Functional State Modelling of Cultivation Processes: Dissolved Oxygen Limitation State

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Abstract: *A new functional state, namely dissolved oxygen limitation state for both bacteria Escherichia coli and yeast Saccharomyces cerevisiae fed-batch cultivation processes is presented in this study. Functional state modelling approach is applied to cultivation processes in order to overcome the main disadvantages of using global process model, namely complex model structure and a big number of model parameters. Alongwith the newly introduced dissolved oxygen limitation state, second acetate production state and first acetate production state are recognized during the fed-batch cultivation of E. coli, while mixed oxidative state and first ethanol production state are recognized during the fed-batch cultivation of S. cerevisiae. For all mentioned above functional states both structural and parameter identification is here performed based on experimental data of E. coli and S. cerevisiae fed-batch cultivations.*

Keywords: *Functional state modelling, Dissolved oxygen limitation state, Fed-batch cultivation, Escherichia coli, Saccharomyces cerevisiae.*

Introduction

Cultivation processes, as representatives of processes in living nature, are characterized by a complicated structure of organization and interdependent characteristics, which determine their non-linearity and non-stationary properties. These processes are known to be very complex and their modelling may be a rather time consuming task. Many mathematical models of cultivation processes have been proposed but just a few have been used to optimize industrial plants. The common modelling approach is the development of an overall non-linear process model that performs satisfactorily through the entire operating range. Unfortunately, this approach has a lot of disadvantages. Complex global models of cultivation processes are characterized with a big number of parameters [5, 16, 20, 21], which complicate the model identification and simulation. Moreover, the global model is not able to describe the metabolic changes during the entire operating range and the parameter non-stationary. As an alternative, an increasingly popular multiple-model approach [2, 4, 6, 7, 14, 15], and in particular – functional state modelling approach can be applied to cope with strongly non-linear and time-varying systems [22, 23]. Using this approach complicated problems are

decomposed into subproblems that can be solved independently. Then the individual solutions of the decomposed problems lead to the global solution of the complex problem.

Functional state modelling approach is an appropriate tool to monitor and control complex processes such as cultivation processes [8, 10, 11, 13, 22, 23]. The process is decomposed into different stages called functional states, thus giving a simplified and transparent process representation. The focus of this investigation is a new functional state – *dissolved oxygen limitation state* – to be identified, both structurally and parametrically, based on the experimental data for two different processes, namely *E. coli* and *S. cerevisiae* fed-batch cultivations.

Theoretical background of functional state modelling approach

The main idea of functional state modelling approach is that the process is divided into macrostates, called *functional states* (*FS*), according to behavioural equivalence. The approach has been originally developed for yeast growth process [22, 23]. In each *FS* the yeast metabolism is dominated by certain metabolic pathways. Based on a lot of investigations, Zhang et al. [22, 23] have supposed that the whole yeast growth process can be divided into at least five functional states in batch and fed-batch cultures depending the critical levels of substrate S (S_{crit}) and dissolved oxygen O_2 (O_{2crit}), and the availability of ethanol E. According to Zhang et al. [22, 23], more functional states might exist in the industrial aerobic yeast growth process where oxygen is often limited. For instance, a state with conditions of $S > S_{crit}$ and $O_2 < O_{2crit}$, and a state with $S = 0$, $O_2 < O_{2crit}$ and $E > 0$ might be possible (here presented as *FS VI* and *FS VII* respectively). Since all of experimental data comes from laboratory scale cultivations, these functional states do not occur frequently. The functional state diagram can be illustrated as it is shown in Fig. 1 [22, 23], slightly complemented with *FS VI* and *FS VII*.

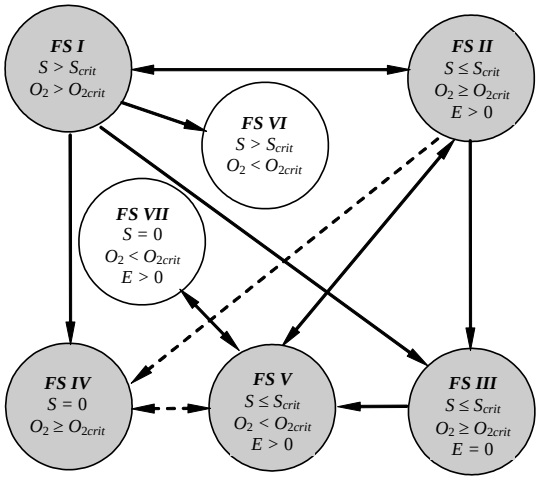

Fig. 1 Functional state diagram

Yeast growth process switches from one *FS* to another when the metabolic conditions are changed. In principle, *FS I* can appear in all batch, fed-batch and continuous yeast growth processes. *FS IV* normally appears only in batch culture. The functional states *FS II*, *FS III* and *FS V* are normally found in fed-batch and continuous cultures [22, 23]. As mentioned above, *FS VI* and *FS VII* do not occur frequently. The solid arrows in Fig. 1 indicate the necessary or normal transition between various functional states of the process. The dotted

arrows indicate that the transitions take place when the mode of culture changes between batch and fed-batch cultures. It should be noted that cultiavtion process could be only in one *FS* at any time. However, a certain *FS* can appear more than once during one cultivation run.

Despite the fact that functional state modelling approach is originally presented for a fed-batch yeast cultivation, it can be applied for modelling *E. coli* cultivation processes based on many proven similarities in microorganisms fermentative metabolism. The analogy in the fermentative metabolism of yeast *S. cerevisiae* and bacteria *E. coli* has been presented in details in [18, 19-21]. Thus, the whole *E. coli* growth process can be divided into at least five functional states, according to the physiological behaviour of the microorganisms in the process. In *E. coli* cultivation process, acetate is produced instead of ethanol in *S. cerevisiae* cultivation process. Table 1 presents the rules for recognition of functional states, according to [22, 23], generalized for both yeast and bacteria cultivation processes.

