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Evaluation of Optimization Techniques for an Extractive Alcoholic Fermentation Process

Aline C. da Costa* **and Rubens Maciel Filho**

DPQ/FEQ/UNICAMP,
Cx. Postal 6066,
Campinas, SP, 13081-970 Brazil,
E-mail:accosta@feq.unicamp.br

Abstract

The mathematical optimization of a continuous alcoholic fermentation process combined with a flash column under vacuum was studied. The objective was to maximize % yield and productivity in the fermentor. The results using surface response analysis combined with modeling and simulation were compared withy those obtained when the problem was written as a nonlinear programming problem and was solved with a successive quadratic programming (SQP) technique. Two process models were evaluated when the process was optimized using the SQP technique. The first one is a deterministic model, whose kinetic parameters were experimentally determined as functions of the temperature, and the second is a statistical model obtained using the factorial design technique combined with simulation. Although the best result was the one obtained using the rigorous model, the values for productivity and % yield obtained using the simplified model are acceptable, and these models can be used when the development of a rigorous model is excessively difficult, slow, or expensive.

Index Entries: Extractive alcoholic fermentation; optimization; successive quadratic programming; factorial design; response surface methodology; productivity.

Introduction

Despite many advantages of using ethanol produced from biomass as a fuel (it is a high-energy, clean-burning, and totally renewable liquid fuel), it will only substitute gasoline if it is economically competitive. Thus, there is an increased interest in the optimization of all the steps of ethanol production.

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

Many aspects related to optimization of the ethanol production process have been addressed in previous works. A key to the optimization of a process is a thorough understanding of the system's dynamics, which can be obtained using an accurate model of the process. Atala et al. *(1)* developed a mathematical model for the alcoholic fermentation based on fundamental mass balances. The kinetic parameters were determined from experimental data and were described as functions of the temperature. The experiments were conducted with high biomass concentration and sugarcane molasses as substrate to simulate the real conditions in industrial units.

Although deterministic models are important to enhance the knowledge of the process, for many bioprocesses they are only valid in specific conditions. Changes in medium composition, process disturbances, or the use of different strains may nullify the modeling predictions. All these changes happen commonly in an industrial unit, and frequent reestimation of the model parameters is usually difficult and time-consuming. Hybrid neural models are a good alternative to deal with this problem. Harada et al. *(2)* proposed a hybrid model for the alcoholic fermentation combining mass balance equations with neural networks that described the kinetics. They proposed the use of functional link networks (FLNs) and obtained an accurate model that can be updated frequently without much effort. Because estimation of the FLN parameters is a linear problem, the training of these neural networks is rapid, requires low computational effort, and the convergence is guaranteed. These characteristics give the FLN a significant potential to be used for on-line control and optimization implementation.

Because ethanol fermentation is inhibited by product, the selective extraction of ethanol during fermentation is also important to improve process performance. Silva et al. *(3)* have reported that the scheme that combines a fermentor with a vacuum flash vessel presents many positive features and good performance when compared to a conventional process *(4)*.

Another important consideration is the development of an efficient control strategy, because this minimizes the costs by maintaining the process in its optimal conditions. Costa et al. *(5)* determined the best control structures and studied the control of the process proposed by Silva et al. *(3)* using a linear predictive controller. Later, a nonlinear predictive controller using FLNs as the internal model was developed and implemented with the same process with promising results *(6)*.

The main objective of the present work was to verify the potential of one of the proposed approaches to optimization: the use of a successive quadratic programming (SQP) technique combined with a statistical model instead of a rigorous model. The results were compared with those obtained by Costa et al. *(5)* using response surface methodology (RSM). In the present work the optimization of the extractive fermentation process proposed by Silva et al. *(3)* is addressed. The objective is to maximize productivity while maintaining high yield. Costa et al. *(5)* optimized the same process using response surface analysis combined with modeling and simulation.

