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Effects of Pore and Film Diffusion Resistances and Deactivation of Enzyme on the Overall Reaction Rate of Immobilized Enzyme

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A method was developed for investigation of the influence of pore and film diffusional resistances on the overall rate of glucose isomerization reaction subject to enzyme deactivation in the immobilized cells reactor. Two kinds of effectiveness factor, η_D and η_{Df}, were used to illustrate the mass transfer aspects of the system. The actual reaction rate can be expressed in terms of a product of the ideal reaction rate by a multiplier, η_D or η_{Df}. η_D is the multiplier for the ideal rate based on the surface concentration and η_{Df} is based on the bulk concentration. The constant of enzyme deactivation was estimated by numerical analysis of the change in η_D with time obtained experimentally in continuous stirred tank reactor. The surface concentration was estimated by iterative calculation method. A calculation procedure of η_{Df} from kinetic data and physical constants determined by experiments was developed. The satisfactory agreement between the calculated values and the experimental results supports the validity of this theoretical analysis.

In heterogeneous immobilized-enzyme catalysis, diffusional resistances inside the particles (pore diffusion resistance) and in the liquid film surrounding the particles (film diffusion resistance) are inevitable. These diffusional effects may reduce the overall reaction rate and make the analysis of reaction kinetics complicated. The effect of pore diffusion has been quantified through the concept of an effectiveness factor.[1] For simplicity, pore diffusion has often been studied separately from film diffusion by many authors.[2,3] In practical reactor performance, however, both the effects of pore and film diffusion often exist simultaneously. In non-linear reaction kinetics the film and pore resistances are too interrelated for separation of these two effects. A theoretical description of film diffusion combined with pore diffusion was given by Lee and Tsao.[4] In their report, the film factor, defined as the ratio of substrate concentration at the particle surface to that in the bulk, was used to account for film diffusion.

Furthermore, a major obstacle to the commercial use of immobilized enzymes is the loss of enzyme activity due to deactivation. From an economic standpoint it is important to minimize this deactivation loss. For the efficient design of an immobilized enzyme reactor, it is essential to develop a model that accounts for simultaneous reaction kinetics and mass transfer. In addition, the effect of deactivation must be considered. The effects of mass transfer on fractional conversion and operational stability of immobilized invertase, β-galactosidase, trypsin and hexokinase in a fixed bed reactor have been studied by Kobayashi and coworkers[5]. In our previous report,[6] the effect of diffusional restriction on the overall reaction rate of immobilized cells in a plate-type reactor under laminar flow operation has been described. In this study, cells of *Streptomyces phaechromogenes*, possessing glucose isomerase activity, were immobilized by the aggregation of chitosan. Besides the conventional effectiveness factor which is used to quantify the extent of pore diffusion resistance,

an overall effectiveness factor was defined as the ratio of actual reaction rate (with pore and film resistances, and deactivation) to the ideal rate without these influences. The overall effectiveness factor can be used as an important design parameter. It was the purpose of this present work to analyse the influence of diffusion on the change in the effectiveness factor of immobilized cells subject to deactivation and to demonstrate quantitatively the correlation between the pore and film resistances so as to provide a better understanding in the design of an enzyme reactor.

Experimental

Preparation of immobilized enzyme Immobilized enzyme beads in which *Streptomyces phaechromogenes* cells containing active glucose isomerase were prepared based on the aggregation of chitosan (obtained from the Toyobo Company). The specific activity of glucose isomerase in the immobilized cells was 350 IGIU/g. One International Glucose Isomerase Unit (IGIU) is defined as the amount of enzyme which initially converts one micromole of glucose to fructose per minute at pH 6.85 and 60°C. The substrate solution contained 2 M glucose, 0.02 M $MgSO_4$ and 0.001 M $CoCl_2$ in 0.2 M maleate buffer. The sizes of the immobilized enzyme beads were determined photographically after the beads were swollen in distilled water for 24 hr.

