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Kinetics of Ethanol Fermentation with High Biomass Concentration Considering the Effect of Temperature

Daniel I. P. Atala,[1] Aline C. Costa,*,[2] Rubens Maciel,[2] and Francisco Maugeri[1]

[1]DEA/FEA/UNICAMP, Cx. Postal 6121, Campinas, SP, Brazil 13081-970; and [2]DPQ/FEQ/UNICAMP, Cx. Postal 6066, Campinas, SP, Brazil 13081-970, E-mail: accosta@feq.unicamp.br

Abstract

A model of ethanol fermentation considering the effect of temperature was developed and validated. Experiments were performed in a temperature range from 28 to 40°C in continuous mode with total cell recycling using a tangential microfiltration system. The developed model considered substrate, product and biomass inhibition, as well as an active cell phase (viable) and an inactive (dead) phase. The kinetic parameters were described as functions of temperature.

Index Entries: Ethanol fermentation; high biomass concentration; temperature.

Introduction

Brazil is one of the greatest ethanol producers in the world as the result of a political strategy initiated in 1975 by the government to cope with the sharp increase in oil prices. Programs in the United States in 1978 and, more recently in Canada, followed this strategy *(1)*. Because of the stabilization of petroleum prices at a low level most of the incentives to the alcohol industries were withdrawn and there was a great interest in the optimization of all the stages of the ethanol production process. Now, a new increase in petroleum prices and improvements in productivity attained in the industries have made alcohol production costs lower than that of gasoline, and, again, there is a good outlook for the alcohol industries in Brazil *(2)*. In addition, alcohol is a clean fuel, producing less harm to the environment.

Operation of the alcoholic fermentation process in a continuous mode is desirable, since higher productivity, improved yields, and better process

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

control are attained *(3)*. However, industrial implementation of a continuous process requires previous study of the process behavior and its use in the development of an efficient control strategy. The influence of temperature on the kinetic parameters must be considered, because it is usually difficult to support a constant temperature during large-scale alcoholic fermentation. The process is exothermic and small deviations in temperature (2–4°C) can dislocate the process from optimal operational conditions. Also, knowledge about the effect of temperature on ethanol fermentation kinetics can be useful in strategies for process optimization *(4)*.

One way of improving productivity in an ethanol production process is to increase cell concentration. Several methods have been proposed to obtain high cell densities in continuous cultures. Cell immobilization *(5)* and cell recycling are common methods for this purpose. Cell recycling by settling *(6)* requires flocculent yeast strains. Despite being the method used in Brazilian industries *(1)*, cell recycling by centrifugation has some disadvantages because aseptic conditions are difficult to achieve and the process is usually too expensive *(7)*. Membrane filter systems have been studied in recent years and seem to be a good alternative to cell recycling. The membrane module can be located inside *(8)* or outside the reactor *(7,9–11)*. The location outside the reactor facilitates washing, cleaning, and regeneration of membranes without interfering with fermentation *(10)*.

In the present study, a mathematical model was developed to describe the alcoholic fermentation process kinetics taking into account the effect of temperature on kinetic parameters. To achieve this goal, experiments were performed at temperatures between 28 and 40°C in a system with cell recycling by tangential microfiltration. The substrate was sugarcane molasses.

Materials and Methods

Microorganism

The yeast used was *Saccharomyces cerevisiae* cultivated in the Bioprocess Engineering Laboratory in the Faculty of Food Engineering/UNICAMP and obtained from an industrial fermentation plant.

Culture Medium

The growth medium for the inoculum consisted of 50 kg/m^3 of glucose, 5 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 kg/m^3 of yeast extract. The production medium was diluted sugarcane molasses to which 1 kg/m^3 of yeast extract and 2.4 kg/m^3 of $(NH_4)_2SO_4$ were added. Sterilization was done at 121°C for 30 min.

