0273-2289/1559–0291 (Online)/07/136–140/753–764/$30.00

Estimation of Temperature Dependent Parameters of a Batch Alcoholic Fermentation Process

Rafael Ramos de Andrade,*[,1] Elmer Ccopa Rivera,*[,1] Aline C. Costa,[1] Daniel I. P. Atala,[2] Francisco Maugeri Filho,[2] and Rubens Maciel Filho[1]

[1]Laboratory of Optimization, Design and Advanced Control, School of Chemical Engineering, State University of Campinas, P.O. Box 6066, 13081-970, Campinas, SP, Brazil, E-mail: rafaelra@feq.unicamp.br, elmer@feq.unicamp.br; and [2]Department of Food Engineering, School of Food Engineering, State University of Campinas, P.O. Box 6121, 13081-970, Campinas, SP, Brazil

Abstract

In this work, a procedure was established to develop a mathematical model considering the effect of temperature on reaction kinetics. Experiments were performed in batch mode in temperatures from 30 to 38°C. The microorganism used was *Saccharomyces cerevisiae* and the culture media, sugarcane molasses. The objective is to assess the difficulty in updating the kinetic parameters when there are changes in fermentation conditions. We conclude that, although the re-estimation is a time-consuming task, it is possible to accurately describe the process when there are changes in raw material composition if a re-estimation of parameters is performed.

Index Entries: Alcoholic fermentation; kinetic parameters estimation; mathematical modeling; Quasi-Newton algorithm; *Saccharomyces cerevisiae*; ethanol production.

Introduction

The interest in renewable energy sources tends to augment with the concern about exhaustion of fossil fuels and the increase in their price. The world meetings make clear that policies for renewable energy are essential to achieve sustainable development in a broad sense. Environmental protection, job creation, alleviation of external debts in developing countries, and security of supply are some of the key issues to mention *(1)*. Bioethanol (ethanol from biomass) is an attractive, sustainable energy source. Modeling potentially reduces the cost of the alcoholic fermentation process development by eliminating unnecessary experimental work. It allows the study of

*Author to whom all correspondence and reprint requests should be addressed.

the various process parameters interactions through simulation. Besides, it provides understanding of the process, which is helpful for operational policy definitions and can be applied for posterior optimization and control.

There are many minor problems associated with the alcoholic fermentation process to be solved nowadays. Among them is the lack of robustness of the fermentation in the presence of fluctuations in operational conditions, which leads to changes in the kinetic behavior, with impact on yield, productivity, and conversion. These changes are very common in plants of alcoholic fermentation; they occur not only because of the variations in the quality of the raw material but also because of variations of dominant yeast in the process. Also, the alcoholic fermentation process is exothermic and small deviations in temperature can dislocate the process from optimal operational conditions.

Temperature has an important influence on the alcoholic fermentation process, because it is usually difficult to support a constant temperature during large-scale alcoholic fermentation and it affects productivity as well as microorganism viability. Besides, terms of temperature influence on ethanol fermentation kinetics can be useful in strategies for process optimization (*2*). Still, there are few works in the literature on the mathematical modeling of the fuel ethanol fermentation considering the effect of temperature on the kinetic parameters. (*2–4*).

In this work we perform kinetic parameters optimization in an alcoholic fermentation process. The kinetics was determined as function of temperature from batch fermentations at temperatures from 30 to 38°C. Based on experimental data, a differential model consisting of rate expressions for cell growth, substrate consumption, and product formation was proposed. The resulting model has eleven parameters, five of which are known to be temperature dependent. In order to describe this dependence, one set of parameters was estimated for each considered temperature and in a subsequent step an equation describing the temperature dependence of each parameter was fitted to the resulting data. The performance of the proposed model in the presence of changes in raw material composition is assessed before and after parameters re-estimation.

Materials and Methods

The microorganism used was *Saccharomyces cerevisiae*, cultivated in the Bioprocess Engineering Laboratory in the Faculty of Food Engineering/State University of Campinas, Campinas, SP, Brazil and obtained from an industrial fermentation plant. The growth medium for the inoculum contained 50.0 kg/m^3 of glucose, 5.0 kg/m^3 of KH_2PO_4, 1.5 kg/m^3 of NH_4Cl, 0.7 kg/m^3 of $MgSO_4 \cdot 7H_2O$, 1.2 kg/m^3 of KCl, and 5.0 kg/m^3 of yeast extract. The growth of microorganisms was performed in 500-mL flasks placed in a shaker at 30°C and 150 rpm for 24 h. The production medium was diluted using sugarcane molasses from an industrial ethanol fermentation plant. Sterilization was performed at 121°C for 20 min in autoclave.