Experimental reactor Figure 1 shows a schematic diagram of the column reactor employed in this study. The immobilized enzyme beads were packed in a glass column (1.5 cm internal diameter, 20 cm height) with a jacket for temperature control. The temperature of the bed was monitored by means of a thermocouple. Three different particle sizes—8–12 mesh, 20–30 mesh and 40–48 mesh (average radii 0.125 cm, 0.046 cm and 0.018 cm, respectively)—were used in the experiments. The flow reactor ran in a temperature range of 60–80°C and at a feed flow rate of 30–120 ml/hr. The substrate solution was pumped via a preheater into the bed by a peristaltic pump.

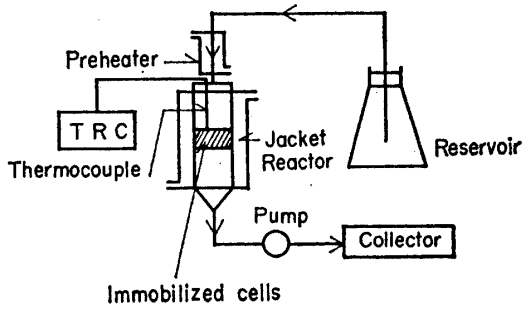

Fig. 1. Schematic diagram of a differential type reactor.

An Amicon ultrafilter (Model 52, working volume 25 ml) was used as a stirred type reactor to yield conditions where the film diffusional effect is small enough to be neglected. The fructose content was assayed at appropriate time intervals.

Substrate solution The substrate solution contained 0.5–5.15 M glucose, and 4×10^{-3} M $MgSO_4\cdot7H_2O$ in 0.1 M Tris-buffer. The pH was adjusted to 8.3.

Analytical methods Fructose and glucose were assayed by the cystine-carbazole method and Glucostat method (Worthington Biochemical), respectively.

Theoretical

Kinetics It is well known that glucose isomerase catalyzes the glucose $\rightleftarrows$ fructose reaction in a reversible manner.[7] According to Peller and Alberty,[8] the rate equation for this mechanism in the steady state, considering enzyme deactivation, can be given as follows,

$$r_A' = \frac{dC_A}{dt} = \psi \frac{(V_A'/K_A)C_A - (V_B'/K_B)C_B}{1 + C_A/K_A + C_B/K_B} \quad (1)$$

where C_A and C_B are the concentrations of glucose and fructose, respectively, V_A' and K_A are the maximum reaction rate and the Michaelis-Menten constant of the forward reaction respectively, and V_B' and K_B are the same constants for the reverse reaction. The activity coefficient, ψ, is the ratio of the reaction rate with deactivating enzyme to that with fresh enzyme. The equilibrium concentration of glucose and fructose is correlated by the following equation:

$$C_{A0}=C_A+C_B=C_{Ae}+C_{Be} \quad (2)$$

where C_{Ae} and C_{Be} are the concentration of glucose and fructose at equilibrium, respectively. C_{A0} is the inlet glucose concentration. The reduced concentration of glucose, C_S, is defined as

$$C_S=C_A-C_{Ae} \quad (3)$$

After rearranging Eqs. (1)–(3), the rate equation is rewritten in an ordinary Michaelis-Menten form following the method described by Lee *et al.*[9]

$$r_A'=\phi\frac{V_m'C_S}{K_m+C_S} \quad (4)$$

where

$$V_m'=\frac{1+(1/K)}{1-(K_A/K_B)}V_A'$$

$$K_m=\frac{1+\{C_{A0}/(K+1)\}\{(1/K_A)+(K/K_B)\}}{1-(K_A/K_B)}K_A$$

$$K=\frac{V_A'/K_A}{V_B'/K_B} \quad (5)$$

and K is the equilibrium constant. The deactivation of enzyme was assumed to be a first order reaction to yield

$$-\frac{d\phi}{dt}=k_f(T)\phi \quad (6)$$

where k_f is the rate constant of deactivation.

