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Discrimination Between Ethanol Inhibition Models in a Continuous Alcoholic Fermentation Process Using Flocculating Yeast

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ABSTRACT

Discrimination between different rival models for describing the inhibitory effect of ethanol both on yeast growth and on fermentation was studied for a continuous process of alcoholic fermentation in a tower reactor with recycling of flocculating cells. Models tested include linear, parabolic, hyperbolic, exponential, and generalized nonlinear power-law types. The best expressions were identified under the criteria that all the kinetic parameters should assume acceptable values in a feasible range and should result in the best fit of the experimental data. The kinetic parameters were estimated from steady-state data of several sugar concentrations in feeding stream (S_0 = 160, 170, 180, 190, 200 g/L), constant dilution rate (D = 0.2 h^{-1}), recycle ratio (α = 13.6), and temperature (T = 30°C). The best model for the yeast growth was of power-law type, whereas for the product formation the best model was of linear type. These models were able to reproduce the trends of the process variables satisfactorily.

Index Entries: Ethanol; alcoholic fermentation; product inhibition; flocculating yeast; model discrimination.

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INTRODUCTION

In the last few years, several different types of fermentation process have been proposed as an alternative to the conventional batch system of alcoholic production, namely continuous stirred-tank reactors with and without cell recycling, reactors in series, reactors under vacuum, immobilized cell reactors, and tower fermenters with flocculating yeasts *(1)*.

Special interest has been directed to the tower reactor with recycling of flocculating cells owing to its high operational stability and high ethanol productivity. This performance is normally ascribed to the high cell concentration achieved in the process (approx 100 g/L dry basis *[2–5]*). As a consequence, media with high glucose concentration levels (100–200 g/L) are rapidly fermented, and bacterial contamination is avoided owing to the low pH and the high ethanol and yeast concentrations *(1)*. Depending on the degree of flocculation and on the mixing intensity, tower fermenters may present hydrodynamic behaviors similar either to tubular reactors or to stirred-tank reactors *(1,3,5,6)*.

Growth and fermentation kinetics of flocculating yeast cells have been usually described through unsegregated and unstructured models. This approach overlooks the functional, structural, and compositional differences in the cell population, and treats the complex growth and fermentation processes by simple kinetic equations *(4)*. For alcoholic fermentation, these kinetics are commonly of the type:

$$\mu_x = f_1(S) \cdot g_1(P) \tag{1}$$

and

$$\mu_P = f_2(S) \cdot g_2(P) \tag{2}$$

where μ_x and μ_P are the specific rates of growth and product formation, S stands for the limitant substrate (sugar) concentration, and P is the product (ethanol) concentration. The effect of sugar concentration on the fermentation kinetics is generally described by the well-known Monod expression *(7)*, except for the cases of high substrate concentrations, or diffusional limitations owing to high cell concentrations. These cases can be described by the expressions of Andrews *(8)* and Contois *(9)*, respectively. The inhibitory effect of ethanol on the specific rates are reported in the literature as noncompetitive for both yeast growth and product formation *(4,10–14)*. Several equations have been proposed for $g_1(P)$ and $g_2(P)$, including linear *(1,3,4,6,10,15)*, exponential *(3,5,13,15,16)*, parabolic *(11,15)*, hyperbolic *(12,14–16)*, and other expressions, such as a generalized nonlinear power-law type *(4,6,15,17)*. The type of inhibition for the growth

