Development of a Kinetic Model for the Alcoholic Fermentation of Must

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We propose a kinetic expression which accounts for the temperature dependence of ethanol yield losses in batch alcoholic fermentation. Moreover, the characteristic parameters of the microbial growth equation have been calculated for *Saccharomyces cerevisiae* under typical wine industry conditions. A substrate consumption equation is established which minimizes possible model deviations in the latter process stages. Experimental data were obtained in the laboratory and the proposed equations were then applied at an industrial level (2.5×10^4 L) where they described the data well.
Key words: alcohol • fermentation • ethanol • *Saccharomyces cerevisiae* • model

INTRODUCTION

Several general fermentation kinetic models have been proposed. However, due to the particular characteristics of alcohol fermentation, it is necessary to use specific models. For alcohol fermentation in the food industry, these particular characteristics include the following[7,26]:

batch fermentations on natural complex media;
anaerobic conditions due to CO_2 generation;
inoculation of a selected strain (*Saccharomyces*);
low media pH (3.0–3.6);
addition of bisulphite ions (50–150 ppm) as an antioxidant and antiseptic;
mixed substrate (120–200 g/L) composed of D-glucose and D-fructose; and
generation of inhibitory product (80–120 g/L ethanol).

The models developed for these cases consequently include certain specific effects. In the first place, substrate inhibition of microbial growth[11,19] has been observed. Secondly, competitive inhibition has been detected among the substrate sugars.[10,23] Lastly, product inhibition and biomass viability reductions have been observed due to ethanol.[1,9,22]

Many models offering good prediction of process variables have been proposed, but most deviate significantly from the final ethanol concentration.[5,12,17,21,28,30] This partly results since some ethanol evaporates during the process, consequently lowering the final concentration below the prediction.[31] However, there are several other factors besides evaporation which can lower alcohol yield, such as substrate assimilation and microbial respiration, as not all substrate is metabolized wholly through fermentation.[3,15,16] These effects are not specifically included in many literature models, which is why erroneous predictions are obtained under certain circumstances, thus limiting their applicability.

To avoid these defects, modifications must be introduced in the kinetic equations to account for factors which reduce ethanol yield apart from evaporation. Furthermore, the equations should reflect the influence of operating temperature on system behavior.

THE MODEL

Bearing in mind the aforementioned ideas, the basic equations are the following:

The general equations for product formation can be expressed as follows:

$$\frac{dE}{dt} = -2f\frac{dS}{dt}\frac{MW(C_2H_6O)}{MW(C_6H_{12}O_6)};$$
$$\frac{dC}{dt} = -2(f + 3r)\frac{dS}{dt}\frac{MW(CO_2)}{MW(C_6H_{12}O_6)} \quad (1)$$

where f and r are the fraction of substrate consumed by fermentation and respiration, respectively. These coefficients are calculated through expressions (2) and (3), which show a temperature dependence as Topiwala and Sinclair suggest for the maximum specific microbial growth rate.[29]

$$f = A_f \exp\left(\frac{E_{Af}}{RT}\frac{T - T_{0f}}{T_{0f}}\right) - B_f \exp\left(\frac{E_{Bf}}{RT}\frac{T - T_{0f}}{T_{0f}}\right) \quad (2)$$

$$r = A_r \exp\left(\frac{E_{Ar}}{RT}\frac{T - T_{0r}}{T_{0r}}\right) - B_r \exp\left(\frac{E_{Br}}{RT}\frac{T - T_{0r}}{T_{0r}}\right) \quad (3)$$

where the A and B terms describe favorable and unfavorable growth, respectively. The reference temperatures for the preexponential parameters are T_{0f} and T_{0r}.