Table 1

FS	**Cultivation process**	**Functional state name**	**Recognition rule**
FS I	*S. cerevisiae*	*First ethanol production state*	$S > S_{crit}$ and $O_2 > O_{2crit}$
	E. coli	*First acetate production state*	
FS II	*S. cerevisiae*	*Mixed oxidative state*	$S \leq S_{crit}$, $O_2 \geq O_{2crit}$ and $E > 0$
	E. coli		$S \leq S_{crit}$, $O_2 \geq O_{2crit}$ and $A > 0$
FS III	*S. cerevisiae*	*Complete sugar oxidative state*	$S \leq S_{crit}$, $O_2 \geq O_{2crit}$ and $E = 0$
	E. coli		$S \leq S_{crit}$, $O_2 \geq O_{2crit}$ and $A = 0$
FS IV	*S. cerevisiae*	*Ethanol consumption state*	$S = 0$ and $O_2 \geq O_{2crit}$
	E. coli	*Acetate consumption state*	
FS V	*S. cerevisiae*	*Second ethanol production state*	$S \leq S_{crit}$, $O_2 < O_{2crit}$ and $E > 0$
	E. coli	*Second acetate production state*	$S \leq S_{crit}$, $O_2 < O_{2crit}$ and $A > 0$
FS VI	*S. cerevisiae*	no name according to [22, 23]	$S > S_{crit}$ and $O_2 < O_{2crit}$
	E. coli		
FS VII	*S. cerevisiae*	no name according to [22, 23]	$S = 0$, $O_2 < O_{2crit}$ and $E > 0$
	E. coli		$S = 0$, $O_2 < O_{2crit}$ and $A > 0$

Results and discussion

Fed-batch cultivation of E. coli

E. coli strain *BL21(DE3)pPhyt109* is used for cultivation experiments. The experiments are performed in the *Department of Fermentation Engineering, Faculty of Technology, University of Bielefeld, Germany*. Experimental data consists of off-line measurements of biomass (bacteria *E. coli*), phytase and acetate and on-line measurements of substrate (glucose) and dissolved oxygen. Full description of cultivation conditions, on-line and off-line analysis, glucose measurement and control system could be found in [18].

The mathematical model for the considered here *E. coli BL21(DE3)pPhyt109* fed-batch cultivation, based on the mass balance of the components (biomass, glucose, acetate, phytase and dissolved oxygen), is presented by the following differential equations [12, 18]:

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

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

$$\frac{dA}{dt} = q_A X - \frac{F}{V} A \tag{3}$$

$$\frac{dPh}{dt} = q_{Ph} X - \frac{F}{V} Ph \tag{4}$$

$$\frac{dO_2}{dt} = -q_{O_2} X + k_L^{O_2} a(O_2^{*} - O_2) - \frac{F}{V} O_2 \tag{5}$$

$$\frac{dV}{dt} = F \tag{6}$$

where X is the concentration of biomass, [g·l^{-1}]; S – concentration of substrate, [g·l^{-1}]; A – concentration of acetate, [g·l^{-1}]; Ph – concentration of phytase, [g·l^{-1}]; O_2 – concentration of dissolved oxygen, [%]; O_2^{*} – dissolved oxygen saturation concentration, [%]; $k_L^{O_2} a$ – volumetric oxygen transfer coefficient, [h^{-1}]; S_{in} – initial concentration of the feeding solution, [g·l^{-1}]; F – feeding rate, [l·h^{-1}]; V – bioreactor volume, [l]. Structures of specific rate functions μ, q_S, q_A, q_{Ph} and q_{O_2} vary in connection to the recognized functional states.

The following values for substrate and dissolved oxygen critical levels are assumed [12, 18]:

S_{crit} = 0.05 g·l^{-1} and O_{2crit} = 32%.

Taking into account the rules in Table 1 and the values for S_{crit} and O_{2crit}, three functional states are recognized for the considered here fed-batch cultivation of *E. coli BL21(DE3)pPhyt109*:

- *FS VI*: *dissolved oxygen limitation state*;
- *FS V*: *second acetate production state*;
- *FS I*: *first acetate production state*.

In the beginning of the cultivation dissolved oxygen concentration is below the corresponding critical level and glucose concentration is above its critical level. Although in laboratory scale cultivations this state does not occur frequently, in the considered here *E. coli* cultivation the state with conditions of $O_2 < O_{2crit}$ and $S > S_{crit}$ is identified. This new state is called sixth functional state (*FS VI*) – *dissolved oxygen limitation state*. The process is in *FS VI* from 4.3 h (start of the fed-batch cultivation) to 5.6 h of the cultivation. In the next nearly four hours – from 5.6 h to 9.2 h of the cultivation, *second acetate production state* (*FS V*) is identified. The process enters this state when the concentrations of dissolved oxygen and substrate are below the corresponding critical levels. At the end of the cultivation, from 9.2 h to 14 h of the cultivation, both dissolved oxygen and glucose concentrations are above the corresponding critical levels, so the process is in the *first acetate production state* (*FS I*).

Dissolved oxygen limitation state (*FS VI*)

Dissolved oxygen limitation state is a new, briefly reported functional state. Due to absence of investigations for description of *FS VI*, various structures of local models are examined.

Some well known kinetics equations [1], used for description of specific rates of biomass growth, glucose utilization, acetate and phytase formation, and dissolved oxygen consumption are summarized in Table 2 (where μ_{max} is the maximum of the specific growth rate, [h^{-1}], and k_S, k, k_i, k_{Ph} and k_{O_2} are constants). The form of specific rates q_S, q_A, q_{Ph} and q_{O_2} is as follows:

$$q_S = \frac{1}{Y_{S/X}}\mu,\ q_A = \frac{1}{Y_{A/X}}\mu,\ q_{Ph} = \frac{1}{Y_{Ph/X}}\mu \text{ and } q_{O_2} = \frac{1}{Y_{O_2/X}}\mu,$$

where $Y_{S/X}$, $Y_{A/X}$, $Y_{Ph/X}$ and $Y_{O_2/X}$ are yield coefficients, [$g \cdot g^{-1}$].

Table 2

Model	μ	q_S	q_A	q_{Ph}	q_{O_2}
1	$\mu = \mu_{max}\frac{S}{k_S + S}$				
2	$\mu = \mu_{max}\frac{S}{kX + S}$				
3	$\mu = \mu_{max}\frac{1}{k_S + S + S^2/k_i}$				
4	$\mu = \mu_{max}\frac{S}{k_S + S/X}$				
5	$\mu = \mu_{max}\frac{S}{k_S + S}$		$\mu = \mu_{max}\frac{A}{k_S + A}$	$\mu = \mu_{max}\frac{S}{k_S + S}$	$\mu = \mu_{max}\frac{O_2}{k_{O_2} + O_2}$
6	$\mu = \mu_{max}\frac{S}{k_S + S}$			$\mu = \mu_{max}\frac{Ph}{k_{Ph} + Ph}$	
7	$\mu = \mu_{max}\frac{S}{k_S + S}$		$\mu = \mu_{max}\frac{A}{k_S + A}$	$\mu = \mu_{max}\frac{S}{k_S + S}$	$\mu = \mu_{max_{O_2}}\frac{O_2}{k_{O_2} + O_2}$
8	$\mu = \mu_{max}\frac{S}{k_S + S}$				$\mu = \mu_{max}\frac{O_2}{k_{O_2} + O_2}$
9	$\mu = \mu_{max}\frac{S}{k + S/X}$				
10	μ_{max}	$\mu = \mu_{max}\frac{S}{k_S + S}$			

Parameter identification of *Models 1÷10* is performed based on available real experimental data. Genetic algorithms (GA) are used as optimization techniques because of their appropriateness in solving complex optimization problems [9]. All model identification procedures are performed in MATLAB environment using Genetic Algorithms Toolbox [3].