Mass transfer aspects subject to deactivation

Pore diffusion The immobilized cells aggregated by chitosan is looked upon as a porous and spherical particle within which enzymes are uniformly distributed. Considering a situation where a liquid film does not exist outside the particle and the surface concentration is identical to the bulk concentration, a mass balance of the steady state on glucose inside the particles gives the following equation in terms of the reduced concentration.

$$D_A\left(\frac{d^2C_S}{dr^2}+\frac{2}{r}\frac{dC_S}{dr}\right)-\phi\frac{V_mC_S}{K_m+C_S}=0 \quad (7)$$

where

$$V_m=V_m'\times\frac{\text{entrapped cell mass (g)}}{\text{volume of gel (ml)}}$$

The boundary condition and the initial condition are

$$\frac{dC_S}{dr}=0 \quad \text{at } r=0$$

$$C_S=C_{SR} \quad \text{at } r=R \quad (8)$$

$$\phi=1 \quad \text{at } t=0$$

Since the immobilized cells were prepared by aggregating the preheated cells with chitosan, the kinetic parameters of the immobilized cells were considered to be the same as that of the preheated cells. Thus, the maximum reaction rate based on the volume of immobilized cells can be evaluated by multiplying the maximum rate based on the weight of the preheated cells by a factor which represents the weight of preheated cells contained in the unit volume of gel.

D_A is the effective diffusivity of glucose and r is the radial distance of the particle. In the case of negligible film diffusion, the effectiveness factor, denoted by η_D, was defined as

$$\eta_D=\frac{\text{Actual reaction rate (without film diffusion resistance)}}{\text{Ideal reaction rate (without enzyme deactivation and diffusion resistance)}}$$

$$=\frac{3}{R}\left(\frac{D_A\frac{dC_S}{dt}\Big|_{r=R}}{r_A\Big|_{\substack{\phi=1\\C_S=C_{SR}}}}\right) \quad (9)$$

where

$$r_A=\phi\frac{V_mC_S}{K_m+C_S}$$

r_A represents the reaction rate based on the volume of immobilized cells. By normalizing the differential equation, Eq. (7), it can be seen that η_D depends on two dimensionless parameters, $\phi=\frac{3}{R}\sqrt{V_m/K_mD_A}$ and $\alpha_R=K_m/C_{SR}$. ϕ is conventionally referred to as the Thiele modulus. Since the reaction is non-linear, analytical solution for η_D cannot be readily obtained from Eqs. (7)–(9). Although the concentration profile can be obtained by solving Eq. (7) numerically, an approximate estimation method for η_D proposed by Kobayashi[10] taking into account enzyme deactivation was applied to this work. According to this method, η_D was represented by the following equation in terms of the effectiveness factors for zero and first order

kinetics, denoted by E_0 and E_1, respectively.

$$\eta_D=\frac{E_0+aE_1}{1+a} \tag{10}$$

where

$$E_0=\begin{cases}1 & m\sqrt{\psi}\leqq 1/\sqrt{3}\\ 1-\left(\frac{1}{2}+\cos\frac{\gamma+4\pi}{3}\right)^3 & m\sqrt{\psi}>1/\sqrt{3}\end{cases}$$

$$\gamma=\cos^{-1}\left(\frac{2}{3m^2\psi}-1\right) \tag{11}$$

$$E_1=\frac{\sqrt{\psi}}{m}\left(\frac{1}{\tanh 3m\sqrt{\psi}}-\frac{1}{3m\sqrt{\psi}}\right)$$

$$m=\frac{\phi}{\sqrt{2}\,(1+\alpha_R)\sqrt{(1/\alpha_R)-\ln\{1+(1/\alpha_R)\}}}$$

$$\psi=e^{-k_f t}$$

$$a=2.6\,\alpha_R^{0.8}$$

Pore diffusion combined with film diffusion

Usually the film resistance is not negligible in the enzyme particles packed in a column reactor. In an attempt to illustrate the influence of both the pore and film diffusion, the overall effectiveness factor, denoted as η_{Df}, is defined as follows:

