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Functional State Modelling of *Saccharomyces cerevisiae* Cultivations

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Abstract: *The implementation of functional state approach for modelling of yeast cultivation is considered in this paper. This concept helps in monitoring and control of complex processes such as bioprocesses. Using of functional state modelling approach for fermentation processes aims to overcome the main disadvantage of using global process model, namely complex model structure and big number of model parameters. The main advantage of functional state modelling is that the parameters of each local model can be separately estimated from other local models parameters. The results achieved from batch, as well as from fed-batch, cultivations are presented.*
Keywords: *Modelling, Functional state modelling approach, Yeast cultivation.*

Introduction

Yeast is an important microorganism, which has been used for industrial applications. Its importance bases on the use in the baking and brewing industries, in single-cell protein production, and as a host in genetic engineering applications. Compared to penicillin fermentation or animal cell cultures, aerobic yeast cultivation is relatively simple. This is caused by the fact that the metabolic mechanism of the process is well known. Therefore, yeast processes are often used as a test process for new methods or ideas and they are also applied in this paper.

The modelling of yeast cultivation has been widely studied and reported. The common modelling approach is to synthesise one global process model such as ones presented from Sonnleitner and Kappeli [8]. The main disadvantage of such approach is the complex model structure and the big number of model parameters, which complicate the model simulation and parameter estimation. The functional state of a process is an alternative concept, which helps in monitoring and control of complex processes such as bioprocesses [10]. The main idea is to use a two-level hierarchy where at the first level the process is divided into macrostates, called *functional states*, according to behavioural equivalence. In a functional state the process is described by a conventional type of model, called *local model*, which is valid in the functional state only. In each functional state, certain metabolic pathways are active enough to dominate the overall behaviour of the process. The biological behaviour in different functional states is quite similar. In many batch-type processes, the functional states would naturally be identified with the different phases of the process. In a fed-batch or continuous process, the situation is more complex, but some functional states can be recognised and some functional state model can be used. The process dynamics in each functional state is described by a simple local model. In principle, the structure of local models in different functional states can be different. At the second hierarchical level some numeric detection algorithms and/or rules based on expert knowledge can be used for the recognition of the functional states and state transitions. A set of local models together with functional state "dynamics" can be used to describe, monitor and control the overall yeast growth process.

The implementation of functional state approach for modelling of aerobic baker's yeast cultivation is developed in this paper. The authors are among the pioneers in using of this approach and they have hard worked to prove the approach advantages [4, 5, 6]. Both batch as well as fed-batch cultivations are considered here to present the applicability of this approach for modelling of aerobic baker's yeast cultivation.

Local models based on the functional state concept

The following assumptions are made in developing the local models of the aerobic baker's yeast growth process in batch and fed-batch cultures [10]:

- The main by-products in an aerobic yeast growth process are water, carbon dioxide and ethanol.

- The bioreactor is completely mixed.
- Ethanol consumption is inhibited when sugar concentration in the broth is higher than zero.
- The elemental composition of yeast in the process does not significantly change.
- Parameters except for the substrate and product concentrations, e.g. pH and temperature, are controlled to certain acceptable constant values during the process.

The rates of cell growth, sugar consumption, ethanol production and oxygen concentration in a yeast fed-batch growth process are commonly described for all functional states according to the mass balance as follows [9, 10]:

$$\frac{dX}{dt} = \mu X - \frac{F}{V} X \tag{1}$$

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

$$\frac{dE}{dt} = q_E X - \frac{F}{V} E \tag{3}$$

$$\frac{dO}{dt} = -q_O X + k_L a(O^* - O) \tag{4}$$

$$\frac{dV}{dt} = F \quad , \tag{5}$$

where:

X is the concentration of biomass, [g/l];

S - concentration of substrate (glucose), [g/l];

E - concentration of ethanol, [g/l];

O - concentration of oxygen, [%];

F - feeding rate, [l/h];

V - bioreactor volume, [l];

k_La - volumetric oxygen transfer coefficient, [h^{-1}];

S_{in} - initial concentration of the feeding solution, [g/l];

μ, q_S, q_E, q_O - parameter functions, varying with the functional state transitions.