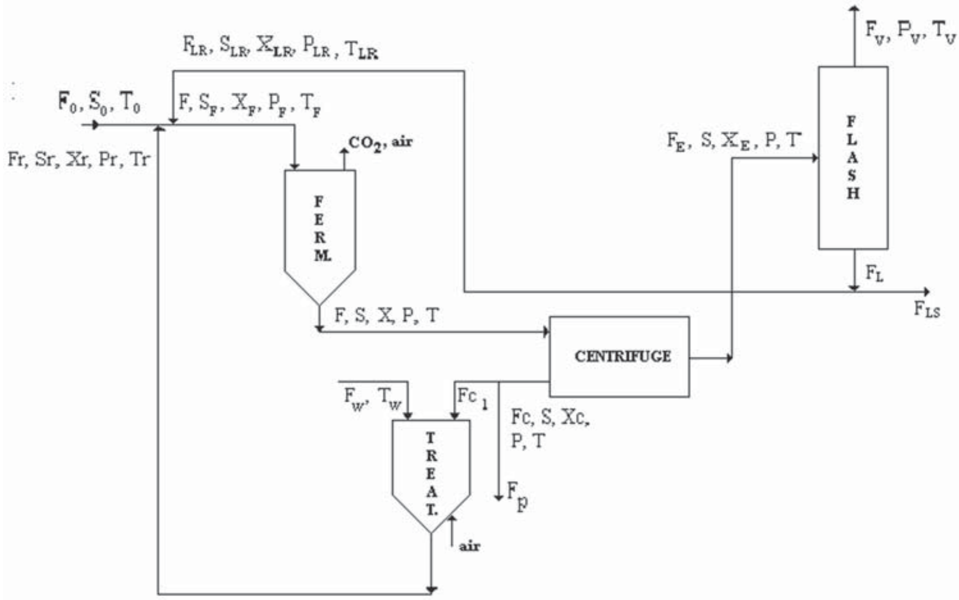

Fig. 1. Extractive alcoholic fermentation.

Although this methodology has the advantage of not requiring model simplifications, it is extremely time-consuming and cannot be used in a real-time optimization scheme.

In the present study, the problem is written as a nonlinear programming problem and is solved with SQP technique. Two process models are evaluated when the process is optimized using the SQP technique. The first one is a deterministic model with the kinetic parameters determined by Atala et al. *(1)*, and the second one is a statistical model obtained using the factorial design technique combined with simulation.

Material and Methods

Extractive Alcoholic Fermentation

A general scheme of the extractive alcoholic fermentation proposed by Silva et al. *(3)* is shown in Fig. 1. The process consists of four interlinked units: the fermentor (ethanol production unit), the centrifuge (cell separation unit), the cell treatment unit, and the vacuum flash vessel (ethanol-water separation unit). A detailed description of the process and mathematical model can be found in ref. *5*.

Assuming constant volume, the mass and energy balance equations for the fermentor using the intrinsic model *(7,8)* are as follows:

Viable cells: $$\frac{dX_v}{dt} = r_x - r_d - \frac{F}{V}\left(X_v - X_{vF}\right) \quad (1)$$

Dead cells:
$$\frac{dX_d}{dt} = r_d - \frac{F}{V}\left(X_d - X_{dF}\right) \tag{2}$$

Substrate:
$$\frac{d\left[\left(1 - \frac{X_t}{\rho}\right) SV\right]}{dt} = F\left(S_F - S\right) - Vr_s \tag{3}$$

Product:
$$\frac{d\left[\left(1 - \frac{X_t}{\rho}\right) PV + \frac{X_t}{\rho}\gamma PV\right]}{dt} = Vr_p + F\left(P_F - P\right) \tag{4}$$

$$\frac{dT}{dt} = D\left(T_F - T\right) + \frac{\Delta H r_s}{\rho_m Cp} \tag{5}$$

ρ and γ in Eqs. 3 and 4 are the ratio of dry cell weight to wet cell volume and the ratio of concentration of intracellular to extracellular ethanol, respectively.

The values of the constants in the energy balance equation (Eq. 5) are given by *(3)*: ΔH = 51.76 kcal/(kg of total reducing sugars [TRS]); ρ_m =1000 kg/m^3, and Cp = 1 kcal/(kg·°C).

