An Improvement of Functional State Local Models of Escherichia Coli MC4110 Fed-batch Cultivation

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Key Words: *Functional state; local models; fed-batch cultivation.*

Abstract. *This paper presents an improvement of functional state local models structures of Escherichia coli fed-batch cultivation. In the previous results it has been already shown how the cultivation process can be divided into functional states and how the model parameters can be obtained using genetic algorithms. The aim of this work is to find better local models structures of E. coli cultivation model based on Zhang investigations. The proposed modification of local models predicts very well the dynamics of the process variables -biomass, substrate, acetate, dissolved oxygen as well as carbon dioxide. Moreover, the modified local models in general are simpler than the previous ones.*

1. Introduction

Zhang et al. [22] introduce the functional state concept to describe and analyze the current biological state of bioprocesses, and apply the approach in expert system-based fault diagnosis and bioprocesses control. Taking into account a lot of applications of such approach [5,6,22] for fermentation processes and reported results [3,14,17], it is obvious that the implementation of the functional state concept has computational advantages and allows direct incorporation of high-level and qualitative plant knowledge into the model. These advantages have proven to be very appealing for industrial applications [4,15,16,19].

Based on many research reports about the changes in *E. coli* process behavior during different cultivation conditions (high or low glucose concentrations, oxygen limitation or oxygen starvation, etc.) [2,8,9,20], it is evident that there are a lot of analogies between the yeast and *Escherichia coli* metabolisms. Due to the similarities of main metabolic pathways of yeast and bacteria [1,7,8,9,10,12,13], the concept of state decomposition could be applied successfully for modelling *E. coli* cultivations. In the previous authors' work [11] the concept of functional state approach was applied and the effectiveness of the proposed identification scheme proved.

The aim of this paper is to present an improvement of the local models structures, compared to [11]. The purpose of the next investigation is to find simpler model structures that still represent accurately real experimental data.

2. Materials and Methods

The mathematical model for the considered *Escherichia coli MC4110* fed-batch cultivation, based on the mass balance of the components (biomass, glucose, acetate, dissolved oxygen and carbon dioxide), is presented by the following differential equations [18]:

$$(1)\quad \frac{dX}{dt} = \mu X - \frac{F}{V}X;$$

$$(2)\quad \frac{dS}{dt} = -q_S X + \frac{F}{V}(S_{in} - S);$$

$$(3)\quad \frac{dA}{dt} = q_A X - \frac{F}{V}A;$$

$$(4)\quad \frac{dO_2}{dt} = -q_{O_2} X + k_L^{O_2} a(O_2^* - O_2) - \frac{F}{V}O_2;$$

$$(5)\quad \frac{dCO_2}{dt} = q_{CO_2} X + k_L^{CO_2} a(CO_2^* - CO_2) - \frac{F}{V}CO_2;$$

$$(6)\quad \frac{dV}{dt} = F$$

where: X, S, S_{in}, A, O_2, CO_2 are the concentrations of biomass [g•l^{-1}], substrate (glucose) [g•l^{-1}], influent glucose [g•l^{-1}], acetate [g•l^{-1}], dissolved oxygen [%] and carbon dioxide [g•l^{-1}] respectively ; F is the influent flow rate, [l•h^{-1}]; V is the bioreactor volume, [l]; $k_L^{O_2} a$ is the volumetric oxygen transfer coefficient, [h^{-1}], $k_L^{CO_2} a$ is the volumetric carbon dioxide transfer coefficient, [h^{-1}]; and μ, q_S, q_A, q_{O_2} and q_{CO_2} are the specific rates of growth, substrate utilization, product (acetate) formation, oxygen consumption and carbon dioxide production, [h^{-1}] respectively. The structures of the specific rates mentioned above vary in connection with the recognized functional states.

