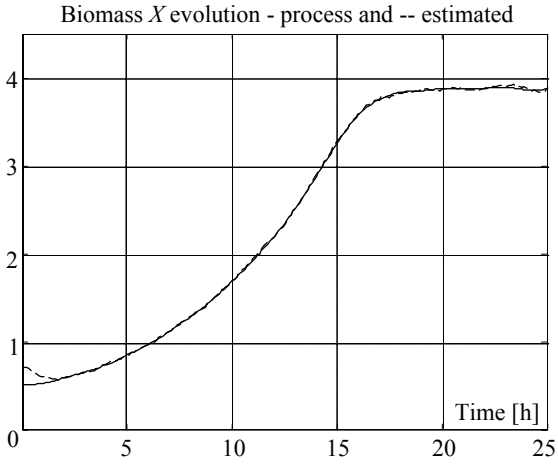

Fig. 15. The biomass (X) concentration (model and estimation)

4.3. *The Extended H∞ Filter for the state and parameter estimation of the biotechnological processes*

In this case the measured variables are S_1 and X and the variables S_1, X, η and μ are estimated. For the filter implementation the matrices A, P,

C_2 and D_1, presented in section 3.5, together with the matrix C_1 and the variable γ, are used:

$$C_1 = \begin{bmatrix} 1 & 1 & 1 & 1 \end{bmatrix};\ \gamma = 0.01$$

The simulation results for the two parameters η and μ are presented in figures 16 and 17.

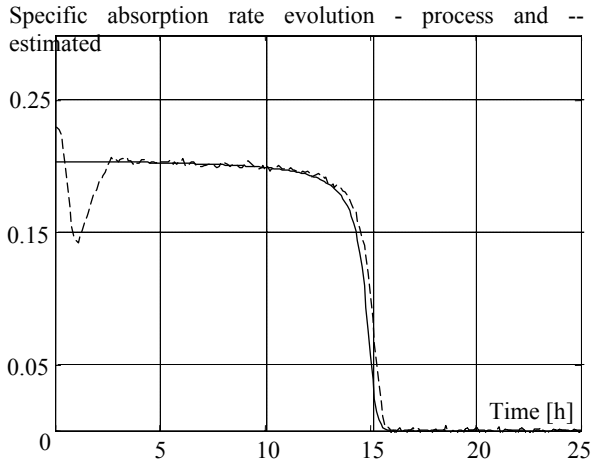

Fig. 16. The specific absorption rate (η) (model and estimation)

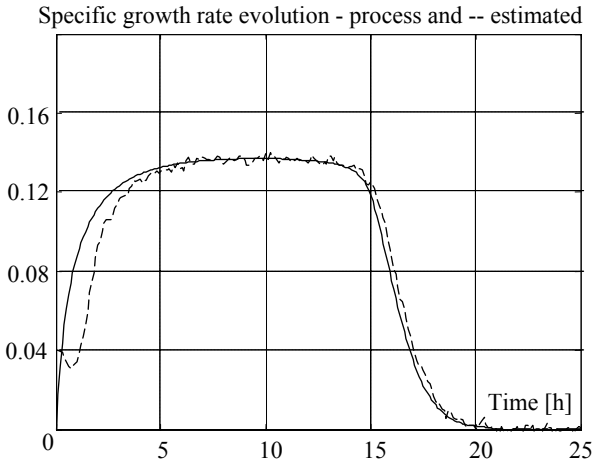

Fig. 17. The specific growth rate (μ) (model and estimation)

5. THE ROBUSTNESS OF THE SOLUTIONS FOR STATE AND PARAMETER ESTIMATION

The two stochastic estimation methods, Kalman and H∞, were tested form the robustness point of view. The parameter values of η^* and μ^*, that appear in η and μ equations, were modified with 10%. The simulation results are presented in figures 18 – 21.

The figures 20 and 21 show the robustness properties of the H∞ Filter, unlike the Kalman Filter for that figures 18 and 19 clearly point out the lack of robustness.