The kinetic rates of growth, death, ethanol formation and substrate consumption are as follows:

$$r_x = \mu_{\max}\frac{S}{K_s + S}\exp\left(-K_i S\right)\left(1 - \frac{X_t}{X_{\max}}\right)^m \left(1 - \frac{P}{P_{\max}}\right)^n X_v \tag{6}$$

$$r_d = \left[K_{dT}\exp\left(-K_{dP}P\right)\right] X_v \tag{7}$$

$$r_p = Y_{px} r_x + m_p X_v \tag{8}$$

$$r_s = \frac{r_x}{Y_x} + m_x X_v \tag{9}$$

The parameters were adjusted as functions of the temperature from the experimental data and are given in Table 1. The proposed model described the dynamic behavior of the alcoholic fermentation *(1)*.

Results

The variables considered for optimization were the same as those used in the work of Costa et al. *(5)*: inlet substrate concentration (S_0), cell recycle rate (R), residence time (t_r), and flash recycle rate (r). They were determined by Silva et al. *(3)* as the relevant variables.

The objective of the optimization problem is to maximize productivity while maintaining a high yield. Productivity and % yield were defined as follows:

$$\text{Prod} = \frac{F_v \cdot P_v + F_{LS} \cdot P_{LR}}{\text{V}} \tag{10}$$

Table 1
Kinetic Parameters as Functions of Temperature (ºC)

Parameter	Expression or value
μ_{max}	$1.57\exp\left(\frac{41.47}{T}\right) - 1.29\cdot 10^4 \exp\left(\frac{-431.4}{T}\right)$
X_{max}	$-0.3279 \cdot T^2 + 18.484 \cdot T - 191.06$
P_{max}	$-0.4421\, T^2 + 26.41 \cdot T - 279.75$
Y_x	$2.704\exp(-0.1225 \cdot T)$
Y_{px}	$0.2556\exp(0.1086 \cdot T)$
K_s	4.1
K_i	$1.393 \cdot 10^{-4}\exp(0.1004 \cdot T)$
m_p	0.1
m_x	0.2
m	1.0
n	1.5
K_{dP}	$7.421 \cdot 10^{-3} \cdot T^2 - 0.4654 \cdot T + 7.69$
K_{dT}	$4 \cdot 10^{13}\exp\left[\frac{41947}{1.987\cdot(T + 273.15)}\right]$
ρ	390
γ	0.78

$$\%\text{Yield} = \frac{F_v \cdot P_v + F_{LS} \cdot P_{LR}}{S_0 \cdot S_0 \cdot 0.511} \tag{11}$$

Conversion was defined as follows:

$$\text{Conv} = \frac{S_0 - S}{S_0} \tag{12}$$

Optimization Using SQP Technique and Deterministic Model

Optimization was conducted with the deterministic steady-state model of the process. It consists of the steady-state mass and energy balances for the fermentor and all the other process units (*see* Fig. 1).

The optimization problem can be written as follows:

$$\text{Maximize Prod} \tag{13}$$

subject to the equality constraints described by the steady-state mass and energy balances for the fermentor and to the following inequality constraints:

$$\%\ \text{Yield} > 0.82 \tag{14}$$

$$28 < T < 40°\text{C} \tag{15}$$

$$80 < S_0 < 280\ \text{kg/m}^3 \tag{16}$$

$$0.2 < R < 0.5 \tag{17}$$

$$1.0 < t_r < 2.5\ \text{h} \tag{18}$$

$$0.2 < r < 0.6 \tag{19}$$

Productivity and % yield are calculated by Eqs. 10 and 11. The constraints for the optimization variables are the same as those used by Costa et al. *(5)*. The balance equations for the other process units were not considered explicitly as constraints but were used to calculate the flow rate and concentrations at the input of the fermentor. The optimization problem was solved by SQP implementation using the routine DNCONF of the IMSL math library of FORTRAN.

When the optimization problem is solved using the SQP technique and the deterministic model, the values of temperature and concentrations in the fermentor have to be considered as optimization variables, so the number of the optimization variables is higher than using RSM. The optimization variables are concentrations of viable cells (X_v), dead cells (X_d), substrate (S), product (P), temperature (T); as well as the variables used by Costa et al. *(5)*: S_0, R, t_r, and r.

