APPLICATION OF FUNCTIONAL STETE MODELLING APPROACH FOR YEAST SACCHAROMYCES CEREVISAE BATCH FERMENTATION STATE ESTIMATION

SANDIS VILUMS[1], OSKARS GRIGS[2]

[1] Biosystems Group, Faculty of Information Technology, Latvia University of Agriculture, Jelgava, Latvia
[2]Faculty of Material Science and Applied Chemistry, Riga Technical University, Riga, Latvia
sandis.vilums@gmail.com

Abstract: *Bioprocess requirements for monitoring, control and identification of process states are increasing to optimize performance and time to avoid disruption that may affect the success of the yeast Saccharomyces cerevisiae fermentation process. Continuous estimation of bioprocess state during fermentation requires to use accurate mathematical model and parameters that can describe the actual state of the yeast growth during the fermentation.*
Application of functional state modelling approach for the mathematical modelling of batch yeast Saccharomyces cerevisiae CEN.PK haploid fermentations is presented. Main advantage of the functional state modelling approach against global model with complex structure is that the parameters of each local model can be estimated separately from the other local models parameters. Functional modelling approach also makes it easier to perform model simulation and parameter estimation as compared to the complex global model. Obtained experimental data from yeast batch fermentations shows sufficiently good match with the curves simulated using functional state model.

Keywords: Bioprocess modelling, batch fermentation, state estimation.

Introduction

The yeast *Saccharomyces cerevisiae* is one of the most relevant microorganisms in the biotechnology industry. It has been used intensively for the production of single cell protein (SCP) for human and animal consumption, and ethanol for alcoholic drink and transport fuel usage from fermentable sugar. In view of increasing importance of ethanol, as an alternative source for chemicals and liquid fuel, a great deal of research interest in ethanol fermentation has been generated (Hunag et al., 2011; Kumoro et al., 2009).
It is valuable to accurately monitor and control biotechnological process (Mednis et al., 2010) to produce the target product (ethanol) for the highest quality and more profitable. For more accurate control it is useful to apply a metabolic model of the yeast in the bioprocess control algorithm that can provide the essential information about ongoing intracellular activities of the bioprocess (Viļums, 2011).
Continuous evaluation of process parameters during the cultivation is a need for a fermentation mathematical model that can simulate the process. The progress of the process can be adequately predicted in a timely manner. Bioreactor control program using the developed model on the basis can handle actuators to stabilize and maintain the progress of the process in the optimal mode. Models cannot only explain and reproduce observed behavior but also predict the evolution of the process. Models are useful for the estimation of parameters and variables. If the anomaly is detected in the functioning process then control system based on model can act on the process to steer and control its variables to the suitable state.
Mostly for bioprocess modeling are used global process models (Nagy, 2007; Renard et al., 2006). The main disadvantage of such approach is the complex model structure and the large number of model parameters, which complicates the model simulation and parameter estimation. The functional state modeling approach is alternative concept of process modeling. In functional state the process is described by local model, which is valid in actual state only. A set of local model together with function state equations can be used to describe, monitor and control the yeast growth process. Several authors have already showed benefits of this approach for more accurate and detailed process modeling (Hristozov et al., 2005; Pencheva and Hristozov, 2005; Pencheva et al.,, 2004; Roeva et al., 2007; Roeva et al., 2006).
This article aims to demonstrate the functional modeling approach benefits of yeast batch fermentation process modeling to use it as base for yeast growth state estimation. This approach in case of good model and experimental data coincide can be used not only for process predictions but also for fermenter control to stabilize process early enough.