According to Zhang investigation [22], cultivation process could be divided into five functional states depending on specific critical values of substrate and dissolved oxygen concentrations (*table 1*). In the considered case of *E. coli* cultivation process the following values for substrate and dissolved oxygen critical levels are assumed [11]:

S_{crit} = 0.1 g•l^{-1} and O_{2crit} = 20.4%.

Taking into account the rules for recognition of the functional states (*table 1*), three functional states are recognized:

- *FS I : first acetate production state;*
- *FS II : mixed oxidative state;*

• *FS V*: *second acetate production state.*

The disposition of the recognized functional states during the process is presented in *figure 1*. In the beginning of the cultivation, the dissolved oxygen and the glucose concentrations are above the corresponding critical levels. The process is in *first acetate production state* (*FS I*) from 6.7 h (the start of the fed-batch cultivation) to 7.2 h. In the next hours - from 7.2 h to 10.5 h, *mixed oxidative state* (*FS II*) is identified. The process enters this state when the sugar concentration decreases to be equal to or below the critical level and when there is sufficient dissolved oxygen in the broth. At the end of the cultivation the process is in *second acetate production state* (*FS V*). From 10.5h to 11.6 h both glucose and dissolved oxygen concentrations are below the corresponding critical levels.

Table 1

Functional state	Rule
FS I	$S > Scrit$ and $O_2 > O_2crit$
FS II	$S \leq Scrit$ and $O_2 \geq O_2crit$ and $A > 0$
FS III	$S \leq Scrit$ and $O_2 \geq O_2crit$ and $A = 0$
FS IV	$S = 0$ and $O_2 \geq O_2crit$
FS V	$S \leq Scrit$ and $O_2 < O_2crit$ and $A > 0$

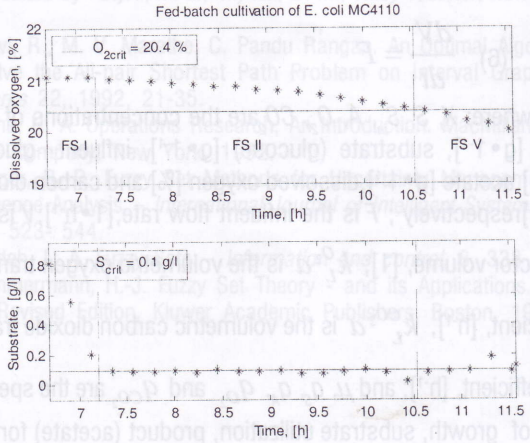

Figure 1

According to the recognized specific functional states, different structures of local models are considered. Parameter identification based on available experimental data is performed. As an optimization procedure, genetic algorithms are used. The genetic algorithm parameters and functions are presented in details in [11]. The optimization criterion is formed as a minimization of a distance measure *J* between the experimental and predicted model values of state vector *y*:

$$(7)\ J = \sum_{i=1}^{n} \sum_{j=1}^{m} \left\{ \left[y_{\exp}(i) - y_{\mathrm{mod}}(i) \right]_j \right\}^2 \rightarrow min$$

where y_{exp} is the measured vector; y_{mod} - modeled vector and $y = [S\ X\ A\ O_2\ CO_2]$ for all recognized functional states.

3. Functional State Modelling Using Modified Local Models

From practical view, modelling studies are performed to identify simple and easy-to-use models that are suitable to support the engineering tasks of process optimization and especially of control. The most appropriate model must satisfy a compromise between the following conditions:

• the model structure should be able to represent the measured data in a proper manner;

• the model structure should be as simple as possible.

Local models presented by Zhang [22] describe very well experimental data, but some of them have complex structures. To obtain the desired results, the available local models [11,22] are changed. Proposed local models are developed based on the specific peculiarities of the considered *E. coli* cultivation process. Furthermore, local models for describing carbon dioxide concentration are proposed. Such local models are not presented in previous investigations [11,22]. Experimental data show that the time curves of dissolved oxygen and carbon dioxide are inversely proportional. For that proportionality, identical local models for the dynamics of O_2 and CO_2 are proposed. This assumption is considered for all identified functional states.