A substrate such as sugar is degrading by yeast to produce a number of carbon intermediates as well as to provide energy. Yeast metabolise the carbon intermediates to synthesise new cell material. If the sugar concentration during an aerobic yeast growth process exceeds a critical level (S_{crit}), a part of the sugar is metabolised to ethanol. The whole yeast growth process can be divided into at least five functional states in batch and fed-batch cultures [10]. In each functional state the yeast metabolism is dominated by certain metabolic pathways.

- *The first functional state (I)* is the *first ethanol production state*. The process is defined to be in this state when the sugar concentration is above the critical level (S_{crit}) and there is sufficient dissolved oxygen. In this state ethanol is produced as described above.
- *The second functional state (II)* is the *mixed oxidative state*. The process enters this state when the sugar concentration decreases to be equal to or below the critical level and there is sufficient dissolved oxygen in the broth. The process remains in this state as long as these conditions are met. Both sugar and produced ethanol are cometabolised through the oxidative pathways in the state.
- *The third functional state (III)* is the *complete sugar oxidative state*. The process is defined to be in this state when there is no ethanol available, the sugar concentration is not higher than the critical level and the dissolved oxygen is above its critical level (O_{crit}). In this state, sugar is completely oxidised to water and carbon dioxide.
- *The fourth functional state (IV)* is the *ethanol consumption state*. The process is defined to be in this state when ethanol is available but no sugar is in the broth, and the dissolved oxygen concentration is above the critical level. Ethanol is the only carbon source for yeast growth.
- *The fifth functional state (V)* is the *second ethanol production state*. The conditions for this state are that both concentrations, for sugar and for dissolved oxygen, are below the corresponding critical levels. When the dissolved oxygen becomes the limiting factor for yeast growth, ethanol is produced.

A yeast growth process switches from one functional state to another like a state machine or automation familiar in computer science. To detect when the process is in a certain functional state might be a non-trivial task. The functional state diagram of the process is illustrated in Fig. 1 [10].

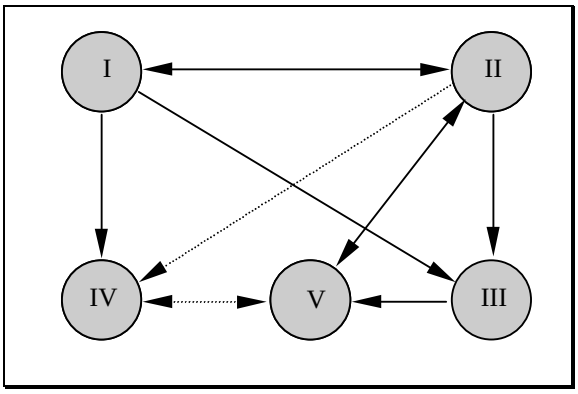

Fig. 1 Functional state diagram of the yeast growth process

In principal the functional state (I) can appear in all batch, fed-batch and continuous yeast growth processes. The functional state (IV) normally appears only in a batch culture. The functional states (II), (III), and (V) are normally found in fed-batch and continuous cultures [10]. The solid lines with arrows in Fig. 1 indicate the necessary or normal transition between various functional states of the process, and the dotted lines with arrows indicate that the transitions take place when the mode of culture changes between batch and fed-batch cultures. It should be noted that a bioprocess could be only in one functional state at any time. However, a certain functional state can appear in the process more than once during one run. Fig. 2 illustrates the metabolic characteristics and interrelationships of the different functional states during fed-batch yeast cultivation [9].

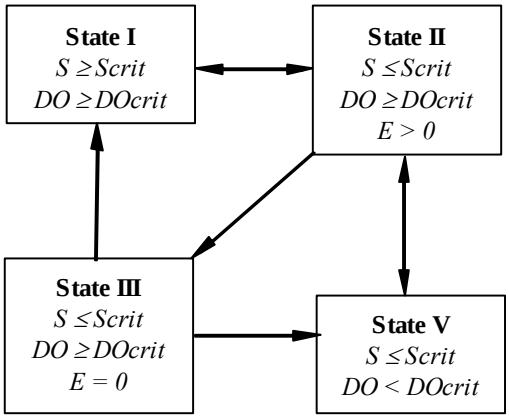

Fig. 2 Functional states and their relations in fed-batch yeast process

Modelling of batch cultivation of *Saccharomyces cerevisiae*

At the beginning the modelling of three batch cultivations of baker's yeast, carried out in *Institut für Technische Chemie, Universität Hannover,* has been considered. The experimental data contain off-line measurements of biomass (yeast), substrate (glucose) and ethanol. The first set of experimental data is used for local models' parameter estimation and two other sets are used to validate the model. It should be noted, that the application of functional state modelling is made for batch process for first time.

