Bioautomation, 2005, 2, 8-16 ISSN 1312-451X

Modelling of Functional States during *Saccharomyces cerevisiae* Fed-batch Cultivation

Tania Pencheva[*1], Iasen Hristozov[1], Dirk Huell[2], Bernd Hitzmann[2], Stoyan Tzonkov[1]

[1]*Centre of Biomedical Engineering "Prof. Ivan Daskalov" - Bulgarian Academy of Sciences*
105 Acad. G. Bonchev Str., 1113 Sofia, Bulgaria
E-mail: tania.pencheva@clbme.bas.bg, hristozov@gbg.bg, tzonkov@clbme.bas.bg

[2]*Institut für Technische Chemie, Universität Hannover*
3 Callinstr., 30167 Hannover, GERMANY
E-mail: huell@iftc.uni-hannover.de, hitzmann@iftc.uni-hannover.de
[*] Corresponding author

Received: December 14, 2004 **Accepted: March 31, 2005**

Published: April 28, 2005

Abstract: *An implementation of functional state approach for modelling of yeast fed-batch cultivation is presented in this paper. Using of functional state modelling approach aims to overcome the main disadvantage of using global process model, namely complex model structure and big number of model parameters, which complicate the model simulation and parameter estimation. This approach has computational advantages, such as the possibility to use the estimated values from the previous state as starting values for estimation of parameters of a new state. The functional state modelling approach is applied here for fed-batch cultivation of Saccharomyces cerevisiae. Four functional states are recognised and parameter estimation of local models is presented as well.*

Keywords: *Yeast fed-batch cultivation, Modelling, Functional state modelling approach.*

Introduction

More complex plants, advances in information technology, and tightened economical and environmental constraints in recent years have lead to practising engineers being faced with modelling and control problems of increasing complexity. When confronted with such problems, there is a strong intuitive appeal in building systems which operate robustly over a wide range of operating conditions by decomposing them into a number of simpler modelling or control problems, even for nonlinear modelling or control problems. This appeal has been a factor in the development of increasingly popular functional state modelling approach to coping with strongly nonlinear and time-varying systems [1]. Such local approaches are directly based on the divide-and-conquer strategy [1], in the sense that the core of the representation is a partitioning of the system's full range of operation into multiple smaller operating regimes each of which is associated with a locally valid model or controller. In addition, the local approach has computational advantages, lends itself to adaptation and learning algorithms and allows direct incorporation of high-level and qualitative plant knowledge into the model.

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 fermentation 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 fermentation 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 [2]. 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. As an alternative approach, functional state modelling approach will be here presented. Four functional states are recognised during fed-batch cultivation of *Saccharomyces cerevisiae* and parameter estimation of local models is presented.

Functional state modelling approach for yeast cultivation

Functional state modelling approach is a concept, which helps in monitoring and control of complex processes such as bioprocesses [1, 3]. The main idea is to use a two-level hierarchy where at the first level the process is divided into macrostates, called *functional states* (FS), according to behavioural equivalence. In each FS certain metabolic pathways are active enough to dominate the overall behaviour of the process. In each FS the process is described by a conventional type of model, called *local model*, which is valid only in this FS. At the second hierarchical level some numeric detection algorithms and/or rules based on expert knowledge can be used for the recognition of the FS and state transitions. A set of local models together with FS "dynamics" can be used to describe, monitor and control the overall yeast growth process.

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 FS in batch and fed-batch cultures [3], namely:

- *FS I - first ethanol production state.*
- *FS II - mixed oxidative state.*
- *FS III - complete sugar oxidative state.*
- *FS IV - ethanol consumption state.*
- *FS V - second ethanol production state.*

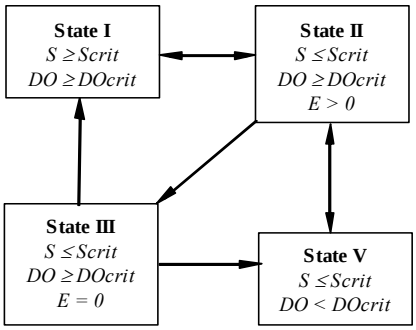

Fig. 1 Functional states and their relations in fed-batch yeast process

Fig. 1 illustrates the metabolic characteristics and interrelationships of the different FS (FS IV normally appears only in batch culture, so that is the reason that it is omitted in that figure).
It is obvious that the bioprocess can be only in one FS at any given time, although a certain FS may appear more than once during the total growth period. An aerobic baker's yeast process switches relatively abruptly from one FS to another when the metabolic conditions change. To detect when the process is in a certain FS might be a non-trivial task.

