Functional State Modelling Approach for Batch Cultivation of *Saccharomyces cerevisiae*

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An application of functional state modelling approach for aerobic batch baker's yeast cultivation is presented in this paper. The functional state approach is appropriate for description, monitoring and control of complex processes such as bioprocesses. The main idea is to divide the process into macrostates, called functional states, according to behavioural equivalence. In each functional state the process is described by a conventional type of model, called a local model, which is valid in this functional state only. Functional state modelling approach is more convenient than using of a global model when parameter estimation should be made. The main advantage of functional state modelling approach is that the parameters of each local model can be separately estimated from the parameters of the other local models. The concept of functional state modelling approach is applied for real aerobic batch baker's yeast cultivation.

Key words:
Batch process, modelling, functional states

Introduction

The modelling of yeast cultivation has been widely studied and reported. The common modelling approach is to be synthesized one global process model.[1] The main disadvantage of such approach is the complex model structure and the big number of model parameters, which complicates the model simulation and parameter estimation.

The functional state approach is a concept, which helps in monitoring and control of complex processes such as bioprocesses.[2,3] The main idea is the process to be divided into macrostates, called functional states, according to behavioural equivalence.[4–7] In a functional state, the process is described by a conventional type of model, called a local model, which is valid in this functional state. In each functional state, certain metabolic pathways are active enough to dominate the overall behaviour of the process. The functional state and state transitions can be recognized with numeric detection algorithms and/or with rules based on expert knowledge. 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 the same states can be often recognized and the same functional states model can be used. In principle, the structure of local models in different functional states can be different. Here the basic forms of the local models are the same, and only the parameters of the models are behaving differently or have different numerical values.

This paper illustrates the concept of functional state approach in connection with the batch aerobic baker's yeast growth process. The process is divided into two functional states. The process dynamics in each functional state is described by a simple local model. A set of local models together with functional state "dynamics" can be used to describe and monitor the overall yeast growth process.

Functional state modelling for batch cultivation

The following assumptions are made in developing of the local models of the aerobic baker's yeast growth process in batch cultures:[2]

– The main by-products in a batch 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 a critical level.

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– The elemental composition of yeast in the process does not significantly change.

– Quantities, with the exception of 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 formation and dissolved oxygen concentration in a batch yeast growth process are commonly[2] described as follows for all functional states according to the mass balance:

$$\frac{\mathrm{d}\gamma_{\mathrm{X}}}{\mathrm{d}t} = \mu\gamma_{\mathrm{X}} \tag{1}$$

$$\frac{\mathrm{d}\gamma_{\mathrm{S}}}{\mathrm{d}t} = -q_{\mathrm{S}}\gamma_{\mathrm{X}} \tag{2}$$

$$\frac{\mathrm{d}\gamma_{\mathrm{E}}}{\mathrm{d}t} = q_{\mathrm{E}}\gamma_{\mathrm{X}} \tag{3}$$

$$\frac{\mathrm{d}w_{\mathrm{O_2}}}{\mathrm{d}t} = -q_{\mathrm{O_2}}w_{\mathrm{X}} + k_{\mathrm{L}}a\,(w_{\mathrm{O_2sat}} - w_{\mathrm{O_2}}), \tag{4}$$

where

γ_{X} – mass concentration of biomass, g $\mathrm{l^{-1}}$

γ_{S} – mass concentration of substrate (glucose), g $\mathrm{l^{-1}}$

γ_{E} – mass concentration of ethanol, g $\mathrm{l^{-1}}$

$w_{\mathrm{O_2}}$ – mass fraction of dissolved oxygen, %

$w_{\mathrm{O_2sat}}$ – saturation mass fraction of dissolved oxygen, %

w_{X} – mass fraction of biomass, %

μ, q_S, q_E, $q_{\mathrm{O_2}}$ – specific rates of, respectively, specific growth rate, substrate utilization, ethanol production and dissolved oxygen consumption, $\mathrm{h^{-1}}$

$k_{\mathrm{L}}a$ – the volumetric oxygen transfer coefficient, $\mathrm{h^{-1}}$

The parameter functions μ, q_S, q_E and $q_{\mathrm{O_2}}$ in Eq. (1)–(4) vary in connection 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 some reducing power and energy. Yeast then utilizes the carbon intermediates to synthesize new cell material. If sugar concentration in the broth in an aerobic yeast growth process exceeds a certain level, called the critical sugar mass concentration (γ_{Scrit}), a part of the sugar is metabolized to ethanol. In the case of batch cultivation, γ_{Scrit} is assumed to be zero. A critical level of dissolved oxygen concentration for yeast growth process is assumed[2] to be 18 %. The whole yeast growth process can be divided into at least five functional states in, both, batch and fed-batch cultures.[2] In each functional state, the yeast metabolism is dominated by certain metabolic pathways.