In the case of batch cultivation the process is described based on balance Eqs. (1-5) when the feeding rate is assumed to be zero. In the case of batch cultivation two phases are identified - first state (I), called the *first ethanol production state*, and second state (IV), called the *ethanol consumption state*. When the functional state is determined to change, the local models are also changed correspondingly. The initial values for simulation in the new functional state (IV) are the last simulated values in the previous functional state (I) so that the trajectories became continuous.

The Runge-Kutta (RK45) integration algorithm [2] is used for numeric simulation of the model. The estimation of the local models' parameters is made with using of *MATLAB Genetic Algorithms Toolbox* and *Optimisation Toolbox procedures*. As the optimisation criterion the function of difference between experimental data and data from simulated model is used. Therefore the optimisation criterion is presented as follows:

$$\begin{aligned} J = {} & c_1(X - X^*)^T(X - X^*) + c_2(S - S^*)^T(S - S^*) + \\ & + c_3(E - E^*)^T(E - E^*) + c_4(O - O^*)^T(O - O^*) \end{aligned}, \qquad (6)$$

where X^*, S^*, E^* and O^* are the column vectors of experimental data, X, S, E and O are the column vectors of simulated data and c_i are the weight coefficients.

The parameter functions of the local models in the states I and IV are presented in Table 1. In the difference of models presented by Zhang [10] some changes are made in the local models. Especially, the specific growth rate is described by Monod' model instead of constant in state I, and correspondingly, the specific oxygen uptake rate is also described with Monod' model.

As well the ethanol production rate in state I is also expressed by Monod kinetics and different yield coefficients in ethanol equations are used for states I and IV.

Table 1

Parameter	State I	State IV
μ	$\mu_1 \frac{S}{S+k_S}$	$\mu_2 \frac{E}{E+k_E}\eta$
q_S	$\mu_1 \frac{S}{S+k_S} Y_{SX}$	0
q_E	$\mu_1 \frac{S}{S+k_S} Y_{ES}$	$-\mu_2 \frac{E}{E+k_E} Y_{EX}\eta$
q_O	$\mu_1 \frac{S}{S+k_S} Y_{OS}$	$\mu_2 \frac{E}{E+k_E} Y_{OE}\eta$

In the Table 1 the following symbols are used:

- μ_i - maximum specific growth rate, [h^{-1}];
- k_S, k_E - saturation constants, [g/l];
- Y_{SX}, Y_{ES}, Y_{EX}, Y_{OS}, Y_{OE} - yield coefficients, [$g.g^{-1}$];
- η - lag term, which is assumed to be as follows:

$$\eta = 1 - \exp\left(-\frac{t - t_m}{t_1}\right) \quad , \tag{7}$$

where t_m shows the time point of involving in lag phase, t_1 is the length of lag phase and t is the current time.

The estimated values of parameters are presented in Table 2 [4].

Table 2

Parameter	Estimated value	Parameter	Estimated value
μ_1	0.3570 h^{-1}	μ_2	0.13832 h^{-1}
k_S	0.0714 gl^{-1}	k_E	0.18128 gl^{-1}
Y_{SX}	6.0162 gg^{-1}	t_1	5.8933 h
Y_{ES}	0.3288 gg^{-1}	t_m	8.4 h
Y_{OS}	1500 gg^{-1}	Y_{EX}	2.0877 gg^{-1}
k_La	83.347 h^{-1}	Y_{OE}	8340 gg^{-1}

Both the cultivation trajectories for the substrate, biomass and ethanol concentrations, and the simulated ones for one of the cultivations are presented in Fig. 3. The variation of the dissolved oxygen concentration is presented in Fig. 4.