Expression (4) describes the substrate consumption used for cell growth. The temperature effect on the substrate yield coefficient Y_s is given by expression (5), which is similar to Topiwala and Sinclair's suggestion for the temperature effect on saturation and inhibition

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constants[29]:

$$-\frac{dS}{dt} = \left(\frac{1}{Y_s}\right) X_v \mu_g \tag{4}$$

$$(1/Y_s) = (1/Y_{s0}) + A_Y \exp - \left(\frac{E_{AY}}{RT}\frac{T - T_{0Y}}{T_{0Y}}\right) \tag{5}$$

Expression (6) describes microbial growth, μ_g, using the saturation and inhibition constants, K_S and K_I. The temperature dependence of K_S and K_I is given by expressions (7):

$$\mu_g = \frac{\mu_{\max} S}{(S + K_S)[1 + (E/K_I)]} \tag{6}$$

$$K_S = K_{0S} \exp - \left(\frac{E_{AS}}{RT}\frac{T - T_{0S}}{T_{0S}}\right);$$

$$K_I = K_{0I} \exp - \left(\frac{E_{AI}}{RT}\frac{T - T_{0I}}{T_{0I}}\right) \tag{7}$$

where $K_{0S} = 112$ g/L; $K_{0I} = 40$ g/L; $T_{0S} = T_{0I} = 293.3$ K; and $E_{AS} = E_{AI} = -11$ kcal/mol.[29]

Equation (8) describes the temperature dependence of the maximum specific microbial growth rate[29]:

$$\mu_{\max} = A_g \exp\left(\frac{E_{Ag}}{RT}\frac{T - T_{0g}}{T_{0g}}\right) - B_g \exp\left(\frac{E_{Bg}}{RT}\frac{T - T_{0g}}{T_{0g}}\right) \tag{8}$$

The net increase in viable biomass concentration was evaluated according to the following balance which accounts for biomass growth μ_g and death μ_d:

$$\frac{dX_v}{dt}\frac{1}{X_v} = \mu = \mu_g + \mu_d \tag{9}$$

The biomass death rate is calculated from Chick's disinfection law[4]:

$$\mu_d X_v = -(dX_v/dt)_d = X_v E/K_D \tag{10}$$

LABORATORY EXPERIMENTS

To evaluate the total fermentation yield losses under different operating conditions, several batch fermentation experiments have been performed in the laboratory at 288, 293, 298, 303, and 308 K. The equipment consisted of a temperature-controlled fermentor (2.5 L) and an absorber charged with water for monitoring the amount of evaporated ethanol. Figure 1 shows a complete plan.

The laboratory fermentor was scaled-down from the industrial fermentor used later (2.5×10^4 L). Since gas–liquid mass-transfer takes place during the evaporation phenomena, the laboratory fermentor was scaled to maintain the same overall mass-transfer coefficient as the industrial fermentor (50–60 h^{-1}). The absorber consisted of a gas countercurrent absorbtion column with

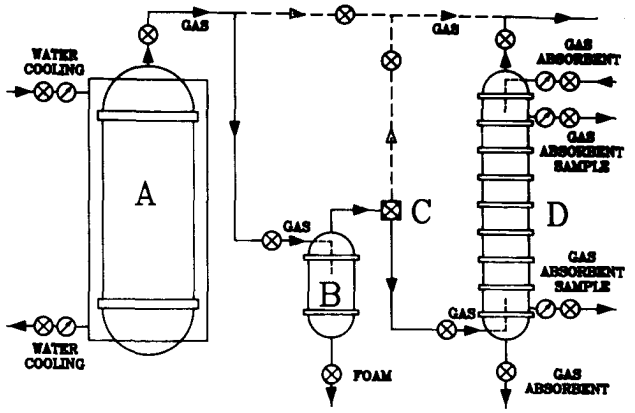

Figure 1. Schematic diagram of the experimental system: (A) batch fermenter (laboratory scale with 2.5 L and industrial scale with 25,000 L); (B) foam knockout trap; (C) safety relief valve; and (D) absorbtion system.

16 real stages, and the operating conditions were optimized to let no ethanol escape in the exit gas.

The fermentation medium was Palomino Fino variety grape must, sterilized and conditioned with 100 ppm SO_2. Before inoculation, it was saturated with oxygen (7–10 ppm O_2) in order to maintain the industrial operating conditions. The yeast strain was *Saccharomyces cerevisiae* var. *cerevisiae*.[18] The inoculum grew on the same fermentor medium, but at 2% of the volume. The initial experimental conditions were the following: substrate (total sugars), S_i, of 214 g/L; viable biomass, X_{vi}, of 1.8×10^6 colony forming units/mL; and ethanol, E_i, of 2 g/L (present in the inoculum).