Fermentation System and Operation

The experimental equipment is shown in Fig. 1. The tangential microfiltration system (Ceraflow model; Millipore) was coupled to a

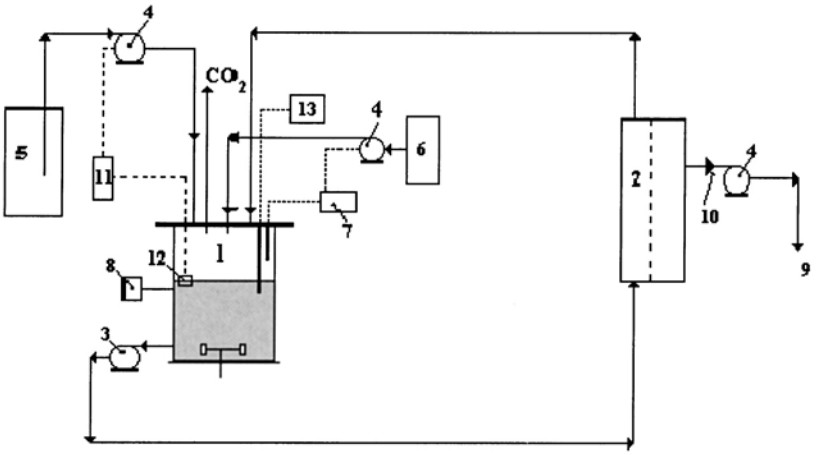

Fig. 1. Fermentation system: 1, fermentor; 2, tangential microfilter; 3, helical pump; 4, peristaltic pumps; 5, feed tank; 6, antifoam tank; 7, foam sensor; 8, temperature indicator; 9, filtrate outlet; 10, reduction pressure valve; 11, level controller; 12, level sensor; 13, turbidimeter.

0.0025-m^3 bioreactor (Bioflow III System; New Brunswick Scientific). Cell broth was recirculated into the microfilter by a helical pump (Netsch 2.NEL.20 A). A reduction pressure valve was installed at the filtrate outlet to avoid outlet rate fluctuation and a turbidimeter was used to monitor cell growth.

Sterilization

Filters were washed with 2 *N* NaOH solution at 40°C for 4 h and then rinsed with water for 4 h. Sterilization was done by circulating saturated steam into the complete system for 2 h.

System Operation

After inoculation, fermentation was operated in batch mode until the end of the exponential phase, monitored by CO_2 production. At this point, the continuous fermentation with total cell recycling was started, by adjusting a peristaltic pump to remove the filtrate in a flow rate corresponding to a dilution rate of 0.1 h^{-1}. Fresh medium was continuously supplied to the fermentor by a peristaltic pump connected to an on–off level controller, so that the fermentor volume was maintained constant. The experiment was finished when the filtrate flow rate could not be maintained constant owing to the decrease in the filtration capacity of the system.

Analytical Methods

Dry cell mass was determined gravimetrically after centrifuging, washing, and drying the cells at 105°C. Viable cells were counted with the methylene blue staining technique *(12)*. Total reducing sugar (TRS) and

ethanol concentrations were determined by high-performance liquid chromatography (Varian 9010 model). A column SHODEX KS 801 at 70°C was used. Ultrapure Milli-Q water was used as the eluent at a flow rate of 0.5 mL/min. The standards were mixed solutions of sucrose, fructose, and ethanol at concentrations from 0.1 to 40 kg/m^3. The software Millennium® v.2.1 was used for integration and quantification.

Results

Experiments

The substrate was fed at a high rate in all the experiments (about 25 kg/[m^3·h] of TRS), so that high substrate and ethanol concentrations were maintained in the fermentor. Then, the kinetics of the fermentation was studied in highly stressing conditions for the microorganism, which enabled the study of inhibition by biomass, substrate, and product.

Figures 2 to 6 show the results of the experiments performed at 28, 31, 34, 37, and 40°C, respectively. They all show the results of the mathematical model developed in the next session. Concentrations of total and viable biomass, substrate (TRS) and ethanol are plotted against time during the operation with total cell recycling. The substrate and ethanol concentrations remained at about a constant value almost since the beginning of the operation. The biomass concentration (total and viable), however, increased with time and, at the end, tended to stabilize near a fixed value. This finding is in agreement with that of other researchers *(10)*, who observed that a pseudo-steady-state biomass concentration was reached in cultures with total cell recycling. Melzoch et al. *(10)* showed that this is not caused by product inhibition, because even at a low product concentration a constant biomass concentration was achieved.