The values of the optimization variables calculated by Costa et al. *(5)* were S_0 = 130 kg/m^3, t_r = 1.3 h, R = 0.3, and r = 0.25, which leads to productivity of 21 kg/(m^3·h), % yield of 0.82, and conversion of 0.96. Costa et al. *(5)* analyzed 16 surfaces to determine the values of the four optimization variables and they cited that it was difficult to determine the best combination of optimization variable values without taking advantage of previous knowledge of the process.

Using the SQP method, solving the optimization problem was straightforward. The calculated values were S_0 = 180 kg/m^3, t_r = 1 h, R = 0.35, and r = 0.4. The values of productivity, % yield, and conversion were 22.7 kg/(m^3·h), 0.82, and 0.96, respectively.

The productivity for the same % yield calculated by the SQP technique was higher than that calculated using the RSM. The RSM calculates the ranges of the optimization variables and not exact values, so it is difficult to determine the exact global maximum point. In addition, the RSM uses statistical models for productivity and % yield and these are simplified models, while the SQP technique uses a rigorous process model.

The conversion values corresponding to optimal conditions calculated may be considered low for industrial units. If one includes a constraint in conversion (conversion > 0.99) and solves the optimization problem using the SQP technique again, the following values are calculated: $S_0 = 180$ kg/m^3, $t_r = 1$ h, $R = 0.42$, and $r = 0.52$. Productivity is 13 kg/(m^3·h), conversion is 0.99, and % yield is 0.89. In this case, the extractive process has productivity only a little higher than the optimized conventional process. Kalil et al. *(9)* optimized the conventional process of Andrietta and Maugeri *(4)* and obtained productivity of 12 kg/(m^3·h), conversion of 0.99, and % yield of 0.86.

Although the productivity calculated using the SQP technique was higher and the result seemed easier to be obtained than using RSM, the results obtained using the SQP technique are highly dependent on the initial guess. For some values of the initial guess, the SQP technique may reach a local maximum or does not converge. In the present work, the initial guess used was $X_v = 30$ kg/m^3, $X_d = 0$ kg/m^3, $S = 2$ kg/m^3, $P = 40$ kg/m^3, $T = 30$ºC, $S_0 = 120$ kg/m^3, $t_r = 1.3$ h, $R = 0.3$, and $r = 0.6$. However, when the initial guess $X_v = 30$ kg/m^3, $X_d = 0$ kg/m^3, $S = 2$ kg/m^3, $P = 40$ kg/m^3, $T = 30$ºC, $S_0 = 120$ kg/m^3, $t_r = 1$ h, $R = 0.2$, and $r = 0.2$, was used, there was no convergence.

Another point to be discussed is the use of the rigorous model compared with the use of simplified models as the statistical models determined by factorial design and used with RSM. Although rigorous models are more realistic and probably lead to better results, for many processes they can be excessively complex, which hinders determination of the optimal result.

Optimization Using SQP Technique and a Simplified Model of the Process

The statistical models determined by factorial design can be used as simplified models with the SQP technique. In this work the results obtained through this approach are compared with the results obtained using a rigorous model of the process. Costa et al. *(5)* determined quadratic models for productivity and % yield as functions of the significant input variables. These equations evaluated productivity and % yield and the SQP technique to determine the optimal values for S_0, t_r, R, and r. The optimization problem is postulated as follows:

$$\text{Maximum Prod} \tag{20}$$

$$\%\ \text{Yield} > 0.82 \tag{21}$$

$$28 < T < 40°\text{C} \tag{22}$$

$$80 < S_0 < 280\ \text{kg/m}^3 \tag{23}$$

$$0.2 < R < 0.5 \tag{24}$$

$$1.0 < t_r < 2.5\ \text{h} \tag{25}$$

$$0.2 < r < 0.6 \tag{26}$$

Productivity and % yield are described by the statistical models, which are written as functions of the optimization variables (S_0, t_r, R, and r).

