MODELLING AND CONTROLLING TECHNIQUES OF MICROORGANISMS MEAN AGE IN BIOTECHNOLOGICAL PROCESSES

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Abstract

This paper deals with the possibility to control the biosynthesis processes using information about microorganisms mean age. The mean age is a very important parameter characteristic to every species and is tightly linked to the mechanism of producing biosynthesis products. The basic idea in controlling the biosynthesis processes using microorganisms mean age information is to bring the most microorganisms developed in the bioreactor to an optimum age. Thus they become capable of producing a large quantity of biosynthesis products. The simulation presented in this paper pertinently illustrates the viability of this method in continuous bioreactors, having as subject a population of Candida Lipolytica, cultivated on an ammonium sulphate sublayer.

1 Introduction

Knowing the dynamics of the microorganisms age distribution is important when estimating the biotechnological processes and has significant effects on the optimal control of certain bioreactors intended for producing biosynthesis products. The traditional methods of biosynthesis processes modelling (methods based on balance equations and equations related to biochemical reactions schemes) generally cover global aspects of the microorganisms physiology. The present paper attempts to achieve some simpler models, physiologically approached, able to account for the qualitative properties of the biosynthesis processes. This is done by making the structural-functional model reflect the "physiological state" of the cells by mean age "m(t)" which can be regarded as a very important state variable in controlling the biosynthesis processes.

In general, optimal controlling techniques aim at extremising a performance criterion, defined in relation with the quantity of biosynthesis products thus obtained. This applies to the enzymatic biosynthesis processes. This criterion may be the quantity of biosynthesis products at the moment "t_f" (final time) considered as unfixed variable or an indicator which expresses the specific cost of the biosynthesis products obtained in the interval $[0, t_f]$. It is very important to determine the optimal command taking into consideration the physiological mechanism specific to the respective species. In [cara1] and [cara2] the authors come to the conclusion that, in the presence of an assimilable sublayer, abundantly present, the microorganisms develop without achieving the biosynthesis of the enzymes (biosynthesis product). When the assimilable sublayer is finished, the cells produce enzymes, with the aim to hydrolyze a sublayer which is not directly assimilable. Thus, from the point of view of cell physiology, it is necessary for the microorganisms to be brought as lengthy as possible to a starvation state, maintaining their viability. We may conclude that, if in a bioreactor the microbial population has a permanent sublayer for an optimal developing - with regard to the biomass - the results concerning the biosynthesis products will be normal. On the contrary, if during the bioreactor batch the supplying with the sublayer has such a rhythm that cells are developed at first and then determined to produce enzymes, valuable results in biosynthesis products will be obtained. In case the microorganisms have all the proper conditions of development the mean age has a minimum value, characteristic to the respective species. If the flow of assimilable sublayer is reduced, the process of formation of new viable cells will take a slower rhythm and the mean age will increase. Consequently, if variable "m(t)" could be evaluated at any moment and the commands were given according to this variable, the following strategy of controlling the biosynthesis processes would be rational: in the initial phase of the process a reduced value of the mean age will be maintained and that will correspond to a faster development of the biomass (later this will fuel the biosynthesis product). Then the nutritional flow will be adjusted so that the mean age should be maintained at an optimal value, from the point of view of the increasing rhythm in the biosynthesis product. This value is evidently bigger than the one in which the maximum increasing rhythm had been obtained.

2 Age models

The dynamics of a microbial population age distribution is described by the state equation [ranta3]:

$$\frac{\partial X(t,\tau)}{\partial t}+\frac{\partial X(t,\tau)}{\partial \tau}+K(t,\tau)X(t,\tau)+$$
$$+D(t)X(t,\tau)=D_i(t)X_i(t,\tau) \qquad (1)$$

to which both the boundary condition (zero-age biomass forming) equation:

$$X(t,0)=\mu_i(t)\int_0^\infty X(t,\tau)d\tau+$$
$$+\int_0^\infty K_F(\tau)X(t,\tau)d\tau \qquad (2)$$

and the initial condition equation:

$$X(0,\tau)=X_0(\tau) \qquad (3)$$

are added.

The following notations were used:

X(t,τ)-concentration of "τ" age biomass;
D-dilution rate, D_i-input age distribution;
X_i(t,τ)-input age distribution;
μ_I--budding rate.