In each experiment, fermentor samples were periodically taken, filtered (0.45 μm), and the ethanol concentration was measured by gas chromatography. In addition, samples were taken from the absorbent liquid to measure the amount of ethanol which evaporated from the fermentor. The amount of CO_2 production was measured with bubble flowmeter from the moment of inoculation until no further gas production was detected. The viable biomass concentration was determined by counting colonies on culture plates containing MPYD agar (2% malt extract, 0.5% peptone, 1% yeast extract, 2% D-glucose, and 2% agar in water).[8]

The experiments were performed in triplicate at each temperature, and the results averaged. The data deviations at the 95% confidence level were in all cases less than 4% of the average value.

LABORATORY RESULTS

Table I shows the final ethanol and CO_2 production from the laboratory fermentors at each temperature. We observe that the ethanol and CO_2 production does not correspond to simple fermentation stoichiometry. From the initial and final substrate concentrations, the stoichiometric ethanol and CO_2 yields by fermentation (E_s and C_{sf}) and the stoichiometric CO_2 yield by respiration (C_{sr}) can be easily calculated from the molecular

Table I. Final ethanol and CO_2 formation at different temperatures for batch laboratory fermentors.

T (K)	t (h)	E_j [a] (g/L)	E_e [a] (g/L)	E_t [b] (g/L)	C_t [a] (g/L)	C_f [b] (g/L)	C_x [b] (g/L)
288	576	102	0.8	101	105.6	96.5	9.1
293	298	104	1.1	103	108.0	98.4	9.6
298	168	104	1.4	103	107.9	98.4	9.5
303	107	100	1.8	100	105.0	95.5	9.5
308	96	85	1.3	84	85.9	80.2	5.7

[a] Measured data.
[b] Calculated results

Note: $E_t = E_j + E_e - E_i$; $C_f = E_t \frac{MW(CO_2)}{MW(C_2H_6O)}$; $C_x = C_t - C_f$; $E_i = 2$ g/L.

weights (MW):

$$E_s = 2(S_i - S_j)\frac{MW(C_2H_6O)}{MW(C_6H_{12}O_6)} = 109.45 \text{ g/L};$$

$$C_{sf} = 2(S_i - S_j)\frac{MW(CO_2)}{MW(C_6H_{12}O_6)} = 104.55 \text{ g/L}$$

$$C_{sr} = 6(S_i - S_j)\frac{MW(CO_2)}{MW(C_6H_{12}O_6)} = 313.65 \text{ g/L} \quad (11)$$

The numerical values could be calculated since the initial substrate concentration was always 214 g/L and the final substrate was essentially zero.

Table I shows the final ethanol concentration in the fermentor, E_j, and the amount evaporated, E_e. Since the initial ethanol concentration in the inoculum, E_i, is known, the total ethanol produced by fermentation, E_t, can be calculated.

The data in Table I show that there are important losses under normal working conditions, since E_t is significantly less than the stoichiometric value, E_s. In extreme conditions (i.e., $T > 303$ K) the losses are more than 10% of the stoichiometric value. However, the ethanol losses by evaporation (E_e) are only about 1% of the total ethanol production and can account for only 10% of the total yield loss. It is therefore obvious that there are other factors, besides evaporation, which lower yield and must be incorporated into kinetic models if we want to obtain accurate theoretical prediction.

Table I also shows the measured carbon dioxide produced during the fermentation, C_t. The amount of carbon dioxide attributed to fermentation processes, C_f, can be calculated from E_t using known stoichiometric relationships. Since C_t and C_f are not equal, there is "excess" carbon dioxide produced. It is assumed that this excess carbon dioxide is produced by respiration. Although ethanol fermentation is considered to be anaerobic, it is actually microaerophilic since the medium was saturated with oxygen prior to the inoculation.

Fermenting and Respiration Yield

To predict the alcoholic yield losses due to respiration and other types of metabolism, it is necessary to consider all possible mechanisms of substrate consumption:

$$\text{cells} + \text{secondary products} \xleftarrow{P} C_6H_{12}O_6 \xrightarrow{R} 6CO_2 + 6H_2O$$
$$\downarrow F$$
$$2C_2H_6O + 2CO_2$$

Route F corresponds to the substrate metabolized by fermentation, route R corresponds to the substrate metabolized by respiration, and route P corresponds to the group of reactions which do not lead to the main products, such as the synthesis of cells (e.g., polysaccharides, amino acids), glycerol, organic acids, etc.[13,24]