Table 1
Initial Substrate Concentrations and Temperature for Experiments

Experiments	T(°C)	S_0 (kg/m^3)
1	30	127.6
2	31.2	86.8
3	34	119.1
4	36.8	84.6
5	38	118.8
6	34	168.1

A bioreactor (Bioflow III System; New Brunswick Scientific Co., Inc., Edison, NJ) with temperature and agitation control systems through proportional and integral differential (PID) controllers was used. The total working volume was 5 L. Agitation was controlled at 300 rpm and performed with two flat blade turbine disk impellers, with six blades each. Temperature was controlled at the fixed value for each fermentation. Dry cell mass was determined gravimetrically after centrifuging for 15 min at 3300 rpm (1219.68*g*), washing, and drying the cells at 70°C. Biomass concentration is given by the weight difference divided by total sample volume.

Viable cells were counted with the methylene blue staining technique (*5*). In this work the cell viability during batch fermentation was always close to 100%. Total reducing sugar and ethanol concentrations were determined by high-performance liquid chromatography (Varian 9010 model, Varian, Inc. Scientific Instruments, Palo Alto, CA). A SHODEX KS 801 column (Showa Denko, Tokyo, Japan) at 30°C was used. Ultrapure water obtained from Milli-Q Purification System (Millipore Corporation, Billerica, MA) containing Millipak membrane of 0.22 μm (diameter of pore) was mixed with H_2SO_4 (pH 1.4) and used as the eluent at a flow rate of 0.7 mL/min. The standards were mixed solutions of sucrose, glucose, fructose, and ethanol at concentrations from 0.1 to 40.0 kg/m^3. The experiments were performed following the initial substrate concentrations and temperatures depicted in Table 1.

Parameter Estimation Problem

Batch Model

For batch fermentation process, the mass balance equations that describe the concentrations of biomass, substrate, and ethanol are:

$$\frac{dX}{dt} = r_x \tag{1}$$

$$\frac{dS}{dt} = -r_s \tag{2}$$

$$\frac{dP}{dt} = r_p \tag{3}$$

where X is the concentration of cell mass (kg/m^3), S is the concentration of substrate (kg/m^3), and P is the concentration of ethanol (kg/m^3). In this study the rates of cell growth, r_x (kg/[m^3·h]), substrate consumption, r_s (kg/[m^3·h]), and product formation, r_p (kg/[m^3·h]), were expressed as functions of temperature, as described below. For fermentation with *S. cerevisiae*, experimental data has shown that cellular, substrate and product inhibitions are of importance *(2)*. In this study, the cell growth rate, r_x, includes terms for such types of inhibitions:

$$r_x = \mu_{max} \frac{S}{K_s + S} \exp(-K_i S) \left(1 - \frac{X}{X_{max}}\right)^m \left(1 - \frac{P}{P_{max}}\right)^n X \quad (4)$$

where μ_{max} is the maximum specific growth rate (h^{-1}), K_s the substrate saturation parameter (kg/m^3), K_i is the substrate inhibition parameter (m^3/kg), X_{max} the biomass concentration when cell growth ceases (kg/m^3), P_{max} the product concentration when cell growth ceases (kg/m^3), and m and n are parameters of cellular and product inhibitions, respectively. Luedeking–Piret expression *(6)* was used to account for the ethanol formation rate, r_p.