GA operators and parameters for parameter identification of considered here cultivation processes are summarized, respectively in Table 3 and Table 4 [18].

Table 3

Operator	Type
encoding	binary
crossover	double point
mutation	bit inversion
selection	roulette wheel selection
fitness function	linear ranking

Table 4

Parameter	Value	
	E. coli	***S. cerevisiae***
generation gap	0.97	0.80
crossover rate	0.70	0.95
mutation rate	0.1	0.05
precision of binary representation	20	20
number of individuals	100	20
number of generations	200	100

The optimization criterion is presented as a minimization of a distance measure J between experimental and model predicted values of state variables, represented by the vector $y = [S, X, A, Ph, O_2]$:

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

To perform reliable identification procedure 30 independent runs of GA are executed for both cultivation processes. Further, the average values of optimization criterion and model parameters estimates are presented. The values of the considered criterion J (Eq. (7)), obtained after the parameter identification of *Models 1÷10*, are listed in Table 5.

Table 5

Model	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
J	1.11	1.11	1.11	1.10	1.11	1.12	1.12	0.95	1.10	1.13

The results indicate that *Model 8* gives better results than other models – this model has the lowest sum of square errors between experimental data and model prediction. On account of that fact, *Model 8* is used for description of *dissolved oxygen limitation state.* Thus, the specific rates of glucose consumption, acetate and phytase production are proportional to the specific bacteria growth rate, i.e. to Monod model. The dissolved oxygen consumption rate depends mainly on the dissolved oxygen concentration using Monod structure. Resulting local models for *FS VI* are presented in Table 6, while Table 7 summarizes values of model parameter estimations. The simulation results of *Model 8* prediction and the experimental data curves are shown in Fig. 2 for substrate and dissolved oxygen concentrations and in Fig. 3 for biomass, acetate and phytase concentrations.

Table 6

FS VI	
Specific rate	**Local model structure**
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_{Ph}	$\frac{1}{Y_{Ph/X}}\mu_{max}\frac{S}{k_S+S}$
q_{O_2}	$\frac{1}{Y_{O_2/X}}\mu_{max}\frac{O_2}{k_{O_2}+O_2}$

Table 7

FS VI	
Model parameter	**Value**
μ_{max}, [h^{-1}]	0.75
k_S, [g·l^{-1}]	0.014
k_{O_2}, [%]	0.015
$Y_{S/X}$, [g·g^{-1}]	0.38
$Y_{A/X}$, [g·g^{-1}]	0.01
$Y_{Ph/X}$, [g·g^{-1}]	0.28
$Y_{O_2/X}$, [g·g^{-1}]	0.28
$k_L^{O_2}a$, [h^{-1}]	179.88
J	0.95

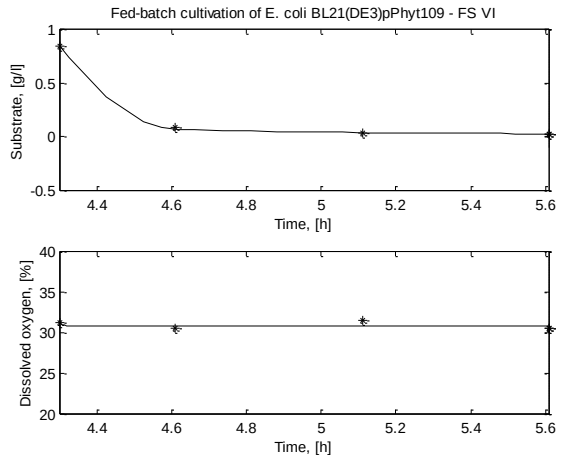

Fig. 2

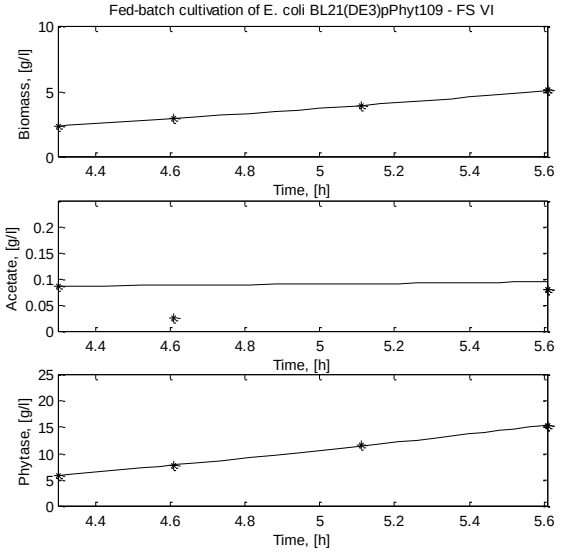

Fig. 3

Second acetate production state (*FS V*)

FS V and *FS I* have been already identified and modelled in fed-batch cultivation of *E. coli MC4110* [18]. Considering *E. coli BL21(DE3)pPhyt109* cultivation, the investigation starts with model structures developed there. The results show an adequate model prediction of experimental data, except for acetate behavior. In this case the typical Monod kinetics can not describe the acetate dynamics. To obtain better results the acetate production should depends mainly on the acetate concentration. Moreover, here a maximum specific growth rate (μ_{maxA}), as well as saturation constant (k_A) are defined, both related to acetate, as it is in the *mixed oxidative state*.

The new set of local models for *FS V* is listed in Table 8. Table 9 presents the estimated values of models parameters. Both the real cultivation trajectories and the simulated ones are presented in Fig. 4 for substrate and dissolved oxygen concentrations, and in Fig. 5 for biomass, acetate and phytase concentrations.

Table 8

FS V	
Specific rate	**Local model structure**
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_{Ph}	$\frac{1}{Y_{Ph/X}}\mu_{max}\frac{S}{k_S+S}$
q_{O_2}	$\frac{1}{Y_{O_2/X}}\mu_{max}\frac{O_2}{k_{O_2}+O_2}$

Table 9

FS V	
Model parameter	**Value**
μ_{max}, [h^{-1}]	0.79
μ_{maxA}, [h^{-1}]	0.10
k_S, [g·l^{-1}]	0.001
k_A, [g·l^{-1}]	0.51
k_{O_2}, [%]	0.001
$Y_{S/X}$, [g·g^{-1}]	0.45
$Y_{A/X}$, [g·g^{-1}]	0.42
$Y_{Ph/X}$, [g·g^{-1}]	0.54
$Y_{O_2/X}$, [g·g^{-1}]	0.55
$k_L^{O_2}a$, [h^{-1}]	179.21
J	4.52