In the case of batch cultivation two phases are identified:

– The first functional state (I) is here called the *first ethanol production state*. The process is defined to be in this state when the sugar mass concentration is above the critical level and there is sufficient dissolved oxygen. In this state ethanol is produced.

– The second functional state (II) is called the *ethanol consumption state*. The process is defined to be in this state when ethanol is available but there is no sugar in the broth, and the dissolved oxygen concentration is above the critical level. Ethanol is the only carbon source for yeast growth.

As it could be seen from the experimental data (i.e. Fig. 2), between 16–th and 18–th cultivation hours there are about two hours, when the concentration of dissolved oxygen decreases below the critical level of 18 %. These values could be assumed as a sensor error. Moreover, there are no conditions for appearing of different functional state. The results achieved for parameter identification confirm that the supposed local model is sufficient to describe the process at this period, so there is no change of the local models at this time.

In principal, the functional state (I) can appear in all batch, fed-batch, and continuous yeast growth processes. The functional state (II) normally appears only in batch culture. 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.

In the first ethanol production state (I) *Zhang* et al.[2] assume that the specific growth rate is a constant due to dissolved oxygen limitation under high sugar mass concentration. Using the Monod's kinetics for the specific growth rate in the state (I) has given better results than constant specific growth rate μ_1. In the first ethanol production state (I) the specific rate of sugar consumption is described by Monod's kinetics. The specific ethanol production rate is directly proportional to the difference between the specific sugar consumption rate and the critical specific sugar consumption rate according to the mass balance and the stoichiometric equation of the fermentation of sugar to ethanol. To obtain more acceptable results, different yield coefficients are used in the ethanol dynamic equation (3), respectively Y_{ES} for state (I) and Y_{EX} in state (II), in the difference from *Zhang* et al.[2] The specific oxygen consumption rate is directly proportional to the specific growth rate, i.e. also constant, according to *Zhang* et al.[2] But, as it was mentioned above, using

the Monod's kinetics for the specific growth rate in the state (I) has given better results than constant specific growth rate μ_1. So, the specific oxygen consumption rate is described also by Monod's kinetics. As sugar is metabolized by yeast, the sugar concentration decreases to the critical level and then the process switches from the first ethanol production state (I) to the ethanol consumption state (II).

After entering state (II), the yeast cells begin to synthesize the enzymes for gluconeogenezis so that cells can utilize ethanol as the carbon-source for growth. It takes some time to synthesize the induced enzymes for gluconeogenezis. This causes a lag in the yeast growth. Hence in the state (II) Monod's kinetics with a lag term is used to describe the specific growth rate. The specific ethanol consumption rate and the dissolved oxygen concentration in state (II) are directly proportional to the specific growth rate. The specific sugar consumption rate is zero in this state. In the previous authors' work[8] the lag term was assumed to be a constant. In this paper, the lag term for specific growth rate of state (II) is assumed to be as follows[2]:

$$\eta = 1 - \exp\left(-\frac{t - t_{\mathrm{m}}}{t_1}\right), \tag{5}$$

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 parameter functions of the local models in the states (I) and (II) are presented in Table 1.

Table 1

Parameter functions	State I	State II
μ	$\mu_1 \frac{\gamma_{\mathrm{S}}}{\gamma_{\mathrm{S}} + K_{\mathrm{S}}}$	$\mu_2 \frac{\gamma_{\mathrm{E}}}{\gamma_{\mathrm{E}} + K_{\mathrm{E}}} \eta$
q_S	$\mu_1 \frac{\gamma_{\mathrm{S}}}{\gamma_{\mathrm{S}} + K_{\mathrm{S}}} Y_{\mathrm{SX}}$	0
q_E	$(q_{\mathrm{S}} - q_{\mathrm{Scrit}}) Y_{\mathrm{ES}}$	$-\mu_2 \frac{\gamma_{\mathrm{E}}}{\gamma_{\mathrm{E}} + K_{\mathrm{E}}} Y_{\mathrm{EX}} \eta$
q_{O_2}	$\mu_1 \frac{\gamma_{\mathrm{S}}}{\gamma_{\mathrm{S}} + K_{\mathrm{S}}} Y_{0\mathrm{X}}$	$\mu_2 \frac{\gamma_{\mathrm{E}}}{\gamma_{\mathrm{E}} + K_{\mathrm{E}}} Y_{0\mathrm{E}} \eta$

Here the mentioned above symbols have saved their meanings and the following new are involved:

μ_1, μ_2 – maximum specific growth rates, h^{-1}

K_S, K_E – saturation constants, g l^{-1}

Y_{SX}, Y_{ES}, Y_{OX}, Y_{EX}, Y_{OE} – yields, g g^{-1}.