Matherials and methods

1. Batch fermentation

The organism used in this study was *Saccharomyces cerevisiae* CEN.PK haploid. 2 litre of production medium was prepared according to the requirements of *S. cerevisae*, containing glucose 20.0 gL-1, peptone “Biolife” 20 gL-1, yeast extract “Sifin” 10 gL-1. The medium was sterilized in autoclave for 30 min at 110 ˚C. At the

beginning of fermentations initial OD was 0.20 – 0.25. The batch fermentations were carried out in a stirred tank fermenter (BTC EDF 5.3.1.), with a working volume of 4.5 litres. Temperature was controlled at 30.0 ± 0.2 ˚C, pH level at 5.5± 0.5, dissolved oxygen partial pressure at 60 ± 5 %. Agitation was ensured with two "rushton" style impellers at 600 rpm and airflow was ensured to 3 slpm. 10 ml of sample were taken every 1.5 hours for the entire fermentation cycle, which was terminated after 9.5 hours. Yeast biomass growth was evaluated by spectrophotometric measurements at 600 nm in a *Helios Epsilon* UV-visible spectrophotometer. The concentration of glucose was determined used glucose measurement device *Accu-Chek Active*. The concentration of ethanol in the medium was determined by liquid chromatography (HPLC).

The pre-culture of the yeast *Saccharomyces cerevisiae* CEN.PK haploid was developed by inoculating 100 ml partially defined medium containing glucose, yeast extract, salts and trace elements with 0,15 ml of frozen culture at 37 ˚C in shaker flasks. 2 L of initial batch culture for fermentation was derived by inoculating overnight 100 ml (14 – 16 h, OD=2.2 – 2.7) flask pre-culture in batch medium. Dry pellets of *S. cerevisiae* were used for inoculation in yeast fermentations directly to batch medium with initial biomass concentrations of ~4 g/L.

2. Fermentation modeling

Dynamic mass balances are the traditional chemical engineering approach to state estimation in bioreactors. Approach uses dynamic balances at the reactor scale and reasonable assumption regarding the regulatory structure of the organism. Making accurate measurements of bioreactor process variables, the possibility of using mass balance models has a high chance of success (Komives and Parker, 2003).

The rates of cell growth, glucose consumption, ethanol production, oxygen concentration and volume are described for all functional states with mass balance equations as follows:

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

$$\frac{dS}{dt} = -q_S X \tag{2}$$

$$\frac{dE}{dt} = q_E X \tag{3}$$

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

$$\frac{dV}{dt} = F_A + F_B + F_{ANT} - F_{SMP} - F_E - F_C \tag{5}$$

where X - the concentration of biomass, g·L-1;
μ – specific cell growth rate, h-1;
S - concentration of substrate (glucose), g·L-1;
q_S – specific substrate consumption rate, h-1;
E - ethanol concentration, g·L-1;
q_E - specific ethanol production rate, h-1;
O – oxygen solubility, g·L-1;
q_O - specific oxygen consumption rate, h-1;
$k_L a$ - volumetric oxygen transfer coefficient, h-1;
O^* - maximal solubility of oxygen, g·L-1;
V - volume, L;
F_A – acid consumption rate, L·h-1;
F_B – base consumption rate, L·h-1;
F_{ANT} – antifoam consumption rate, L·h-1;
F_{SMP} - sampling rate, L·h-1;
F_E – evaporation rate, L·h-1];
F_C – carbon loss rate, L·h-1.

A substrate such as glucose is consumed by yeast to produce a number of carbon intermediates as well as to provide energy. The yeast then utilizes the carbon intermediates to synthesize a new cell material. If the sugar concentration in the broth in an aerobic yeast growth process exceeds a certain level, called the critical sugar mass concentration (S_{crit}), a part of the sugar is metabolized in ethanol. In the case of batch cultivation S_{crit} is assumed to be zero. A critical level of dissolved oxygen concentration for yeast growth process is assumed to be 18%. The whole yeast growth process can be divided into at least five functional states in batch and fed-batch cultures (Pencheva et al., 2004; Roeva et al., 2006).

In the case of batch cultivation two phases are identified (Fig. 1.):

1. The first functional state (I) is 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.
2. 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.

