Kalkwarf and Antoine equations.

For all three benzoates, the polynomial with four parameters, i.e., the Cragoe equation gives a good correlation as well.

The constants of the Cragoe equation are listed in **Table 4**. The constants of the Antoine and Frost-Kalkwarf equations are not listed, because these equations have larger errors than the Cragoe equation. The constants of the Chebyshev polynomial are not shown because the polynomial with four parameters is actually the Cragoe equation, as described above.

Conversely, the temperatures were calculated from the pressures by using the Cragoe, Frost-Kalkwarf, and Antoine equations. The mean temperature differences of the Cragoe, Frost-Kalkwarf and Antoine equations with the experimental values were taken as 0.03, 0.11 and 0.18 K, respectively, for isopropyl benzoate; 0.04, 0.05 and 0.22 K, respectively, for *t*-butyl benzoate; and 0.04, 0.05 and 0.28 K, respectively, for 2-chloroethyl benzoate.

Nomenclature

A_a, B_a, C_a	=	Antoine constants defined by Eq. (7)	
D_a	=	$A_aC_a-B_a$	
A_c, B_c, C_c, D_c	=	Cragoe constants defined by Eq. (3)	
A_f, B_f, C_f, D_f	=	Frost-Kalkwarf constants defined by Eq. (5)	
a_0, a_i	=	constants of Chebyshev polynomial defined by Eq. (1)	
$E_1(x)$	=	$-x$	[—]
$E_2(x)$	=	$-2x^2-1$	[—]
$E_i(x)$	=	$-2xE_{i-1}(x)-E_{i-2}(x)$	[—]
P	=	pressure	[kPa]
T	=	temperature	[K]
t	=	$T-273.15$	[°C]
x	=	$(2T-(T_{max}+T_{min}))/(T_{max}+T_{min})$	[—]

Literature Cited

1) Ambrose, D., J. F. Counsell and A. J. Davenport: *J. Chem. Thermodyn.*, **2**, 283 (1970).
2) Bender, M. L.: *J. Am. Chem. Soc.*, **73**, 1626 (1951).
3) Brown, W. G. and J. L. Garnett: Inst. J. Appl. Radiation Isotopes, **5**, 114 (1959).
4) Cohen, S. G.: *J. Am. Chem. Soc.*, **66**, 1395 (1944).
5) Cohen, S. G. and A. Schneider: *J. Am. Chem. Soc.*, **63**, 3382 (1941).
6) Cragoe, C. S.: "International Critical Tables of Numerical Data, Physics, Chemistry and Technology," first ed., ed by National Research Council of the United States of America, Vol. 3, pp. 228–233, McGraw-Hill, New York (1928).
7) Dorris, T. B., F. J. Sowa and J. A. Nieuwland: *J. Am. Chem. Soc.*, **56**, 2689 (1934).
8) Frost, A. A. and D. R. Kalkwarf: *J. Chem. Phys.*, **21**, 264 (1953).
9) Goldfarb, Ya. L. and L. M. Smorgonskii: *J. Gen. Chem. (U.S.S.R.)*, **8**, 1516 (1938).
10) Katayama, H. and Y. Harada: *J. Chem. Eng. Data*, **29**, 373 (1984).
11) Katayama, H.: *J. Chem. Eng. Data*, **33**, 75 (1988).
12) Matsuno, K. and K. Han: *Bull. Chem. Soc. Japan*, **8**, 333 (1933).
13) Miller, D. G.: *Ind. Eng. Chem.*, **56**, No. 3, 46 (1964).
14) Millington, J. E. and F. L. M. Pattison: *Can. J. Chem.*, **34**, 1532 (1956).
15) Murakami, K.: *Kobunshi Kagaku (Japan)*, **8**, 85 (1951).
16) Pfannl, M.: *Monatshefte fur Chemie*, **32**, 509 (1911).
17) Schroeder, M. R., B. E. Poling and D. B. Manley: *J. Chem. Eng. Data*, **27**, 256 (1982).
18) Tommila, E.: *Ann. Acad. Sci. Fenn. Ser. A*, **59**, No. 3, 3 (1942).
19) Vavon, G., M. Barbier and G. Thiebaut: *Bull. Soc. Chim. (France)*, **1**, 806 (1934).
20) Zaki, A.: *J. Chem. Soc. (of London)*, 2269 (1930).