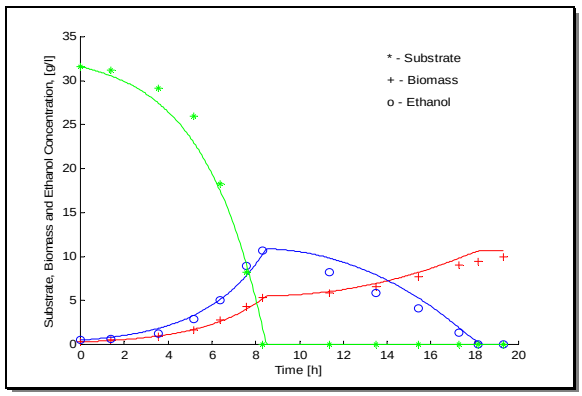

Fig. 3 Measured and simulated aerobic batch yeast cultivation using local models: substrate, biomass and ethanol concentrations

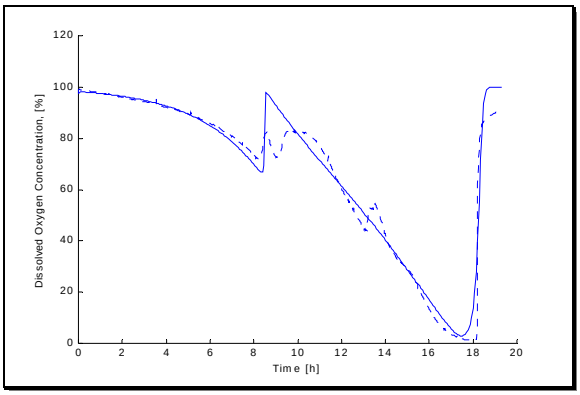

Fig. 4 Measured and simulated aerobic batch yeast cultivation using local models: dissolved oxygen concentration

To verify the identified model the simulation with rest two data sets from the real yeasts' cultivations is made. Due to the identical results, here the results only from one of the data sets will be presented. Both the real cultivation trajectories and the simulated ones are presented respectively in Fig. 5 and Fig. 6.

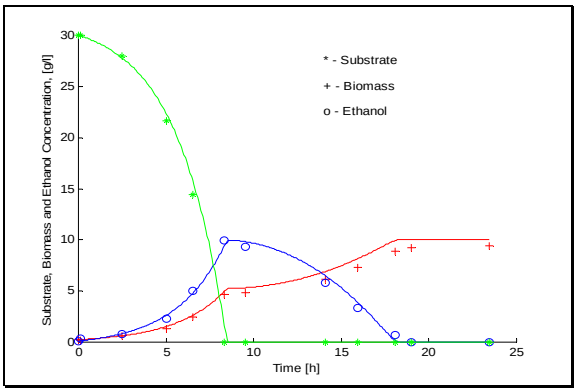

Fig. 5 Measured and simulated aerobic batch yeast cultivation using local models: substrate, biomass and ethanol concentrations

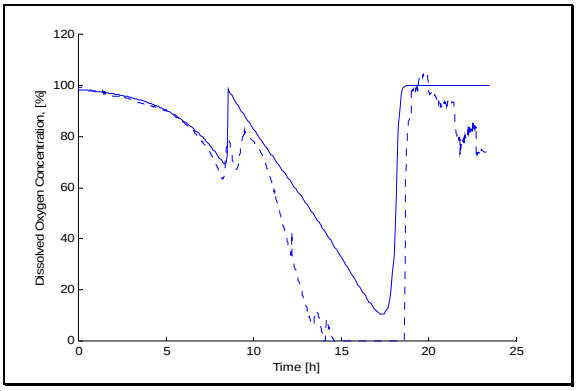

Fig. 6 Measured and simulated aerobic batch yeast cultivation using local models: dissolved oxygen concentration

Fig. 5 shows that the results obtained for model parameters are verified for biomass, substrate and ethanol concentration. Better result for dissolved oxygen is obtained if the same model is used and just the yield coefficient Y_{OE} is changed to 15340gg^{-1} (Fig. 7).

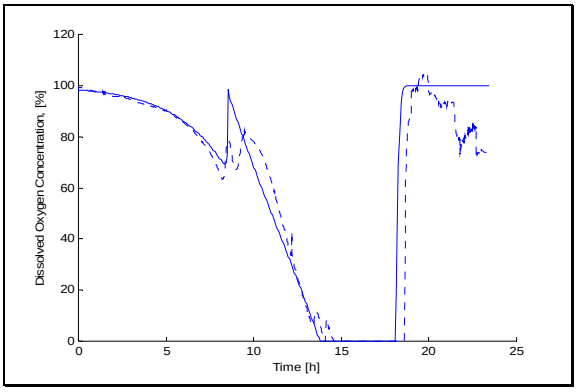

Fig. 7 Measured and simulated aerobic batch yeast cultivation using local models: dissolved oxygen concentration

As a conclusion of this application, the local models developed by Zhang et al. are first time successfully applied for description of real aerobic batch yeast growth process. In order to achieve better results, some changes, toward the local models presented by Zhang [10], are made.