Modelling of functional states in fed-batch cultivation of *S. cerevisiae*

Materials and Methods

The cultivation of the yeast *Saccharomyces cerevisiae* is performed in a 2 l reactor, developed at the *Institute of Technical Chemistry, University of Hannover, Germany*, using a *Schatzmann medium*. For the process control a *BiostatB unit* from *B. Braun* is used. Before the fed-batch, a batch cultivation was carried out to get an appropriate amount of biomass. After the end of the batch, a break of 4 hours is made to get a defined metabolic state. The values of the process parameters used for both cultivations are listed in Table 1. In the exhaust gas carbon dioxide and oxygen are measured using the Process-analyze system *"Advance Optima/ Uras 14E"* (*Hartmann & Braun*). Off-line samples are collected almost every hour. From the off-line samples the dry biomass concentration, as well as the concentration of ethanol and glucose, are measured. The glucose concentration is measured using a glucose analyzer (YSI 2700, Yellow Springs Instruments). For the determination of ethanol a gas chromatograph (GC-14B, Shimadzu) is used. The dry biomass concentration is measured by separating the cells by centrifuge. After drying for 24h at 110°C the biomass is measured by weighing the tubes.

Table 1. Process parameters

Parameter	Value
Aeration rate	300 L/h
Stirrer speed	1200 rpm
Temperature	30 °C
pH	5.5
Start volume	1.3 l
Initial value of biomass	4.5 g/l
Glucose concentration in feed	35 g/l

For glucose measurements a flow injection analysis (*FIA*) system, developed by *ANASYSCON Hannover, Germany*, is employed, which uses a sampling probe (*Flownamics E19*) to get cell-free samples for the FIA. Employing an extended Kalman filter the state variables biomass, glucose concentration as well as μ_{max} (*Monod* model) and volume are estimated. Based on the glucose estimation a feedforward-feedback controller is implemented to establish the different setpoints between 0.04 and 0.09 g/l.

The determination of the time-delay is carried out in a simple reactor by measuring the changes of the conductivity. The reactor contains 200ml distilled water. At the start of the measurement 20ml of 1mol KCl solution are added as a pulse. The change of the conductivity was measured. From the plots of the conductivity vs. time the time delay is calculated.

The bioreactor, as well as FIA measurement system is presented in Fig. 2. Fig. 3 show the computers used for the data measurement from the FIA system, as well as computer for the control of the process.

Fig. 2

Fig. 3

Model of fed-batch cultivation of S. cerevisiae

Experimental data from fed-batch cultivation of baker's yeast is used. The used data set consists of off-line measurements of biomass (yeast) and ethanol and on-line measurements of substrate (glucose).

The rates of cell growth, sugar consumption and ethanol production in a yeast fed-batch growth process are commonly described for all FS according to the mass balance as follows:

$$\frac{dX(t)}{dt} = \mu(S)X(t) - \frac{\dot{V}(t)_{feed}}{V(t)}X(t) + \frac{\dot{V}_{sample}}{V(t)}X(t) \quad (1)$$

$$\frac{dS(t)}{dt} = -q_S X(t) + \frac{\dot{V}(t)_{feed}}{V(t)}\left(S_{in} - S(t)\right) \quad (2)$$

$$\frac{dE(t)}{dt} = q_E X(t) - \frac{\dot{V}(t)_{feed}}{V(t)}E(t) \quad (3)$$

$$\frac{dV}{dt} = \dot{V}(t)_{feed} - \dot{V}_{sample} \quad , \tag{4}$$

where:
X, S and E are the concentrations of biomass, substrate (glucose) and ethanol, [g/l];
V - bioreactor volume, [l];
$\dot{V}(t)_{feed}$ - feeding rate, [l/h];
$\dot{V}_{sample}$ - taking on-line samples, [l/h];
S_{in} - initial concentration of the feeding solution, [g/l];
μ, q_S, q_E - parameter functions, varying with the functional state transitions.