$$\eta_{Df}=\frac{\text{Actual reaction rate (with deactivation of the enzyme and effects of diffusion resistances)}}{\text{Ideal reaction rate (without deactivation of the enzyme and effects of diffusion resistances)}}$$

$$=\frac{\eta_D\dfrac{V_mC_{SR}}{K_m+C_{SR}}}{\dfrac{V_mC_{SS}}{K_m+C_{SS}}} \tag{12}$$

where

$$C_{SS}=C_{AS}-C_{Ae}$$

$$C_{SR}=C_{AR}-C_{Ae} \tag{13}$$

C_{AS} and C_{AR} represent the bulk concentration and the surface concentration, respectively. When the film resistance cannot be neglected, a concentration difference between the particle surface and the bulk is significant. As mentioned before, the actual reaction rate is expressed in terms of a product of η_D by the ideal reaction rate based on the surface concentration. But in the case where the surface concentration is smaller than the bulk concentration due to the film resistance, the overall mass transfer process cannot sufficiently be illustrated by η_D alone. The correlation between pore diffusion and film diffusion can be interpreted as follows. A material balance at the solid-liquid interface gives:

$$k_L\cdot(C_{SS}-C_{SR})=\eta_D\cdot\frac{R}{3}\cdot\frac{V_mC_{SR}}{K_m+C_{SR}} \tag{14}$$

Combining Eq. (12) and Eq. (14), a dimensionless equation for η_{Df} is obtained as

$$\eta_{Df}=\eta_D\cdot\beta\cdot\frac{1+\alpha_S}{\beta+\alpha_S} \tag{15}$$

where

$$\beta=\frac{C_{SR}}{C_{SS}}=\frac{1}{2}\left\{1-\alpha_S\left(\frac{\eta_Dm_f}{3}+1\right)+\sqrt{\left[1-\alpha_S\left(\frac{\eta_Dm_f}{3}+1\right)\right]^2+4\alpha_S}\right\}$$

$$m_f=\frac{V_mR}{K_mk_L}\;;\quad \alpha_S=\frac{K_m}{C_{SS}} \tag{16}$$

m_f, referred to as the film modulus, is regarded as a parameter to express the effect of film resistance. β, the so-called film factor,[4] is an index for the film resistance. Equation (16) shows that the relation between the film and pore resistance is implicit because η_D depends on C_{SR}. This situation occurs in non-first order reactions such as Michaelis-Menten kinetics. From the above description, η_{Df} can illustrate the overall effects of diffusion and enzyme deactivation in the immobilized cell system.

Iterative calculation of the surface concentration

From Eqs. (10) and (15), η_{Df} depends on the bulk concentration as well as the surface concentration, on which η_D depends. Therefore, calculation of the effectiveness factors, η_{Df} and η_D, needs the value of the surface concentration. Since η_D depends on C_{SR}, the surface concentration cannot be explicitly expressed from Eq. (15). Thus, an iterative calculation method for the surface concentration using Eqs. (10) and (15) was proposed. First, the surface concentration was assumed to be a reasonable value, say, C_{SR1}. The values of α_R and α_S was calculated from their definitions, respectively. The degree of activity ψ was calculated from Eq. (6). The value of η_D was obtained from Eq. (10) for a given ϕ value. When η_D was obtained, another surface concentration referred to as C_{SR2} was computed for a given m_f value from Eq. (16). Then a comparison between C_{SR1}

and C_{SR2} was made to determine whether the assumed value was adequate. If a good agreement was seen upon comparison, the assumed value C_{SR1} offered a good estimate for the surface concentration; otherwise, a new value for C_{SR1} was assumed and the computation was repeated until C_{SR2} agreed with C_{SR1} satisfactorily.

Results and Discussion

Theoretical analysis for effectiveness factor Consider a special case where enzyme deactivation does not take place. In such a situation, the effectiveness factor, η_{D0}, is used to characterize a pore diffusion process without film resistance, and the effectiveness factor, η_{Df0}, can be used to quantify the overall transfer process including the pore and film diffusion resistances simultaneously.

