Model Based Control of Large Scale Fed-Batch Baker's Yeast Fermentation

Akif Hocalar and Mustafa Türker

Pakmaya, P.K. 149, 41001 İzmit, Kocaeli, Türkiye
akifh@pakmaya.com.tr
mustafat@pakmaya.com.tr

Abstract : Two different control methods are applied to the technical scale (25 m^3) fed-batch baker's yeast fermentation. Feedback linearizing control design is used to manipulate the substrate feeding rate in order to maximize the biomass yield and minimizing the production of ethanol. Firstly, the specific growth rate controller is developed and applied to maintain the specific growth rate at specified trajectory. Secondly, the minimal ethanol controller is developed to maximize biomass productivity, by controlling specific growth rate just above the maximum oxidative growth rate by controlling ethanol concentration. The both controllers worked successfully and can be combined to follow required specific growth rate trajectory and respond successfully to disturbances in overflow fermentations such as *Saccharomyces cerevisiae.*

Keywords: Nonlinear control, feedback linearizing control, fed-batch, baker's yeast, specific growth rate, ethanol concentration, biocalorimetry.

1. INTRODUCTION

Fed-batch bioprocesses have extensive applications in industry for production of baker's yeast, enzymes, antibiotics, growth hormones, microbial cells, vitamins, amino acids and other organic acids (Perulekar and Lim, 1985; Yamane and Shimizu, 1984). *Saccharomyces cerevisiae* is used in many applications such as beverage products (beer, wine), baker's yeast for bread production, heterologous protein production, bio-transformations, flavour components, single cell protein, bio-ethanol, glycerol and food additives (Walker, 1998; Renard and Wouwer, 2008). The specific growth rate is a key variable for the growth-associated biotechnological processes and determines the physiological state of the cells and the capacity of cell's protein-synthesizing machinery that is important for recombinant protein production or biomass production in several fermentations (Cannizzaro et. al., 2004; Gnoth et. al., 2008). Similarly, *Escherichia coli* show similar metabolic behaviour in the presence of excess substrate and shortage of oxygen. In the production of recombinant proteins with *E. coli*, acetate is produced as an overflow metabolite both when *E.coli* grown under anaerobic or oxygen limiting conditions. It is important to maintain the specific growth rate below a certain threshold in order to avoid the accumulation of acetate throughout the fermentation (Rocha and Ferreira, 2002; Jana and Deb, 2005).

In the literature, many works have been reported by several authors for the control of fed-batch fermentation, most of these studies report experimental results either at laboratory scale or simulation results (Chen and Bastin, 1995; Pomerleau and Viel, 1992; Soons et al., 2006; Rocha and Ferreira, 2002; Cannizzaro et al., 2004; Valentinotti et al., 2003).

The main objective of this study is to develop a robust control scheme to cope with changing process dynamics during fermentation, set point tracking, required minimum process measurements and batch-to-batch consistency for the technical scale fed-batch baker's yeast fermentation. The control methods are based on previously developed and verified state estimation model and reliable measurement system (Hocalar et. al., 2006).

In this work, different key process variables are controlled with the state feed-back linearizing control scheme at technical scale fermentations: 1. nonlinear control of specific growth rate, 2. nonlinear control of ethanol concentration. In the first part, the derivation of nonlinear specific growth rate controller ant its results are discussed. The restrictive conditions of the controlling of specific growth rate are given at the end of first section. In the second part, the control of nonlinear ethanol concentration is presented. By means of controlling of overflow metabolite concentration at minimal concentration, specific growth rate can be maintained near the maximum values.

2. STOICHIOMETRY OF THE PROCESS

General stoichiometry of the baker's yeast fermentation process can be written with respect to reaction rates as (Türker, 2003; Türker, 2004):

$$r_s CH_2ON_{0.0125} + r_n NH_3 + r_o O_2 \rightarrow r_x CH_{1.8}O_{0.5}N_{0.2} + r_p CH_3O_{0.5} + r_c CO_2 + r_w H_2O + r_q \quad (1)$$

and the rate vector is

$$r = (-r_s\ ,\ -r_n\ ,-r_o\ ,\ r_x\ ,\ r_p\ ,\ r_c\ ,\ r_w\ ,\ r_q)^T \quad (2)$$

Metabolic heat production rate is added to reaction rates. The unknown process states are determined by the metabolic black-box modeling and the integration of estimated reaction rates. The redundant reaction rates are used in the derivation of reconciliated reaction rates *(Hocalar et. al., 2006)*.