Estimation of the local models parameters

Experimental data from three batch cultivations of *Saccharomyces cerevisiae*, obtained in *Institut für Technische Chemie, Universität Hannover, Germany,* are used. The experimental data contain off-line measurements of biomass (yeast), substrate (glucose) and ethanol and online measurements of dissolved oxygen. The first set of experimental data is used for local models' parameter estimation and the other two are used for models' verification. It is well-known that the kinetic parameters of the Monod type model could not be uniquely identified, especially from the noisy batch measurements. Due to the limited number of experimental data (between 10 and 15 points, taken approximately every half an hour) it is inappropriate some kind of filter to be applied because of possible loss of important information. In order to obtain more accurate estimations, the *genetic algorithms in MATLAB environment* are applied for the estimation of the local models' parameters.

As an optimization criterion, the residual sum of squares of the differences between the experimental data and data from simulated model is used:

$$\begin{aligned} J = {} & c_1(\gamma_{\mathrm{X}} - \gamma_{\mathrm{X}}^*)^T(\gamma_{\mathrm{X}} - \gamma_{\mathrm{X}}^*) + \\ & + c_2(\gamma_{\mathrm{S}} - \gamma_{\mathrm{S}}^*)^T(\gamma_{\mathrm{S}} - \gamma_{\mathrm{S}}^*) + \\ & + c_3(\gamma_{\mathrm{E}} - \gamma_{\mathrm{E}}^*)^T(\gamma_{\mathrm{E}} - \gamma_{\mathrm{E}}^*) + \\ & + c_4(w_{\mathrm{O}_2} - w_{\mathrm{O}_2}^*)^T(w_{\mathrm{O}_2} - w_{\mathrm{O}_2}^*) \end{aligned} \tag{6}$$

where

γ_{X}^*, γ_{S}^*, γ_{E}^* and $w_{\mathrm{O}_2}^*$ are the column vectors of experimental data;

γ_{X}, γ_{S}, γ_{E} and w_{O_2} – column vectors of simulated data;

c_i – weight coefficients of the differences between experimental data and model output.

The Runge-Kutta (namely *RK45*) integration algorithm is used for numeric simulation of the model. The switching between two functional states (I and II) is determined at the reaching of γ_{S} to zero. 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 (II) are the last simulated values in the previous functional state (I) so that the trajectories became continuous.

The values of the estimated parameters are presented in Table 2. Both the real cultivation trajectories and the simulated ones are presented in the Fig. 1 and Fig. 2.

Table 2

Quantities of state I	Estimated value	Quantities of state II	Estimated value
μ_1	0.3570 h^{-1}	μ_2	0.13832 h^{-1}
K_S	0.0714 g l^{-1}	k_E	0.18128 g l^{-1}
Y_{SX}	6.0162 g g^{-1}	Y_{EX}	2.0877 g g^{-1}
Y_{ES}	0.3288 g g^{-1}	Y_{OE}	8340 g g^{-1}
Y_{OX}	1500 g g^{-1}	t_1	5.8933 h
–	–	t_m	8.4 h
k_La	83.347 h^{-1}	k_La	83.347 h^{-1}