In (1), K(t,τ) (biomass growth rate) has the form:

$$K(t,\tau)=K_F(\tau)+K_D(\tau)-\mu(t,\tau) \qquad (4)$$

where the following parameters represent:

K_F(τ) - the fission rate;
K_D(τ) - the decay rate;
μ(t,τ) - the growth rate of the "τ" age biomass.

Defining the mean age by the relation

$$m(t)=\frac{1}{\int_0^\infty X(t,\tau)d\tau}\int_0^\infty \tau X(t,\tau)d\tau \qquad (5)$$

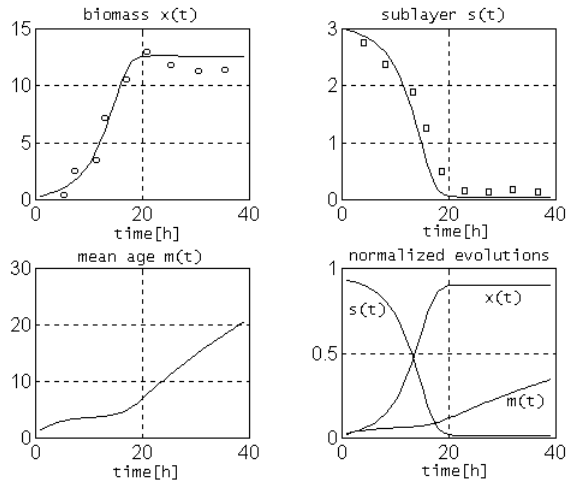
and making assumptions on functions K_F(τ) and K_D(τ), various model variants can be obtained able to explain the mean age dynamics [cara4] and [cara5]. The mathematical model M_1 is described by the following state equations:

$$\frac{dX(t)}{dt}=\left[\mu(t)-D(t)\right]X(t) \qquad (6)$$

$$\frac{dS(t)}{dt}=D(t)\left[S_0-S(t)\right]-\frac{v_m S(t)X(t)}{k_m+S(t)} \qquad (7)$$

$$\frac{dm(t)}{dt}=1-\mu(t)m(t) \qquad (8)$$

This model admits that the biomass growing processes, by forming zero age cells, take place only by budding. The M_1 model does not contain parameters K_F(τ) and K_D(τ); the model does not consider the cells fission and autoliza phenomena (figure 1).



-Figure 1: The simulation of M_1 model-

The mathematical model M_2 is described by the following state equations:

$$\frac{dX(t)}{dt}=\left[\mu(t)-D(t)-K_D\right]X(t) \qquad (9)$$

$$\frac{dS(t)}{dt}=D(t)\left[S_0-S(t)\right]-\frac{v_m S(t)X(t)}{k_m+S(t)} \qquad (10)$$

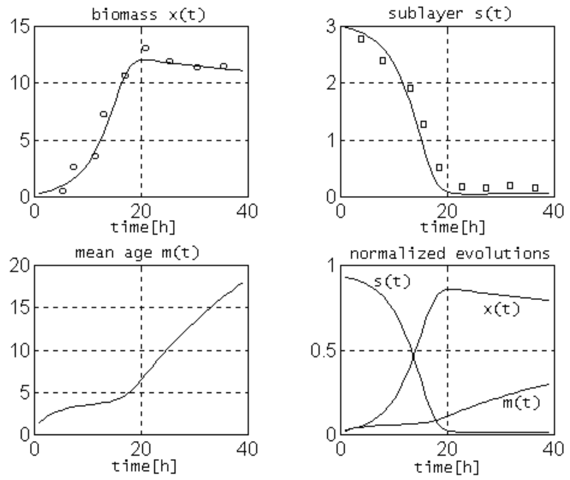
$$\frac{dm(t)}{dt}=1-[\mu(t)+K_F]m(t) \qquad (11)$$

where K_D=const. and K_F=const. (figure 2).

The model does consider the both phenomena of biomass growing by the formation of zero age cells:

- fission
- budding.

The autoliza phenomenon is also taken into consideration.