3. RESULTS AND DISCUSSIONS

3.1. Nonlinear Control of Specific Growth Rate

The feedback linearizing control of the specific growth rate is based on the assumption of the presence of sufficient oxygen concentration and the absence of ethanol in the fermentation broth (Claes, 1999). In order to implement the control approach, the oxygen concentration has to be maintained high enough not to run into oxygen limitation throughout fermentation and the specific growth rate has to be below the critical value in order not to form ethanol.

The starting point for the derivation of the controller expression is the general dynamical mass balance equation for the substrate feeding as shown in Eq. 3.

$$\frac{dS}{dt} = D\,(S_{in} - S) - \left(\frac{\mu_x^{ox}}{Y_{X/S}^{ox}} + \frac{q_{e,pr}}{Y_{E/S}^{red}} + m \right) X \quad (3)$$

By rearranging the Eq. 3, Eq. 4 can be written as;

$$\frac{dC_s}{dt} = -\sigma X + \frac{F_s}{V}(C_{s,in} - C_s) \quad (4)$$

where $\sigma = \left(\frac{\mu_x^{ox}}{Y_{X/S}^{ox}} + \frac{q_{s,red}}{Y_{E/S}^{red}} + m \right)$, C_s is substrate concentration, X biomass, F substrate feed rate. The second step is to set up a stable linear reference model for tracking error. The reference model determines to the decreasing trajectory of the tracking error.

$$\frac{d}{dt}(C_s' - C_s) + \lambda\,(C_s' - C_s) = 0 \quad (5)$$

The λ is arbitrary adjustment coefficient and have to be chosen such that the differential equation (Eq. 5) is stable. At steady state conditions, the substrate concentration can be accepted zero, $(\frac{dC'}{dt} \approx 0)$ and Eq. 5 can be written as $\lambda\,(C_s' - C_s) = \frac{dC_s}{dt}$ and by substituting the Eq. 4 in the Eq. 5,

$$F_s = \frac{\sigma X - \lambda(C_{s,in} - C_s')}{C_{s,in} - C_s'} V \quad (6)$$

obtained as a final controller equation. Under oxidative conditions and in the absence of ethanol in the fermentation broth, specific growth rate is a function of substrate concentration. Therefore, specific growth rate (μ) can be written instead of substrate concentration term in Eq. 6. By rearranging the Eq. 6, the expression for substrate feed rate controller can be written as follows;

$$F_s = \frac{\frac{\mu_x}{Y_{X/S}^{ox}} X - \lambda\,(\mu_s - \mu')}{C_{s,in}} V \quad (7)$$

where λ is the arbitrary adjustment coefficient for the decrease of tracking error. When the Eq. 7 is applied to the fermentation, steady state errors are observed between the estimated specific growth rate and set profiles. In order to eliminate this difference, an integral term is added to the Eq. 7. and the obtained results are presented in Fig. 1 for the controlling of time varying specific growth rate profile.

$$F_s = \frac{\frac{\mu_x}{Y_{X/S}^{ox}} X - \lambda_P \left\{ (\mu_s - \mu') - \frac{1}{\lambda_i} \Sigma (\mu_s - \mu') \right\}}{C_{s,in}} V \quad (8)$$

The results of the implementation of Eq. 8 in a fed-batch fermentation are given Fig. 1. The adjustment parameters are $\lambda_p = 0.14$, $\lambda_i = 1800$ for the ascending and $\lambda_p = 0.27$, $\lambda_i = 1800$ for the descending specific growth rate region.