KINETIC MODELING OF SIMULTANEOUS SACCHARIFICATION AND FERMENTATION OF CELLULOSE

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Introduction

Cellulose is an abundant, renewable resource. However, chemical or biological treatment is required to convert this polymeric form of glucose into useful materials. Takagi *et al.*[12] proposed an one-stage process involving the enzymatic saccharification of cellulose and simultaneous fermentation of the glucose by yeast in the one vessel (SSF). The courses of the concentration change of glucose and ethanol in

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SSF have been shown[3,5,8,9,11] and models have also been proposed.[5,8,9,11] However, attention has been focused primarily on the hydrolysis reaction alone. We proposed in this study a model accounting for both hydrolysis and fermentation reactions. Experiments were also carried out to assess the validity of the model.

1. Experimental

The cellulase, cellulose and yeast utilized were Meicelase CEPB-5081, filter paper powder (under 300 mesh), and *Saccharomyces cerevisiae* Kyokai 7, respectively. Three kinds of experiments, hydrolysis of cellulose, fermentation of glucose, and SSF, were carried out at 303 K. In the SSF experiments, cellulose powder and the yeast were added to the enzyme solution. The cellulase attained its maximum activity at pH = 5.0, and thus the pH for all the experiments was kept at 5.0 by use of acetate buffer. The initial and final pH values were respectively 5.02 and 5.03 for hydrolysis and 5.01 and 5.05 in the case of SSF. The concentrations of two kinds of sugars, glucose and reducing sugars, were analyzed in a preliminary experiment. The concentrations of both sugars were almost the same, suggesting that the main constituent of the reducing sugars was glucose. The course of hydrolysis reaction was followed by measuring the glucose concentration, and that of glucose fermentation was followed by measuring the concentrations of glucose, ethanol, and yeast cells. The glucose oxidase method was employed to determine the concentration of glucose. The dicromate method of Zimmermann and the agar plate method were used to determine the concentrations of ethanol and yeast cells, respectively.

2. Kinetic Modeling

The kinetics of cellulose hydrolysis was assumed to be of the form:[7]

$$\mathrm{E}+\mathrm{S} \rightleftarrows \mathrm{E}-\mathrm{S} \longrightarrow \mathrm{E}+\mathrm{G} \tag{1}$$

$$\mathrm{E}+\mathrm{G} \rightleftarrows \mathrm{E}-\mathrm{G} \tag{2}$$

where S refers to cellulose. Equation (2) implies that the enzyme, E, is competitively inhibited by the glucose, G. The rates of cellulose reduction and glucose production can be expressed from Eqs. (1) and (2) as follows:

$$-d(\mathrm{S})/dt = d(\mathrm{G})/dt = V_{\max}(\mathrm{S})/[K_m+(K_m/K_{ig})(\mathrm{G})+(\mathrm{S})] \tag{3}$$

where the substances in parentheses refer to concentrations, and K_{ig}, K_m and $V_{\max}$ are constants.

In the fermentation of glucose, the rates of glucose consumption,[1] ethanol production and cell growth may be described by Eqs. (4), (5) and (6), respectively.

$$d(\mathrm{G})/dt = -[\mu/Y_{\mathrm{X/G}}+m](\mathrm{X}) \tag{4}$$

$$d(\mathrm{P})/dt = Y_{\mathrm{P/G}}[\mu/Y_{\mathrm{X/G}}+m](\mathrm{X}) \tag{5}$$

$$d(\mathrm{X})/dt = \mu(\mathrm{X}) \tag{6}$$

For a specific growth rate, μ, Eq. (7) is adopted.[10]

$$\mu = \mu_{\max}(\mathrm{G})/[K_{\mathrm{G}}+(\mathrm{G})]/[1+(\mathrm{P})/K_{ip}] \tag{7}$$

where m, $Y_{\mathrm{X/G}}$, $Y_{\mathrm{P/G}}$, K_{ip} and K_{G} are constants. (P) and (X) are the ethanol and the cell concentrations, respectively.