$$r_p = Y_{px} r_x + m_p X \quad (5)$$

where Y_{px} is Luedeking–Piret growth associated constant (kg/kg) and m_p is the Luedeking–Piret nongrowth associated constant (kg/[kg·h]). The substrate consumption rate, r_s, is given by:

$$r_s = \left(r_x / Y_x\right) + m_x X \quad (6)$$

This equation describes the sugar consumption during fermentation, which leads to cell mass and ethanol formation. Y_x and m_x are the limit cellular yield (kg/kg) and maintenance parameter (kg/[kg·h]), respectively. The first term of Eq. 6 considers that part of substrate is used for cell growth and the second one, for cellular maintenance that is the energy used for basic cell functions. According to the above description, there are 11 parameters to be estimated from experimental observations and some of them are temperature-dependent parameters (μ_{max}, X_{max}, P_{max}, Y_x, and Y_{px}). This temperature dependence can be described by the following expression *(7)*:

$$\text{Temperature-dependent parameter} = A \cdot \exp\left(B/T\right) + C \cdot \exp\left(D/T\right) \quad (7)$$

Optimization Using the Quasi-Newton Algorithm

Let θ specify the parameters vector, which contains all the temperature-dependent parameters. The objective of the optimization problem is to find out θ by minimizing the objective function *(8,9)*, min $E(\theta)$:

$$E(\theta)=\sum_{n=1}^{np}\left[\frac{\left(X_n-Xe_n\right)^2}{Xe_{max}^2}+\frac{\left(S_n-Se_n\right)^2}{Se_{max}^2}+\frac{\left(P_n-Pe_n\right)^2}{Pe_{max}^2}\right]=\sum_{n=1}^{np}\varepsilon_n^2(\theta) \quad (8)$$

The optimization is performed using the experimental data from fermentations in the temperature range of 30–38°C. Xe_n, Se_n, and Pe_n are the measured concentrations of cell mass, substrate, and ethanol at the nth sampling time. X_n, S_n, and P_n are the concentrations computed by the model at the nth sampling time. Xe_{max}, Se_{max}, and Pe_{max} are the maximum measured concentrations and the term np is number of sampling points. $\varepsilon_n(\theta)$ is the error in the output owing to the nth sample. Equations 1–6 were solved using a FORTRAN program with integration by an algorithm based on the fourth-order Runge–Kutta method. In order to model the fermentation experiments, the temperature dependent parameters (μ_{max}, X_{max}, P_{max}, Y_x, and Y_{px}) in Eqs. 4–6 were determined by minimizing Eq. 8 using a Quasi-Newton (QN) algorithm. The FORTRAN IMSL routine DBCONF was used for this purpose. The optimization problem was implemented as a nonlinear programming problem written as:

Minimize Eq. 8

Subject to $l_p \le x_p \le u_p$, $p = 1, \ldots, 5$

where, x_p is the temperature-dependent parameters. The l_p and u_p are specified lower and upper bounds on the variables, with $l_p \le u_p$. The parameters that are not temperature dependent had their values fixed according to the work of Atala et al. *(2)* as follows: K_s = 4.1 kg/m^3, K_i = 0.004 m^3/kg, m_p = 0.1 kg/(kg·h), m_x = 0.2 kg/(kg·h), m = 1.0, and n = 1.5. The optimization procedure was repeated for each temperature value (30, 31.2, 34, 36.8, and 38°C), resulting in five sets of the temperature dependent parameters (μ_{max}, X_{max}, P_{max}, Y_x, and Y_{px}).

Influence of Temperature on the Kinetics

After the temperature dependent parameters were optimized by using the QN algorithm, they were described by Eq. 7 as functions of temperature. The Levenberg–Marquardt algorithm was used for fitting all constants (A, B, C, and D), whose values are presented in Table 2. This table also shows the coefficient of determination (r^2) of the parameters fitting. Figure 1 shows the resulting dependence of μ_{max}, X_{max}, P_{max}, Y_x, and Y_{px} with temperature. The symbols (■) are the optimized values determined for each temperature using the QN algorithm and the lines represent the temperature dependence description of Eq. 7. It can be observed that the maximum specific growth rate occurs at a temperature of 34°C, considered the optimum condition for growth. Ethanol tolerance, limit cellular yield and X_{max} decreases with temperature as expected.