Analysing experimental data and assuming that *Scrit* has been changed during the cultivation, there are sufficient conditions for availability of four FS during this cultivation, namely:
A: complete sugar oxidative state (FS III)
B: first ethanol production state (FS I)
C: first ethanol production state (FS I), after changing of Scrit
D: mixed oxidative state (FS II)

All parameter functions of the local models for four recognised FS are presented in Table 2.

Table 2. Local models in different functional states

	FS A	**FS B**	**FS C**	**FS D**
μ	$\mu_3 \frac{S}{S+k_S}$	$\mu_1 \frac{S}{S+k_S}$	$\mu_1 \frac{S}{S+k_S}$	$\mu_{2S} \frac{S}{S+k_S} + \mu_{2E} \frac{E}{E+k_E}$
q_S	$\frac{\mu_3}{Y_{SX}} \frac{S}{S+k_S}$	$\frac{\mu_1}{Y_{SX}} \frac{S}{S+k_S} \frac{E}{E+k_E}$	$\frac{\mu_1}{Y_{SX}} \frac{S}{S+k_S}$	$\frac{\mu_{2S}}{Y_{SX}} \frac{S}{S+k_S}$
q_E	0	$\frac{\mu_1}{Y_{ES}} \frac{S}{S+k_S}$	$(q_S - q_{Scrit}) Y_{ES}$	$-\frac{\mu_{2E}}{Y_{EX}} \frac{E}{E+k_E}$

In the Table 2 the following symbols are used:

- μ_i - maximum values of the corresponding specific growth rates, [h^{-1}];
- k_S, k_E - saturation constants, [g/l];
- Y_{SX}, Y_{ES}, Y_{EX} - yield coefficients, [$g.g^{-1}$].

The estimation of the local models' parameters is made with using of *MATLAB Optimisation Toolbox procedures*. The *RK45 integration algorithm* is used for numeric simulation of the model. 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:

$$J = c_1 \left(X - X^*\right)^T \left(X - X^*\right) + c_2 \left(S - S^*\right)^T \left(S - S^*\right) + c_3 \left(E - E^*\right)^T \left(E - E^*\right), \tag{5}$$

where X^*, S^* and E^* are the column vectors of experimental data, X, S and E are the column vectors of simulated data and c_i are weight coefficients. The values of estimated parameters are presented in Table 3.

Table 3. Values of the estimated parameters in different functional states

Name	***FS A***	***Name***	***FS B***	***Name***	***FS C***	***Name***	***FS D***
μ_3	0.14 h^{-1}	μ_1	0.29 h^{-1}	μ_1	0.43 h^{-1}	μ_{2S} μ_{2E}	0.45 h^{-1} 0.14 h^{-1}
k_S	0.05 gl^{-1}	k_S Y_{SX}	0.05 gl^{-1} 0.18 gg^{-1}	k_S	0.05 gl^{-1}	k_S k_E	0.06 gl^{-1} 0.88 gl^{-1}
Y_{SX}	0.54 gg^{-1}	k_E Y_{ES}	0.31 gl^{-1} 5.84 gg^{-1}	Y_{SX} Y_{SE}	0.46 gg^{-1} 34.14 gg^{-1}	Y_{SX} Y_{EX}	0.31 gg^{-1} 32.43 gg^{-1}

Description of recognized functional states

FS A: The process is defined to be in this state when there is no ethanol available, the sugar concentration is no more than the critical level and the dissolved oxygen is above its critical level. During parameter identification of this state, the original local models, presented by Zhang [3] have been used. Local model functions for this state (as well as for the other states) are presented in Table 2. The values of estimated parameters for this state (as well as for the other states) are listed in Table 3. Both the real cultivation trajectories and the simulated ones for each FS are presented in the following figures, respectively Fig. 4 for FS A, Fig. 5 for FS B, Fig. 6 for FS C and Fig. 7 for FS D. With small letters *a, b* and *c* are noted consequently substrate, biomass and ethanol.