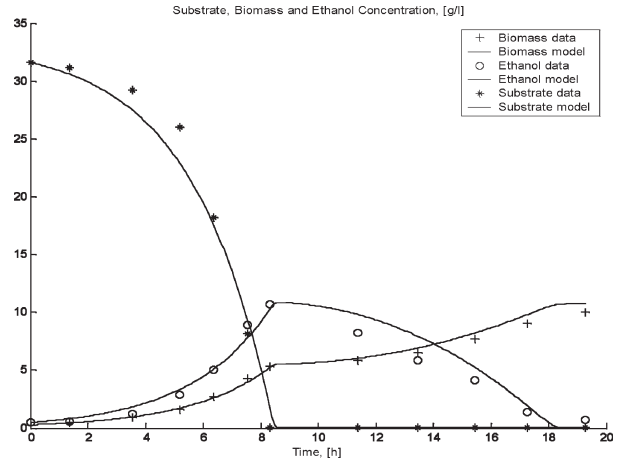


Fig. 1 – *Measured and simulated data for substrate, biomass and ethanol.*

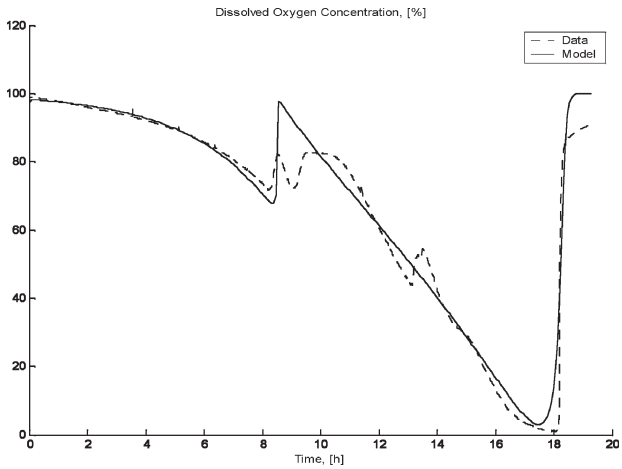


Fig. 2 – *Measured and simulated data for dissolved oxygen*

Presented results from the parameter identification of the developed model of batch cultivation of *S. cerevisiae*, based on the functional state modelling, show high degree of adequacy. As a proof for the model validity, the values of *Fisher criterion*[9] are presented in Table 3.

Table 3 shows the values of *Fisher criterion* for all considered process variables are below the table values. This fact confirms the model adequacy and the efficiency of the applied approach of functional state modelling.

Table 3

Value of *Fisher criterion* for substrate	1.2253
Table value at confidence probability 95 % (error probability 5 %)	1.75
Value of *Fisher criterion* for biomass	1.0066
Table value at confidence probability 95 % (error probability 5 %)	1.75
Value of *Fisher criterion* for ethanol	1.0720
Table value at confidence probability 95 % (error probability 5 %)	1.75

Moreover, to verify the obtained results, the simulation of the obtained model is compared with other two data sets from real batch cultivations of *S. cerevisiae*. Initial values of the used process variables are listed in Table 4.

Table 4

Initial conditions	Substrate γ_S/g l^{-1}	Biomass γ_X/g l^{-1}	Ethanol γ_E/g l^{-1}	Oxygen w_{O_2}/%
data set for identification	31.5992	0.2704	0.4702	97.47
data set 1 for verification	30.00	0.2576	0.1091	98.00
data set 2 for verification	32.5987	0.2002	0.3502	–

Both, the real cultivation trajectories and the simulated ones from the model are presented respectively in the Fig. 3 and Fig. 4 for substrate, biomass

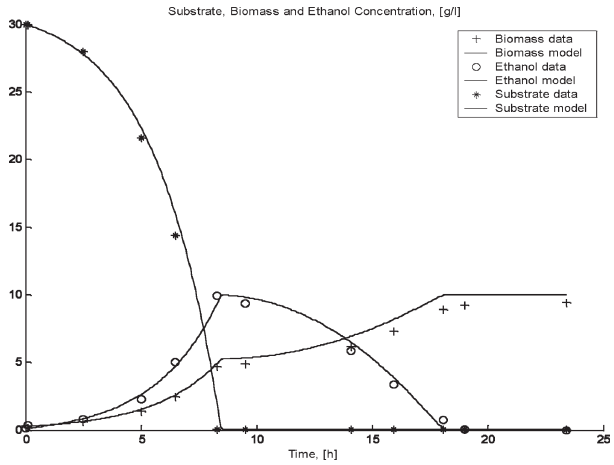
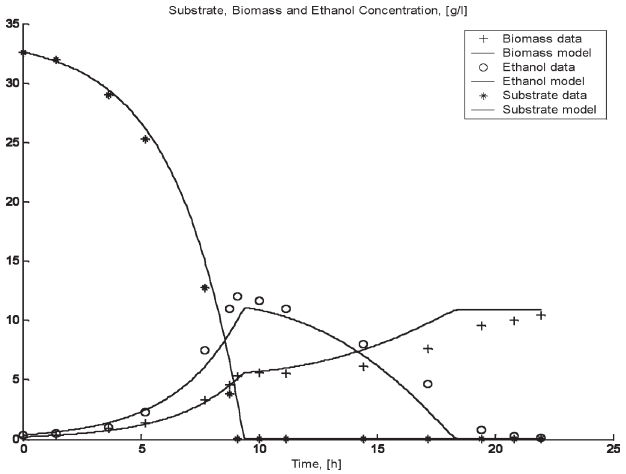
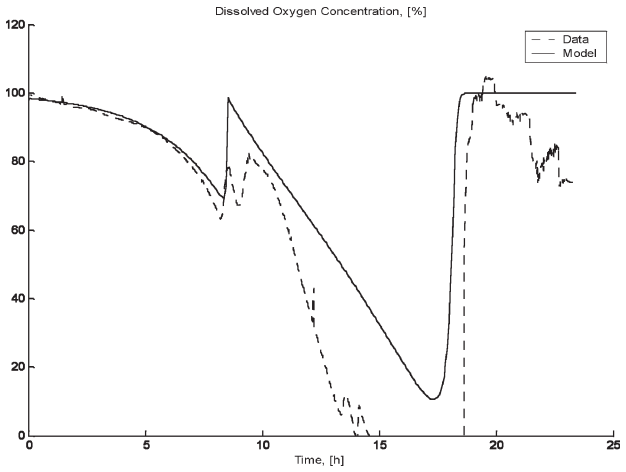