Table 2
Constant Values in the Eq. 7 and Coefficient of Determination (r^2) of Parameters Fitting as Functions of Temperature for Batch Model Optimized by QN

Parameter	(r^2)	*A*	*B*	*C*	*D*
μ_{max}	0.999	-2.98×10^5	–304.44	2.82×10^5	–302.18
X_{max}	0.999	7.20×10^{-18}	1284.40	10.24	48.58
P_{max}	0.999	6.01×10^{-22}	1588.05	106.33	–12.02
Y_x	0.999	-2.98×10^{-17}	1194.15	11.73	–5.60
Y_{px}	0.997	-4.10×10^{-78}	5111.34	0.01	33.72

Results

The mathematical model to be computed consists of Eqs. 1–6. The temperature dependent parameters μ_{max}, X_{max}, P_{max}, Y_x, and Y_{px} are given by Eq. 7 with the constants (A, B, C, and D) shown in Table 2, the parameters, which are not temperature dependent are fixed according to Atala et al. *(2)*. The profiles for ethanol, substrate, and biomass are shown in Fig. 2. It can be observed that the estimated model was able to fit batch experimental observations very satisfactorily and, therefore, the model can be applied for posterior optimization and controller process design. However, it should be stressed that when there are changes in operational conditions, the model kinetic parameters have to be re-estimated. It is worthwhile mentioning that, although the re-estimation is a time-consuming task, it is necessary to accurately describe the process when there are changes in raw material composition. Figure 3 shows the results for experiment 6 described in Table 1. The experimental data of this experiment were not used in the estimation procedure, but only to validate the model.

The residual standard deviation (RSD), Eq. 9, written as a percentage of the average of the experimental values, was the measurement used for characterizing the quality of the prediction of the model.

$$\mathrm{RSD}(\%) = \left(\frac{\sqrt{\mathrm{RSD}}}{\bar{d}_p} \right) 100 \tag{9}$$

where $\mathrm{RSD} = \frac{1}{np} \sum_{p=1}^{np} (d_p - x_p)^2$ in which x_p and d_p are, respectively, the value predicted by the mathematical model and experimental value, $\bar{d}_p$ is the average of the experimental values, and *np* is the number of experimental points. The RSDs (%) for the batch model optimized by QN are shown in Table 3. The concentrations of biomass, substrate, and ethanol concentrations calculated using the resulting mathematical presented deviations of 4.9–23.3% from the experimental data.