The optimization variables values are $S_0 = 118.3$ kg/m^3, $t_r = 1$ h, $R = 0.2$, and $r = 0.2$. The values for % yield and productivity calculated by the statistical models are 0.82 and 26.73 kg/(m^3·h), respectively. Using the values calculated for S_0, t_r, R, and r in the rigorous model, however, the % yield calculated was 0.69 and productivity was 26.39 kg/(m^3·h). Note that although the statistical model for yield developed by Costa et al. (5) has a high correlation coefficient and passed the F-test with 99% confidence, at the calculated optimal conditions, it presents a great deviation from the rigorous model.

We also tested the influence of the initial guess on the optimal values calculated. The results shown above were obtained with the following initial guess: $S_0 = 130$ kg/m^3, $t_r = 2$ h, $R = 0.2$, and $r = 0.6$. Many other values were tested and the optimal result was almost always obtained. The exception was when we used a high initial value for S_0. For example, the initial guess $S_0 = 230$ kg/m^3, $t_r = 2$ h, $R = 0.2$, and $r = 0.6$ led to the following values calculated for the optimization variables: $S_0 = 280$ kg/m^3, $t_r = 2.5$ h, $R = 0.2$, and $r = 0.597$. Yield and productivity calculated by the statistical models are 0.82 and 20.63 kg/(m^3·h), respectively. These results show one of the disadvantages of the SQP technique: the optimization algorithm can reach a local maximum. The values for % yield and productivity calculated using the rigorous model are 0.77 and 16.20 kg/(m^3·h), respectively. In this case, the statistical model for productivity also presents a high deviation from the result calculated from the rigorous model.

The statistical models developed by Costa et al. *(5)* were functions only of the inputs considered statistically significant by a test performed with the software Statistica (Statsoft, v. 5.0). Since the results obtained by these models presented deviations from the rigorous model, complete models were tested. The following models were developed using the software Statistica:

$$\begin{aligned}&\text{Rend} =\\ &72.10 - 0.40S_0 - 6.56\cdot 10^{-4}S_0^2 + 23.83t_r - 4.76t_r^2 + 53.55R - 47.73R^2 + 33.49r - 38.83r^2\\ &+ 7.09\cdot 10^{-2}S_0t_r + 0.42S_0R + 0.59S_0r - 11.42t_rR - 22.64t_rr - 64.85Rr\end{aligned} \tag{27}$$

$$\begin{aligned}&\text{Prod} =\\ &27.44 + 0.14\text{S}_0 - 2.84\cdot 10^{-4}\text{S}_0^2 - 23.04\text{t}_\text{r} + 3.77\text{t}_\text{r}^2 + 20.50\text{R} - 48.18\text{R}^2 + 13.63\text{r} - 41.61\text{r}^2\\ &- 7.75\cdot 10^{-3}\text{S}_0\text{t}_\text{r} + 9.58\cdot 10^{-4}\text{S}_0\text{R} + 9.44\cdot 10^{-2}\text{S}_0\text{r} + 7.34\text{t}_\text{r}\text{R} + 3.22\text{t}_\text{r}\text{r} - 78.49\text{Rr}\end{aligned} \tag{28}$$

Table 2 depicts the analysis of variance for % yield and productivity. Both responses present a high correlation coefficient, and the model can be considered statistically significant according to the F-test with 99% confidence. As a practical rule, a model has statistical significance if the calculated F value is at least three to five times greater than the listed value *(9)*.

Table 2
Analysis of Variance

	Sum of squares		Mean square		Degrees	F-value	
Source of variation	Rend	Prod	Rend	Prod	of freedom	Rend	Prod
Regression	2326.5	726.3	166.2	51.9	14	35.8	28.1
Residual	46.4	18.5	4.64	1.85	10		
Total	2372.9	744.8			24		
Correlation coefffecient	0.980	0.975					
F listed value					$4.56 < F_{14,10} = 4.71$ (99%)		

Table 3
Results Using RSM, SQP Technique + Rigorous Model,
and SQP Technique + Simplified Model

	RSM	SQP + rigorous model	SQP + simplified model
S_0 (kg/m^3)	130.00	180.00	103.00
t_r (h)	1.30	1.00	1.00
R	0.30	0.35	0.34
r	0.25	0.40	0.20
Prod (kg/[m^3·h])	21.00[a]	22.70	21.60[a]
% yield	0.82[a]	0.82	0.83[a]

[a]Calculated using the rigorous model.