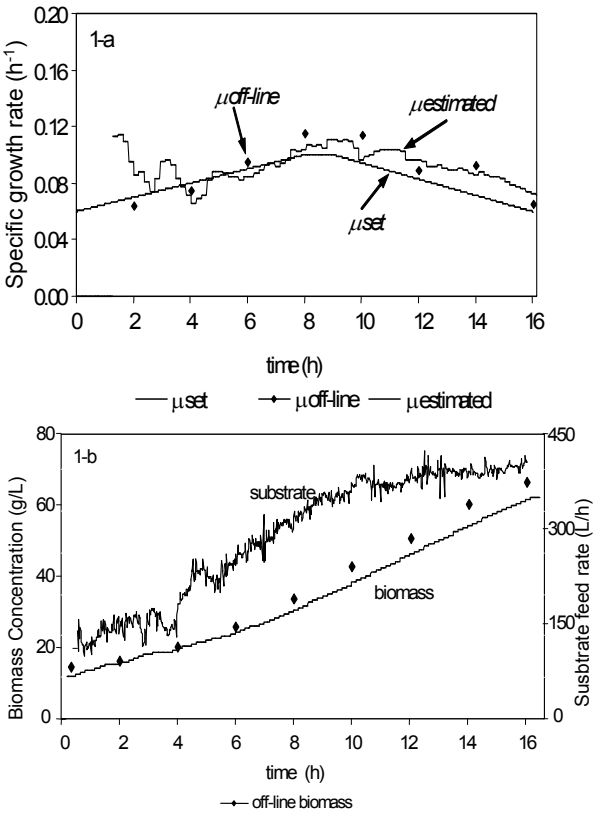

Figure 1.a- Specific growth rate b- biomass concentration and substrate.

The estimation of biomass concentration can be accepted successfully (Fig. 1-b) and is used in the calculation of specific growth rate (fig. 1-a). The specific growth rate estimations and off-line measurements are close to each other

with acceptable accuracy. The time varying specific growth rate profile is controlled successfully by the controller and obtained substrate feed rate resemble the predetermined substrate feeding profiles widely used in practice.

In Fig. 2, the results of different specific growth rate controlled fermentation are given. In this fermentation, ethanol formation is observed at the different times during the process and cause's decrease in the specific growth rate. The controller increased the substrate feed rate in order to compensate the decrease in the specific growth rate that caused more ethanol formation (Fig. 2-a). The unexpected decreases in the specific growth rate estimation are given in Fig. 2-b. The excess in the substrate feed rate puts the process more instability and results in failure of the control of specific growth rate. As a result, the substrate feed rate is manually intervened to consume the ethanol in the broth.

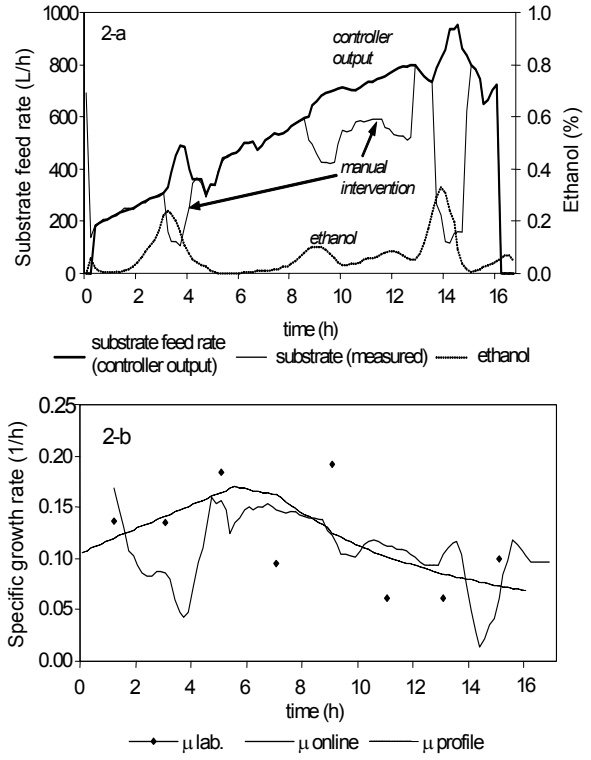

Figure 2.a- substrate feed rate and ethanol concentration, b- specific growth rate.