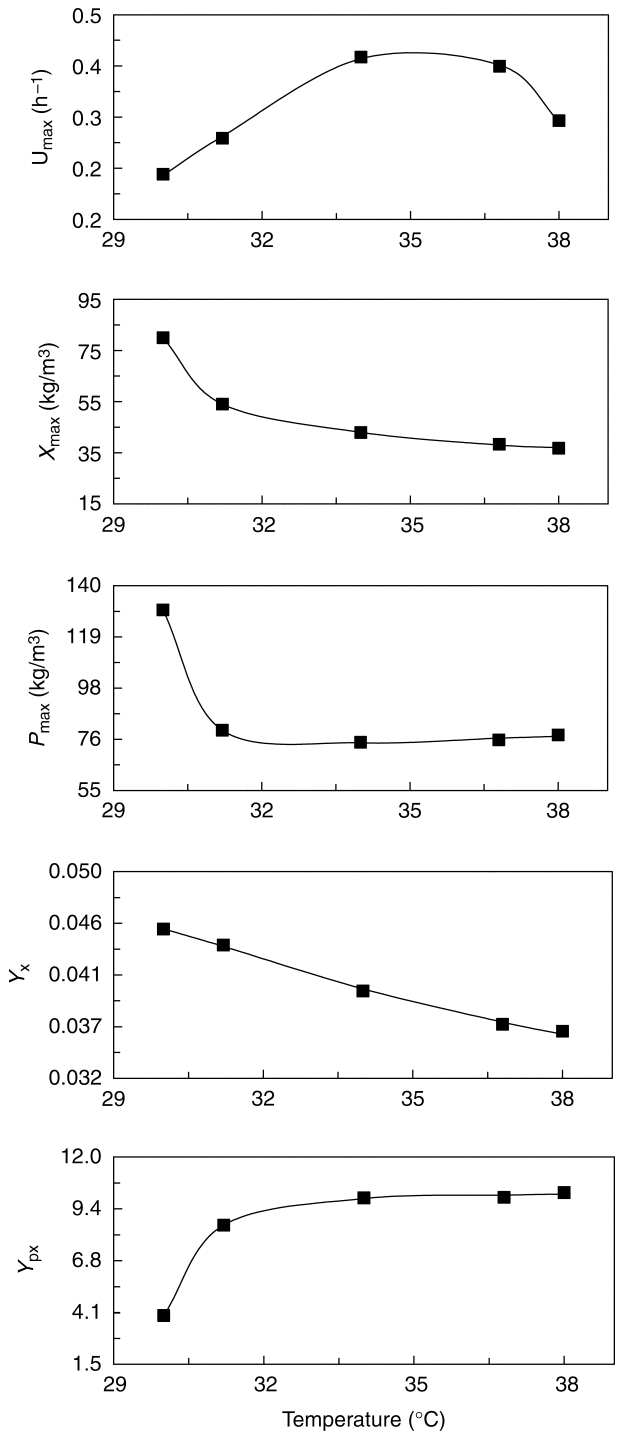

Fig. 1. Kinetic parameters optimized by QN algorithm at 30, 31.2, 34, 36.8, and 38°C, respectively.

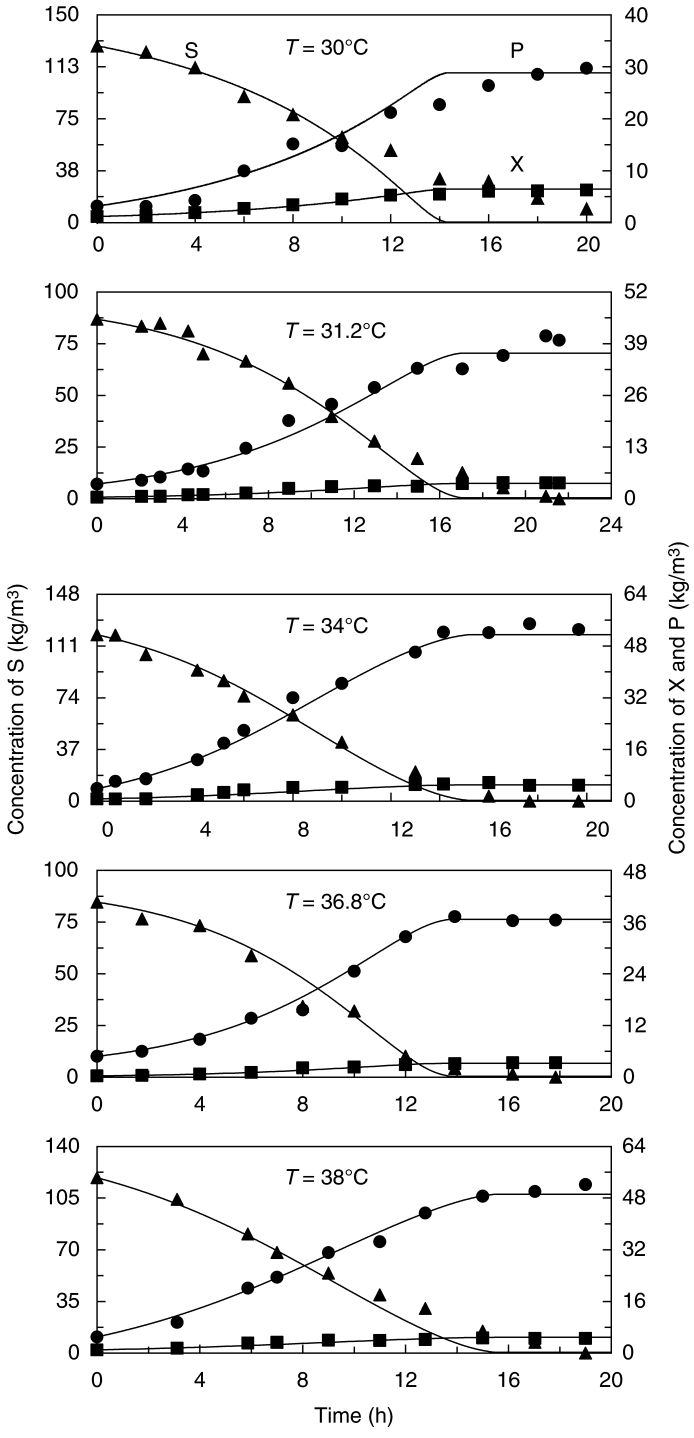

Fig. 2. Experimental and simulated data (QN—) from 30 to 38°C. Experimental data are for concentration of substrate, *S* (▲); cell mass, *X* (■); and ethanol, *P* (●).