The optimization problem described by Eqs. 20–26 was solved using Eqs. 27 and 28 to calculate % yield and productivity. At the low S_0 initial guess (S_0 = 130 kg/m^3, t_r = 2 h, R = 0.2, and r = 0.6) the optimal values calculated are S_0 = 103 kg/m^3, t_r = 1 h, R = 0.344, and r = 0.2. Yield and productivity calculated by the statistical models are 0.82 and 21.63 kg/(m^3·h), respectively, and calculated using the rigorous model are 0.83 and 21.62 kg/(m^3·h), respectively. If the high S_0 initial guess is used (S_0 = 230 kg/m^3, t_r = 2 h, R = 0.2, and r = 0.6), the optimization variables calculated are S_0 = 186 kg/m^3, t_r = 1 h, R = 0.247, and r = 0.6, resulting in % yield and productivity of 0.82 and 21.57 kg/(m^3·h), respectively, calculated by the statistical model, and 0.82 and 21.01 kg/(m^3·h), respectively, calculated by the rigorous model. Note that when the complete statistical models were used, the results for % yield and productivity are very close to those calculated by the rigorous model. It can also be observed that different initial guesses can lead to different results. A comparison of the optimization variables and responses calculated by the three approaches is shown in Table 3.

Discussion

Comparison of the performance of the three approaches (RSM, SQP + rigorous model, and SQP + statistical model) shows that the SQP + rigorous model approach led to the highest productivity (22.7 kg/[m^3·h]), while maintaining a high % yield (at least 0.82). Comparison of the two approaches with the simplified model demonstrates that the one using the SQP technique led to the highest productivity value (21.6 kg/[m^3·h]). Using the RSM, the productivity was 21 kg/m^3·h. In this case, many surfaces are analyzed at the same time and the determination of the global maximum is more difficult.

Note that although the productivity and % yield values are similar using the three approaches, the optimization variables values calculated are different, which shows that there are many combinations of values of the optimization variables that lead to high productivity and % yield. This conclusion had already been drawn in the work of Costa et al. *(5)* by analysis of the response surfaces. One of the advantages of the RSM is that it is possible to picture the behavior of the optimization variables in the region of interest.

Although the best result was the one obtained using the rigorous model, the values for productivity and % yield obtained using the simplified model are acceptable, and these models can be used when the development of a rigorous model is excessively difficult, slow, or expensive. Several simulations were carried out with the SPQ optimizer, which was shown to be very robust despite some initial condition dependence.

Another situation when the use of the statistical model can be a good choice over the RSM is when the deterministic model is excessively complex. For example, when the process is described by a distributed parameters model, the steady-state mass and energy balances are differential equations. The use of differential equations as constraints in an optimization problem makes its solution difficult and increases the incidence of convergence problems. In this case, solving the optimization problem using the statistical model is much simpler. The statistical model can also be used when the computational effort to solve the optimization problem using the deterministic model is too high, as can be the case for real-time optimization problems.

The simulations were performed using a mathematical model validated experimentally *(5)*, and their results can be said to have physical meaning. The next step is to perform experiments using the optimal conditions determined using the three methods shown in Table 3. In fact, a simulation study and analysis is a good tool to discriminate among possible techniques as well as to determine the possible operating range to be tested in an experimental apparatus.