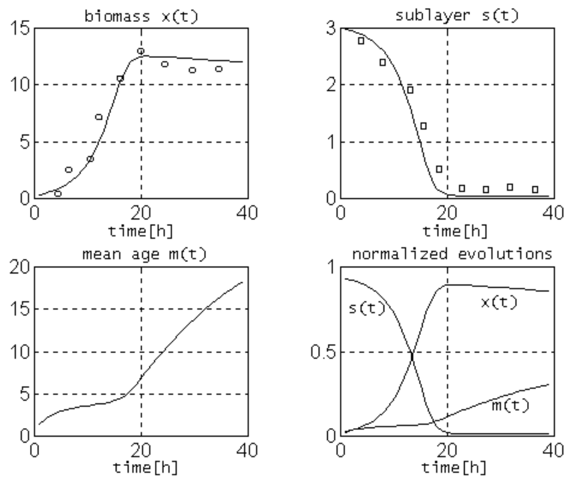


-Figure 2: The simulation of M_2 model-

The mathematical model M_3 (figure 3) is described by the state equations (9)-(11) where:

$$K_D(t) = K_{D_1} m(t) \quad (12)$$

$$K_F(t) = K_{F_1} m(t) \quad (13)$$



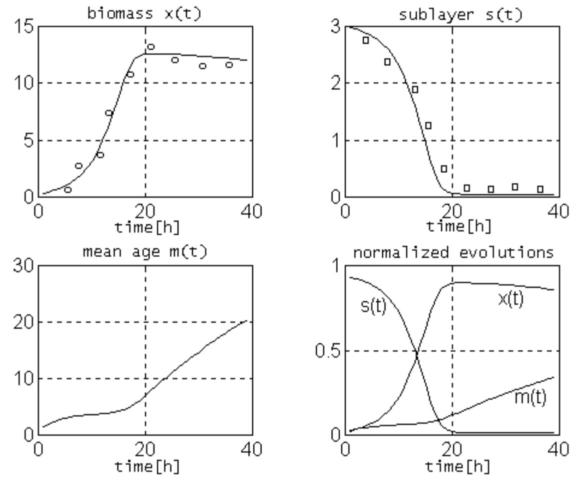
-Figure 3: The simulation of M_3 model-

We admit that the fission rate is variable and it depends on the mean age. As we can see in (12) and (13) the dependence of K_D and K_F on m(t) is linear.

The mathematical model M_4 (figure 4) is also described by the state equations (9)-(11) where:

$$K_D(t) = K_{D_1} m^2(t) \quad (14)$$

$$K_F(t) = \begin{cases} K_{F_0} m(t) & \text{if } \mathrm{m(t)} < m_0 \\ K_{F_1} & \text{if } \mathrm{m(t)} \geq m_0 \end{cases} \quad (15)$$

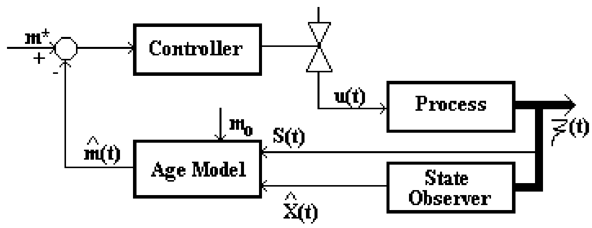


-Figure 4: The simulation of M_4 model-

The models presented before were validated based on real data about biomass and sublayer and using qualitative information about the microorganisms age (form of the microorganisms, color and odor of the culture, etc.).

3 The control system of the microorganisms mean age

This bioreactor controlling technique by the use of mean age will apply with good results in the case of the continuous bioreactors. It can be applied also for discontinuous bioreactors but the nature of the command is different [cara1]. While for the continuous bioreactors the mean age is controlled by the nutritional flow, in discontinuous bioreactors the age may be increased through a mechanism of starvation obtained by nutritive stress factor (for example: stirring interruption). In this manner, the sublayer not being uniformly spread in all the bioreactor volume, the microorganisms have no access to food, appearing thus the phenomenon of aging (increase of the mean age). The decrease of the mean age may be achieved by stirring again or, in case of fed-batch bioreactors, by periodically adding sublayer in the bioreactor.



-Figure 5:The control system of the microorganisms mean age-

Further on we shall consider the case of a continuous bioreactor when the mean age is controlled by means of the nutritional flow. Figure 5 presents the system of the mean age controlling.