In the SSF, the glucose which is produced due to hydrolysis is consumed by the yeast.

$$-d(\mathrm{S})/dt = d(\mathrm{G})/dt = V_{\max}(\mathrm{S})/[K_m+(K_m/K_{ig})(\mathrm{G})+(\mathrm{S})] - [\mu/Y_{\mathrm{X/G}}+m](\mathrm{X}) \tag{8}$$

Equations (5), (6), (7) and (8) were solved simultaneously via numerical integration with the initial conditions of (G) = (P) = 0, (S) = (S_0), (X) = (X_0) at t = 0. Equations (1) and (2) and Eqs. (4) through (7) have been treated independently, but they have never before been solved simultaneously.

3. Results and Discussion

3.1 Hydrolysis

The constants K_m and $V_{\max}$ were determined by

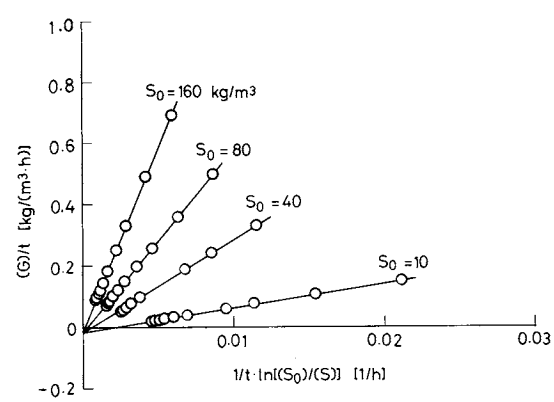

Fig. 1. Foster-Niemann plot of hydrolysis data

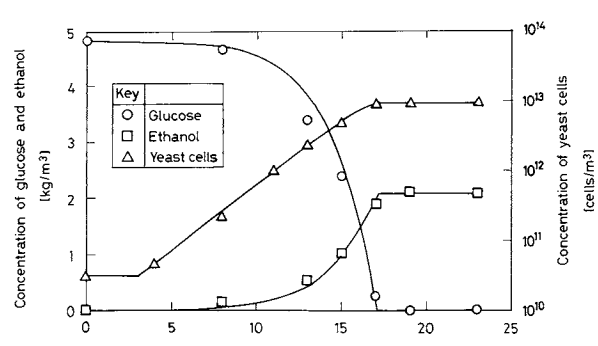

Fig. 2. Time courses of glucose and ethanol concentrations and number of yeast cells in fermentation

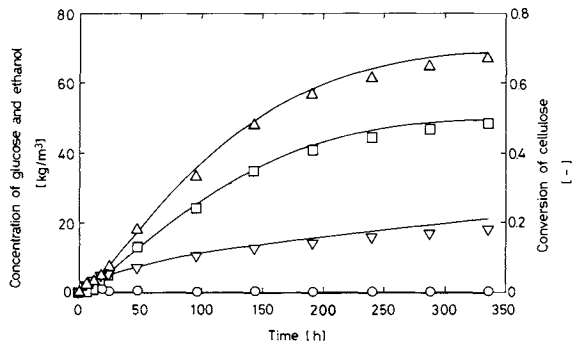

Fig. 3. Time courses of glucose and ethanol concentrations in SSF, and cellulose conversion in both SSF and hydrolysis. ○, glucose concentration; □, ethanol concentration; △, cellulose conversion in SSF; ▽, cellulose conversion in hydrolysis

means of the Lineweaver-Burk plot using the initial hydrolysis data with various substrate concentrations. It was found in preliminary experiments that the cellulose was not completely degradable: approximately 30 percent of the cellulose remained undegraded. Therefore, the initial concentration of cellulose was multiplied by 0.7 in the calculation. The Foster-Niemann plot[8] of hydrolysis data is shown in **Fig. 1**, from which K_{ig} was evaluated.