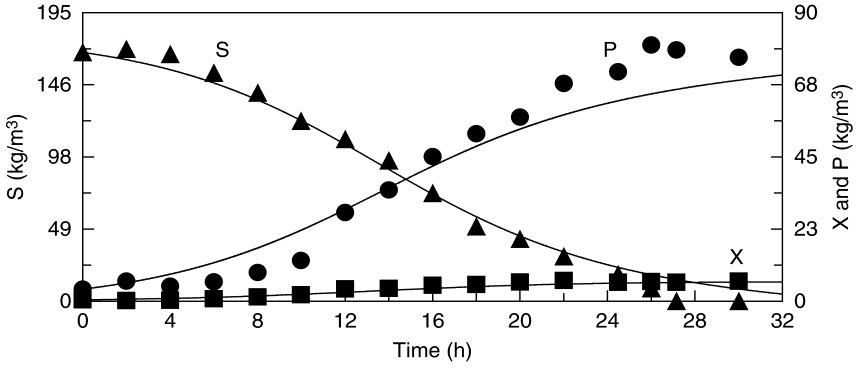

Fig. 3. Validation of the model. Experimental and simulated data for experiment 6 in Table 1. Experimental data are for concentration of substrate, *S* (▲); cell mass, *X* (■); and ethanol, *P* (●).

Table 3
RSDs (%) for the Batch Model

	RSD (%)					
Output variable	T = 30°C	T = 31.2°C	T = 34°C	T = 36.8°C	T = 38°C	T = 34°C (validation)
X	9.5	15.6	18.6	9.6	12.1	16.6
S	23.3	10.4	8.2	12.9	14.1	8.0
P	14.6	11.0	6.3	4.9	4.9	8.4

In order to test the model performance in the presence of fluctuations in raw material and culture medium compositions, an experiment performed at 34°C was considered. The only difference between this experiment and the experiments at 34°C shown in Figs. 2 and 3 are the molasses origin (molasses from a different harvesting) and the production medium, which, in this case, was diluted sugarcane molasses with addition of 1.0 kg/m^3 of yeast extract and 2.4 kg/m^3 of $(NH_4)_2SO_4$. The results are shown in Fig. 4A. The results after re-estimation of kinetic parameters are shown in Fig. 4B. The RSD (%) values for the model with and without parameters re-estimation are shown in Table 4.

Discussion

The main objective of this work is to evaluate mathematical models to describe the alcoholic fermentation process in the presence of changes in operational conditions and fluctuations in the quality of raw material. These models are important to enable dynamic behavior studies, determination of control structures, optimization, and design of process controllers. It is