Fig. 3 – *Measured and simulated data for substrate, biomass and ethanol: verification (set 1)*



F i g . 4 – *Measured and simulated data for substrate, biomass and ethanol: verification (set 2)*



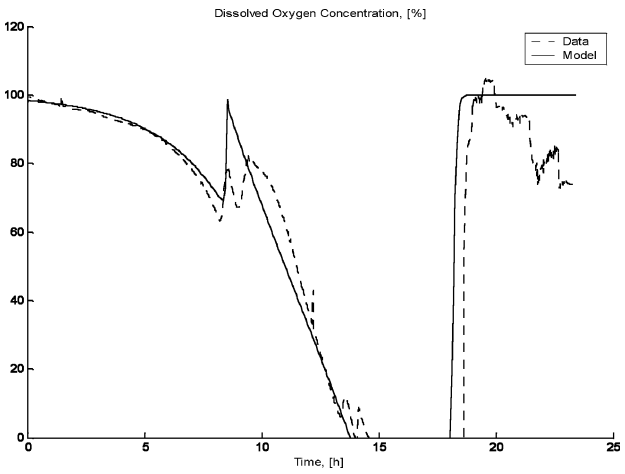
F i g . 5 – *Measured and simulated data for dissolved oxygen: verification (set 1)*

and ethanol, and in Fig. 5 for dissolved oxygen (for the last data set there is no experimental data for dissolved oxygen). The difference between the measured and simulated dissolved oxygen is mainly caused by the sensitivity of the volume transfer coefficient k_La from different physical parameters. The model simulation with different value of k_La or Y_{OE} gives more appropriate results (Fig. 6).

Analysis and conclusions

Functional state modelling approach, developed by *Zhang* et al., is successfully applied for description of real aerobic batch yeast growth process. Some changes in the local models' structures, in the comparison with the original ones presented by *Zhang* et al., are made in order to obtain better overlapping between experimental data and model.

Functional state modelling approach is more convenient than using of global model when parameter estimation should be made. The main advantage of functional state modelling is that the parameters of each local model can be separately estimated from the parameters of the other local models.



F i g . 6 – *Measured and simulated data for dissolved oxygen: verification (set 1) with different yield coefficient*

Functional state modelling approach is applied, based on the three experimental data sets of batch cultivation of *S. cerevisiae*. One of the experimental data sets is used for local models' parameter estimation and the other two are used for models' verification. Two functional states are recognized, based on the real data. The results obtained from the parameter identification and verification of the models show a good efficiency of the applied approach. As a proof for the model adequacy, the values of *Fisher criterion* are presented.

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List of symbols

γ_X – mass concentration of biomass, g l^{-1}
γ_S – mass concentration of substrate (glucose), g l^{-1}
γ_E – mass concentration of ethanol, g l^{-1}
w_{O_2} – mass fraction of dissolved oxygen, %
w_{O_2sat} – saturation mass fraction of dissolved oxygen, %
c_i – weight coefficients
μ, q_S, q_E, q_{O_2} – specific rates of, respectively, growth rate, substrate utilization, ethanol production and dissolved oxygen consumption, h^{-1}
μ_1, μ_2 – maximum specific growth rates, h^{-1}
K_S, K_E – saturation constants, g l^{-1}
Y_{SX}, Y_{ES}, Y_{OX}, Y_{EX}, Y_{OE} – yields coefficients, g g^{-1}
t_m – time point of involving in lag phase, h
t_l – length of lag phase, h
t – current time, h

Dimensionless symbols

J – optimization criterion

Superscripts and subscripts

* – experimental data

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