Modelling of fed-batch cultivation of *Saccharomyces cerevisiae*

The modelling of fed-batch cultivation of *S. cerevisiae* has been also developed. Experimental data from two fed-batch cultivations of baker's yeast, obtained in *Institut für Technische Chemie, Universität Hannover,* are used. The experimental data consists of off-line measurements of biomass (yeast), substrate (glucose) and ethanol and on-line measurements of substrate (glucose) and oxygen. For glucose measurements a special flow injection analysis (FIA) system is employed [1, 3, 7], which uses a glucose oxidase solution instead of immobilised enzymes. Employing an extended Kalman filter the biomass, glucose concentration as well as μ_{max} (Monod model) are estimated. Based on the glucose estimation a PI-control with a set point of 0.08 and 0.05 g/l respectively is carried out.

The rates of cell growth, sugar consumption, ethanol formation and dissolved oxygen concentration in a fed-batch yeast growth process are commonly described by Eq. (1-5).

For the one data set only the *first ethanol production state* (state I) is identified [5]. The parameter functions of the local model in this state are presented in Table 3. In the difference from batch cultivation, no changes are made and local models for this state are as presented in [10]. The estimation of the local model' parameters is again made with using *MATLAB Genetic Algorithms Toolbox* and *Optimisation Toolbox procedures,* based on the optimisation criterion (Eq. (6)) and applying RK45 integration algorithm for numeric simulation of the model. The parameters values are presented in Table 4.

Table 3

Parameter	Local model
μ	μ_{max}
q_S	$\mu_{max}\frac{S}{S+k_S}Y_{SX}$
q_E	$(q_S - q_{Scrit})Y_{ES}$
q_O	$Y_{OX}\mu_{max}$

Table 4

Parameter	Estimated value
μ_{max}	0.29804 h^{-1}
k_S	0.27976 gl^{-1}
Y_{SX}	14.099 gg^{-1}
Y_{ES}	0.42678 gg^{-1}
k_La	80 h^{-1}
Y_{OX}	7.6342e+003 gg^{-1}

Both the real cultivation trajectories for the substrate, biomass and ethanol concentrations, and the simulated ones for this cultivation are presented in Fig. 8, while the variation of the dissolved oxygen concentration is presented in Fig. 9.

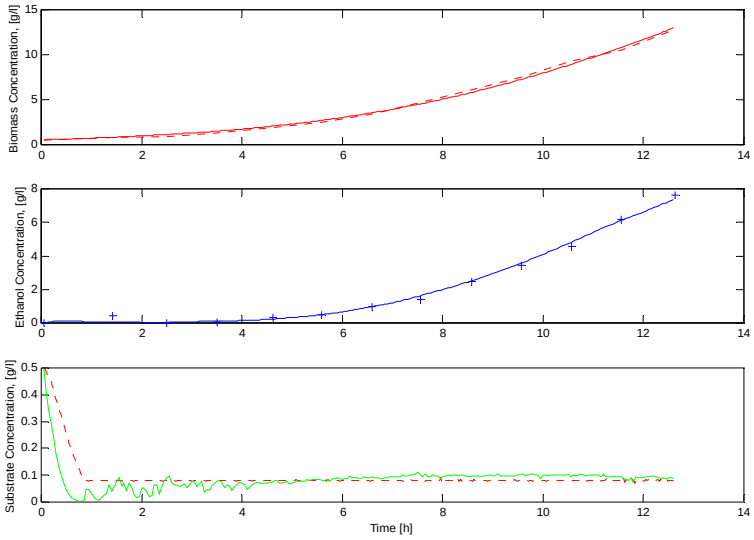

Fig. 8 Measured and simulated aerobic fed-batch yeast cultivation using local model: biomass, ethanol and substrate concentrations