It is well known that a series of variables are not directly measurable and in this case the state estimators can be used [bast7], [kato8]. In figure 5 $\xi(t)=[S(t)\ X(t)\ P(t)]$, S(t) being the assimilable sublayer, X(t) biomass and P(t) the biosynthesis product. Variable "S(t)", is a directly measurable variable, representing a concentration of a chemical substance. Using the sublayer, an observer can be built and this will estimate the variable "X(t)", viable biomass [cara6]. Once the biomass has been already estimated, we can determine the mean age by using a simple model of the age as was presented in section 2 (for example: the model given by equation 8). Thus the model of the feedback reaction of controlling the mean age system, in a continuous bioreactor, is as follows:

$$\frac{d\hat{S}}{dt} = -\mu(\hat{S})\hat{X} - D(S_0 - \hat{S}) + \omega_1(\hat{X},\hat{S})(S - \hat{S}) \qquad (16)$$

$$\frac{d\hat{X}}{dt} = \left[\mu(\hat{S}) - D\right]\hat{X} + \omega_2(\hat{X},\hat{S})(S - \hat{S}) \qquad (17)$$

The equations (16) and (17) present the observer's equations for the viable biomass. The model of the process is described by one of the forms presented in subchapter 2 of this paper. The efficiency of the estimator is treated in [cara2]. The control value "u(t)" is the dilution rate, defined as u(t)=F(t)/V(t), where "F(t)" is the inflow of the bioreactor and "V(t)" is the volume of the bioreactor. This method has been tested through simulation in the case of a bioreactor process with a population of Candida Lipolytica, cultivated on a sublayer, which contains ammonium sulfate.

4 Graphical results of the microorganisms mean age controlling

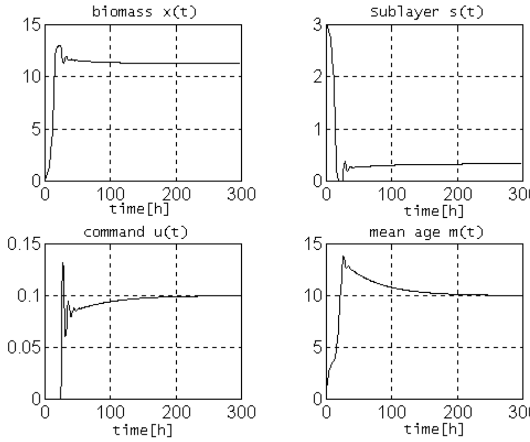
For simulation several models have been used, as presented in subchapter 2 of this paper. In every case the setpoint has been considered 10 hours and the mean age controller is of "PI" type [cara4]. The evolution of the main state variables is presented in figures 6, 7, 8 and 9.

A fuzzy logic controller to control the microorganisms mean age was also put to test. Let us consider the model M_4. The best results were obtained considering a PI fuzzy logic controller having two inputs (e(t) and de(t)/dt) and one output (Δu(t)). To every variable of the fuzzy logic controller were assigned seven linguistic values. The membership functions of the fuzzy logic controller are of triangular type and are presented in figure 10. The linguistic terms have the following significance:

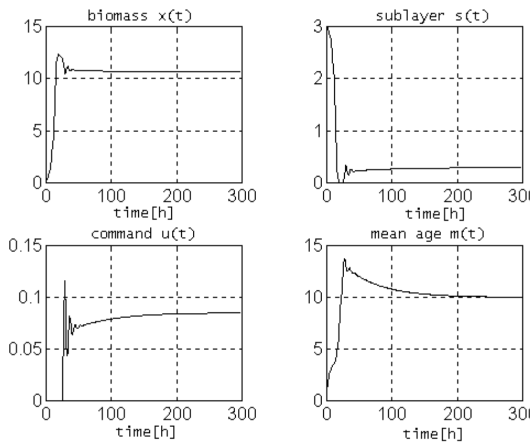
- NB - negative big;
- NM – negative medium;
- NS – negative small,;
- Z – zero;
- PS – positive small;
- PM – positive medium;
- PB – positive big.

In the same picture, the following notations were used:

- μ_e – error membership functions;
- μ_{de} – derivative error membership functions;
- μ_u – command membership functions;

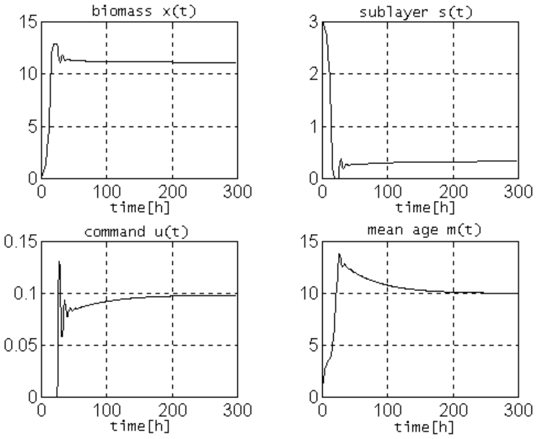


-Figure 6: The controlling of the mean age using the model M_1-



-Figure 7: The controlling of the mean age using the model M_2-

The simulation presented in figure 11 shows the response of the control system using the fuzzy logic controller, described above, in comparison with the response of a control system having a classic PI controller. The best response was obtained in the case of the fuzzy logic controller, the response obtained with the classic PI controller having a big overshoot (27%).