3.1. Modelling *First Acetate Production State (FS I)*

The model structures used for describing *FS I* are presented in *table 2*. The specific growth rate μ is a constant. The structure of the specific rate of sugar consumption q_S is the same for all discussed functional states. A specific acetate production rate q_A proportional to Monod kinetics is preferred here. The specific dissolved oxygen consumption rate q_{O_2} and specific carbon dioxide accumulation rate q_{CO_2} are directly proportional to the specific growth rate. Based on the considered models structures, parameter identification is fulfilled. The results are listed in *table. 3*.

Table 2

	FS I
Parameter function	Local model
μ	μ_{max}
q_S	$\frac{1}{Y_{S/X}} \mu_{max} \frac{S}{k_S + S}$
q_A	$\frac{1}{Y_{A/X}} \mu_{max} \frac{S}{k_S + S}$
q_{O_2}	$\frac{1}{Y_{CO_2/X}} \mu_{max}$
q_{CO_2}	$\frac{1}{Y_{O_2/X}} \mu_{max}$

Table 3

FS I	
Parameter	Value
μ_{max} , [h^{-1}]	0.45
k_S , [g·l^{-1}]	0.03
$Y_{S/X}$, [g·g^{-1}]	0.46
$Y_{A/X}$, [g·g^{-1}]	0.019
$Y_{O_2/X}$, [g·g^{-1}]	0.096
$Y_{CO_2/X}$, [g·g^{-1}]	0.16
$k_L^{O_2}a$, [h^{-1}]	52.49
$k_L^{CO_2}a$, [h^{-1}]	42.06
J_{FSI}	1.13

The simulation results are depicted in *figure 2* for modeled (designated by -) and measured (designated by *) biomass and acetate concentrations and in *Figure 3* for predicted and measured substrate, dissolved oxygen and carbon dioxide concentrations.

The investigations show that the modified simpler local models for *FS I* reflect the experimental data better than the previous models [11].

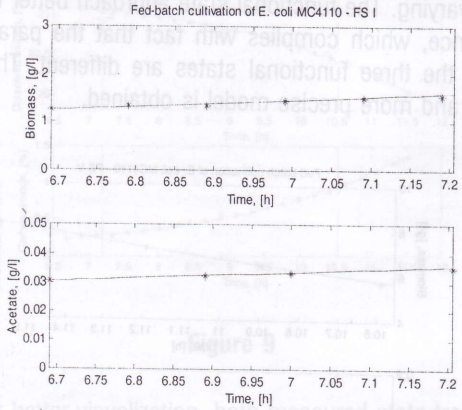

Figure 2

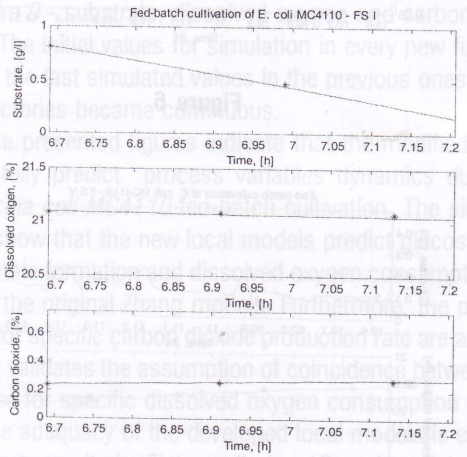

Figure 3

3.2. Modelling *Mixed Oxidative State (FS II)*

The following changes are made to improve the model quality: the specific acetate production rate, specific dissolved oxygen consumption rate and specific carbon dioxide production rate are expressed by Monod kinetics with regard to the acetate concentration, dissolved oxygen concentration and carbon dioxide concentration respectively. The modified local models structures for *FS II* are listed in *table 4*. *Table 5* represents the results from the model parameter identification.

Simulation results for model behavior in *FS II* are presented in *fgure 4* (for biomass and acetate concentrations) and *figure 5* (for substrate, dissolved oxygen and carbon dioxide concentrations).