The estimated K_m, V_{max} and K_{ig} values were 0.40 kmol-glucose/m^3, 7.0×10^{-3} kmol-glucose/(m$^3\cdot$h) and 7.5×10^{-3} kmol-glucose/m^3, respectively. These values are in accord with those in the literature.[2,4,6,8,9,12]

3.2 Fermentation

The course of fermentation is shown in **Fig. 2**. The symbols stand for experimental data, the solid lines for calculations. Parameter estimation by using curve-fitting method resulted in: $m=8.0\times10^{-16}$ kmol-glucose/(cell$\cdot$h), $K_G=4.0\times10^{-3}$ kmol-glucose/m^3, $K_{ip}=0.20$ kmol-ethanol/m^3, $Y_{X/G}=1.8\times10^{12}$ cells/mol-glucose, $Y_{P/G}=1.7$ mol-ethanol/mol-glucose and $\mu_{max}=0.5$ 1/h.

3.3 SSF

The courses of the glucose and ethanol concentrations and the cellulose conversion are illustrated in **Fig. 3** for the SSF of 160 kg/m^3 cellulose concentration. The conversion is the ratio of the concentration of an effective glucose produced to that of the initial cellulose. The effective glucose refers to the sum of residual glucose and ethanol divided by $Y_{P/G}$. The cellulose conversion in the case of hydrolysis reaction alone (without the presence of yeast) is smaller than that of the SSF as shown in Fig. 3. The solid lines stand for predictions by the model, the symbols for experiments. Good agreement can be observed between predictions and measurements.

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Nomenclature

(E)	=	concentration of enzyme	[kmol/m^3]
(G)	=	concentration of glucose	[kmol/m^3]
K_{ip}	=	inhibition constant of ethanol	[kmol/m^3]
K_{ig}	=	inhibition constant for cellulose hydrolysis	[kmol/m^3]
K_G	=	constant concerning growth rate of yeast	[kmol/m^3]
K_m	=	Michaelis-Menten constant	[kmol/m^3]
m	=	constant of glucose consumption	[kmol/(cell$\cdot$h)]
(P)	=	concentration of ethanol	[kmol/m^3]
(S)	=	concentration of cellulose	[kmol/m^3]
t	=	time	[h]
V_{max}	=	maximum rate of hydrolysis	[kmol/(m$^3\cdot$h)]
$Y_{P/G}$	=	stoichiometric factor of production yield	[mol-ethanol/mol-glucose]
$Y_{X/G}$	=	stoichiometric factor of growth yield	[cells/mol-glucose]
(X)	=	concentration of cells	[cells/m^3]
μ_{max}	=	maximum specific growth rate of cells	[1/h]

Literature Cited

1) Aiba, S., A. E. Humphrey and N. F. Millis: "Biochemical Engineering," 2nd ed., University of Tokyo Press and Academic Press (1973).
2) Amemura, A. and G. Terui: *J. Ferment. Technol.*, **43**, 281 (1965).
3) Arai, M., T. Ooi, H. Hayashi and S. Murao: *Hakkokougaku Kaishi* **63**, 427 (1985).
4) Fan, L. T., Y. Lee, and H. David: *Biotechnol. Bioeng.*, **22**, 177 (1980).
5) Ghosh, P., N. B. Pamment and W. R. B. Martin: *Enzyme Microb. Technol.*, **4**, 425 (1982).
6) Holtzapple, M. T., H. S. Caram and A. E. Humphrey: *Biotechnol. Bioeng.*, **26**, 753 (1984).
7) Howell, J. A.: *Biotechnol. Bioeng.*, **20**, 847 (1978).
8) Ooshima, H., Y. Ishitani and Y. Harano: *Biotechnol. Bioeng.* **27**, 389 (1985).
9) Ooshima, H. and Y. Harano: *Kagaku Kojo.*, **27**, 99 (1983).
10) Novak, M., P. Strehaiano, M. Moreno and G. Goma: *Biotechnol. Bioeng.*, **23**, 201 (1981).
11) Ryu, D. D. Y. and S. B. Lee: *Biotechnol. Bioeng.*, **24**, 1047 (1982).
12) Takagi, M., S. Abe, S. Suzuki, G. H. Emert and N. Yata: Proc. Bioconv. Sympo., Delhi, p. 551 (1977).