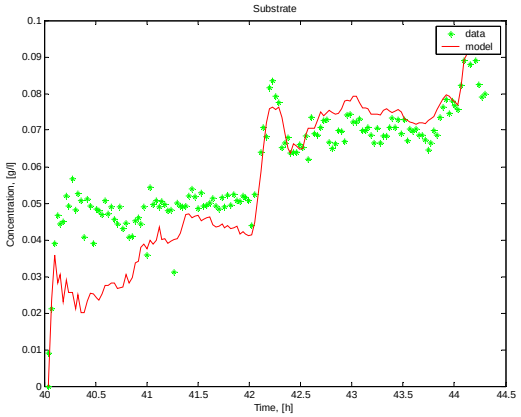

Fig. 4.a Substrate

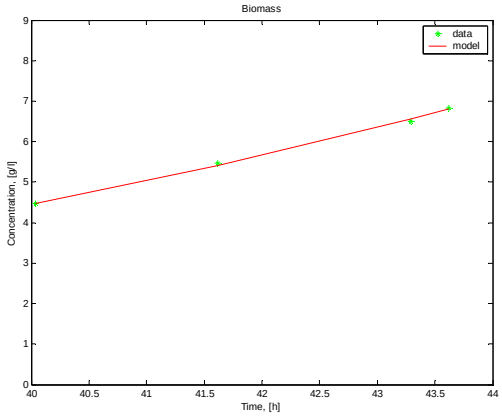

Fig. 4.b Biomass

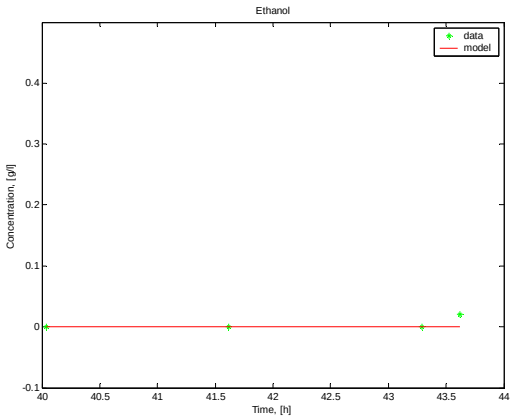

Fig. 4.c Ethanol

Fig. 4.a,b,c Measured and simulated data for FS A

FS B: The process is defined to be in this state when the sugar concentration is above its critical value and there is sufficient dissolved oxygen. During parameter identification of this state, all original local models, presented by Zhang [3] have been changed. Using *Monod*' kinetics instead of proposed constant value for biomass concentration gives more meaningful results. Using of *Monod*' kinetics for the specific rate of sugar consumption does not succeed to describe the process behaviour. Many other specific rates had been applied without a big success. At the moment using of *Aborhey and Williamson's* kinetics describes in a sufficient degree the process behaviour in this state. Perhaps some other decisions have to be further discussed. Better results are achieved by using of *Monod* kinetics also for specific ethanol production rate, instead of proposed by Zhang rate, directly proportional to the difference between the specific sugar and the critical sugar specific consumption rate.