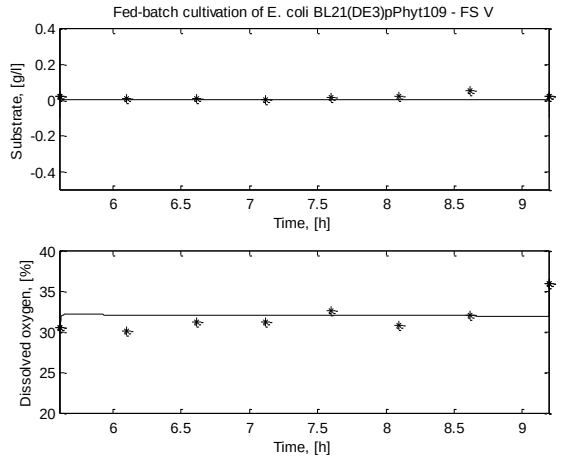

Fig. 4

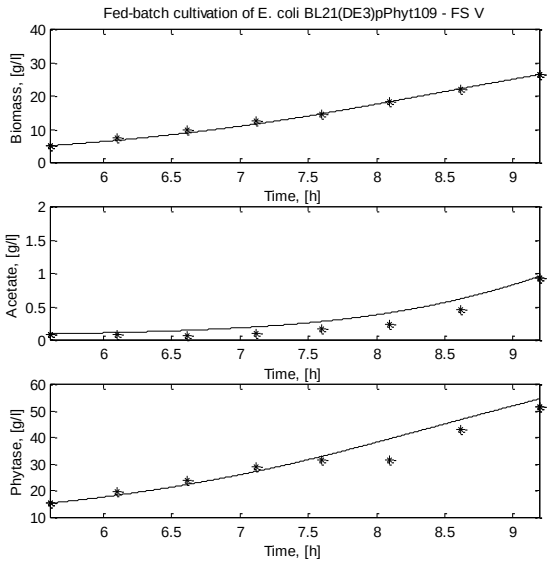

Fig. 5

First acetate production state (*FS I*)

In cultivation process of *E. coli BL21(DE3)pPhyt109* two products are examined in contrast to considered in [18] cultivation of *E. coli MC4110*. In the *first acetate production state*, according to Zhang et al. [22, 23], the specific growth rate is assumed to be a constant. In this

case better results are obtained with Monod type of specific growth rate. Taking into account this fact, the specific dissolved oxygen rate is not a constant too.

On the other hand, although the specific phytase production rate is expressed successfully with Monod model in *dissolved oxygen limitation* and *second acetate production states*, in *FS I* Monod kinetics is unable to fit the phytase date. To achieve higher model accuracy some changes in the local model describing the phytase variation are done. The specific phytase production rate is described using Monod structure depending on phytase concentration.

The modified set of local models for *FS I* is listed in Table 10. Results from the identification of model parameters are shown in Table 11.

Table 10

FS I	
Specific rate	**Local model structure**
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_{Ph}	$\frac{1}{Y_{Ph/X}}\mu_{max}\frac{Ph}{k_{Ph}+Ph}$
q_{O_2}	$\frac{1}{Y_{O_2/X}}\mu_{max}\frac{O_2}{k_{O_2}+O_2}$

Table 11

FS I	
Model parameter	**Value**
μ_{max}, [h^{-1}]	0.54
k_S, [g·l^{-1}]	0.098
k_{O_2}, [%]	0.011
k_{Ph}, [g·l^{-1}]	0.01
$Y_{S/X}$, [g·g^{-1}]	0.16
$Y_{A/X}$, [g·g^{-1}]	0.09
$Y_{Ph/X}$, [g·g^{-1}]	0.90
$Y_{O_2/X}$, [g·g^{-1}]	0.13
$k_L^{O_2}a$, [h^{-1}]	178.01
J	27.52

The simulation results are depicted in Fig. 6 for predicted and real substrate and dissolved oxygen concentrations and in Fig. 7 for predicted and real biomass, acetate and phytase concentrations.

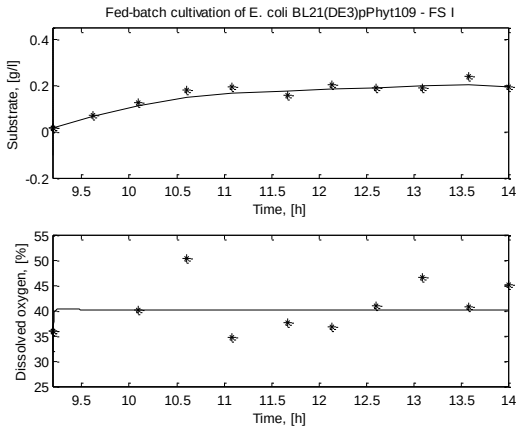

Fig. 6

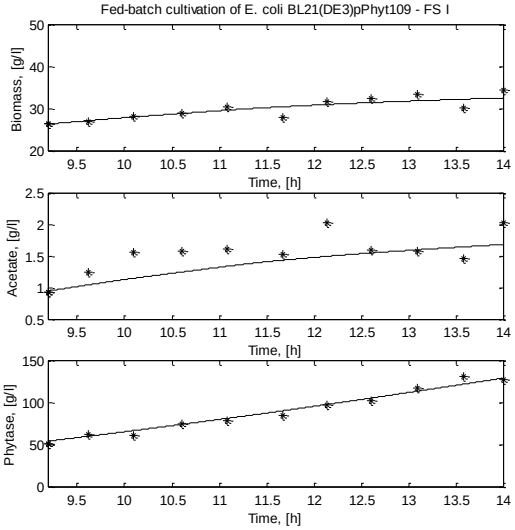

Fig. 7

Both the measured cultivation trajectories and the simulated ones are presented for all recognized functional states (*FS VI*, *FS V* and *FS I*) in Fig. 8 for substrate (glucose) and dissolved oxygen concentrations, and in Fig. 9 for biomass, acetate and phytase concentrations.