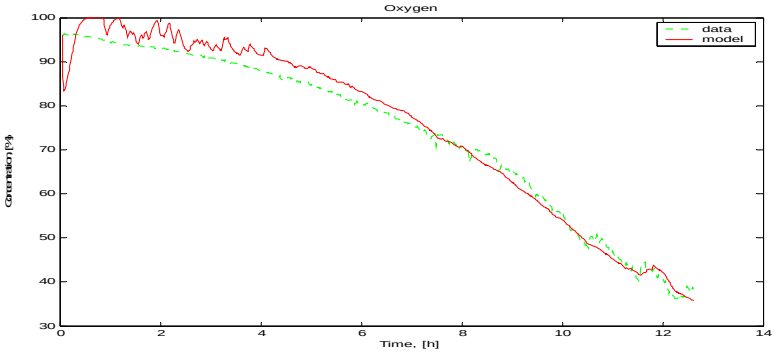

Fig. 9 Measured and simulated aerobic fed-batch yeast cultivation using local model: dissolved oxygen concentration

For another data set from aerobic fed-batch yeast cultivation only the *mixed oxidative state* (state II) is identified [5]. The peak around 12-th hour in the ethanol data is accounted as a data error because the substrate is below the critical level, so there is no conditions ethanol to be produced. The parameter functions of the local model in this state are presented in Table 5. The estimation of the local model' parameters is again made with using *MATLAB Genetic Algorithms Toolbox* and *Optimisation Toolbox procedures,* based on the optimisation criterion (Eq. (6)) and applying

RK45 integration algorithm for numeric simulation of the model. The estimated values of parameters are presented in Table 6.

Table 5

Parameter	Local model
μ	$\mu_S \frac{S}{S+k_S} + \mu_E \frac{E}{E+k_E}$
q_S	$\mu_S \frac{S}{S+k_S} Y_{SX}$
q_E	$-\mu_E \frac{E}{E+k_E} Y_{EX}$
q_O	$q_E\ Y_{OE} + q_S Y_{OS}$

Table 6

Parameter	Estimated value
μ_S	0.95204 h^{-1}
μ_E	0.33917 h^{-1}
k_S	0.11068 gl^{-1}
k_E	5.0052 gl^{-1}
Y_{SX}	2.2135 gg^{-1}
Y_{EX}	1.4946 gg^{-1}
k_La	98.2707 h^{-1}
Y_{OS}	799.4950 gg^{-1}
Y_{OE}	0.0013 gg^{-1}

Both the real cultivation trajectories for the substrate, biomass and ethanol concentrations, and the simulated ones for this cultivation are presented in Fig. 10, while the variation of the dissolved oxygen concentration is presented in Fig. 11.

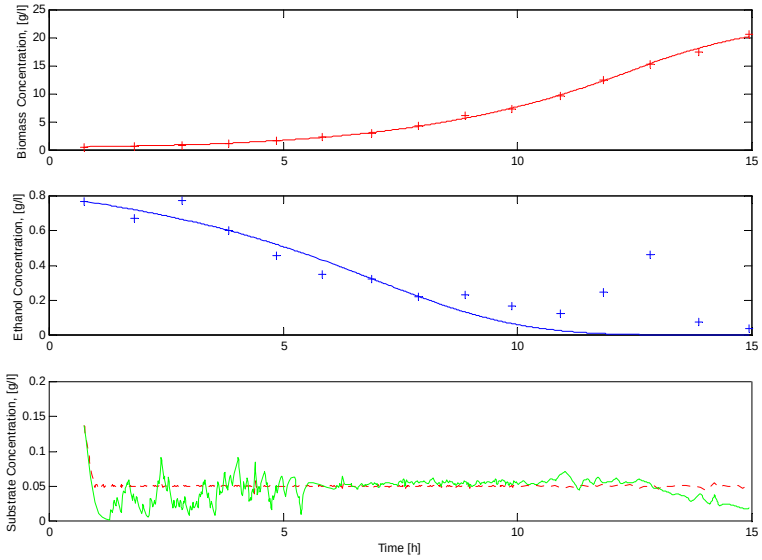

Fig. 10 Measured and simulated aerobic fed-batch yeast cultivation using local model: biomass, ethanol and substrate concentrations