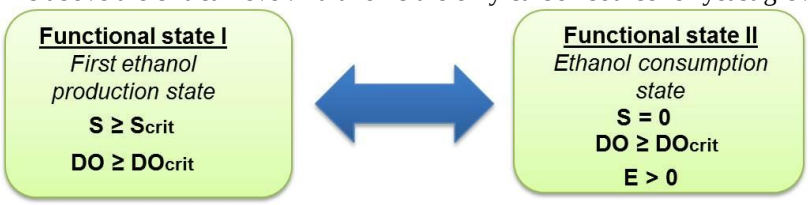

Fig. 1. **Identified functional states of the yeast batch cultivation**

(Roeva et al., 2006) assume that 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. Parameter functions of the local models in the states (I) and (II) are presented in Table 1.
After entering state II yeast cell begin to synthesize the enzymes for gluconeogenezis so that cells can utilize ethanol as the carbon-source for growth. This causes a lag in the yeast growth. The lag term for functional state can be calculated by equation (1).

Table 1

Parameter functions of the local models (Tania Pencheva et al., 2004)

Parameter functions	**State I**	**State II**
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	$\mu_1 \frac{S}{S+k_S}Y_{OX}$	$\mu_2 \frac{E}{E+k_E}Y_{OE}\eta$

where μ_i – maximum specific growth rates, h-1;
k_S, k_E – saturation constants, g·L-1;
Y_{SX}, Y_{ES}, Y_{EX}, Y_{OX}, Y_{OE} – yield coefficients, g·g-1;
η – lag term, h.

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

where t – the current process time, h;
t_m – time point of involving in lag phase, h;
t_1 – the length of lag phase, g·g-1.

Results and discussion

The functional state model of yeast batch process was developed in MATLAB environment. The model consists of script M-Files and experimental data CSV files. Experimental data obtained from two batch fermentations of yeast *Saccharomyces cerevisiae* CEN.PK haploid. The experimental data contains on-line measurements of pO2. Off-line data include measurements of biomass, substrate (glucose) and ethanol.
The first batch experiment measured and model simulated curves are presented in the Fig 2.