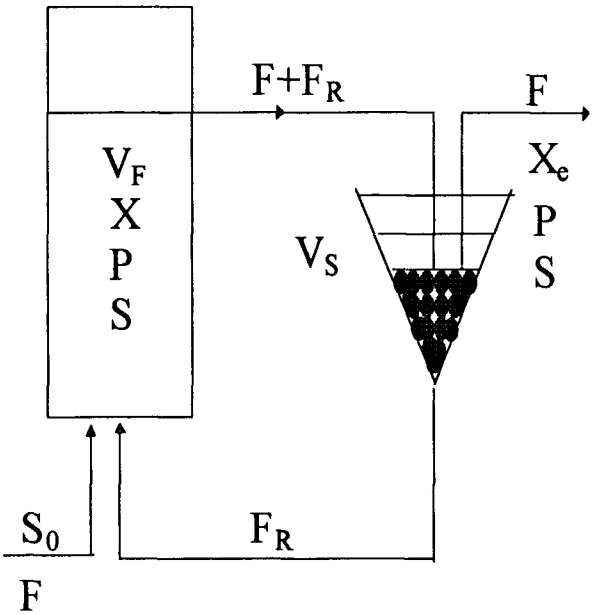

Fig. 1. Schematic diagram of the experimental system used in the continuous fermentation runs.

kinetics is not necessarily the same as for the product formation. Thus, it is necessary to identify the best form for each effect.

In a previously reported work, Paiva et al. *(2)* evaluated the performance of a continuous alcoholic fermentation process in a tower reactor with recycling of flocculating yeasts. The aim of the present article is to develop a mathematical model of the kinetics of this process, focusing on the discrimination between the expressions for the inhibitory effect of ethanol. Linear, hyperbolic, parabolic, exponential, and generalized nonlinear expressions were tested to describe the inhibitory effect of ethanol on both growth and fermentation kinetics.

MATERIALS AND METHODS

The data analyzed in the present article were obtained by Paiva et al. *(2)* using the experimental system shown schematically in Fig. 1. The fermentation runs were carried out in a continuously operated tower fermenter (volume V_F = 0.22 L), under constant dilution rate (D = 0.2 h^{-1}), recycle ratio (α = 13.6), and temperature (T = 30°C). Dilution rate is defined as $D = F/V_F$ (the feed volumetric flow rate divided by the fermenter volume), and recycle ratio, as $\alpha = F_R/F$ (the ratio between the recycle and feed volumetric flow rates). Sugar concentrations in the feeding stream were varied for each different run (S_0 = 160, 170, 180, 190, 200 g/L). The feed medium was constituted of sugarcane juice supplemented with

Table 1
Yield Factor $Y_{P/S}$ for Each Continuous Fermentation Run

Run	S_0 (g/L)	S (g/L)	P (g/L)	$Y_{P/S}$ ($g-P/g-S$)
1	160	1.0	72.5	0.46
2	170	1.1	77.1	0.46
3	180	1.8	79.6	0.45
4	190	2.0	82.4	0.44
5	200	4.2	86.6	0.44

mineral nutrients ($CaCl_2$, KH_2PO_4, $[NH_4]_2SO_4$, $MgSO_4$) and yeast extract. A flocculating yeast strain, *Saccharomyces cerevisiae* IR-2, isolated from fermented foodstuff and supplied from Fermentation Research Institute (Tsukuba-Japan), was used. The effluent from the reactor followed to a settler (volume V_S = 0.025 L), where the cells were separated and recycled to the fermenter. The process was monitored by periodical samplings both of the fermenting medium, in order to determine cell concentration inside the reactor, and of the effluent of the system for determination of cell, ethanol, and residual sugar concentrations. More details about the experiments can be found in the paper by Paiva et al. *(2)*.

MATHEMATICAL MODEL

Model Development

The development of the mathematical model was based on a preliminary analysis of the experimental data, as well as on the often observed phenomena in alcoholic fermentation, such as the inhibition by ethanol. The following hypothesis were assumed:

- The hydrodynamic behavior of the tower fermenter is described as a perfectly mixed reactor, owing to the high degree of mixing observed in the system.
- Fermentation in the settler is neglected, owing to the low residence times in the separation vessel caused by high recycle volumetric flow rates and small settler volume.
- Inhibitory effect of the substrate is neglected, owing to the low residual sugar concentrations measured in the experiments.
- Cell concentration inhibitory effect on μ_x was considered, owing to the high cell densities attained in the experiments *(18)*.

An apparent yield factor from sugar to ethanol $Y_{P/S}$ is defined by the relation of the mass of ethanol produced and the mass of sugar consumed.