From the data in Table I, the yields of the different routes can be calculated; that is to say, the fraction of substrate metabolized by fermentation (f), respiration (r), and residue (p). In this model, it is assumed that carbon dioxide is only produced by routes F and R, not route P. Thus, the "excess" carbon dioxide is attributed solely to route R. Even though there are several metabolic routes which yield carbon dioxide, besides fermentation and respiration, the amount obtained can be negligible in normal conditions. Under microaerophilic conditions, the specific oxygen demand for *Saccharomyces cerevisiae* respiration is 8 mmol O_2/h g biomass at industrial operating conditions.[25] This value lead to a respirative CO_2 production rate consistent with the order as excess CO_2 obtained.

The fraction of substrate converted via fermentation (f) is calculated as the actual amount of ethanol produced, E_t, divided by the stoichiometric amount which could have been produced from the substrate, E_s. The fraction of substrate converted via respiration (r) is estimated as the excess carbon dioxide, C_x, divided by the stoichiometric amount which could have been produced by respiration, C_{sr}. The fraction of substrate converted to other products (p) is estimated as $1 - (f + r)$.

Table II shows that the conversion coefficients (f, r, and p) depend on temperature, so it is desirable to develop a functional relationship. The data do not fit simple functions of T (linear, logarithmic, exponential, etc.), exhibiting low regression coefficients for these cases ($r^2 < 0.75$). Besides, these types of functions would be difficult to justify theoretically. Consequently, an expression analogous to the general equation of microbial temperature dependence has been chosen, which does have a theoretical basis.[29] Two exponential terms are thus considered; the first refers to favorable reac-

Table II. Experimental values of conversion coefficients: fermentative (f), respirative (r) and residual (p) coefficients at different temperatures.

T	f	r	p
288	0.923	0.029	0.048
293	0.941	0.031	0.028
298	0.941	0.030	0.029
303	0.914	0.030	0.056
308	0.767	0.018	0.215
$f = E_t/E_s$	$r = C_x/C_{sr}$		$p = 1 - (f + r)$

tions to the route in question and the second refers to unfavorable reactions. The proposed expressions are therefore (2) and (3).

The adjustable parameters were determined by the least-squares multiple regression (see Table III), at a reference temperature of 300.0 K. The resulting functions are shown together with the experimental data in Figure 2.

The values obtained for the parameters are consistent with those in the growth equations. That is, the unfavorable activation energies (E_B) are higher than the favorable ones (E_A). This means that below a certain critical value, temperature increases favor both microbial growth and the fermentation and respiration processes; once the critical temperatures are surpassed, all the processes are suddenly halted.

On the other hand, it is observed that the activation energy favorable to fermentation (E_{Af}) is lower than that favorable to respiration (E_{Ar}), which means that below the critical value, a temperature increase favors the fermentation process in relation to the respiration process, under the experimental oxygen conditions used.

Substrate Consumption

Another consideration which can make the experimental product formation values lower than the theoretical values is the form of the general substrate consumption equation. For alcoholic fermentation, expression (12) has been applied[4]; consumed substrate is invested in biomass growth and biomass maintenance:

$$-\frac{dS}{dt} = \left(\frac{\mu_g}{Y_g} + m\right)X_v \qquad (12)$$

Parameters Y_g and m are the substrate yield for growth and biomass maintenance coefficients, respectively, and the specific growth rate expression (μ_g) can differ according to the model (e.g., Monod).

According to eq. (12), substrate consumption depends on both viable biomass concentration and biomass growth rate; consumption is therefore only zero when there is no viable biomass in the medium. The literature parameters for *Saccharomyces cerevisiae* are $(1/Y_g)$ = 10 g substrate/g biomass and m = 0.01 g substrate/h g biomass.[5] These values lead to a maintenance substrate consumption lower than 10% of total substrate consumption, for the industrial process under study (time process of 100 h). It is therefore possible to use eq. (4) for substrate consumption in this case and assume $Y_s \approx Y_g$.