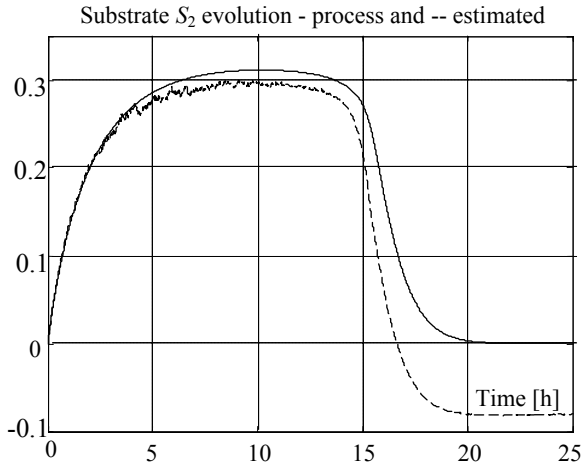

Fig. 18. The substrate S_2 concentration (model and estimation using Extended Kalman Filter)

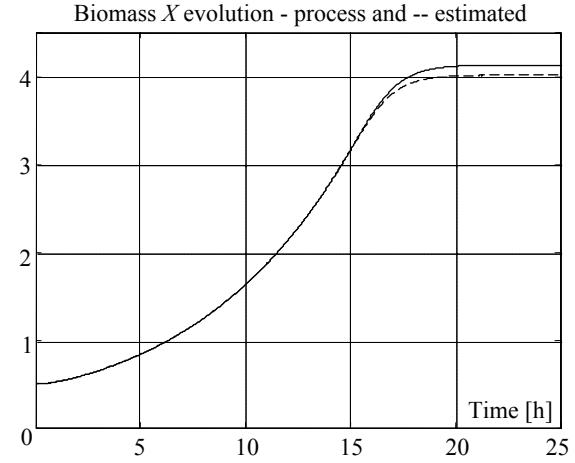

Fig. 19. The biomass X concentration (model and estimation using Extended Kalman Filter)

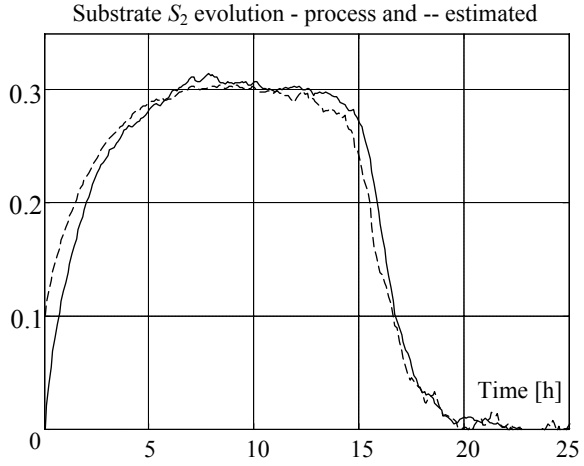

Fig. 20. The substrate S_2 concentration (model and estimation using Extended H∞ Filter)

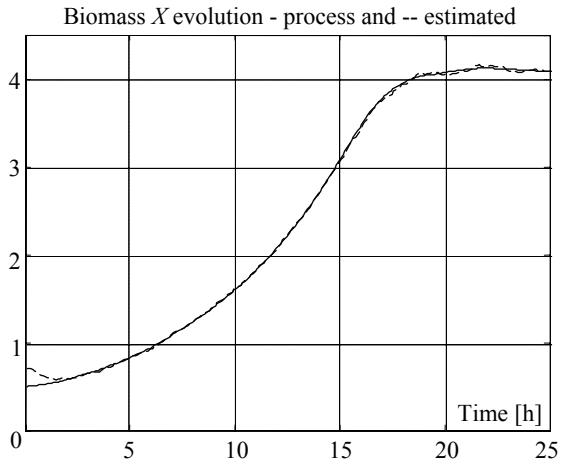

Fig. 21. The biomass *X* concentration (model and estimation using Extended H∞ Filter)

6. CONCLUSIONS

The following conclusions can be drawn from this study:

- As is well known from the literature the state variables and the parameters that characterize the proper biosynthesis process are not accessible to the direct [5], [10]. This is why the authors advance the use of the stochastic estimators (Extended Kalman and H∞ Filters) to estimate the state variables and parameters of the biotechnological processes.
- The results can be compared to those obtained using Extended Luenberger Observer in [15]. We consider that the stochastic estimators offer better filtering properties and the results are also better.
- The Extended Kalman Filter does not guarantee the global convergence of the estimation error. Local stability can be proved, however, assuming some bounds on the nonlinearities [14].
- The Extended H∞ Filter assures filtering properties as Kalman Filter does and robustness properties with respect to the parameters uncertainties.

7. REFERENCES

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