Table 1 shows that this yield coefficient is almost independent of the operating conditions in the range studied. Thus, the average value of $Y_{P/S}$ (0.45 g of ethanol/g sugar) was adopted. Therefore, the specific substrate consumption rate (μ_S) can be correlated with the specific ethanol production rate (μ_P) by this yield coefficient. This approach has been used by several authors *(1,4–6,10,13)*.

Considering the previous assumptions and the scheme shown in Fig. 1, the mathematical model for the process in steady-state is described by the following material balance equations:

- Material balance of cells:

$$\mu_X X - DX_e = 0 \tag{3}$$

- Material balance of ethanol:

$$\mu_P X - DP = 0 \tag{4}$$

- Material balance of sugar:

$$D(S_0 - S) - (\mu_P / Y_{P/S}) X = 0 \tag{5}$$

where S and P are sugar and ethanol concentrations on both inside the reactor and in the effluent, and X and X_e are cell concentrations inside the reactor and effluent, respectively.

The specific growth rate is given by Eq 1 multiplied by term $(1 - X/X_m)$ to take into account cell-concentration inhibitory effect. Cell inhibitory effect was considered, because at high cell concentrations, growth and metabolism conditions are less favorable owing to space and mass-transfer limitations, and cell interaction *(19)*.

The specific ethanol production rate is given by Eq 2. In Eqs 1 and 2, the functions f_1 (S) and f_2 (S) are given by the well-known Monod expression:

$$f_1(S) = \mu_{max,i} S / (K_{S,i} + S); \; i = 1,2 \tag{6}$$

The following expressions were tested to describe the inhibitory effect of ethanol on the specific rates, g_1 (P) and g_2 (P):

Linear (L) $$g_i(P) = [1 - (P/P_{m,i})] \tag{7}$$

Generalized nonlinear (GN) $$g_i(P) = [1 - (P/P_{m,i})]^{n_i} \tag{8}$$

Parabolic (P) $$g_i(P) = [1 - (P/P_{m,i})]^{0.5} \tag{9}$$

Hyperbolic (H) $$g_i(P) = [K_{P,i}/(K_{P,i} + P)] \quad (10)$$

Exponential (E) $$g_i(P) = [\exp(-K_{p,i}P)] \quad (11)$$

PARAMETER ANALYSIS

Models (H) and (E) predict growth and fermentation in all range of product concentration, even though several experimental studies have shown that these processes stop at some high ethanol concentration. On the other hand, inhibition models (L), (GN), and (P) consider that there exists a given ethanol concentration above which the growth and fermentation stop. Thus, in these models, the $P_{m,i}$ parameters are ethanol concentrations above which growth and product formation do not occur.

In models (L), (GN), and (P), the exponents of the term $(1 - P/P_{m,i})$ are called by Levenspiel *(17)* "toxic power." The toxic power values are indicative of how strongly the inhibition term $(1 - P/P_{m,i})$ affects the specific growth and ethanol production rates. As toxic power increases, the inhibition intensity increases for a constant ethanol concentration.

The parameter X_m is the maximum cell concentration that would be reached under ideal growth conditions, such as an adequate supply of nutrients and the absence of inhibitory effects.

The $K_{P,i}$ parameters do not admit a physical meaning and can be regarded as simple empirical constants, which apparently depend on the cultivation procedure (batch or continuous) *(14)*.

Table 2 presents typical values for the parameters as reported in the literature *(1,3–6,10–21)*.

PARAMETER ESTIMATION

The parameter estimation of the kinetic models was made using Marquardt's algorithm *(22)* by minimizing the summation of normalized-residues squares, ϕ. The normalization of the residues was performed owing to the different orders of magnitude of the variables X, S, and P *(20)*:

$$\begin{aligned}\phi = &\sum_{i=1}^{n} [(S_{exp,i} - S_{calc,i})/S_{exp,max}]^2 \\ &+ \sum_{i=1}^{n} [(P_{exp,i} - P_{calc,i})/P_{exp,max}]^2 \\ &+ \sum_{i=1}^{n} [(X_{exp,i} - X_{calc,i})/X_{exp,max}]^2\end{aligned} \quad (12)$$

where n is the number of runs ($n = 5$), subscripts exp,i and calc,i stand for the experimental and calculated values of the variables, and subscript exp,max stands for the maximum experimental values.