Effect of ϕ and α_R on η_{D0} η_{D0} was calculated from Eq. (10) for various ϕ and α_R under the condition $\psi=1$. In Fig. 2, η_{D0} was plotted against ϕ with α_R as a parameter. The enzyme particles having high activity tend to have low η_{D0}, while ones with low activity tend to have high η_{D0}.

Effect of ϕ and m_f on η_{Df0} Estimating surface concentration, η_{Df0} was calculated from Eq. (15) in which η_D was replaced by η_{D0}. With varying ϕ and m_f, the calculation of η_{Df0} was carried out. In Fig. 3, η_{Df0} was plotted against m_f with various values of ϕ as a parameter. Obviously, in the large ϕ region, η_{Df0} changes little as m_f varies; while in the small ϕ region, η_{Df0} decreases with increasing m_f. It is suggested that film diffusion resistance plays an important role in the overall transfer process as pore resistance is weak. In the case of strong pore resistance (large particle), the change of η_{Df0} was not very noticeable even at large m_f.

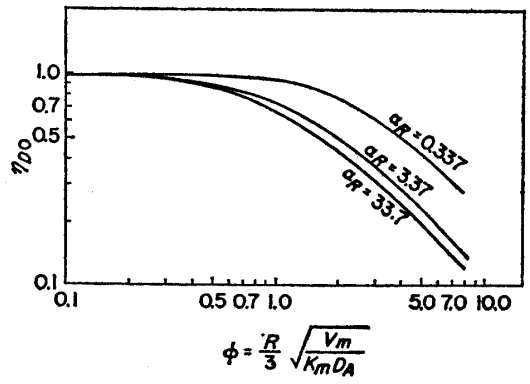

Fig. 2. Effectiveness factor η_{D0} as function of Thiele modulus and dimensionless Michaelis constant.

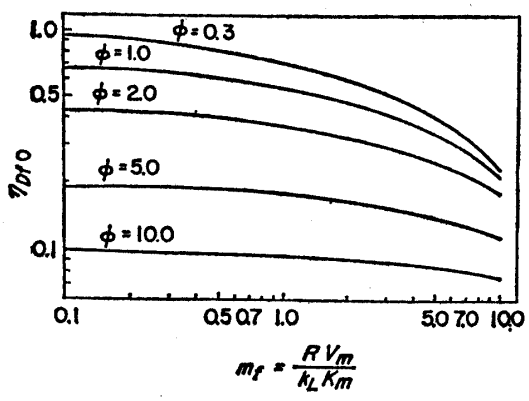

Fig. 3. Overall effectiveness factor η_{Df0} as function of moduli ϕ and m_f.

Commonly as an enzyme is deactivated with time in a continuous process, η_D and η_{Df} decrease. The behavior of η_D and η_{Df} during the process time will be elucidated below.

Variation of η_D with process time Figure 4 represents the effect of particle radius on the variation of η_D against process time. Here, the process time is represented by a dimensionless term, $k_f t$. For a large particle

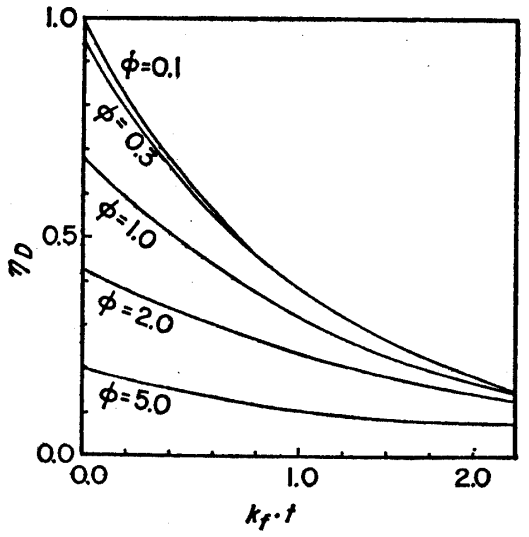

Fig. 4. Effect of the modulus ϕ on the change in the effectiveness factor η_D.
C_{A0}=5.15 M, $k_f=2.58\times10^{-3}$ 1/hr.