This controller has successfully controlled the specific growth rate at trajectory under defined conditions as shown in Fig. 1. Once the fermentation went to beyond the restrictive conditions the controller failed as shown in Fig. 2.

3.2. Nonlinear Control of Minimum Ethanol Concentration

An alternative way to control the specific growth rate at maximum oxidative rate is to use the overflow metabolite as an indicator of how close the actual value to critical growth rate to maximize biomass production. If ethanol concentration can be controlled at constant minimal concentration, it is possible to keep the specific growth rate slightly above the critical value (Cannizzaro et. al., 2004). In order to control the ethanol concentration, the regulator design is based on a feedback linearization of a reduced-order model of the process obtained by singular perturbation of the state space model under the following assumptions: the stoichiometric (yield) coefficients are known, the gaseous outflow rates (ethanol, CO_2, O_2) are measured on-line, the influent substrate concentration S_{in} is fixed and known, the specific growth rate is unknown. The singular perturbation techniques can be used for systems in which some reactions proceed at much faster rates than the others (Bastin and Dochain, 1990; Pomerleau and Viel, 1992; Chen et. al., 1995).

The dynamical process equations for five process states with known yield coefficients can be given as follows;

$$\frac{d}{dt}\begin{bmatrix} X \\ S \\ E \\ O \\ C \end{bmatrix} = \begin{bmatrix} 1 & 1 & 1 \\ -Y_{X/S}^{ox} & -Y_{X/S}^{red} & 0 \\ 0 & Y_{X/E}^{red} & -Y_{X/E}^{eth} \\ -Y_{X/O}^{ox} & 0 & -Y_{X/O}^{eth} \\ Y_{X/C}^{ox} & Y_{X/C}^{red} & Y_{X/C}^{eth} \end{bmatrix} \begin{bmatrix} \mu_x^{ox} \\ \mu_x^{red} \\ \mu_e^{ox} \end{bmatrix} X - D \begin{bmatrix} X \\ S \\ E \\ O \\ C \end{bmatrix} + \begin{bmatrix} 0 \\ DS_{in} \\ 0 \\ r_o \\ -r_c \end{bmatrix} \tag{9}$$

The general state space dynamical model can be written as follows (Bastin and Dochain, 1990);

$$\frac{d\xi}{dt} = K\,\varphi(\xi) - D\,\xi + F - Q \tag{10}$$

$$\xi^T = [X, S, E, O, C]$$

where ξ, Q, F involves n components, φ involves m reaction rates ve K is (NxM) size yield coefficient matrix. In Eq. 9, the first term $K\varphi(\xi)$ describes the kinetics of microbiological reactions, the remaining term $-D\xi + F - Q$ describes the transport dynamics of the components through the bioreactor. The yield coefficients used in the design is shown in Table 1.

Table 1. Parameters used in nonlinear controller design (Cmol/mol) (Beşli et. al. 1995).

k_1, $Y_{X/S}^{ox}$	3.65	k_2, $Y_{X/S}^{red}$	0.36
k_3, $Y_{X/E}^{red}$	0.19	k_4, $Y_{X/E}^{eth}$	1.35
k_5, $Y_{X/O}^{ox}$	1.56	k_6, $Y_{X/O}^{eth}$	0.83
k_7, $Y_{X/C}^{ox}$	1.45	k_8, $Y_{X/C}^{red}$	0.2
k_9, $Y_{X/C}^{eth}$	1.99		

By the systematic application of singular perturbation technique, fully reduced model can be established and in the case of $\dim(\xi_F) = M$ and K_F full rank, the process states can be partitioned as slow $\xi_S^T = [X, E]$ and fast varying state variables $\xi_F^T = [S, O, C]$. The substrate, oxygen and carbon-dioxide are fast varying state variables and biomass and ethanol are slow varying state variables for the fed-batch yeast fermentation process. The general dynamical model can be written as given in Eq. 11 by the assumption of the fast

varying state variables dynamics allow the singular perturbation (Bastin and Dochain, 1990);

$$\frac{d\xi_S}{dt} = K_S\,\varphi - D\,\xi_S + F_S - Q_S$$
$$K_F\,\varphi + F_F - Q_F = 0 \tag{11}$$