Comparison of the results from the different experiments is shown in Table 1. Productivity showed a maximum at 31°C and then decreased with temperature; conversion (defined as $100 \times [S_F - S]/S_F$) always decreases with temperature. The highest biomass concentrations were obtained at the lowest temperatures (28 and 31°C) and then diminished with temperature, presenting a very low value at 40°C. Final viability (defined as X_V/X_T) remained constant at about 60% at the three first temperatures studied and then increased at 37 and 40°C. This increase in viability is probably owing to the lower biomass and ethanol concentrations when compared to the experiments at lower temperatures.

The biomass concentrations achieved were low when compared with the values obtained by other investigators. Lafforgue et al. *(9)* obtained 300 kg/m^3 in a continuous fermentation with cells recycling by tangential microfiltration. Lee and Chang *(7)* reached 210 kg/m^3 using a cell recycle system with a hollow-fiber membrane filter. The biomass concentration attained by Melzoch et al. *(10)* was nearer to that obtained in the present work (60 kg/m^3 of biomass using a separation module composed of ultrafiltration tubular membranes). One of the reasons for the low biomass con-

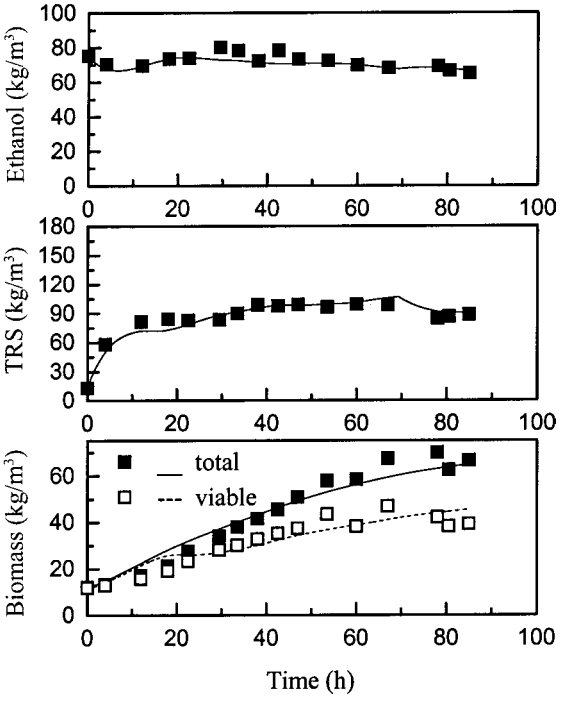

Fig. 2. Experimental (■, □) and modeling (——; – – –)results at 28°C.

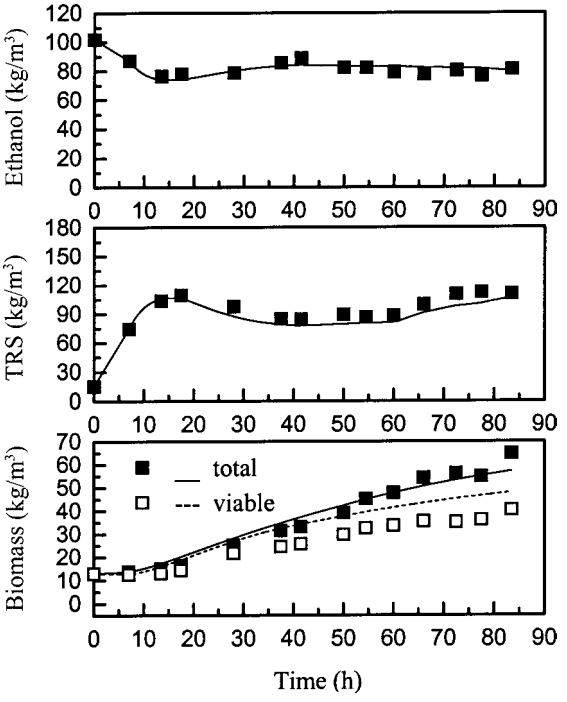

Fig. 3. Experimental (■, □) and modeling (——; – – –) results at 31°C.