Table 2
Typical Values of the Kinetic Parameters for Alcoholic Fermentation

		Inhibition				
		L	GN	P	H	E
$\mu_{max,1}(h^{-1})$	0.11–0.56					
$\mu_{max,2}$ (g-P/g − X·h)	0.21–1.90					
$K_{s,1}$(g − S/L)	0.07–0.48					
$K_{s,2}$(g − S/L)	0.33–60.0					
$P_{m,1}$(g − P/L)		87.0–95.0	73.0–87.5	93.6		
$P_{m,2}$(g − P/L)		114.0–135.0	87.5	99.0		
n_1			0.41–2.0			
n_2			0.41			
$K_{p,1}$(L/g − P)					16.0–105.2	0.016–0.029
$K_{p,2}$(L/g − P)					12.5–71.5	0.015–0.094
X_m(g − X/L)	100.0–330.0					

During the parameter estimation, the toxic power values in the models (L) and (P) are fixed in 1.0 and 0.5, respectively, whereas in the model (GN), this value is an adjustable parameter.

The values of the dependent variables (X, S, P) for one given set of values of kinetic parameters, independent variables (D, S_0), and X_e were calculated according to the following procedure. First, Eqs 3–5 were written in transient state, and then, the resulting ordinary differential equations were integrated numerically until steady-state was achieved. Numerical integration was carried out by a variable-step fourth-order Runge-Kutta-Gill method. The final values of the dependent variables (S, P, X) correspond to the solution of the steady-state material balances. This so-called false-transient method was found to be robust and avoids the typical convergence difficulties owing to inappropriate initial guesses of the classical methods for solving systems of nonlinear algebraic equations (*23*).

RESULTS AND DISCUSSION

Table 3 shows the fitting results for all the 25 possible combinations of $g_1(P)$ and $g_2(P)$. The best fitting was obtained for the combination of generalized nonlinear (GN) for $g_1(P)$ with linear (L) for $g_2(P)$. The other 24 combinations produced either poorer fitting or unacceptable values for the parameters. For example, the combination (GN) − (E) gives lower ϕ, but at the expense of an unsuitably high value for $\mu_{max,1}$.

An analysis of the parameters obtained for the (GN) − (L) combination reveals that the $K_{S,1}$ value is higher than those commonly found in the literature. This high value can be ascribed to diffusional limitations owing

Table 3
Parameter Estimation Results for All 25 Possible Combinations of $g_1(P)$ and $g_2(P)$