Table 4

FS II	
Parameter function	Local model
μ	$\mu_{max}\frac{S}{k_S + S}$
q_S	$\frac{1}{Y_{S/X}}\mu_{max}\frac{S}{k_S + S}$
q_A	$\frac{1}{Y_{A/X}}\mu_{maxA}\frac{A}{k_A + A}$
q_{O_2}	$\frac{1}{Y_{O_2/X}}\mu_{max}\frac{O_2}{k_{O_2} + O_2}$
q_{CO_2}	$\frac{1}{Y_{CO_2/X}}\mu_{max}\frac{CO_2}{k_{CO_2} + CO_2}$

Table 5

FS II	
Parameter	Value
μ_{max} , [h^{-1}]	0.52
μ_{maxA} , [h^{-1}]	0.20
k_S , [g·l^{-1}]	0.023
k_A , [g·l^{-1}]	0.59
k_{O_2} , [%]	0.023
k_{CO_2} , [%]	0.02
$Y_{S/X}$, [g·g^{-1}]	0.50
$Y_{A/X}$, [g·g^{-1}]	0.013
$Y_{O_2/X}$, [g·g^{-1}]	0.20
$Y_{CO_2/X}$, [g·g^{-1}]	0.10
$k_L^{O_2}a$, [h^{-1}]	155.87
$k_L^{CO_2}a$, [h^{-1}]	53.41
J_{FSII}	2.79

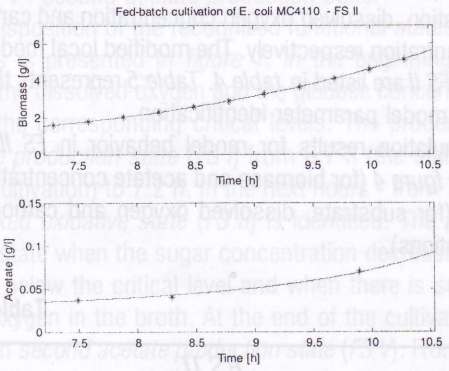

Figure 4

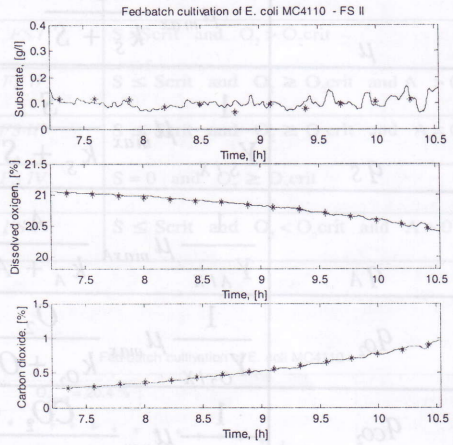

Figure 5

3.3. Modelling *Second Acetate Production State (FS V)*

The specific rates of glucose consumption and acetate production are proportional to the specific bacteria growth rate, i.e. to a Monod model. The dissolved oxygen consumption rate depends on the dissolved oxygen concentration. The model structures for *FS V* and the parameter estimations are respectively presented in *table 6* and *table 7*.

Table 6

FS V	
Parameter function	Local model
μ	$\mu_{max}\frac{S}{k_S+S}$
q_S	$\frac{1}{Y_{S/X}}\mu_{max}\frac{S}{k_S+S}$
q_A	$\frac{1}{Y_{A/X}}\mu_{max}\frac{S}{k_S+S}$
q_{O_2}	$\frac{1}{Y_{O_2/X}}\mu_{max}\frac{O_2}{k_{O_2}+O_2}$
q_{CO_2}	$\frac{1}{Y_{CO_2/X}}\mu_{max}\frac{CO_2}{k_{CO_2}+CO_2}$

The model prediction for biomass and acetate concentrations could be seen in *figure 6*, and for substrate, dissolved oxygen and carbon dioxide concentrations in *figure 7*.