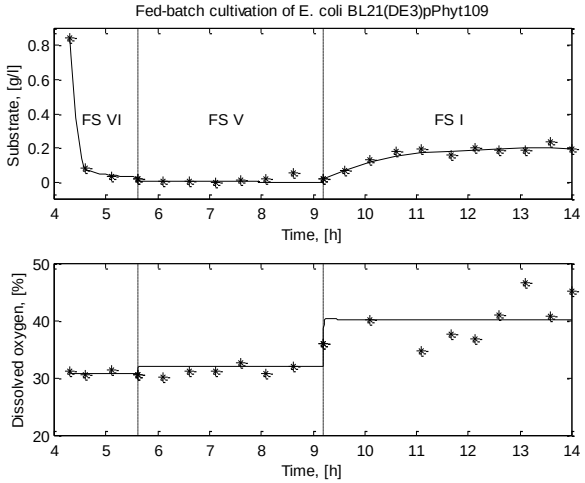

Fig. 8

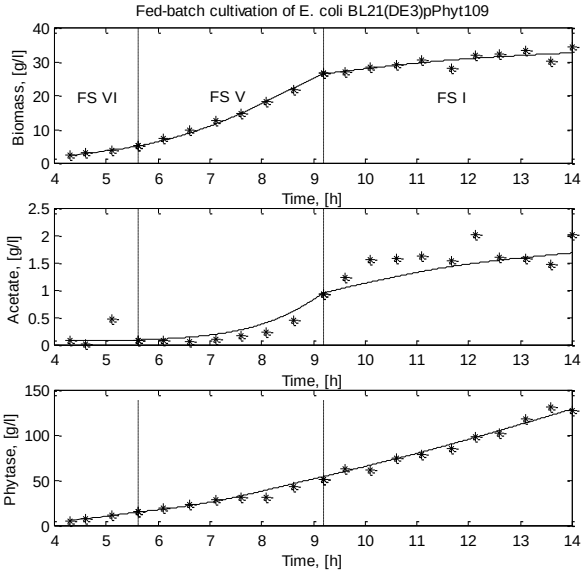

Fig. 9

The figures show that the model predicts successfully the variation of glucose utilization, biomass concentration, acetate and phytase formation, and dissolved oxygen consumption during the fed-batch cultivation of *E. coli BL21(DE3)pPhyt109*.

Fed-batch cultivation of S. cerevisiae

Experimental data from a fed-batch cultivation of *S. cerevisiae*, carried out in the *Institute of Technical Chemistry, Leibniz University of Hannover, Germany*, consists of off-line measurements of biomass (yeast) and ethanol and on-line measurements of substrate (glucose) and dissolved oxygen. Full description of cultivation conditions, on-line and off-line analysis, glucose measurement and control system could be found in [17].

The rates of cell growth, sugar utilization, ethanol production and dissolved oxygen consumption in yeast fed-batch cultivation are commonly described for all functional states and according to the mass balance as follows [22, 23]:

$$\frac{dX}{dt} = \mu X - \frac{F}{V} X \tag{8}$$

$$\frac{dS}{dt} = -q_S X + \frac{F}{V}\left(S_{in} - S\right) \tag{9}$$

$$\frac{dE}{dt} = q_E X - \frac{F}{V} E \tag{10}$$

$$\frac{dO_2}{dt} = -q_{O_2} X + k_L a\left(O_2^* - O_2\right) \tag{11}$$

$$\frac{dV}{dt} = F \tag{12}$$

where X, S, O_2, O_2^*, k_La, S_{in}, F and V keep their meaning as presented after Eqs. (1-6), and additionally E is the concentration of ethanol, [g·l^{-1}]. Here again the structures of specific rate functions μ, q_S, q_E and q_{O_2} vary in connection with recognized functional states.

Based on Zhang et al. [22, 23] and the experience with the *S. cerevisiae* strain and cultivations, the following critical values for substrate and dissolved oxygen concentrations are assumed:

S_{crit} = 0.05 g·l^{-1} and O_{2crit} = 18%.

As it is mentioned above, since most of experimental data comes from laboratory scale cultivations, functional state with $O_2 < O_{2crit}$ and $S > S_{crit}$ does not occur frequently. Although, in the considered here experimental data set conditions for *FS VI* recognition appear even twice.

Taking into account the rules in Table 1 and the values for S_{crit} and O_{2crit}, three functional states, but two of them repeated twice, have been recognized for the discussed here fed-batch cultivation of *S. cerevisiae*:

- *FS II*: *mixed oxidative state*;
- *FS I*: *first ethanol production state*;
- *FS VI*: *dissolved oxygen limitation state*.

In this investigation the focus is pointed only on the last five *FS*, starting from about the middle of the 25-th hour of considered cultivation process, since there are two appearances of *dissolved oxygen limitation state*, which is the main subject of current investigation. About that time the process enters in the *mixed oxidative state* (*FS II*), because the concentration of the substrate is below or equal to its critical level S_{crit}, while the concentration of the dissolved oxygen is above its critical level O_{2crit}, and there is ethanol in the broth. At the beginning of the 26-th hour, the process switches to *first ethanol production state* (*FS I*) due to there is still dissolved oxygen above its critical level O_{2crit}, but the substrate concentration also increases to values above its critical level S_{crit}. Keeping the substrate concentration above S_{crit}, but having dissolved oxygen lower than its critical level O_{2crit}, the process enters in *dissolved oxygen limitation state* (*FS VI*). That happens at the end of 26-th hour of the cultivation process. In the middle of the 27-th hour, the process enters once again in *FS I*, and in the middle of the 29-th hour – again in *FS VI*.

Model identification procedures for all recognized *FS* are again performed in MATLAB environment using Genetic Algorithms Toolbox and based on available off-line and on-line measurements of state variables. GA operators and parameters are as shown in [18]. The optimization criterion is as presented in Eq. (7), only taking into consideration that in the case of yeast cultivation the vector of state variables has the form $y = [S, X, E, O_2]$.

Mixed oxidative state (*FS II*)

During parameter identification of this state, the original local models, presented by Zhang et al. [22, 23] have been used, showing satisfied results in both structural and parameter identification. Local model parameter functions for *FS II* are presented in Table 12, where μ_{2S} and μ_{2E} are the maximum growth rates of substrate and ethanol respectively, [1·h^{-1}];

k_S and k_E are saturation constants of substrate and ethanol respectively, [g·l^{-1}] and $Y_{S/X}$, $Y_{E/X}$, $Y_{O/E}$ and $Y_{O/S}$ are yield coefficients, [g·g^{-1}]. Table 13 lists the values of estimated parameters.

Table 12

FS II	
Specific rate	**Local model structure**
μ	$\mu_{2S}\frac{S}{S+k_S}+\mu_{2E}\frac{E}{E+k_E}$
q_S	$\frac{\mu_{2S}}{Y_{S/X}}\frac{S}{S+k_S}$
q_E	$-\frac{\mu_{2E}}{Y_{E/X}}\frac{E}{E+k_E}$
q_{O_2}	$q_E\ Y_{O/E}+q_S Y_{O/S}$

Table 13

FS II	
Model parameter	**Value**
μ_{2S}, [h^{-1}]	0.12
μ_{2E}, [h^{-1}]	0.65
k_S, [g·l^{-1}]	0.06
k_E, [g·l^{-1}]	0.43
$Y_{S/X}$, [g·g^{-1}]	0.74
$Y_{E/X}$, [g·g^{-1}]	67.42
$Y_{O/E}$, [g·g^{-1}]	0.001
$Y_{O/S}$, [g·g^{-1}]	97.53
k_La, [h^{-1}]	4.57
J	12.22

Both the real cultivation trajectories and the simulated ones are presented in Figs. 10-13, respectively for biomass, ethanol, substrate and dissolved oxygen concentrations.