$f_1(S)$		$f_2(S)$		$g_1(P)$				$g_2(P)$					
$\mu_{max,1}$	$K_{S,1}$	$\mu_{max,2}$	$K_{S,2}$		$P_{m,1}$	n_1	$K_{P,1}$		$P_{m,2}$	n_2	$K_{P,2}$	X_m	ϕ
0.76	2.79	0.91	1.57	(L)	98.0			(L)	126.9			135.6	0.0303
1.02	0.10	0.75	1.73	(L)	94.6			(GN)	146.5	0.98		110.4	0.0174
0.41	1.65	0.98	2.05	(L)	101.2			(P)	97.2			152.5	0.0515
0.51	1.53	0.98	1.07	(L)	101.5			(H)			31.1	130.3	0.0279
1.26	0.98	1.64	2.16	(L)	92.6			(E)			0.018	103.6	0.0139
0.39	1.94	1.16	2.60	(GN)	88.1	0.16		(L)	126.5			114.0	0.0083
0.50	4.60	0.97	2.47	(GN)	88.8	0.33		(GN)	107.4	0.60		130.7	0.0131
0.49	1.96	1.29	4.12	(GN)	89.3	0.43		(P)	99.7			120.1	0.0156
0.47	3.73	1.10	2.04	(GN)	89.6	0.42		(H)			40.6	134.7	0.0239
0.94	4.66	1.96	2.33	(GN)	88.1	0.11		(E)			0.02	109.0	0.0073
0.46	2.18	0.84	1.93	(P)	90.4			(L)	140.1			126.8	0.0171
1.00	1.43	0.47	1.37	(P)	88.9			(GN)	167.2	0.66		109.1	0.0122
0.78	0.67	0.58	1.42	(P)	89.1			(H)			87.5	109.1	0.0126
0.42	3.17	0.60	1.57	(P)	91.0			(P)	115.0			139.8	0.0214
0.62	2.10	1.16	1.79	(P)	89.7			(E)			0.015	119.1	0.0137
0.44	2.19	1.31	2.81	(H)			79.7	(L)	118.4			113.7	0.116
0.38	1.74	1.23	2.64	(H)			53.3	(GN)	129.5	1.13		118.0	0.136
0.27	1.35	1.02	2.28	(H)			80.0	(H)			40.9	114.7	0.180
0.50	3.05	1.01	2.58	(H)			127.9	(P)	96.9			109.5	0.0771
0.56	0.23	0.32	0.46	(H)			29.5	(E)			0.0051	106.9	0.0531
0.55	0.92	0.86	1.76	(E)			0.024	(L)	135.1			105.9	0.0452
0.42	0.97	0.82	2.62	(E)			0.021	(GN)	94.5	0.34		106.4	0.0266
0.46	0.97	0.77	2.00	(E)			0.023	(P)	104.7			106.9	0.0265
0.31	0.37	0.88	1.92	(E)			0.021	(H)			54.2	137.1	0.0982
0.44	0.97	0.73	1.36	(E)			0.022	(E)			0.011	107.0	0.0281

to the high cell concentrations achieved in the experiments. Thus, the estimated $K_{S,1}$ value would be in fact, as suggested by Contois *(9)*, an apparent value representing the product of the true $K^*_{S,1}$ times the yeast concentration ($K_{S,1} = K_{S,1}^* \cdot X$). Since the yeast concentration did not vary significantly for the different runs ($\bar{X} = 96.8 \pm 8.6$), a rough estimate of the true $K^*_{S,1}$ value would be 1.94/96.8 = 0.02 g/L, a quite acceptable value for the pair substrate-microrganism used.

Figure 2 shows the predictive capability of the mathematical model developed. According to this figure, the model predictions agree well with experimental data. In addition, the deviations between experimental and calculated values of the variables *X*, *S*, and *P* are within the experimental error observed for the measurements, as can be seen in Table 4. Therefore, the proposed model with generalized nonlinear inhibition for yeast growth and linear inhibition for ethanol production is able to represent adequately the steady-state behavior of the system considered.

The fact that dilution rate and recycle ratio were constant for all fermentation runs does not restrict the validity of the model. Experimental data obtained with different values of D and α can be added to the remaining data and the parameter estimation can be updated.

Although not considered in this work, it is interesting to speculate how the model could be altered to accommodate the effects of loss of cell viability during the processing. Cell population would be divided into two distinct groups: viable cells and nonviable or dead cells, which would be inactive or nongrowing, but nevertheless, intact cells, not subject to lysis. Specific rates of cell growth, ethanol production, substrate consumption, and cell death would be defined with respect to concentration of viable cells, which are actually responsible for the growth and fermentation processes. For cell population description, material balance equations of viable and nonviable cells would be needed. Material balance equations of ethanol and substrate would be basically the same with the previous modifications in the terms involving μ_P and μ_S. For model identification, measurements of the concentration of viable and nonviable cells, on both inside the reactor and in the effluent of the system, would be required. As a consequence, the requirements for monitoring the process would increase. The consideration of loss of cell viability in the process modeling is, at present, the main potential of further development of the current model.