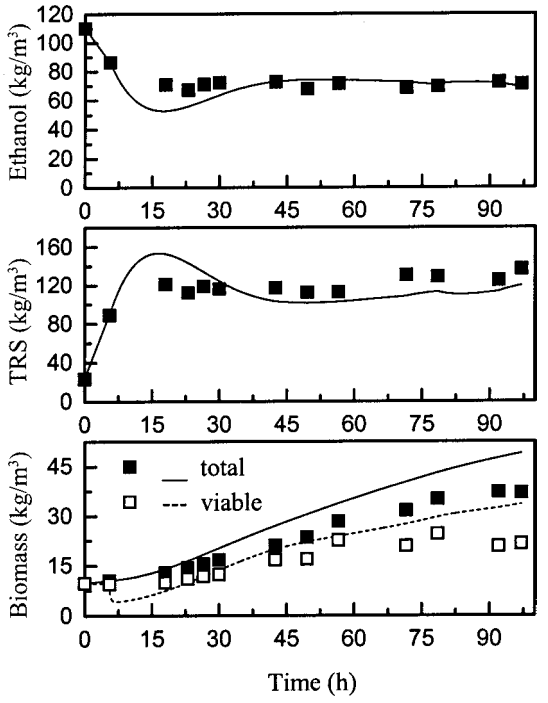

Fig. 4. Experimental (■, □) and modeling (——; – – –) results at 34°C.

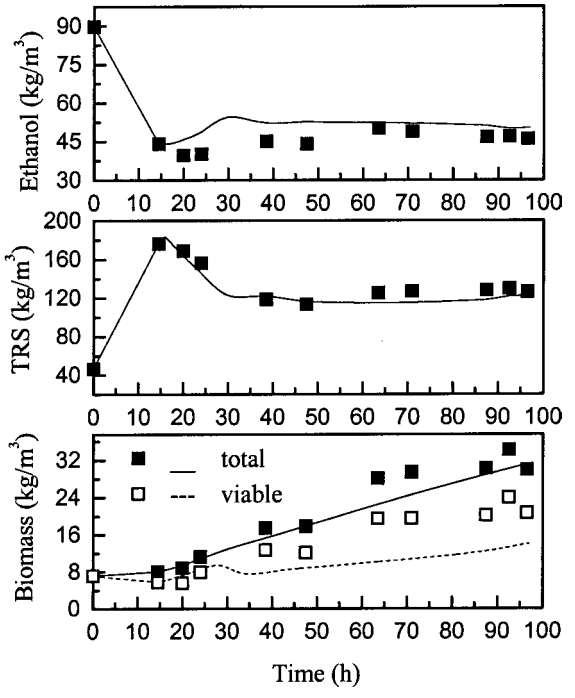

Fig. 5. Experimental (■, □) and modeling (——; – – –) results at 37°C.

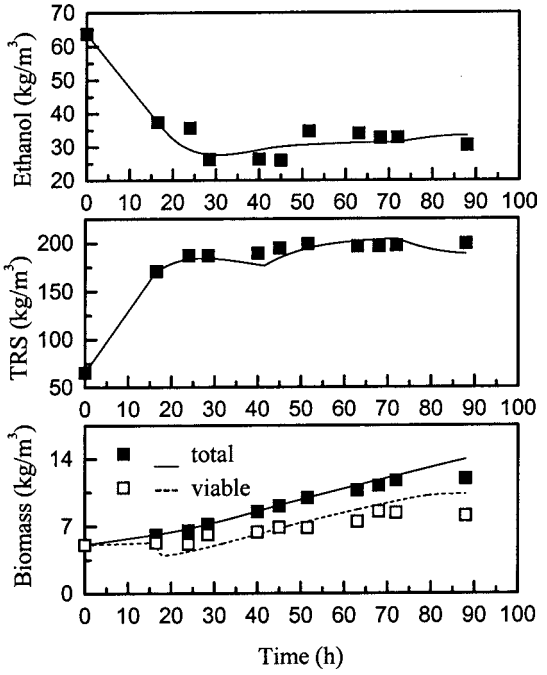

Fig. 6. Experimental (■, □) and modeling (——; – – –) results at 40°C.