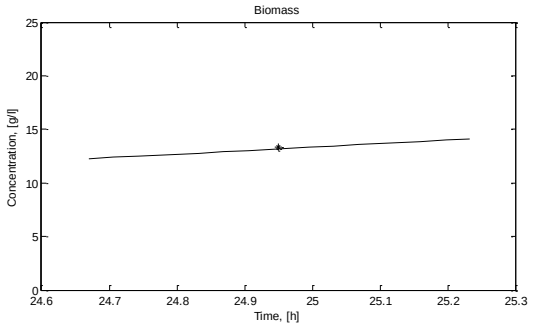

Fig. 10

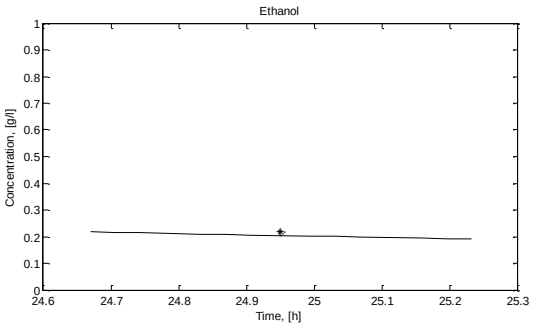

Fig. 11

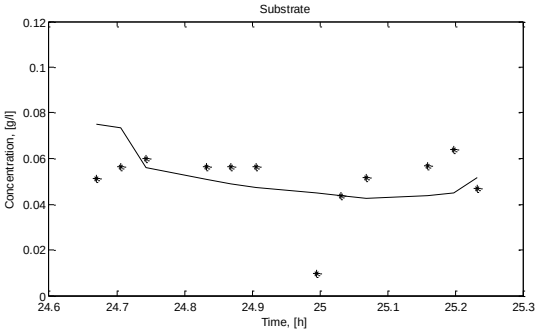

Fig. 12

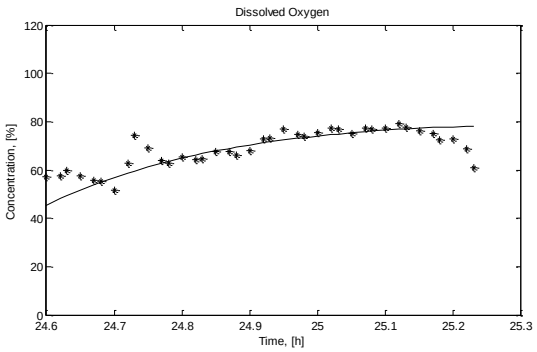

Fig. 13

First acetate production state (*FS I*)

During parameter identification of this state, all local models have been kept as originally presented in [22, 23], except the specific rate of dissolved oxygen consumption. Using Monod kinetics towards to dissolved oxygen instead of proportional to growth rate value gives more meaningful results. Local model functions for *FS I* are presented in Table 14 (where μ_1 is the maximum of the specific growth rate, [h^{-1}]) while the values of estimated parameters are listed in Table 15.

Table 14

FS I	
Specific rate	**Local model structure**
μ	μ_1
q_S	$\frac{\mu_1}{Y_{S/X}} \frac{S}{S+k_S}$
q_E	$(q_S - q_{S_{crit}})Y_{E/S}$
q_{O_2}	$\frac{\mu_1}{Y_{O/S}} \frac{O_2}{O_2+k_{O_2}}$

Table 15

FS I	
Model parameter	**Value**
μ_1, [h^{-1}]	0.11
k_S, [$g \cdot l^{-1}$]	0.57
k_{O_2}, [%]	0.973
$Y_{S/X}$, [$g \cdot g^{-1}$]	0.16
$Y_{E/S}$, [$g \cdot g^{-1}$]	0.001
$Y_{O/S}$, [$g \cdot g^{-1}$]	0.07
k_La, [h^{-1}]	3.84
J	6.37

Both the real cultivation trajectories and the simulated ones for one of two recognized *FS I* are presented in Figs. 14-17, respectively for biomass, ethanol, substrate and dissolved oxygen concentrations.

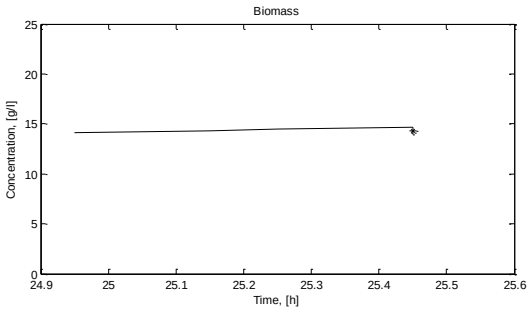

Fig. 14

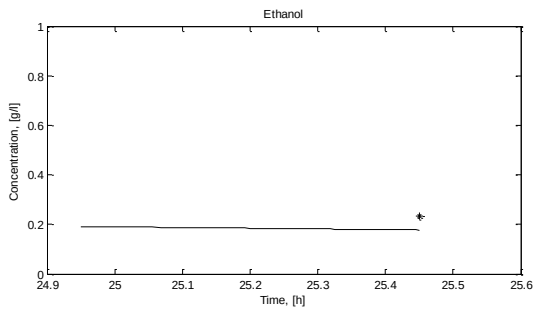

Fig. 15

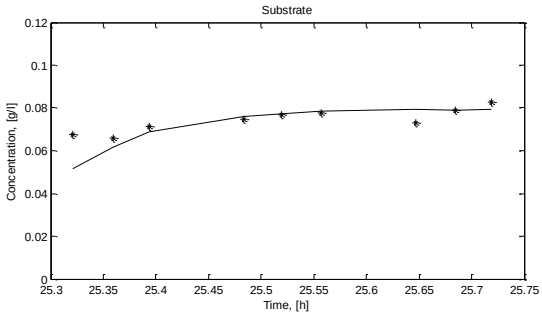

Fig. 16

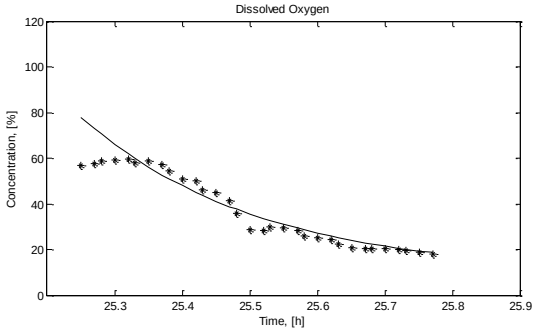

Fig. 17

Dissolved oxygen limitation state (*FS VI*)

Dissolved oxygen limitation state is a new, unstudied functional state for yeast fed-batch cultivation. As shown in the previous section, such functional state has been recognized for a fed-batch cultivation of *E. coli*. In this section various structures of local models are again examined in order to identify the best model structure and parameters estimations for *FS VI* in *S. cerevisiae* cultivation process. Considered mathematical models are presented in Table 16.