CONCLUSIONS

The inhibitory effect of ethanol on the yeast growth and fermentation was studied in a continuous process of alcoholic fermentation with recycling of flocculating cells. Different kinetic models for this inhibition were tested, and the best expressions were identified. The criteria used were

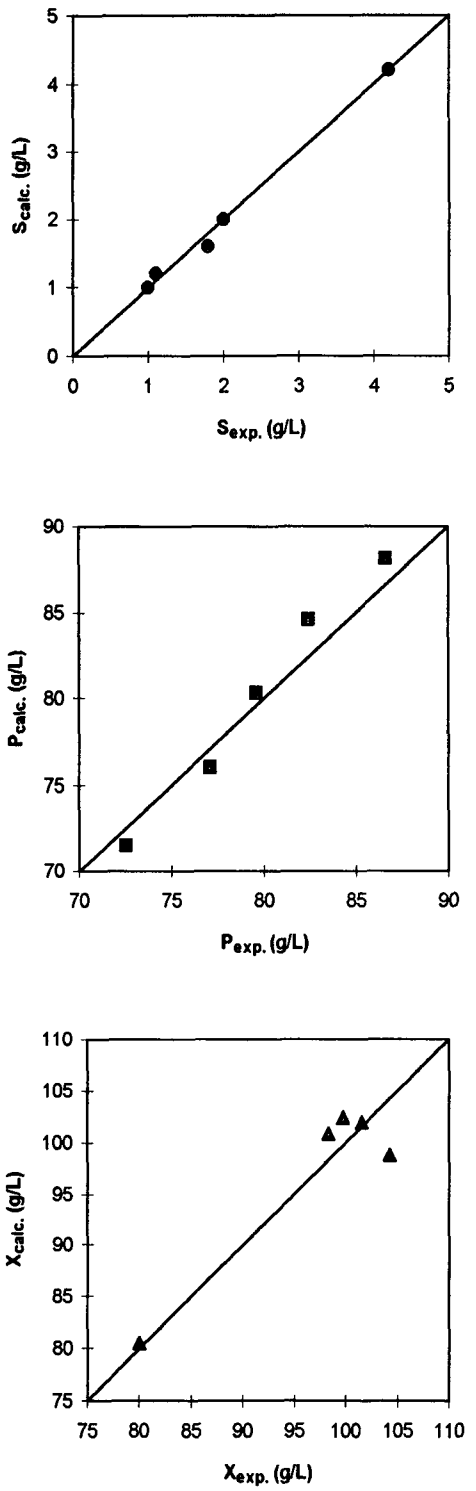

Fig. 2. Distribution of the points: ● = ($S_{exp.}$, $S_{calc.}$), ■ = ($P_{exp.}$, $P_{calc.}$), and ▲ = ($X_{exp.}$, $X_{calc.}$) around the ideal straight lines $S_{calc.} = S_{exp.}$, and $X_{calc.} = X_{exp.}$, respectively.

Table 4
Comparison Between Measured Sugar, Ethanol, and Cell Concentrations with the Calculated Values by the Model

			S(g/L)		P(g/L)		X(g/L)	
Run	S_0(g/L)	X_e(g/L)	Exp.	Calc.	Exp.	Calc.	Exp.	Calc.
1	160	6.0 ± 3.3	1.0 ± 0.1	1.0	72.5 ± 1.6	71.5	98.3 ± 6.4	100.8
2	170	5.7 ± 3.8	1.1 ± 0.2	1.2	77.1 ± 3.5	76.0	99.7 ± 2.6	102.4
3	180	7.9 ± 6.1	1.8 ± 0.6	1.6	79.6 ± 3.7	80.3	104.3 ± 8.9	98.8
4	190	6.3 ± 4.1	2.0 ± 0.7	2.0	82.4 ± 5.1	84.6	101.6 ± 6.3	102.0
5	200	7.1 ± 4.0	4.2 ± 3.3	4.2	86.6 ± 3.5	88.0	80.0 ± 11.7	80.5

that all the kinetic parameters should lay in an acceptable and feasible range of values and, additionally, should result in the best fit of the experimental data.

The model developed was able to reproduce satisfactorily the trends of the process variables. However, the validity of further control and optimization studies using this model will be restricted to the range of variables studied and hypothesis assumed here.

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