The reaction rate vector , $\varphi(\xi)$, can be written as;

$$\varphi(\xi) = -K_F^{-1}\,(F_F - Q_F) \tag{12}$$

By substituting Eq. 12 in Eq. 11, the dynamics of slow varying state variables can be obtained as in Eq. 13.

$$\frac{d}{dt}\begin{bmatrix} X \\ E \end{bmatrix} = -D\begin{bmatrix} X \\ E \end{bmatrix} + \begin{bmatrix} 1 & 1 & 1 \\ 0 & k_4 & -k_3 \end{bmatrix} * inv(K_F) \begin{bmatrix} DS_{in} \\ r_o \\ -r_c \end{bmatrix} \tag{13}$$

In steady state, singular perturbation allows the fast varying state's dynamics to consider to equal to zero and unknown reaction rates can be determined by simple matrixes operations as shown below if the inverse of the yield coefficient matrices can be calculated (Hocalar, 2007).

$$\begin{bmatrix} \mu_s^{ox} \\ \mu_s^{red} \\ \mu_e^{ox} \end{bmatrix} = -K_F^{-1} \begin{bmatrix} DS_{in} \\ r_o \\ -r_c \end{bmatrix} \tag{14}$$

By inserting the Eq. 14 in Eq. 13, slow varying process states can be calculated by means of basic matrice operations:

$$\psi = det(K_F) = \left(k_7 k_6 k_2 - k_5 k_9 k_2 - k_1 k_8 k_6\right)$$
$$\upsilon_1 = \left(-k_4 k_1 k_6 + k_3 k_2 k_5\right)\psi^{-1}$$
$$\upsilon_2 = \left(-k_4 k_1 k_9 + k_3 k_2 k_7 - k_3 k_1 k_8\right)\psi^{-1}$$
$$\upsilon_3 = \left(k_4 k_5 k_9 - k_4 k_6 k_7 + k_3 k_5 k_8\right)\psi^{-1}$$
$$\frac{dE}{dt} = -\,D\,E + \upsilon_3\,DS_{in} + \upsilon_2\,r_o - \upsilon_1\,r_c \tag{15}$$

By inserting the Eq. 15 into the first order reference model equation in Eq. 16, the controller law for substrate feed rate can be obtained as in Eq. 17.

$$\frac{d}{dt}(y^* - y) + (\lambda_1 + \lambda_2 x)(y^* - y) = 0 \quad (\lambda_1, \lambda_2 > 0) \tag{16}$$

$$F_s = \frac{1}{\upsilon_3}\left\{\frac{dE^*}{dt} + (\lambda_1 + \lambda_2 \hat{X})(E_s - E) + DE + \upsilon_1 r_c - \upsilon_2 r_o\right\} \tag{17}$$

The tracking ethanol error is tried to minimize using λ adjustment parameters. Several fed-batch experiments were conducted in a 25 m^3 fermentor to validate the control strategy. The results are given in Fig. 3.