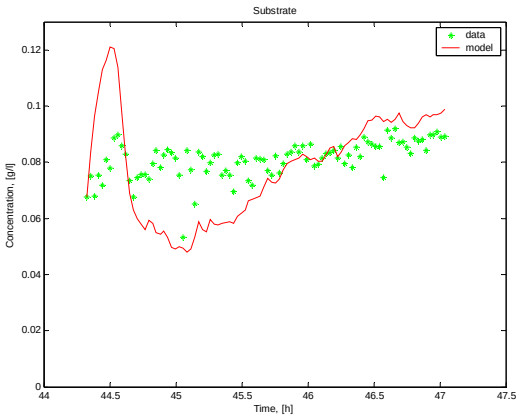

Fig. 5.a Substrate

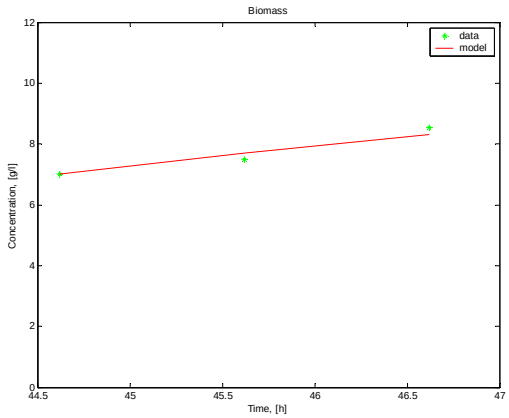

Fig. 5.b Biomass

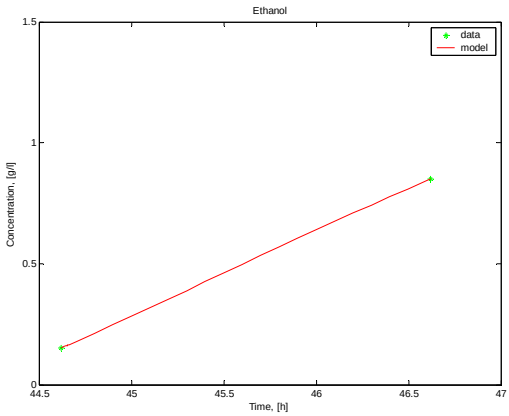

Fig. 5.c Ethanol

Fig. 5.a,b,c Measured and simulated data for FS B

FS C: The process follows to be at the same conditions as FS B. It is supposed that because of changing of *Scrit* this state should be considered as new. Moreover, the local models, which describe this state, are different from those, used in FS B, and the rate of ethanol production is different in both states (Table 2 and Table 3). Only the specific growth rate of biomass has been changed again with *Monod*' kinetics instead of constant value (as it was in FS B as well). The specific substrate consumption rate as well as specific ethanol production rate is equal to the originals presented in Zhang [3].

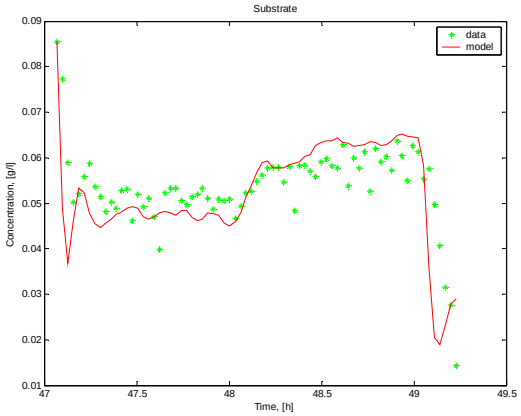

Fig. 6.a Substrate

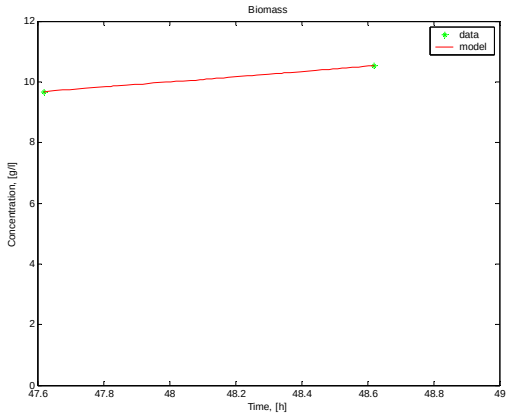

Fig. 6.b Biomass

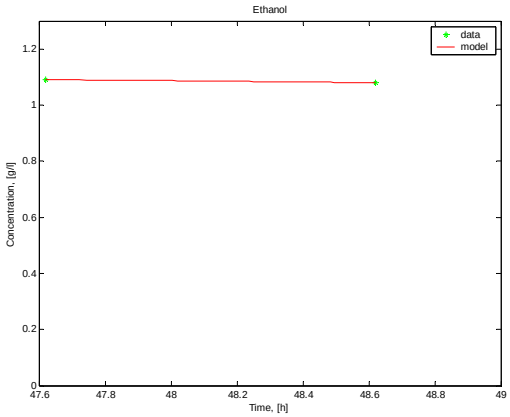

Fig. 6.c Ethanol

Fig. 6.a,b,c Measured and simulated data for FS C

FS D: 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. During parameter identification of this state, the original local models, presented by Zhang [3] have been used.