Table 16

<table>
<tr><th>Model</th><th>μ</th><th>q_S</th><th>q_E</th><th>q_{O_2}</th></tr>
<tr><td>1</td><td colspan="4">$\mu = \mu_{max}\frac{S}{k_S + S}$</td></tr>
<tr><td>2</td><td colspan="4">$\mu = \mu_{max}\frac{S}{kX + S}$</td></tr>
<tr><td>3</td><td colspan="4">$\mu = \mu_{max}\frac{1}{k_S + S + S^2/k_i}$</td></tr>
<tr><td>4</td><td colspan="4">$\mu = \mu_{max}\frac{S}{k_S + S/X}$</td></tr>
<tr><td>5</td><td>$\mu = \mu_{max}\frac{S}{k_S + S}$</td><td colspan="2">$\mu = \mu_{max}\frac{E}{k_S + E}$</td><td rowspan="4">$\mu = \mu_{max}\frac{O_2}{k_{O_2} + O_2}$</td></tr>
<tr><td>6</td><td colspan="3">$\mu = \mu_{max}\frac{S}{k_S + S/X}$</td></tr>
<tr><td>7</td><td>$\mu = \mu_{max}\frac{S}{k_S + S}$</td><td colspan="2">$\mu = \mu_{max}\frac{S}{kX + S}$</td></tr>
<tr><td>8</td><td colspan="3">$\mu = \mu_{max}\frac{S}{k_S + S}$</td></tr>
<tr><td>9</td><td>μ_{max}</td><td colspan="3">$\mu = \mu_{max}\frac{S}{k_S + S}$</td></tr>
</table>

The values of the criterion *J* (Eq. (7)) obtained after the parameter identification of examined models are listed in Table 17.

Table 17

Model	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
J	21.35	27.81	22.39	24.82	25.12	27.19	22.21	20.07	24.13

As seen from Table 17, *Model 8* achieves the best results, respectively the lowest sum of square errors between experimental data and model prediction, in comparison to other models. Therefore *Model 8* is chosen to describe *dissolved oxygen limitation state*. Thus, the specific rates of glucose consumption and ethanol production are proportional to the specific growth rate, i.e. to Monod model. The dissolved oxygen consumption rate depends on the

concentration of dissolved oxygen using Monod kinetics structure. The selected local models for *FS VI* are listed in Table 18, while Table 19 presents the parameter values.

Table 18

FS VI	
Specific rate	**Local model structure**
μ	$\mu_{max}\frac{S}{k_S+S}$
q_S	$\frac{1}{Y_{S/X}}\mu_{max}\frac{S}{k_S+S}$
q_E	$\frac{1}{Y_{E/X}}\mu_{max}\frac{S}{k_S+S}$
q_{O_2}	$\frac{1}{Y_{O_2/X}}\mu_{max}\frac{O_2}{k_{O_2}+O_2}$

Table 19

FS VI	
Model parameter	**Value**
μ_{max}, [h^{-1}]	0.37
k_S, [g·l^{-1}]	0.06
$Y_{S/X}$, [g·g^{-1}]	2.61
$Y_{E/X}$, [g·g^{-1}]	8.80
$Y_{O_2/X}$, [g·g^{-1}]	0.0006
k_{O_2}, [%]	16.69
k_La, [h^{-1}]	40.29
J	20.07

The simulation results of *Model 8* and the experimental data curves for one of two recognized *FS VI* are shown in Figs. 18-21, respectively for biomass, ethanol, substrate and dissolved oxygen concentrations.

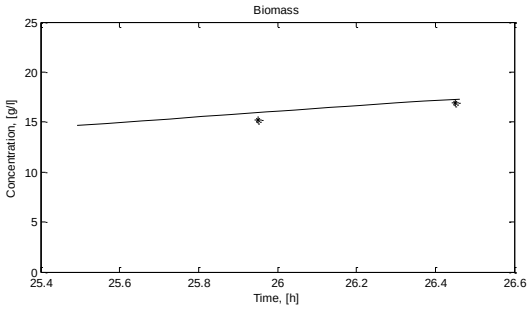

Fig. 18

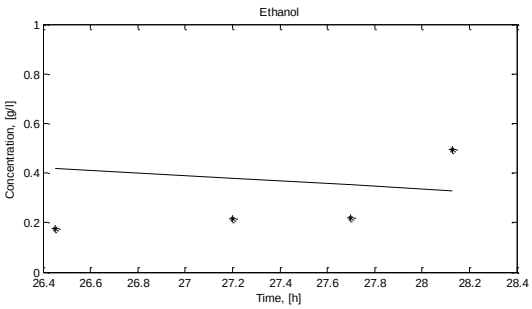

Fig. 19

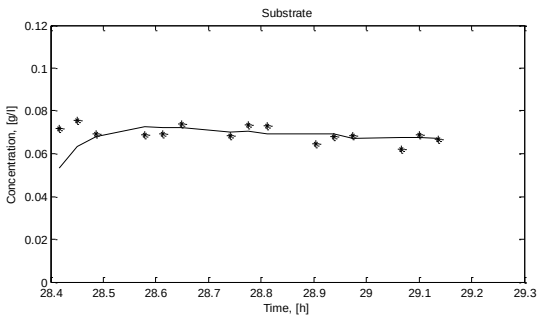

Fig. 20

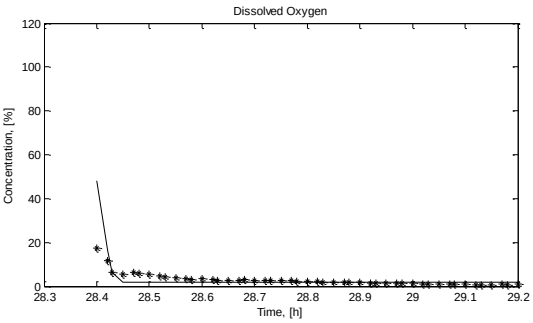

Fig. 21

The results presented show good prediction of the developed model, especially for substrate and biomass. This model has been validated based on the second appearance of *FS VI* during this cultivation. Results are not shown because of the similarity.

Both the measured cultivation trajectories and the simulated ones for all recognized functional states (*FS II*, *FS I*, *FS VI*, *FS I* and *FS VI*) are presented in Figs. 22-25, respectively for biomass, ethanol, substrate and dissolved oxygen concentrations.