Table 1
Comparison of Experimental Results for Different Temperatures

	28°C	31°C	34°C	37°C	40°C
Conversion (%)	60–75	59–67	49–58	29–50	23–30
Productivity (kg/[m^3·h])	6.5–7.5	7.1–8.4	6.5–7.2	4.1–4.7	2.5–3.5
Final total biomass (kg/m^3)	65–70	65	37	30	8
Viability (%)	60	62	59	70	67

centrations obtained could be the use of sugarcane molasses as substrate, since all the other works cited used synthetic media. It is well known that sugarcane molasses, although a good substrate, has some components that can act as inhibitors for ethanol fermentation. In this work, the objective was to study fermentation kinetics under industrial conditions, so only sugarcane molasses was used as substrate.

Mathematical Modeling

The following approximations were made for the development of an unstructured mathematical model for the alcoholic fermentation process: the fermentor volume is constant, the bubble volume is negligible, and the microfilter operation is ideal (i.e., the filtrate stream contains no cells).

Monbouquette *(13)* showed that the use of models developed from low biomass concentration cultures leads to errors if they are extrapolated to high cell concentrations. He emphasized the need to use intrinsic models (models that take into account cell volume fraction) if the volume fraction occupied by biomass is >10%.

In agreement with the results obtained by many researchers *(9,14)*, the experimental data showed that there is a loss of cell viability during fermentation. Taking this into account, it is assumed that the total biomass comprises a viable (active) phase, X_v, and an inactive (dead) phase, X_d.

The mass balance equations using the intrinsic model are as follows:

$$\frac{d(X_v V)}{dt} = Vr_x - Vr_d \tag{1}$$

$$\frac{d(X_d V)}{dt} = Vr_d \tag{2}$$

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

$$\text{product: } \frac{d\left[\left(1 - \frac{X_t}{\rho}\right) PV + \frac{X_t}{\rho}\gamma P V\right]}{dt} = Vr_p - FP \tag{4}$$

In Eqs. 3 and 4, ρ is the ratio of dry cell weight per wet cell volume and γ is the ratio of concentration of intracellular to extracellular ethanol. Assuming constant volume and simplifying, the following equations are obtained:

$$\text{viable cells: } \frac{dX_v}{dt} = r_x - r_d \tag{5}$$

$$\text{dead cells: } \frac{dX_d}{dt} = r_d \tag{6}$$

$$\text{substrate: } \frac{dS}{dt} = \frac{D(S_F - S) - r_s + (r_x/\rho)/S}{1 - (X_t/\rho)} \tag{7}$$

$$\text{product: } \frac{dP}{dt} = \frac{r_p - dP + \frac{(1-\gamma)}{\rho} r_x P}{1 - (X_t/\rho) + (\gamma/\rho)/X_t} \tag{8}$$

Table 2
Kinetic Parameters as Functions of Temperature (°C)

Parameter	Expression or value
μ_{max}	$1.57 \exp(\frac{-41.47}{T}) - 1.29 \times 10^4 \exp(\frac{-431.4}{T})$
X_{max}	$-0.3279 \times T^2 + 18.484 \times T - 191.06$
P_{max}	$-0.4421 \times T^2 + 26.41 \times T - 279.75$
Y_x	$2.704 \exp(-0.1225 \times T)$
Y_{px}	$0.2556 \exp(0.1086 \times T)$
K_s	4.1
K_i	$1.393 \times 10^{-4} \exp(0.1004 \times T)$
m_p	0.1
m_x	0.2
m	1
n	1.5
K_{dP}	$7.421 \times 10^{-3} \times T^2 - 0.4654 \times T + 7.69$
K_{dT}	$4.10^{13} \exp\left[-\frac{41{,}947}{1.987 \times (T + 273.15)}\right]$
ρ	390
γ	0.78

Many different models have been proposed to describe the kinetic rates of ethanol fermentation. Herein, the growth rate was described by

$$r_x = \mu_{max} \frac{S}{K_s + S} \exp(-K_i S) \left(1 - \frac{X_t}{X_{max}}\right)^m \left(1 - \frac{P}{P_{max}}\right)^n X_v \quad (9)$$

which is similar to the equation proposed by Jarzebski et al. *(14)* plus a term to describe substrate inhibition.