-Figure 8: The controlling of the mean age using the model M_3-

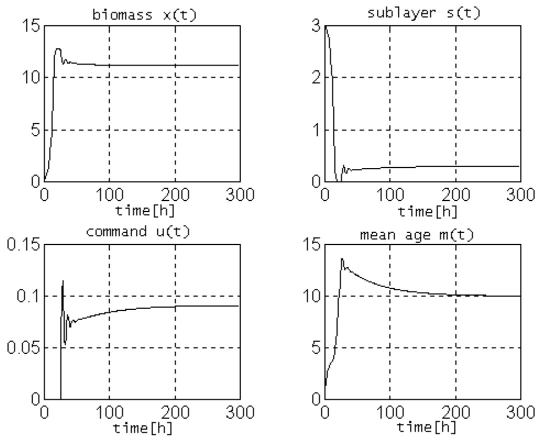
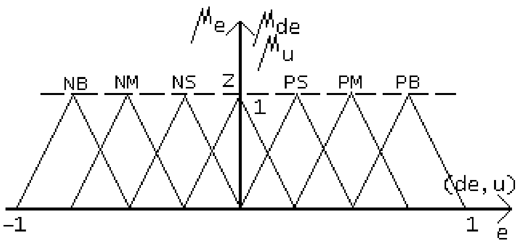
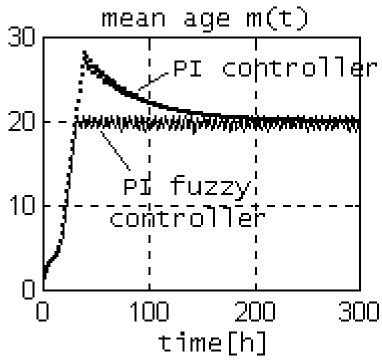


Figure 9: The controlling of the mean age using the model M_4-

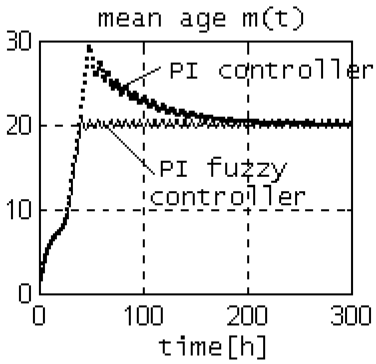


-Figure 10: The membership functions of the fuzzy logic controller

The simulation presented in figure 12 shows the robust behavior of the control system with fuzzy logic controller in comparison with the control system with a classic PI controller. The specific growth rate (μ_m) was modified at a half of the previous value. In the case of the control system with PI controller the overshoot increased at 30%, while the response of the control system with fuzzy logic controller is almost unmodified.



-Figure 11: The response of the control system with FLC in comparison with a control system with a PI classical one-



-Figure 12: The robust behavior of the control system with FLC (fuzzy logic controller)-

5 Considerations about the connection between the product quantity, mean age and sublayer

Let us consider the mathematical model of a biosynthesis process that takes place in a continuous bioreactor, given by equations (9)-(11), where the coefficients K_D and K_F are linear functions of mean age "m(t)" (relations (12) and (13)). Equation (18), which represents the evolution of the product, was added to the equations (9)-(11).

$$\frac{dP(t)}{dt} = \frac{v_m S(t)}{k_m + S(t)} X(t) - D(t)P(t) \tag{18}$$

In equation (18), the term "D(t)P(t)" represents the quantity of the product extracted from bioreactor in a unit of time.