The yield coefficient, Y_s, establishes the relationship between microbial grow rate, cell concentration and substrate consumption rate [see equation (4)]. Equation (5) describes a relationship for Y_s and temperature, proposed exponential, similar to a maintenance coefficient relationship described in the literature.[29] The parameters of eq. (5) have been taken from this reference $(1/Y_{0s})$ = 10 g substrate/g biomass and E_{AY} = 9 kcal/mol); however, the A_Y parameter has been determined from our laboratory data using the same reference temperature (T_{0Y} = 293.3 K), resulting in A_Y = 2.1 g substrate/g biomass.

Table III. Values of the calculated parameters for eqs. (2) and (3).

A_f = 1.1390	E_{Af} = 2.4 kcal/mol	
B_f = 0.2049	E_{Bf} = 20.0 kcal/mol	r^2 = 0.985
A_r = 0.0555	E_{Ar} = 6.7 kcal/mol	
B_r = 0.0255	E_{Br} = 18.0 kcal/mol	r^2 = 0.940
	$T_{0f} = T_{0r}$ = 300.0 K	

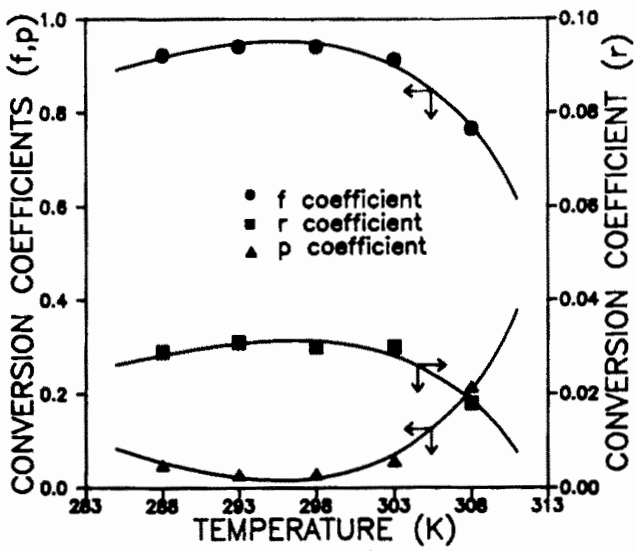

Figure 2. Conversion coefficients vs. temperature: (f) fermentative, (r) respirative, and (p) residual. The points show experimental values and the lines show fitted curves.

Microbial Growth Rate

The maximum specific microbial growth rate, μ_{max}, was obtained from the viable biomass concentration. The data are shown in Table IV, together with the theoretical literature values calculated from eq. (8). This growth equation was originally proposed for a yeast of the Montrachet strain grown on Colombard grape must,[14] where the values of the parameters are the

Table IV. Maximum specific growth rate at different temperatures.

Temperature (K)	μ_{max} (h^{-1})	
	Literature	Experimental
288	0.068	0.024 ±0.004
293	0.104	0.090 ±0.008
298	0.154	0.182 ±0.016
303	0.185	0.390 ±0.030
308	—	0.708 ±0.084

following:

$$A_g = 0.18\ h^{-1}; \quad E_{Ag} = 14.2\ kcal/mol;$$
$$B_g = 5.4 \times 10^{-3}\ h^{-1}; \quad E_{Bg} = 121.0\ kcal/mol;$$
$$T_{0g} = 300.0\ K \quad (13)$$

It is clear that there is no agreement between the theoretical literature values and our experimental data using a *Saccharomyces*. The differences confirm that the equation parameters depend strongly on the species of microorganism used, as well as other factors independent of operating temperature.

Readjusting the parameters in eq. (8) from the experimental data in Table IV gives the following results for the same reference temperature:

$$A_g = 0.2405\ h^{-1}; \quad E_{Ag} = 28.4\ kcal/mol;$$
$$B_g = 5.74 \times 10^{-4}\ h^{-1}; \quad E_{Bg} = 121.0\ kcal/mol;$$
$$r^2 = 0.999 \quad (14)$$

Figure 3 shows the theoretical curves corresponding to both cases. The readjusted parameters predicts microbial growth at 308 K, accurately reflecting the thermal behavior of the yeast. In addition, μ_{max} goes up to 0.7 h^{-1}, which agrees well with growth rates generally exhibited by yeasts (0.3–0.9 h^{-1}).[27]

The calculated activation energies of eq. (14) are approximately the same order as the initially reported values of eq. (13). However, the growth rate is generally higher for the microorganism and conditions of this study.