(large ϕ), η_D shows a low value and changes little with time, while for a small particle, η_D has a high value and decreases rapidly with time. Hence, for a large particle, the decrease of reaction rate due to deactivation is partly compensated for by a relatively slow decrease of η_D. Thus, it is apparent why the overall reaction rate changes slowly in this case. For a small particle, since η_D was initially near 1.0 because of a small ϕ (0.1–0.5), even a small decrease in $m\sqrt{\psi}$ causes a big decrease of η_D. Thus, the overall reaction rate decreases substantially as the enzyme is deactivated.

Variation of η_{Df} with the process time The flow chart shown in Fig. 5 is a procedure for calculating η_{Df} during the process time. The parameters, K_m, V_m, ϕ and m_f were calculated using the kinetic constants determined from the experimental data. By means of the previously described method for estimation of the surface concentration, a proper surface concentration was attained. Then the value of η_{Df} was calculated from Eq. (15) at small time intervals until the required

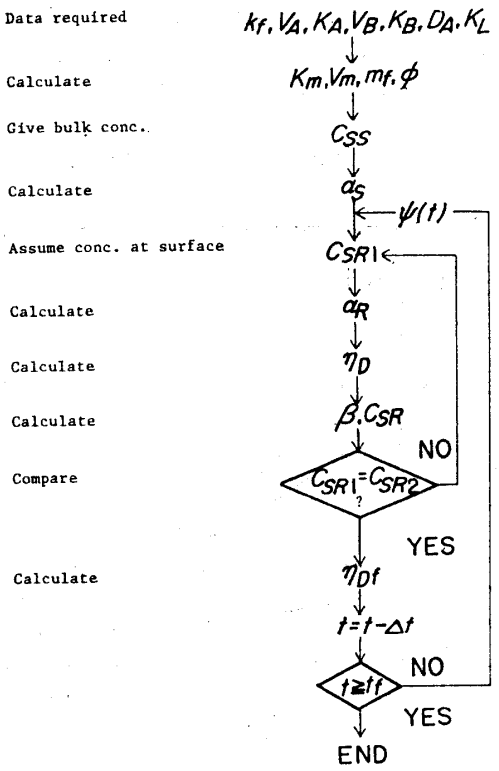

Fig. 5. Estimation of the value of η_{Df} during the process time.

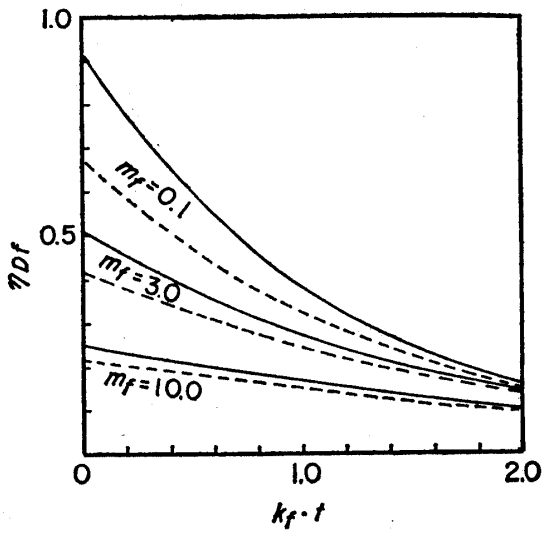

Fig. 6. Effect of the moduli ϕ and m_f on the change in overall effectiveness factor η_{Df}.
C_{A0}=5.15 M, k_f=2.58×10^{-5}/hr.
—— ϕ=0.3, ---- ϕ=1.0.

time range is covered. The computed results are presented in Fig. 6, which shows how the moduli ϕ and m_f exerted an effect on η_{Df} as the process time elapsed. In the small m_f region where the film resistance was small, ϕ has a strong influence on the variation of η_{Df}; in contrast, in the large m_f region, the difference between the η_{Df} value for a large ϕ and that for a small ϕ was negligible. In addition, the change of η_{Df} with time became gradual. It is indicated that in such a situation, the film diffusion resistance is dominant in the overall transport process.