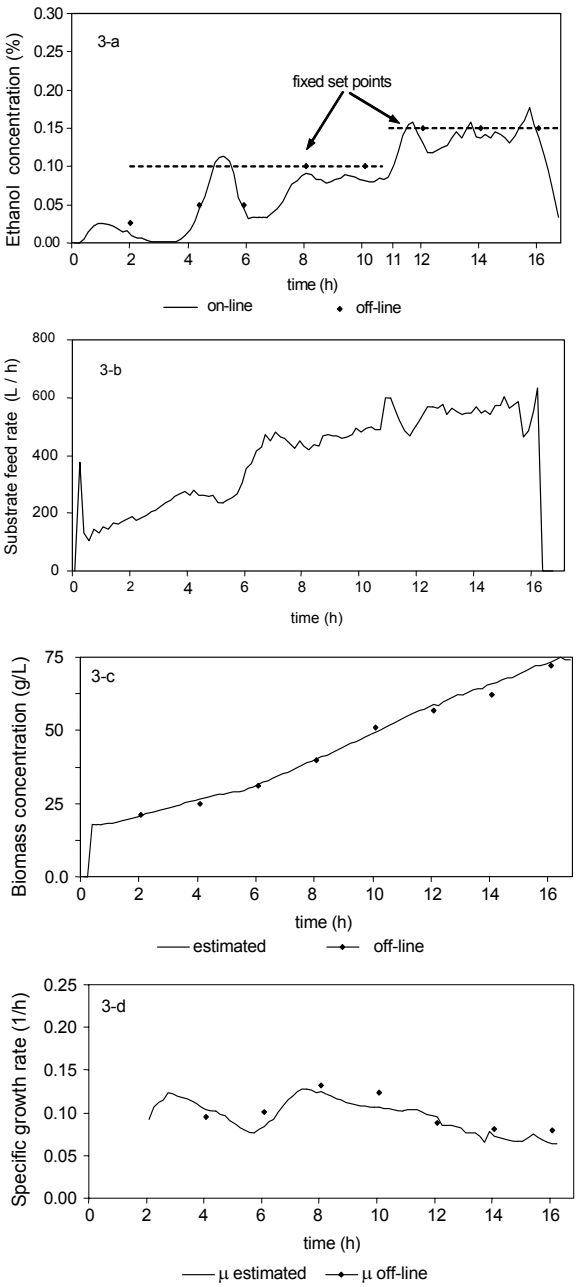

Figure 3: a- Ethanol concentration, b- substrate feed rate, c- biomass concentration and d- specific growth rate curves obtained from the industrial fermentation.

The controller was started at second hour and two fixed set points were tried to control for certain periods with ethanol set values E_s= % 0.10 and then with E_s= % 0.15. The ethanol concentration was successfully controlled at different set values from the 7th hour to the end of fermentation. The manipulated variable substrate feed rate and biomass concentration are given in Fig. 3-b and 3-c respectively. The biomass concentration increased exponentialy (Fig. 3.c) and the specific growth rate estimation is given in Fig. 3-d and quite close to experimental results. The controller developed stable response to the step change in the ethanol set point. The controller automatically adapted the feed rate of substrate to compensate for step changes. The difficulty of controlling the ethanol concentration can be seen in first

hours of fermentation (exponential growth phase). During the first hours, the controller increased the substrate feed rate and because of the time delay of the ethanol formation, slightly excess substrate feeding suggested by the controller.

4. CONCLUSION

The state feedback linearizing control strategy is applied to the industrial fed-batch baker's yeast fermentations. The control of specific growth rate and minimal ethanol concentration are attempted at technical scale fermentations. The control of specific growth rate at specificed trajectory is required in many fermentation processes. In this work, this approach has been successfully applied to baker's yeast fermentation. In order to maximize biomass concentration and productivity, the process has to be controlled at its maximum oxidative growth rate, minimizing by-product ethanol formation. This strategy is applied in second controller and specific growth rate was maintained slightly above maximum oxidative growth rate by maintaining and controlling by product ethanol at minimal concentration. This approach can also be applied to similar overflow processes such as the growth of *E. coli*. The ethanol concentration was controlled successfully at minimal concentrations. Both controllers can be combined to control specific growth rate at any trajectory and to minimize ethanol production.

NOMENCLATURE

C_i	concentration of i (kg/m^3)
D	dilution rate (1/h)
F_i	flow rate of i (m^3 / h)
K	yield coefficient matrices
M_i	molar weight of i (kg)
q_i	specific conversion rates of i (kg/kgh, C-mol/ C-mol h)
S	substrate concentration (kg/m^3)
X	biomass (kg/m^3)
V	volume (m^3)
$Y_{i/j}$	yield of i over j

Subscripts

ox	oxidative
red	reductive
eth	ethanol
m	maintenance
ae	aerobic
in	inlet
out	outlet
T	transpose
n	nitrogen
o	oxygen
e	ethanol
c	carbon
q	metabolic heat production
s	substrate
p	product
x	biomass
w	water

Greek Letters

μ	specific growth rate, (h^{-1})
ξ	state variable
λ	adjustment coefficient

REFERENCES

Bastin, G. and Dochain, D. (1990) On-line estimation and adaptive control of bioreactors. *Elsevier*.