Let us define the following parameter:

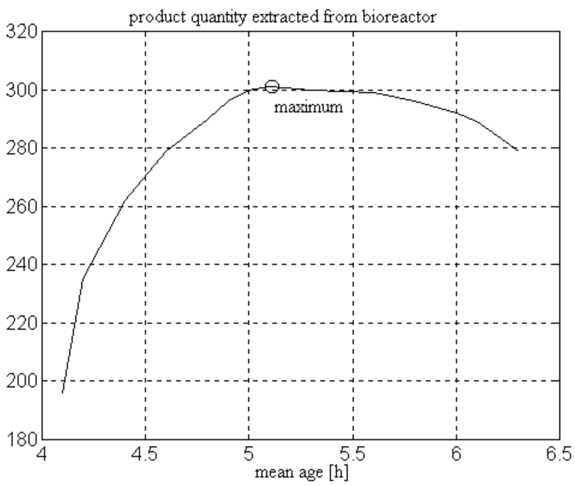
$$I = \int_0^{t_f} D(t)P(t)dt \tag{19}$$

"I" represents the quantity of product obtained in a stationary regime considering a time horizon [0, t_f] of 200 hours. By integrating the set of equations (9)-(11) and (18) on the time horizon mentioned above, for distinctive values of the mean age (which is easily done by the mean age controller), the results presented in table 1 were obtained:

Table 1

M(t)	4.1	4.2	4.4	4.6	4.8	4.9	5	5.1	5.3	5.6	5.8	6	6.1	6.2	6.3
I(t)	196	235	262	279	290	296	300	301	300	299	296	292	289	284	279

Figure 13 shows that the control of the mean age by means of dilution rate "D", produces a maximum of the product quantity for a given mean age.



-Figure 13: The dependence of the product quantity on the microorganisms mean age-

Observations

1. Unfortunately, the product quantity thus obtained depends on the age and on the limitative character of the sublayer as well. There is no guarantee for the fact that there is no other combination between mean age and sublayer, which could lead to a better productivity.
2. The maximum that can be obtained of the product is reached for the mean age of 5.1 hours. It has been noticed that between values of 4.8 h and 5.8 h the maximum is flat enough. This means that we have a relatively large range of age values for which "good" results may be obtained from the point of view of product quantity.

6 Conclusions

This paper takes into consideration an extremely important factor in controlling the biosynthesis bioreactors. This factor characterizes the physiological state of the microorganisms, namely the mean age. This is specific to the respective species and is tightly linked to the mechanism of producing, by the microorganisms, of the biosynthesis products. The simulations presented in this paper pertinently illustrate the viability of this method in continuous bioreactors, having as subject a population of Candida Lipolytica, cultivated on an ammonium sulphate sublayer [kato8].

References

[cara1] Caraman S., Ceanga E., Bumbaru S., "Some Results Regarding Modeling and Control of Alpha-Amilase and Protease Biosynthesis Process in Discontinuous Bioreactors", *Proceedings of Symposium SIMSIS'96, Galati*, pp. 6-11, (1996)

[cara2] Caraman S. "Contributions regarding the achievement of the expert systems used to control batch bioreactors", *Doctoral thesis, "Dunarea de Jos" University of Galati*, pp.220, (1997)

[rant3] Ranta I. et al., "Modelling and Simulation Of the Bacterial Age Distribution in an Activated Sludge Plant*", Proc. IFAC Workshop on System Analysis Application to Complex Programs, London, Pergamon Press*, pp. 93-101, (1978)

[cara4] Caraman, S., Ceanga, E. and Tulvan, L., "Controlling Techniques of Microorganisms Mean Age in Biotechnological Processes", *International Symposium on System Theory, Robotics, Computers & Process Informatics, SINTES 9, Craiova*, **Vol. 1**, pp. 133-139, (1998)

[cara5] Caraman, S., Ceanga, E., "Mathematical Modeling of the Biosynthesis Processes using age information", *Journal of "Gas-Oil" University of Ploiesti*, **Vol. XLVII-L**, no. 12, pp. 202-207, (1998)

[cara6] Caraman, S., Ceanga, E., "Estimation Techniques of the State Parameters in Biotechnological Processes", *Journal of "Gas-Oil" University of Ploiesti*, **Vol. XLVII-L**, no. 12, pp. 208-211, (1998)

[bast7] Bastin G., Dochain D., "Ön-line Estimation and Adaptive Control of Bioreactors", *Elsevier*, pp. 375, (1990)

[kato8] Katoh M., Masubuchi M., "Analisys and State Estimation of Enzyme Kinetic Model for Microbial Growth Process", *8-th Triennial World Congress, Kyoto, Japan*, 1981, pp. 2803-2808, (1981)