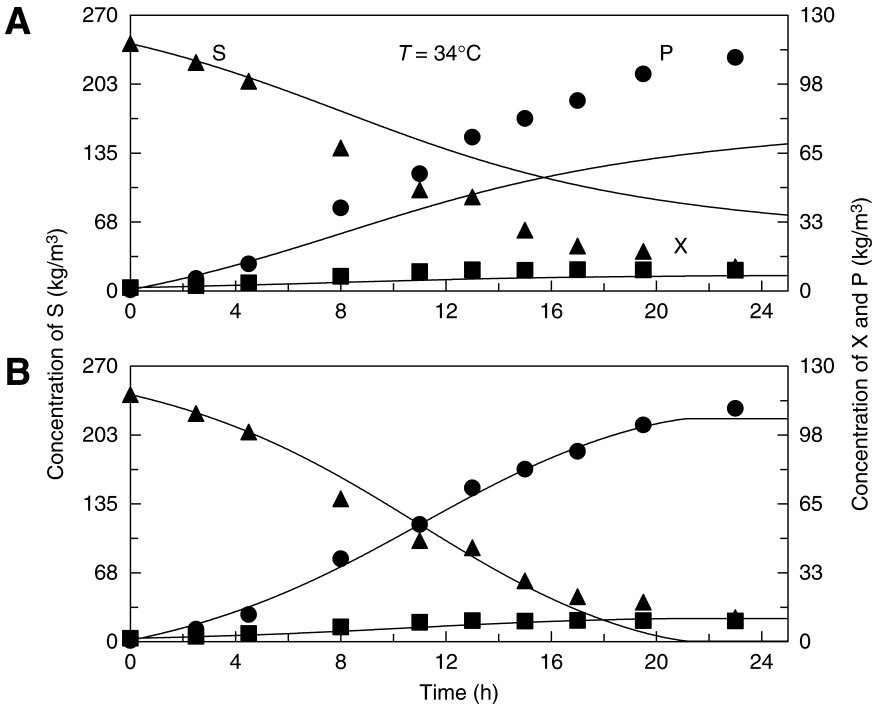

Fig. 4. Model performance in the presence of fluctuation in raw material composition. **(A)** Model without parameters re-estimation. **(B)** Model with parameters re-estimation. Experimental data are for concentration of substrate, *S* (▲); cell mass, *X* (■); and ethanol, *P* (●).

Table 4
RSD (%) for the Batch Model in the Presence of Fluctuations in Raw Material and Production Medium Compositions With and Without Parameters Re-estimation

Output variable (T = 34°C)	RSD (%)	
	Without re-estimation	With re-estimation
X	39.7	17.4
S	36.1	13.2
P	45.3	4.8

well-known from past studies that models, which do not have their parameters re-estimated when changes in operational conditions occur, do not describe the process accurately. Also, the description of the temperature dependence by the model is very important, although it transforms the parameter re-estimation problem into a much more complicated optimization problem.

In this work a procedure is presented for parameters re-estimation to follow more accurately the process behavior when changes in operational

conditions take place. The general methodology consists on keeping the parameters that are not temperature dependent and estimating those ones that are affected by temperature. The fitting procedure makes use of a double exponential function to find out the best relationship between parameters and temperature. The profiles of biomass, substrate, and ethanol concentrations calculated using the resulting mathematical presented deviations from 4.9 to 23.3% of the experimental data.

Acknowledgments

The authors acknowledge Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP) and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) for financial support.

Nomenclature

K_i	substrate inhibition coefficient (m^3/kg)
K_s	substrate saturation parameter (kg/m^3)
m	parameter used to describe cellular inhibition
m_p	ethanol production associated with growth (kg/[kg·h])
m_x	maintenance parameter (kg/[kg·h])
n	parameters used to describe product inhibitions
P	product concentration (kg/m^3)
P_{max}	product concentration when cell growth ceases (kg/m^3)
r_p	kinetic rate of product formation (kg/[m3·h])
r_s	kinetic rate of substrate consumption ($kg/[m^3 \cdot h]$)
r_x	kinetic rate of growth ($kg/[m^3 \cdot h]$)
S	substrate concentration (kg/m^3)
T	temperature into the fermentor (°C)
X	biomass concentration (kg/m^3)
X_{max}	biomass concentration when cell growth ceases (kg/m^3)
Y_{px}	yield of product based on cell growth (kg/kg)
Y_x	limit cellular yield (kg/kg)
μ_{max}	maximum specific growth rate (h^{-1})

References

1. Goldemberg, J., Coelho, S. T., Nastari, P. M., and Lucon, O. (2004), *Biomass Bioenergy* **26,** 301–304.
2. Atala, D. I. P., Costa, A. C., Maciel, R., and Maugeri, F. (2001), *Appl. Biochem. Biotechnol.* **91–93(1–9),** 353–366.
3. Aldiguier, A. S., Alfenor, S., Cameleyre, X., et al. (2004), *Bioproc. Biosyst. Eng.* **26,** 217–222.
4. Kalil, S. J., Maugeri, F., and Rodrigues, M. I. (2000), *Proc. Biochem.* **35(6),** 539–550.
5. Lee, S. S., Robinson, F. M., and Wang, H. Y. (1981), *Biotechnol. Bioeng.* **11,** 641–649.
6. Luedeking, R. and Piret, E. L. (1959), *J. Biochem. Microbiol. Technol. Eng.* **1,** 431–450.
7. Akerberg, K., Hofvendahl, K., Zacchi, G., and Hahn-Hägerdal, B. (1998), *Appl. Microbiol. Biotechnol.* **49,** 682–690.
8. Wang, F. S. and Sheu, J. W. (2000), *Chem. Eng. Sci.* **55,** 3685–3695.
9. Wang, F. S., Su, T. L., and Jang, H. J. (2001), *Ind. Eng. Chem. Res.* **40,** 2876–2885.