INDUSTRIAL LEVEL FERMENTATION

To check the validity of the changes introduced in the kinetic equations, several fermentation experiments have been carried out at an industrial level (2.5×10^4 L), at a controlled temperature (300 K), using the same kind of substrate and microorganism as in the laboratory tests. In this case, the initial conditions imposed are the usual ones for the type of industrial process under study: substrate (total sugars), S_i, of 172 g/L; viable biomass, X_{vi}, of 6.8×10^6 cfu/mL; and ethanol, E_i, of 15 g/L (present in the inoculum).

To integrate the kinetic equations, the Euler method was implemented on a computer. The differential time interval was 3.6 s, and the total integration was extended until the product concentrations stabilized.

The experimental results for substrate, ethanol, and carbon dioxide are shown in Figures 4, 5, and 6, respectively, together with the predicted values calculated from our kinetic model. As may be observed, the theoretical predictions agree well with all the experimental data for the industrial process under study. In addition, the calculated yields are within allowable stoichiometric limits.

It has been proven that there are other factors which affect the kinetics of the process, such as medium pH, dissolved oxygen concentration, inhibitor concentration, etc.[2,6,20]; therefore, the values of the proposed parameters must be different as these operating conditions change. However, the conditions studied are the usual ones in alcoholic fermentation processes in the wine industry, and the proposed model can therefore give good results for many typical fermentation processes.

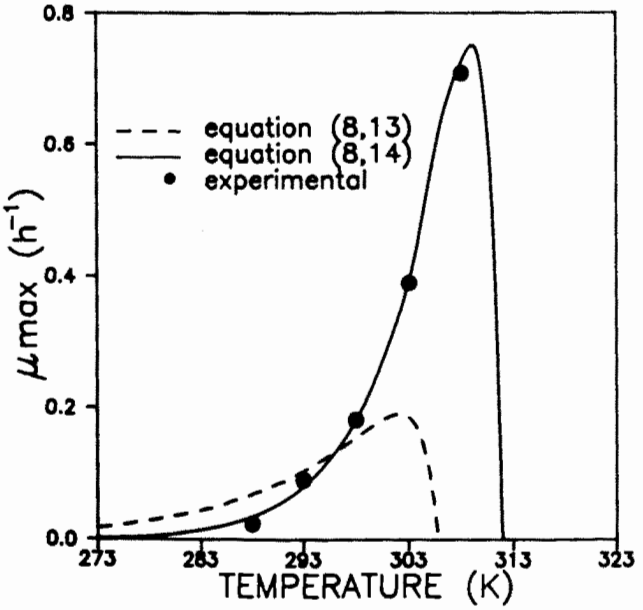

Figure 3. Variation of μ_{max} with temperature. The dashed line shows literature values for Montrachet strain and the continuous line shows the proposed equation for *Saccharomyces cerevisiae*.

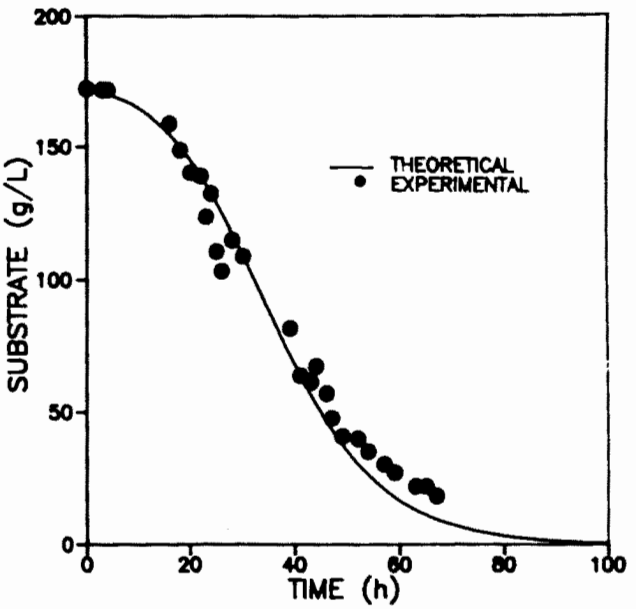

Figure 4. Variation of substrate concentration (total sugars) with time: (○) experimental values from industrial plant; (——) theoretical values from the proposed model.