Table 7

FS V	
Parameter	Value
μ_{max}, [h^{-1}]	0.59
k_S, [g·l^{-1}]	0.039
k_{O_2}, [%]	0.04
k_{CO_2}, [%]	0.037
$Y_{S/X}$, [g·g^{-1}]	0.49
$Y_{A/X}$, [g·g^{-1}]	0.013
$Y_{O_2/X}$, [g·g^{-1}]	0.21
$Y_{CO_2/X}$ [g·g^{-1}]	0.21
$k_L^{O_2}a$, [h^{-1}]	69.93
$k_L^{CO_2}a$, [h^{-1}]	32.27
J_{FSV}	1.79

The estimation for *FS I*, *FS II* and *FS V* are in the admissible domain. The new local models fit better the experimental data according to the obtained criterion values for *FS I* and *FS II*: J_{FSI} =7.5213, J_{FSII}=11.1843 and J_{FSV} =9.3916 in [11] compared to the present results-J_{FSI}=1.13 (*table 3*), J_{FSII}=2.79 (*table 5*) and J_{FSV}=1.79 (*table 7*). The model accuracy of modified structures increases many times.

As it is well known, the parameters of the fermentation processes models, and particularly in the cultivation of *E. coli*, are time-varying. The functional state approach better reflects this variance, which complies with fact that the parameters values in the three functional states are different. Thus, an adequate and more precise model is obtained.

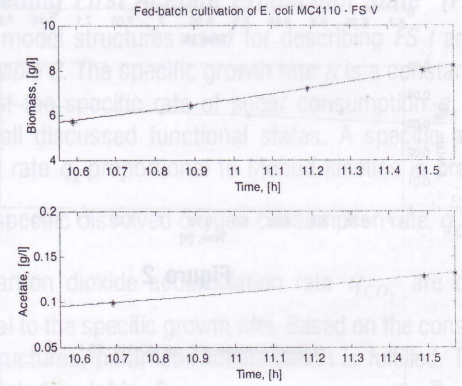

Figure 6

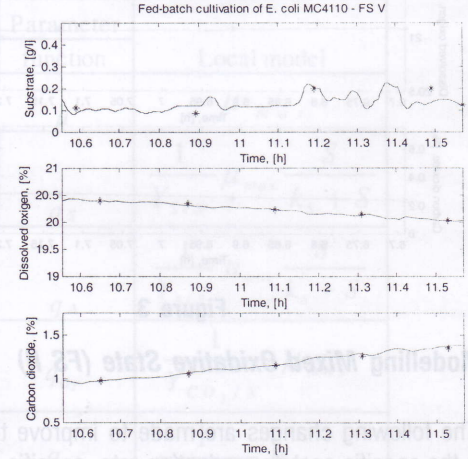

Figure 7

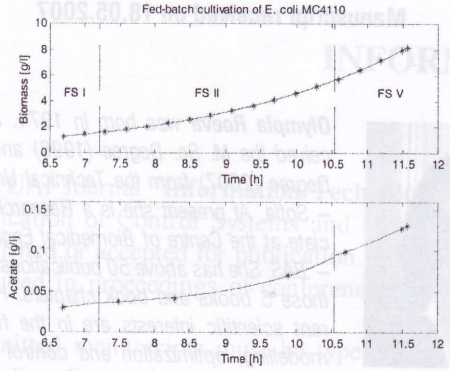

Figure 8

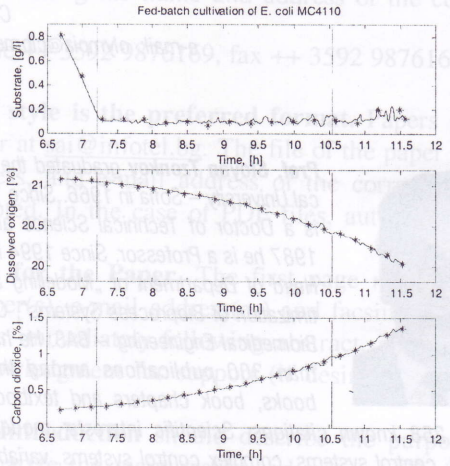

Figure 9

For better visualization, both measured state trajectories and modeled ones are presented together for all states in the next two figures. *figure 8* shows biomass and acetate curves and *figure 9* - substrate, dissolved oxygen and carbon dioxide curves. The initial values for simulation in every new functional sate are the last simulated values in the previous ones, so that the trajectories became continuous.