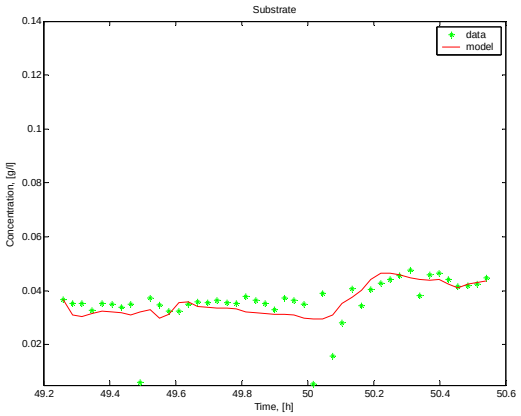

Fig. 7.a Substrate

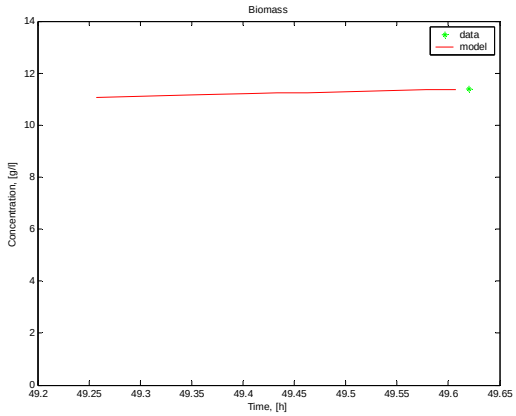

Fig. 7.b Biomass

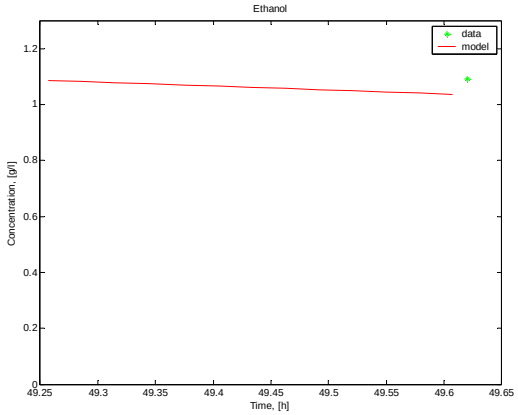

Fig. 7.c Ethanol
Fig. 7.a,b,c Measured and simulated data for FS D

Conclusions

The concept of functional state approach and its application for modelling of aerobic fed-batch yeast cultivation are presented in this paper. The concept's implementation leads to the process description with simpler and more transparent local models. Moreover, the implementation of FS modelling approach has computational advantages and allows direct incorporation of high-level and qualitative plant knowledge into the model. These advantages have proven to be very appealing for industrial applications.

The work process shows that the FS modelling approach should be applied for such a complex cultivation, because of proved changes of the process parameters during the time of the cultivation. Four FS have been recognized and the estimation of state parameters has been presented. This fact proves the implementation of FS modelling approach as more convenient for parameter estimation than the global models of this process. The main advantage of FS modelling is that parameters of each local model could be separately estimated from other local models. The results obtained in the simulation of models show a good efficiency of the approach for functional state modelling.

Acknowledgements

This work is partially supported from DFG Project №113/135, NSF-TN-1314/2003 and Project №03-1046/2003 with Thracian University- Stara Zagora.

References

1. Murray-Smith R., T. A. Johansen (1997). Multiple Model Approaches to Modelling and Control, Taylor and Francis, London.
2. Sonnleitner B., O. Kappeli (1986). Growth of Saccharomyces cerevisiae is controlled by its limited respiration capacity: formulation and verification of a hypothesis, Biotechnology and Bioengineering, 28, 927-937.
3. Zhang X.-Ch., A. Visala, A. Halme, P. Linko (1994). Functional State Modelling Approach for Bioprocesses: Local Models for Aerobic Yeast Growth Processes, Journal of Process Control, 4(3), 127- 134.