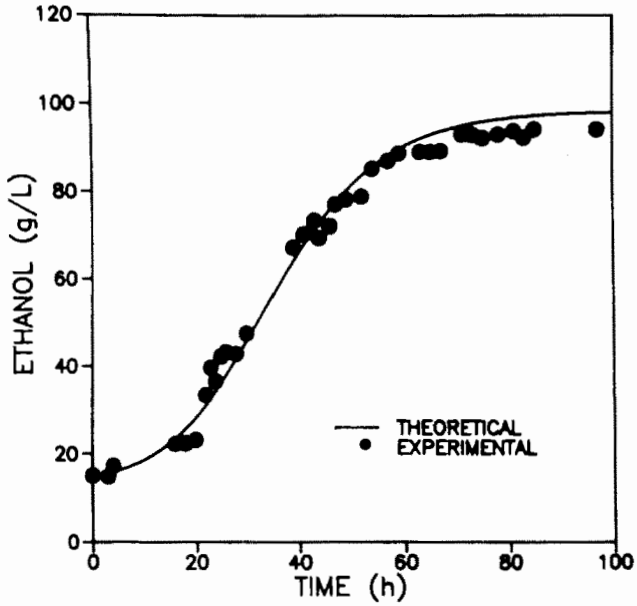

Figure 5. Variation of ethanol concentration with time: (○) experimental values from industrial plant; (——) theoretical values from the proposed model.

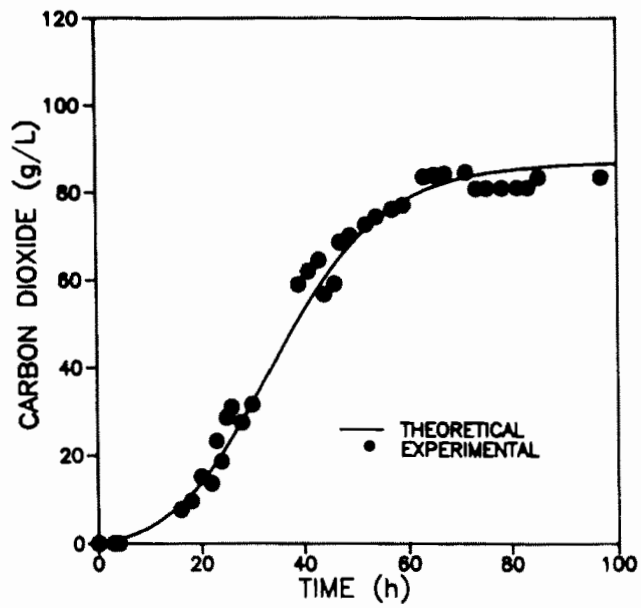

Figure 6. Total yield of carbon dioxide with time: (○) experimental values from industrial plant; (——) theoretical values from the proposed model.

CONCLUSIONS

From the results obtained in this study, we may conclude it is necessary to incorporate microbial functions other than fermentation in kinetic equations. Respiration, for example, can explain up to 50% of the total losses in fermenting yield (between 5 and 20 g ethanol/L), whereas the evaporation of the product (ethanol) does not account for more than 10% of the total losses, under normal conditions. Other processes, such as synthesis of cells and secondary products, are also important loss mechanisms.

The maximum growth rate of the selected strain depends on operating conditions which must be known exactly. In addition, there may be variations between different strains growing under the same operating conditions. In this sense, it is important to define the thermal behavior of each species, as the results obtained under extreme temperature conditions can be completely different, depending on the microorganism used. Finally, for an alcohol fermentation, we have shown it is possible to use a kinetic model obtained from laboratory data, to predict an industrial-scale process, provided similar operating conditions are employed at each scale.

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NOMENCLATURE

A	favorable reference coefficient (h^{-1})
B	unfavorable reference coefficient (h^{-1})
C	amount of carbon dioxide (g/L)
E	amount of ethanol (g/L)
E_A	favorable activation energy (kcal/mol)
E_B	unfavorable activation energy (kcal/mol)
f	fermentative conversion coefficient (g/g)
K_D	reciprocal death constant (g/L h)
K_I	ethanol inhibition constant (g/L)
K_S	modified saturation constant (g/L)
m	maintenance coefficient (g substrate/g biomass h)
MW(i)	molecular weight of compound i (g/mol)
p	residual conversion coefficient (g/g)
r	respirative conversion coefficient (g/g)
R	gas constant (kcal/mol K)
S	substrate concentration (g/L)
t	time process (h)
T	operation temperature (K)
X_v	viable biomass concentration (g/L)
Y_s	biomass yield factor (g biomass/g substrate)
μ	specific growth rate (h^{-1})