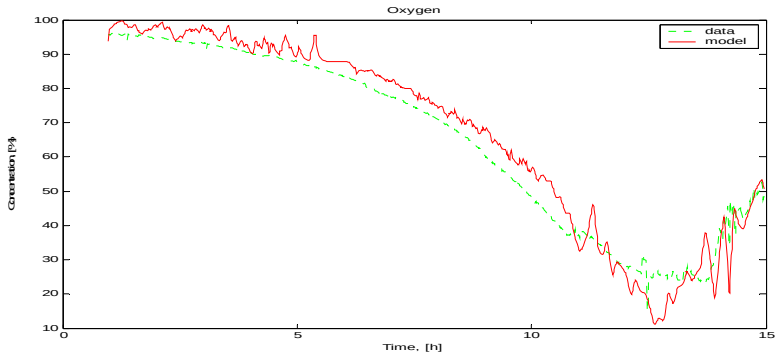

Fig. 11 Measured and simulated aerobic fed-batch yeast cultivation using local model: dissolved oxygen concentration

Although the fact that in both considered fed-batch cultivations only one functional state has been identified, the step of parameter identification of considered two functional states is very important for the further development of functional state approach for modeling of fed-batch yeast cultivation. The recognition of more than one functional state during the fed-batch cultivation is in a big interest and importance for the authors, but it depends on the way of carrying out of the cultivation.

Conclusions

The functional state approach, developed by Zhang et al., has been applied for modelling of three batch and two fed-batch yeast cultivations. The work process shows the functional state modelling approach as more convenient for parameter estimation than the global models of this process. The main advantage of functional state modelling is that parameters of each local model can be separately estimated from other local models' parameters.

The results obtained from the parameter identification and verification of models show a good efficiency and the applicability of functional state approach for modeling of aerobic yeast growth process. The authors will use the obtained results as a stable basis for further implementation of functional state approach for modeling of aerobic yeast growth process with recognition of more than one functional state.

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References

1. Arndt M., B. Hitzmann (2001). Feed forward/feedback control of glucose concentration during cultivation of *Escherichia coli*, Proc. of the 8th IFAC Int. Conf. on Comp. Appl. in Biotechn., Quebec, Canada, pp. 425-429.
2. Bronstein I. N., K. A. Semendjaev (1986). Guide on mathematics for engineers and students, Nauka (in Russian).
3. Hitzmann B., O. Broxtermann, Y.-L. Cha, O. Sobieh, T. Scheper (2000). The Control of Glucose Concentration during Yeast Fed-batch Cultivation Using a Fast Measurement Complimented by an Extended Kalman Filter.
4. Hristozov I., T. Pencheva, E. Staerk, B. Hitzmann, T. Scheper, St. Tzonkov (2001). Modelling of Functional States of Aerobic Batch Yeast Growth Process, International Symposium "Bioprocess Systems'2001-BioPS'2001", Sofia, Bulgaria, pp. I.49-I.52.
5. Hristozov I., T. Pencheva, M. Arndt, B. Hitzmann, St. Tzonkov (2001). Modelling of Functional States of Aerobic Fed-Batch Yeast Growth Process, International Symposium "Bioprocess Systems'2001-BioPS'2001", Sofia, Bulgaria, pp. I.57-I.60.
6. Pencheva T., S. Vassileva, T. Ilkova, Y. Georgieva, B. Hitzmann, St. Tzonkov (2004). Multimodel Approach for Modelling of Biotechnological Processes, Biotechn. and Biotechn. Equipm., *2(18),* pp. *206-214*.
7. Schoengarth K., K. Friehs, B. Hitzmann (1998). A Fast Glucose Measurement for Bioprocesses Using a Flow Injection Analysis System, International Symposium "Bioprocess Systems'98-BioPS'98", Sofia, Bulgaria, pp. IV.5-IV.12.
8. Sonnleitner B., O. Kappeli (1986). Growth of Saccharomyces cerevisiae is controlled by its limited respiration capacity: formulation and verification of a hypothesis, Biotechnol. Bioeng., 28, pp. 927-937.
9. Zhang X.-C., A. Visala, A. Halme, P. Linko (1994), Functional State Modeling and Fuzzy Control of Fed-batch Aerobic Baker's Yeast Process, J. of Biotechn., v. 37, pp. 1-10.
10. Zhang X.-Ch., A. Visala, A. Halme, P. Linko (1994). Functional State Modelling Approach for Bioprocesses: Local Models for Aerobic Yeast Growth Processes, J. Proc. Cont., 4(3), pp. 127- 134.