Analysis of experimental data Determination of the kinetic and physical constants of glucose isomerization with immobilized cells is now described.

Estimation of kinetic constants The maximum rate of forward and reverse reactions and their Michaelis-Menten constants for various temperatures were estimated from Lineweaver-Burk plots of the data obtained with preheated cells. The results are listed in Table 1. The temperature dependence of the maximum reaction rate and the Michaelis-Menten constants are shown by Arrhenius-type plots in Figs. 7 and 8. The glucose concentration was varied over the range of 0.50 M to 5.15 M and no marked change in the values of these con-

Table 1. Kinetic constants of glucose isomerization by preheated cells at various temperatures.

Kinetic constants	60°C	65°C	70°C	75°C	80°C
V_A' (mol/hr·g cell)	4.07×10^{-2}	5.56×10^{-2}	7.53×10^{-2}	1.01×10^{-1}	1.34×10^{-1}
V_B' (mol/hr·g cell)	3.71×10^{-2}	4.85×10^{-2}	6.29×10^{-2}	8.09×10^{-2}	1.03×10^{-1}
K_A (mol/ml)	1.73×10^{-3}	1.61×10^{-3}	1.50×10^{-3}	1.41×10^{-3}	1.33×10^{-3}
K_B (mol/ml)	1.80×10^{-3}	1.70×10^{-3}	1.62×10^{-3}	1.53×10^{-3}	1.45×10^{-3}
K (—)	1.15	1.22	1.29	1.36	1.43
V_m' (mol/hr·g cell)	1.65	1.81	2.04	2.33	2.73
K_m (mol/ml)	1.47×10^{-1}	1.18×10^{-1}	9.91×10^{-2}	8.52×10^{-2}	7.48×10^{-2}

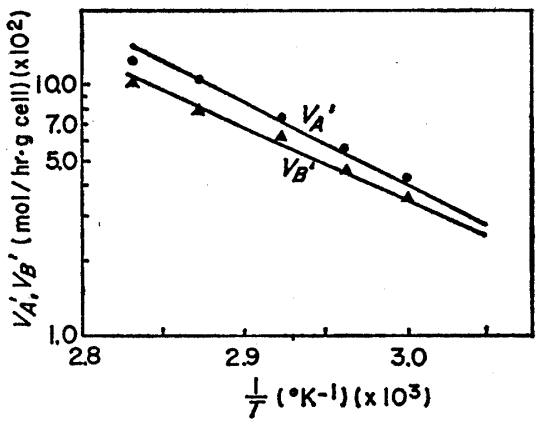

Fig. 7. Temperature dependence of the maximum reaction rates.

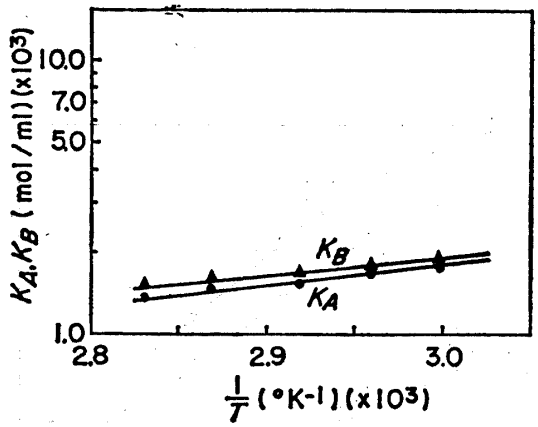

Fig. 8. Temperature dependence of the Michaelis constants.

stants were observed. In the experimental conditions the magnitude of K_m is much greater than C_{ss}, thus, η depends slightly on C_{ss}.