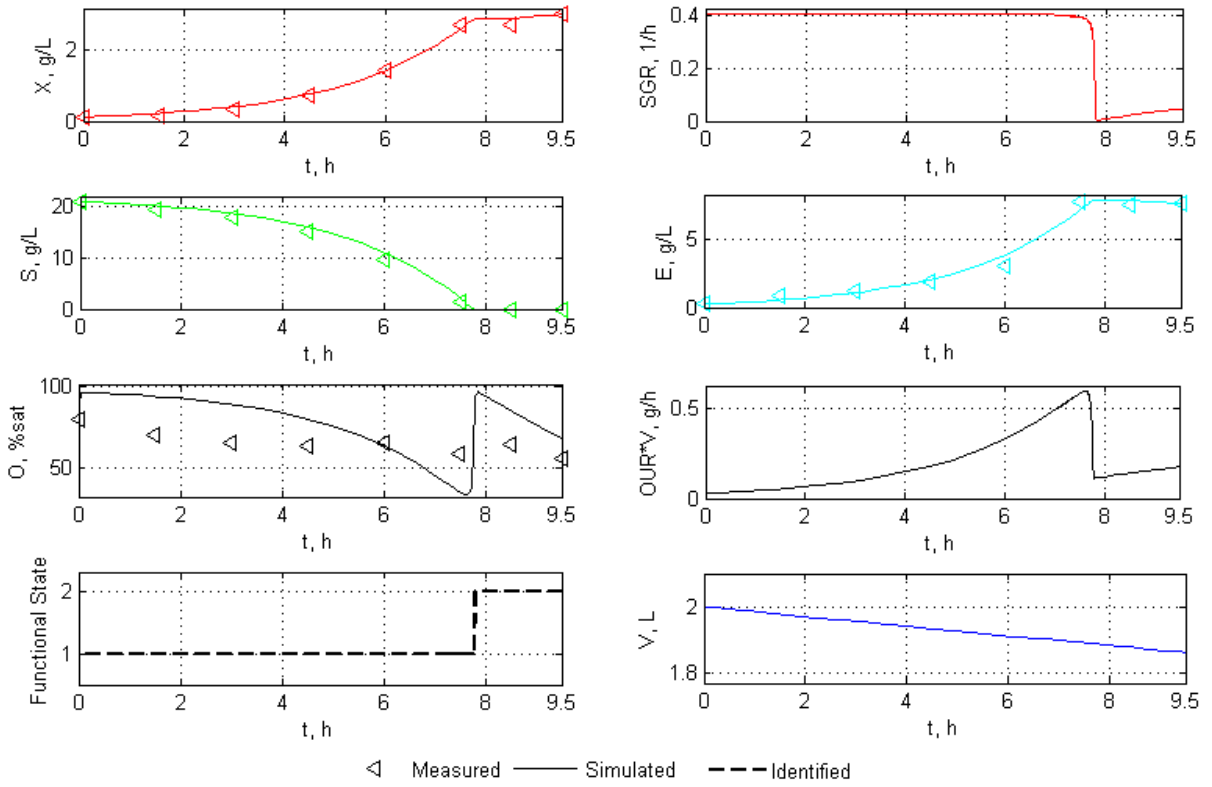

Fig. 2. **1st Batch fermentation measured, model simulated and identified curves**

The process was carried out in 9.5 hours. Model simulated curves X (biomass), S (substrate), E (ethanol) shows good accordance with a measured experimental data. The actual functional state is identified automatically from actual substrate and dissolved oxygen saturation concentration. The functional state plot (Fig. 2) shows that switching between the 1st to the 2nd functional state is approximately at 7.8 process hour. Then substrate concentration has reached the critical value. The estimated model parameters can be seen in Table 2.

Table 2

1st batch experiment estimated values of functional state parameters

Parameters of State I	Estimated value	Parameters of State II	Estimated value
$mumax_1$	0.41	$mumax_2$	0.158
K_S	0.0714	K_E	0.181
Y_{SX}	7.7	Y_{EX}	2.07
Y_{ES}	2.8	Y_{OE}	0.9
Y_{OX}	0.25	t_1	3.7
k_La	55	t_m	7.8
-	-	k_La	55

The second batch experiment measured and model simulated curves are presented in the Fig 3. The process was carried out in 11.5 hours. Model simulated curves X, S, E shows good accordance to a measured experimental data. The actual functional state is identified automatically from actual substrate and dissolved oxygen saturation concentration. The functional state plot (Fig. 3) shows that switching from the 1st to the 2nd functional state didn't occur because process substrate and dissolved oxygen concentration was all the process time above critical values.