The presented figures indicate that the modified models successfully predict process variables dynamics during the *Escherichia coli MC4110* fed-batch cultivation. The simulation results show that the new local models predict glucose utilization, acetate formation and dissolved oxygen consumption, better than the original Zhang models. Furthermore, the proposed models for specific carbon dioxide production rate are adequate. This fact validates the assumption of coincidence between them and those for specific dissolved oxygen consumption rate.

The adequacy of the developed local models is evaluated based on two criteria: Fisher criterion (*F*) and model selection criterion (*MSC*). The MSC is defined as [21]:

$$(8)\qquad MSC = \ln\left(\frac{\sum_{i=1}^{N} w(i)\left[y_{\exp}(i) - \overline{y}_{\exp}(i)\right]^2}{\sum_{i=1}^{N} w(i)\left[y_{\exp}(i) - y_{\mathrm{mod}}(i)\right]^2}\right) - \frac{2\dim(p)}{N},$$

where N is the number of points, $w(i)$ is the weight applied to each points, $\overline{y}_{\exp}$ is the weighted mean of the observed data, $y_{\exp}(i)$ is the weighted value of observed data, $y_{\mathrm{mod}}(i)$ represents the weighted value of calculated data and p is the level of significance of the simulation (parameter vector). The *MSC* attempts to represent the "information content" of a given set of parameter estimates by relating the coefficient of determination to the number of parameters (or equivalently, the number of degrees of freedom) that were required to obtain the fit.

In the *table 8* the values of the *F* and *MSC* criteria are given. The results show that the modified local models are adequate.

Table 8

Criterion	Value
F	4.2939
MSC	6.821

4. Conclusion

Based on the application of the functional state approach, modified local models of *E. coli MC4110* fed-batch cultivation are proposed. Three functional states are recognized, namely *first acetate production state*, *mixed oxidative state* and *second acetate production state*. For further high quality control of the cultivation specific local models structures are identified. This implementation of functional state modelling approach describes a process with local models which are simpler but nevertheless adequate and precise.

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References

1.Enfors, S.-O. Teknisk Mikrobiologi. 2003, available at http://www.biotech.kth.se/courses/gru/courselist/3A1313/Downloads%20copy/Overflowmetabolism.pdf.

2.Enfors-Häeggströem. Bioprocess Technology – Fundamentals and Applications, KTH, Stockholm, 2000, available at http://www.biotech.kth.se/courses/gru/courselist/3A1313/ Downloads%20copy/Chapt%204_Metabolic%20basis.pdf.

3.Feng, M. and J. Glassey. Physiological State-Specific Models in Estimation of Recombinant *Escherichia coli* Fermentation Performance. – *Biotechnology and Bioengineering*, 69 (5), 2000, 495-503.

4.Fukudome, K., M. Sato, Y. Takata, H. Kuroda, J. Watari, M. Takashio. Evaluation of Yeast Physiological State by Alcian Blue Retention. – *Journal American Society of Brewing Chemists*, 60 (4), 2002, 149-152.

5.Knop, D. R., K. Hu R. M. Worden. Physiological-state Control of a Fed-batch Quinic Acid Fermentation. Annual Meeting of American Institute of Chemical Engineers, 8 November 2002, Indiana, USA.

6.Murray-Smith, R. and T. A. Johansen. Multiple Model Approaches to Modelling and Control. Taylor and Francis, 1997.