Subscripts

0	reference value
d	death
e	evaporation
f	fermentation
g	growth
i	initial value
j	final value
max	maximum value
r	respiration
s	stoichiometric value
t	total value
x	excess value
Y	substrate yield
I	inhibition constant
S	saturation constant

References

1. Aiba, S., Shoda, M., Magatani, M. 1968. Biotechnol. Bioeng. **10**: 845.
2. Andreyeva, L. N., Biryukov, V. V. 1973. Biotechnol. Bioeng. Symp. **4**: 61.

3. Bacila, M., Horii, J. 1979. Trends. Biochem. Sci. **4**: 59.
4. Bailey, J. E., Ollis, D. F. 1977. Biochemical engineering fundamentals. McGraw–Hill, New York.
5. Boulton, R. 1979. Biotechnol. Bioeng. Symp. **9**: 167.
6. Gontcharova, E. M., Potoroko, G.V., Martakov, A. A. 1971. Prikl. Biokhim. Mikrobiol. **7**: 313.
7. Hanswerner, D., Sevastiano, F. L. 1988. Process Biochem. **8**: 100.
8. Harrigan, W. F., McCance, M. E. 1976. Laboratory methods in food and dairy microbiology. Academic, London.
9. Holzberg, I., Finn, R. K., Steinkraus, K. H. 1967. Biotechnol. Bioeng. **9**: 413.
10. Hopkins, R. H. 1931. Biochem. J. **26**: 245.
11. Hopkins, R. H., Roberts, R. H. 1935. Biochem. J. **29**: 931.
12. Hoppe, G. K., Hansford, C. S. 1982. Biotechnol. Lett. **4**: 39.
13. Ingraham, J. L., Guymon, J. F. 1960. Arch. Biochem. Biophys. **88**: 157.
14. Jacob, F. C., Archer, T. E., Castor, J. G. B. 1964. Am. J. Enol. Viticult. **15**: 69.
15. Lagunas, R. 1979. Mol. Cell. Biochem. **27**: 139.
16. Lagunas, R. 1981. Trends. Biochem. Sci. **6**: 201.
17. Levenspiel, O. 1980. Biotechnol. Bioeng. **22**: 1671.
18. Lodder, J. 1984. The Yeast. A Taxonomic Study. North Holland, Amsterdam.
19. Kunkee, R. E., Amerine, M. A. 1968. Appl. Microbiol. **16**: 1067.
20. Morgan, M. S., Edwards, V. H. 1971. Chem. Eng. Prog. Symp. Ser. **114**: 51.
21. Novak, M., Strehaiano, P., Moreno, M. 1981. Biotechnol. Bioeng. **23**: 201.
22. Ough, C. S. 1966. Am. J. Enol. Viticult. **17**: 74.
23. Ough, C. S., Amerine, M. A. 1963. Am. J. Enol. Viticult. **14**: 194.
24. Oura, E. 1977. Process Biochem. **12**: 19.
25. Pirt, S. J. 1975. Principles of microbe and cell cultivation. Blackwell Scientific, Oxford.
26. Ribéreau-Gayon, J., Peynaud, E., Ribéreau-Gayon, P., Sudraud, P. 1976. p. 3. Traité d'oenologie. Sciences et techniques du vin. Dunod, Paris.
27. Schanderl, H. 1959. Die Mikrobiologie des mostes und weins. Eugen Ulmer, Stuttgart.
28. Steinmeyer, D. E., Shuler, M. L. 1989. Chem. Eng. Sci. **44**: 2017.
29. Topiwala, H. H., Sinclair, C. G. 1971. Biotechnol. Bioeng. **13**: 795.
30. Tyagi, R. D., Ghose, T. K. 1982. Biotechnol. Bioeng. **24**: 39.
31. Williams, L. A., Boulton, R. 1983. Am. J. Enol. Viticult. **34**: 234.
32. Zines, D. O., Rogers, T. L. 1971. Biotechnol. Bioeng. **13**: 293.