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

$$r_d = [K_{dT} \exp(K_{dP} P)] X_v \quad (10)$$

$$r_p = Y_{px} r_x + m_p X_v \quad (11)$$

$$r_s = (r_x / Y_x) + m_x X_v \quad (12)$$

The kinetic parameters were adjusted as functions of the temperature from the experimental data and are shown in Table 2. Equations 5–12 were solved using a Fortran program with integration by an algorithm based on the fourth-order Runge-Kutta method. A comparison of the results of the mathematical model and the experimental is presented in Figs. 2–6. The proposed model described well the dynamic behavior of the alcoholic fermentation.

Table 3
RSD of the Average of Experimental Values

Temperature (°C)	RSD X_t (%)	RSD X_V (%)	RSD ART (%)	RSD ethanol (%)
28	9.4	13.3	6.6	4.5
31	8.8	26.6	8.3	3.5
34	27.9	34.0	15.6	10.0
37	13.3	43.9	5.6	10.7
40	7.5	14.8	3.9	9.0

The quality of the prediction of the model can be characterized using the residual standard deviation (RSD), Eq. 13, which provides an indication of the accuracy of the prediction, as suggested by Cleran et al. *(15)*:

$$\text{RSD} = \frac{\sqrt{\sum_{i=1}^{n} (y_i - y_{pi})^2}}{n} \tag{13}$$

in which y_i is the experimental value, y_{pi} is the value predicted by the mathematical model, and n is the number of experimental points.

Because the magnitude of the RSD will vary depending on the magnitude of the variable to be predicted, it is easier to analyze the RSD written as a percentage of the average of the experimental values $\overline{y_i}$:

$$\text{RSD}(\%) = \frac{\text{RSD}}{\overline{y_i}} \times 100 \tag{14}$$

The results are shown in Table 3. The deviations are from 3.5% to more than 40%. In general, the model does not predict well the process at 34°C and the data for X_V at all the temperatures. The bad results for X_V are mainly owing to the difficulty in finding a function to describe parameter K_{dP} in all of the studied temperature range. Deviations below 10% can be considered acceptable regarding bioprocess engineering. Therefore, the model can be used to predict system performance and to design process controllers.

Discussion

As reported by Jarzebski et al. *(14)*, at high yeast cell concentrations, conditions for growth are less favorable owing to hindered access to nutrients, space limitations, and cell interaction. These facts, together with the prolonged residence time of cells, lead to a kinetic behavior different from that usually encountered, and an adequate mathematical model must take these factors into account. A model was developed by taking into

account the loss of viability during fermentation and the volume fraction occupied by biomass, as proposed by Monbouquette *(13)*.

The experimental data have shown that the alcoholic fermentation kinetics is strongly dependent on temperature. It was shown that the increase in temperature decreases productivity, conversion, and final biomass concentration. These values at 40°C are extremely low: conversion is 23–30%, productivity is 2.5–3.5 kg/(m^3·h), and final biomass concentration is about 8 kg/m^3. The effect of temperature on viability is not so clear, because it increased at 37 and 40°C when it was expected that the increase in temperature would make it decrease. This increase is probably owing to the low biomass and ethanol concentrations attained in the experiments performed at these temperatures.

Because the experimental data show that temperature has a strong influence on the kinetics of the process, in the mathematical model developed in this work the kinetic parameters are described as functions of temperature. The proposed model was shown to describe the experimental data well.

Cell viability decreased greatly with an increase in fermentation time, reaching values of about 60 and 70% at the end of the experiments. This can be explained by the highly stressing conditions to the microorganism in the experiments, owing to high substrate, ethanol, and biomass concentrations and to the friction forces in the recirculation pump and filters. Higher viability could be obtained if, instead of total cell recycling, partial recycling were used. The use of a purge permits cell renovation and the withdrawal of secondary products accumulated in the fermentor (which could be toxic to the microorganism).