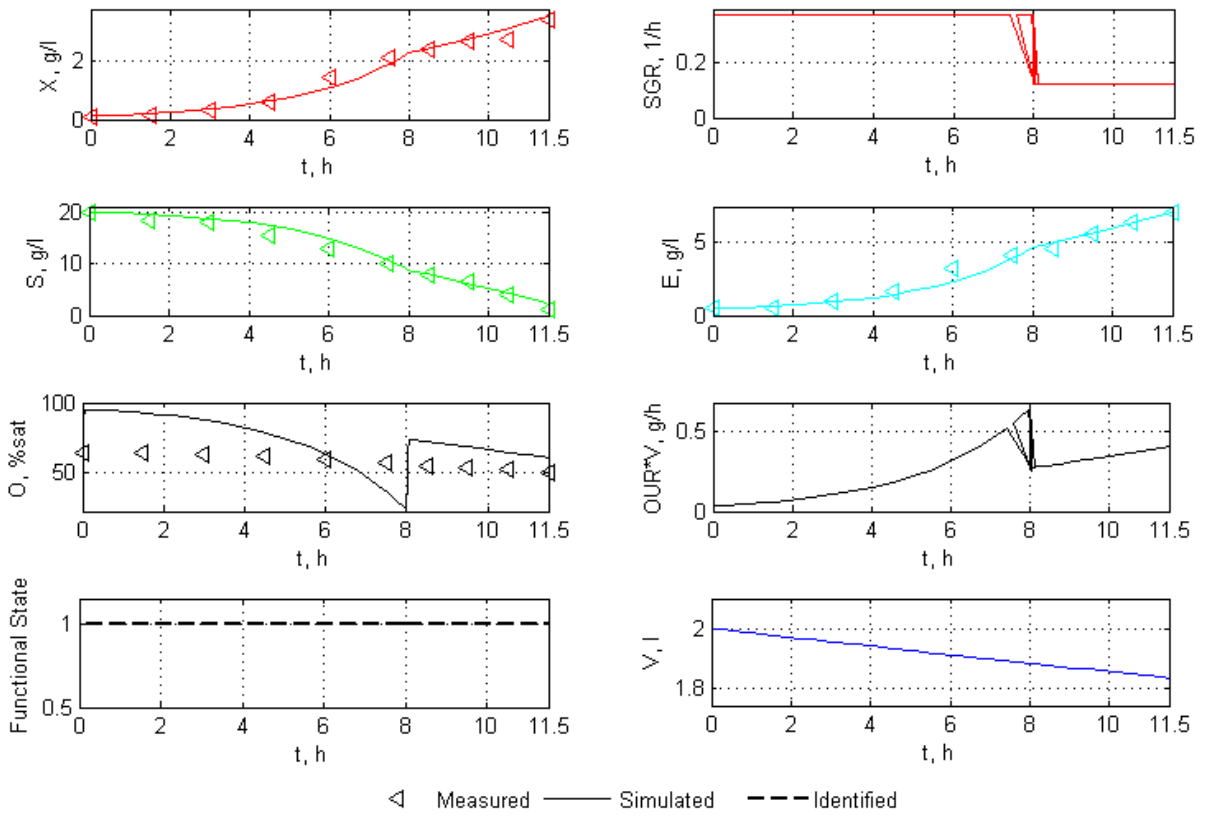

Fig. 3. **2nd batch fermentation measured, model simulated and identified curves**

Parameters of the actual functional state were manually estimated by changing parameter value to reach the best experimental data and model simulated curve consistency. The estimated model parameters are shown in Table 3. The 2 mumax coefficients where estimated in the 1st functional state. Beginning of the process effective growing was observed and mumax value was estimated 0.376 but after the 8th hour the growing was decreased and was estimated mumax value 0.125. Other parameters were similar to the 1st batch process (see Fig 2.).

Table 3

2nd batch experiment estimated values of functional state parameters

Parameters of State I	Estimated value
$mumax_{11}$	0.376
$mumax_{12}$	0.125
K_S	0.0714
Y_{SX}	5.15
Y_{ES}	1.9
Y_{OX}	0.35
k_La	55

Conclusion

The application of the functional state modeling approach for two yeast batch fermentations modeling were presented in this paper. This approach shows good benefits for using it as base for the yeast batch fermentation model based control system development. The yeast growth functional state estimation is valuable to find out appropriate parameters that can adequately describe the actual physiological state of yeast growth process. Model simulated curves for X (biomass), S (substrate) and E (ethanol) showed good accordance to measured experimental and model simulated data for both batch fermentations. The greater deviation from measured and model simulated curves was observed with dissolved oxygen partial pressure value due to problems to identify a correct mass transfer coefficient value and functional state parameters. The functional state modelling approach parameters for actual functional state were manually estimated by changing parameter value to reach the best experimental data and model simulated curve consistency. In future is planned to improve yeast batch fermentation model with automatic functional state parameter estimation.

Acknowledgements

Paper becomes written by financial support of European Structural Fund – Project "Establishment of Latvia interdisciplinary interuniversity scientific group of systems biology" – realized by Latvia University of Agriculture (contract no. 2009/0207/1DP/1.1.1.2.0/09/IPIA/VIAA/128).

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