Nomenclature

Cp = heat capacity (kcal/[kg·°C])
$D = F/V$ = dilution rate, (h^{-1})

F	=	feed stream flow rate (m^3/h)
F_0	=	fresh medium flow rate (m^3/h)
F_L	=	liquid outflow from vacuum flash tank (m^3/h)
F_{LR}	=	liquid phase recycling flow rate (m^3/h)
F_{LS}	=	liquid phase flow to rectification column (m^3/h)
F_r	=	cell recycling flow rate (m^3/h)
F_V	=	vapor outflow from the vacuum flash tank (m^3/h)
K_{dP}	=	coefficient of death by ethanol (m^3/kg)
K_{dT}	=	coefficient of death by temperature (h^{-1})
K_{ei}	=	equilibrium constant
K_i	=	substrate inhibition constant (m^3/kg)
K_s	=	substrate saturation constant (kg/m^3)
m	=	constant in Eq. 6
m_p	=	ethanol production associated to growth (kg/[kg·h])
m_x	=	maintenance coefficient (kg/[kg·h])
n	=	constant in Eq. 6
P	=	product concentration in fermentor (kg/m^3)
P_F	=	feed product concentration (kg/m^3)
P_{LR}	=	product concentration in light phase from centrifuge (kg/m^3)
$P_{\max}$	=	product concentration when cell growth ceases (kg/m^3)
P_V	=	product concentration in vapor phase from flash tank (kg/m^3)
$r = F_{LR}/F_L$	=	flash recycle rate
r_d	=	kinetic rate of death (kg/[m^3·h])
r_p	=	kinetic rate of product formation (kg/[m^3·h])
r_s	=	kinetic rate of substrate consumption (kg/[m^3·h])
r_x	=	kinetic rate of growth (kg/[m^3·h])
$R = Fr/F$	=	cells recycle rate
S	=	substrate concentration in fermentor (kg/m^3)
S_0	=	inlet substrate concentration (kg/m^3)
S_F	=	feed substrate concentration (kg/m^3)
T	=	temperature in fermentor (°C)
T_F	=	feed temperature (°C)
t_r	=	residence time (h)
V	=	reactor volume (m^3)
X_d	=	dead biomass concentration in fermentor (kg/m^3)
X_{dF}	=	dead biomass concentration in feed stream (kg/m^3)
X_F	=	feed biomass concentration (kg/m^3)
$X_{\max}$	=	biomass concentration when cell growth ceases (kg/m^3)
$X_t = X_v + X_d$	=	total biomass concentration in fermentor (kg/m^3)
X_v	=	viable biomass concentration in fermentor (kg/m^3)
X_{vF}	=	viable biomass concentration in feed stream (kg/m^3)
Y_{px}	=	% yield of product based on cell growth (kg/kg)
Y_x	=	limit cellular yield (kg/kg)
ΔH	=	reaction heat (kcal/[kg TRS])

γ = ratio of concentration of intracellular to extracellular ethanol (kg/m^3)
μ_{max} = maximum specific growth rate (h^{-1})
ρ = ratio of dry cell weight per wet cell volume (kg/m^3)
ρ_m = density (kg/m^3)

References

1. Atala, D. I. P., Costa, A. C., Maciel, R., and Maugeri, F. (2001), *Appl. Biochem. Biotechnol.* **91(3),** 353–365.
2. Harada, L.H.P., Costa, A. C., Maciel Filho, R. (2002), *Appl. Biochem. Biotechnol.* **98(1–3),** 1009–1024.
3. Silva, F. L. H., Rodrigues, M. I., and Maugeri, F. (1999), *J. Chem. Tech. Biotechnol.* **74,** 176–182.
4. Andrietta, S. R. and Maugeri, F.(1994), *Adv. Bioprocess Eng.*, 47–52.
5. Costa, A. C., Atala, D. I. P., Maciel Filho, R., and Maugeri Filho, F. (2001), *Process Biochem.* **37(2),** 125–137.
6. Costa, A. C., Meleiro, L. A. C., and Maciel Filho, R. (2002), *Process Biochem.* **38(5),** 743–750.
7. Monbouquette, H. G. (1987), *Biotechnol Bioeng.* **29,** 1075–1080.
8. Monbouquette, H. G. (1992), *Biotechnol Bioeng.* **39,** 498–503.
9. Kalil, S. J., Maugeri, F., and Rodrigues, M. I. (2000), *Process Biochem.* **35,** 539–550.