The biomass concentrations were not as high as those reported by some other researchers *(7,9)*, probably because molasses was used as the substrate. Although the experiments were interrupted owing to the decrease in the system's filtration capacity, experimental data have shown that in all the cases the biomass concentration had stabilized near a constant value, which suggests that even if higher-capacity filters were used the attained biomass concentration would not increase.

Dilution rate and feed substrate concentration are important parameters in ethanol fermentation. Their influence on the final biomass concentration should be studied in order to determine the possibility of increasing biomass concentration, conversion, and yields. This study can be made experimentally or by computer simulation using the mathematical model developed herein.

Acknowledgments

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Nomenclature

$D = F/V$ = dilution rate (h^{-1})
F = substrate feed flow rate (m^3/h)
K_{dP} = coefficient of death by ethanol (m^3/kg)
K_{dT} = coefficient of death by temperature (h^{-1})
K_i = substrate inhibition coefficient (m^3/kg)
K_s = substrate saturation constant (kg/m^3)
m = constant in Eq. 5
m_p = ethanol production associated with growth (kg/[kg·h])
m_x = maintenance coefficient (kg/[kg·h])
n = constant in Eq. 5
P = product concentration (kg/m^3)
P_{max} = product concentration when cell growth ceases (kg/m^3)
r_d = kinetic rate of death ($kg/[m^3 \cdot h]$)
r_p = kinetic rate of ethanol formation ($kg/[m^3 \cdot h]$)
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)
S_F = feed substrate concentration (kg/m^3)
V = reactor volume (m^3)
X_d = dead 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 (kg/m^3)
X_v = viable biomass concentration (kg/m^3)
Y_{px} = yield of product based on cell growth (kg/kg)
Y_x = limit cellular yield (kg/kg)
γ = ratio of concentration of intracellular to extracellular ethanol
μ_{max} = maximum growth rate (h^{-1})
ρ = ratio of dry cell weight per wet cell volume (kg/m^3)

References

1. Wheals, A. E., Basso, L. C., Alves, D. M. G., and Amorim, D. M. G. (1999), *Tibtech.* **17,** 482–487.
2. Furtado, M. R. (1999), *Química Derivados* 8–17.
3. Kargupta, K., Datta, S., and Sanyal, S. K. (1998), *Biochem. Eng. J.* **1,** 31–37.
4. Kalil, S. J., Maugeri, F., and Rodrigues, M. I. (2000), *Process Biochem.* **35,** 539–550.
5. Dale, M. C., Chen, C., and Okos, M. R. (1990), *Biotechnol. Bioeng.* **36,** 983–992.
6. Ghose, T. K. and Tyagi, R. D. (1979), *Biotechnol. Bioeng.* **21,** 1401–1420.
7. Lee, C. W. and Chang, H. N. (1987), *Biotechnol. Bioeng.* **29,** 1105–1112.
8. Chang, H. N., Lee, W. G., and Kim, B. S. (1993), *Biotechnol. Bioeng.* **41,** 677–681.
9. Lafforgue, C., Malinowski, J., and Goma, G. (1987), *Biotechnol. Lett.* **9,** 347–352.
10. Melzoch, K., Rychtera, M., Markivichov, N. S., Pospíchalová, V., Basařová, G., and Manakov, M. N. (1991), *Appl. Microbiol. Biotechnol.* **34,** 469–472.
11. Lafforgue-Delorme, C., Delorme, P., and Goma, G. (1994), *Biotechnol. Lett.* **16,** 741–746.
12. Lee, S. S., Robinson, F. M., and Wang, H. Y. (1981), *Biotechnol. Bioeng. Symp.* **11,** 641–649.

13. Monbouquette, H. G. (1992), *Biotechnol. Bioeng.* **39,** 498–503.
14. Jarzebski, A. B., Malinowski, J. J., and Goma, G. (1989), *Biotechnol. Bioeng.* **34,** 1225–1230.
15. Cleran, Y., Thibault, J., Chéruy, A., and Corrieu, G. (1991), *J. Ferment. Bioeng.* **71,** 356–362.