7. Nielsen, J. and J. Villadsen. Bioreaction Engineering Principles. Plenum Press, New York and London, 1994.

8.O'Beirne, D. and G. Hamer. The Utilization of Glucose/Acetate Mixtures by *Escherichia coli W3110* Under Aerobic Growth Conditions. – *Bioprocess Engineering*, 23, 2000, 375-380.

9.O'Beirne, D. and G. Hamer. Oxygen Availability and Growth of *Escherichia coli W3110*: Dynamic Responses to Limitation and Starvation. –

Bioprocess Engineering, 23, 2000, 381-387.
10.Otterstedt, K., C. Larsson, R. M. Bill, A. Stehlberg, E. Boles, S. Hohmann, L. Gustafsson. Switching the Mode of Metabolism in the Yeast *Saccharomyces Cerevisiae*. European Molecular Biology Organization, Scientific EMBO Report, 5 (5), 2004, 532-537.
11.Roeva, O., T. Pencheva, Y. Georgieva, B. Hitzmann, St. Tzonkov. Implementation of Functional State Approach for Modelling of *Escherichia coli* Fed-batch Cultivation. – *Biotechnology and Biotechnological Equipment*, 18 (3), 2004, 207-214.
12.Roeva, O., T. Pencheva, St. Tzonkov. Functional State Approach to Modelling of *Escherichia coli* Fed-batch Cultivation: An Analysis. International Symposium Bioprocess Systems 2005 – BioPS'05, Sofia, Bulgaria, October 25-26, 2005, I.29-I.36.
13.Roeva, O., T. Pencheva, U. Viesturs, St. Tzonkov. Modelling of Fermentation Processes based on State Decomposition. – *Bioautomation*, 5, 2006, 1-13.
14. Ruenglertpanyakul, W. and K.-H. Bellgardt. Physiological Phase Models for Bioprocesses. - Comp. Appl. in Biot. 7th, Osaka, Japan, May 31 - June 4, 1998, 119-122.
15.Shimizu, H., K. Miura, S. Shioya, K. Suga. On-line State Recognition in a Yeast Fed-batch Culture Using Error Vectors. – *Biotechnology and Bioengineering*, 47, 1995, 165-173.
16.Takiguchi, N., H. Shimizu, S. Shioya. An On-line Physiological State Recognition System for the Lysine Fermentation Process Based on a Metabolic Reaction Model. – *Biotechnology and Bioengineering*, 55 (1), 1997, 170-181.
17.Tartakovsk,y B., M. Sheintuch, J.-M. Hilmer, T. Scheper. Modelling of *E. coli* Fermentations: Comparison of Multicompartment and Variable Structure Models. – Bioprocess Engineering, 16, 1997, 323-329.
18.Tzonkov, St. (Ed.) Bioprocess Systems: Modelling, Control and Optimization. Sofia, East-West, 2004 (in Bulgarian).
19.Venkat, A. N., P. Vijaysai, R. D. Gudi. Identification of Complex Nonlinear Processes Based on Fuzzy Decomposition of the Steady State Space. – *J. Proc Cont.*, 13, 2003, 473-488.
20.Xu, B., M. Jahic, G. Blomsten, S.-O. Enfors. Glucose Overflow Metabolism and Mixed-acid Fermentation in Aerobic Large-scale Fed-batch Processes with *Escherichia Coli*. – *Appl. Microbiol. Biotechnol.*, 51, 1999, 564-571.
21.Zelic, B., D. Vasic-Racki, C. Wandrey, R. Takors. Modeling of the Pyruvate Production with *Escherichia Coli* in a Fed-batch – *Bioreactor, Bioprocess Biosyst. Eng.*, 26, 2004, 249-258.
22.Zhang, X.-Ch., A. Visala, A. Halme, P. Linko. Functional State Modelling Approach for Bioprocesses: Local Models for Aerobic Yeast Growth Processes. – *J. Proc. Cont.*, 4 (3), 1994, 127-134.

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