Beşli, N., Türker, M., Gül, E. (1995). Design and simulation of a fuzzy controller for fedbatch yeast fermentation. *Bioprocess Engineering*. 13:141-148.

Cannizzaro, C., Valentinotti, S., von Stockar, U. (2004). Control of yeast fed-batch process through regulation of extracelluler ethanol metabolite. *Bioprocess Biosystem Engineering*. 26: 377-383.

Chen, L. (1992). Modelling, identifiability and control of complex biotechnological systems. *PhD Thesis*. Faculte des Sciences Appliquees. Universite Catholique de Louvan.

Chen, L., Bastin, G., Van Breusegem, V. (1995). A case study of adaptive nonlinear regulation of fed-batch biological reactors. *Automatica*. 31:1: 55-65.

Claes, J.E. (1999). Optimal adaptive control of the fed-batch bakers yeast fermentation process. *PhD Thesis*. Katholieke Universiteit Leuven.

Gnoth, S., Jenzsch, M., Simutis, R., Lubbert, A. (2008). Control of cultivation processes for recombinant protein production: a review. *Bioprocess and Biosystems Engineering*. 31: 21–39.

Hocalar, A. (2007). Model based control of industrial fed-batch baker's yeast fermentation process. *PhD Thesis*. University of Kocaeli, Turkey.

Hocalar, A., Türker, M., Öztürk, S. (2006). State estimation and error diagnosis in industrial fed-batch yeast fermentation. *AIChE Journal*. 52:11: 3967-3980.

Jana, S., and Deb, J. (2005). Strategies for efficient production of heterologous proteins in

Escherichia coli. *Applied Microbiology and Biotechnology*. 67: 289-298.

Perulekar, S.J. and Lim, H.C. (1985). Modeling, optimization and control of semi-batch bioreactors. *Advances in Biochemical Engineering*. 32: 207–258.

Pomerleau, Y., Viel, G. (1992). Industrial application of adaptive nonlinear control for baker's yeast production. *IFAC Modelling and Control of Biotechnological Processes*. Colorado, USA.

Renard, F. and Wouwer, A.V. (2008). Robust adaptive control of yeast fed-bacth cultures. *Computers and Chemical Engineering*. 32: 1246–1256.

Rocha, I. and Ferreira, E.C. (2002). Model-based adaptive control of acetate concentration during the production of recombinant protein with *E. Coli*. *IFAC 15th Triennial World Congress*. Barcelona, Spain.

Soons, Z.I.T.A., Voogt, J.A., Van Straten, G., Van Boxtel, A.J.B. (2006). Constant specific growth rate in fed-batch cultivation of *Bordetella Pertussis* using adaptive control. *Journal of Biotechnology*. 125: 252-268.

Türker, M. (2003). Measurement of metabolic heat in a production scale bioreactor by continuous and dynamic calorimetry. *Chemical Engineering Communication*. 190: 573-598.

Türker, M. (2004). Development of biocalorimetry as a technique for process monitoring and control in technical scale fermentations. *Thermochimica Acta*. 419: 73-81.

Valentinotti, S., Srinivasan, B., Holmberg, U., Bonvin, D., Cannizzaro, C., Rhiel, M., Von Stockar, U. (2003). Optimal operation of fed-batch fermentations via adaptive control of overflow metabolite. *Control Engineering Practice*. 11: 665-674.

Walker, G.M. (1998). *Yeast Physiology and Biotechnology*. Wiley. ISBN: 978-047196446-9.

Yamane, T. and Shimizu. S. (1984). Fed-batch techniques in microbial processes. *Advances in Biochemical Engineering*. 30: 148–194.