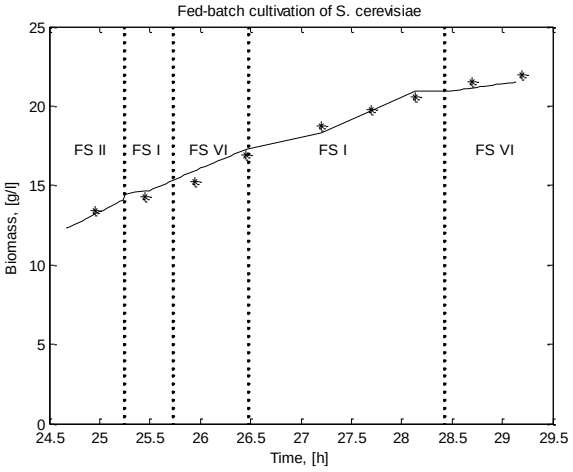

Fig. 22

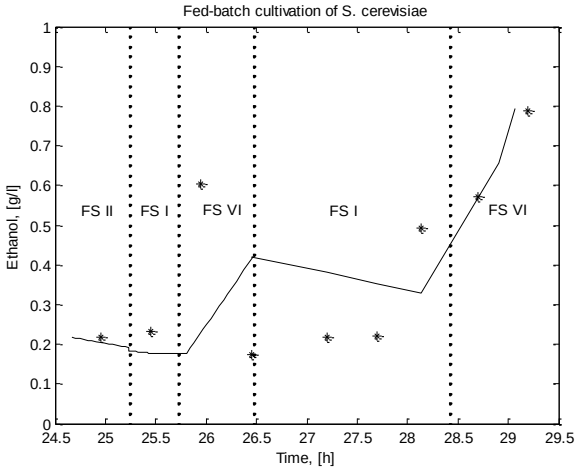

Fig. 23

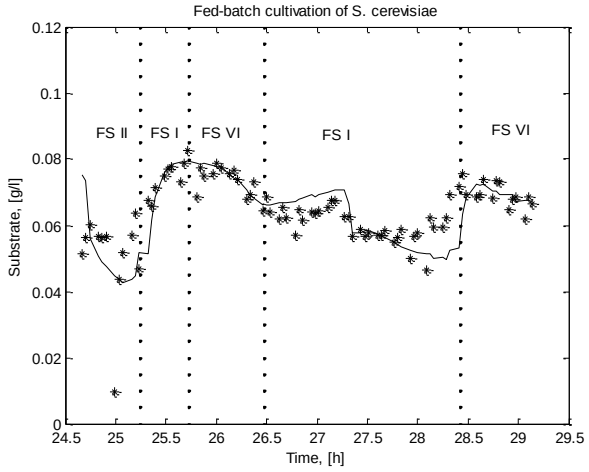

Fig. 24

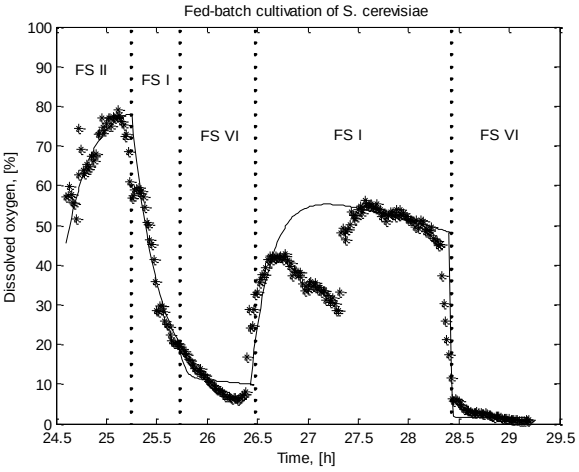

Fig. 25

The figures show that the model predicts successfully the variation of biomass concentration, glucose utilization, ethanol production and dissolved oxygen consumption during the fed-batch cultivation of *S. cerevisiae*.

Conclusion

In this paper the concept of functional state modelling is applied for description of *E. coli* and *S. cerevisiae* fed-batch cultivations. Identification of a new functional state, namely *dissolved oxygen limitation state* is here presented. Both structural and parameter identification of recognized functional states are performed based on the experimental data of considered fed-batch cultivation processes. As *dissolved oxygen limitation state* is a new functional state, a group of various local models have been examined in order to be found an appropriate model structure to describe this state.

In the case of modelling of *E. coli*, for *dissolved oxygen limitation state* ten groups of various structures of local models are examined. The results indicate that better prediction shows the model that uses specific rates of glucose consumption, acetate and phytase production proportional to the specific bacteria growth rate. The dissolved oxygen consumption rate depends mainly on the dissolved oxygen concentration using Monod structure. For the *second acetate production state* the local models developed for fed-batch cultivation of *E. coli MC4110* showed an adequate model prediction of experimental data, except for acetate behavior. To obtain better results for description of acetate production rate a modified local model is proposed, which depends mainly on the acetate concentration. For the *first acetate production state* better results are obtained with Monod type of specific growth rate. To improve the model prediction some changes in the local model described the phytase variation are done. The specific phytase production rate is described using Monod structure towards the phytase concentration. The simulation results show that the developed models predict successfully the variation of glucose utilization, biomass concentration, acetate and phytase formation and dissolved oxygen consumption during the fed-batch cultivation of *E. coli BL21(DE3)pPhyt109*.

In the case of *S. cerevisiae* fed-batch cultivation, based on the values for substrate and dissolved oxygen critical levels, three functional states are recognized, but two of them appearing twice: *mixed oxidative state*, *first ethanol production state* and *dissolved oxygen limitation state.* No changes of the original local models have been done in *mixed oxidative state*, due to satisfied results obtained during the structural and parameter identification. For the *first ethanol production state* all local models, except the specific rate of dissolved oxygen consumption, have been kept as proposed by Zhang et al. [22, 23]. More meaningful results are obtained using Monod kinetics towards to dissolved oxygen instead of proportional to growth rate value. For the *dissolved oxygen limitation state* nine groups of various local models have been examined in order to be found an appropriate model to describe *FS VI* since this state is recognized for a first time in yeast cultivation. The results indicate that better prediction is obtained when using specific rates of glucose consumption and ethanol production proportional to the specific growth rate. The dissolved oxygen consumption rate depends on the concentration of dissolved oxygen using Monod kinetic structure. Another part of experimental data, where the conditions for *FS VI* are fulfilled, allows the developed model to be validated.

As a conclusion, functional state modelling approach has been successfully applied for two cultivation processes – of bacteria *E. coli BL21(DE3)pPhyt109* and yeast *S. cerevisiae*. In both cases three different functional states have been recognized and structurally and parametrically identified based on available experimental data. Moreover, a new functional state, namely *dissolved oxygen limitation state*, is presented and thus, an extension of functional state modelling approach is proposed.

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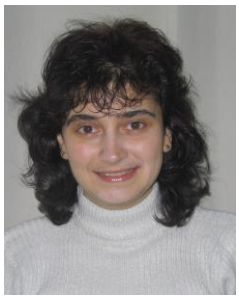

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