Estimation of effective diffusivity D_A A simple estimation method to get an effective diffusion coefficient for the immobilized enzyme was devised as follows. The effect of film diffusion was eliminated by using a mixing type reactor in the experiment. The value of η_{D0} was calculated from the ratio of the actual reaction rate obtained using the immobilized cells to the ideal rate based on the kinetics of the preheated cells. Then, from the profile presented in Fig. 2, a value of ϕ which corresponds to the experimental value of η_{D0} was obtained. After V_m and K_m, defined by Eqs. (7) and (5), were calculated from the kinetic data, and estimated value of D_A was obtained from the definition of ϕ. The estimation results for the cases of high and low substrate concentrations are shown in Table 2. The effective diffusion coefficient, D_A, measured at low concentrations of glucose, was about 40% of the molecular diffusivity of glucose and about 60% for the high concentration value. The low value of D_A in the case of the high concentration is considered to result from

Table 2. Estimation of effective diffusion coefficient.

Concentration of glucose (M)	R (cm)	η_{D0}	ϕ	D_A (cm²/hr)	Concentration of glucose (M)	R (cm)	η_{D0}	ϕ	D_A (cm²/hr)
5.15	0.018	0.82	0.62	1.47×10^{-3}	0.50	0.018	0.97	0.18	1.72×10^{-2}
5.15	0.046	0.50	1.55	1.54×10^{-3}	0.50	0.046	0.86	0.50	1.50×10^{-2}
			average	1.50×10^{-3}				average	1.62×10^{-2}

increased viscosity. The viscosities of glucose at 0.5 M and 5.15 M were found to be 0.5 cp and 26.1 cp at 70°C, respectively. Consequently, the glucose solution of a high concentration tends to have a low value of η_{D0} because of the high value of ϕ.

Estimation of film mass transfer coefficient, k_L A simple method of estimating k_L is shown in Fig. 9. After η_{Df0} was calculated from the ratio of the actual reaction rate of the immobilized cells in a column reactor to the ideal rate based on the kinetics of the preheated cells, a value of m_f corresponding to η_{Df0} and ϕ was obtained from Fig. 3. The estimated value of k_L was obtained from the definition of m_f as illustrated in Fig. 10. The estimation of k_L for $R=0.018$ cm and $R=0.046$ cm led to values of 0.33 cm/hr and 0.27 cm/hr, respectively. The value of k_L estimated by this method was compared with that obtained by following the McCune type correlation[11] for a packed bed.

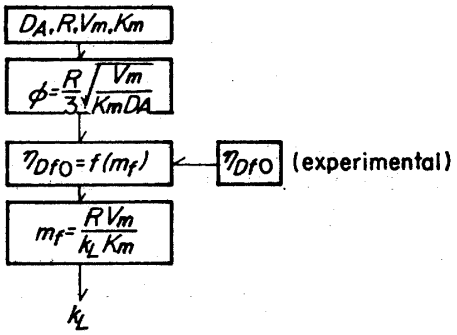

Fig. 9. Estimation of the external mass transfer coefficient.

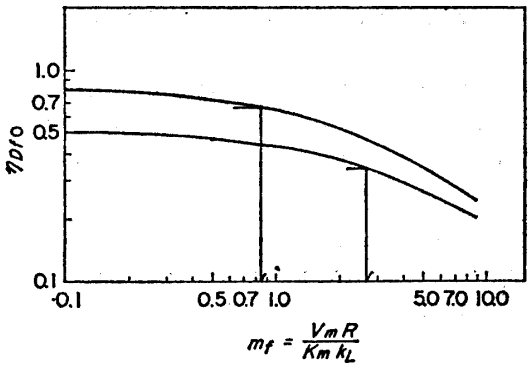

Fig. 10. Estimation of k_L.

	ϕ	R (cm)	η_{Df0}	m_f	k_L (cm/hr)
(A)	0.62	0.018	0.67	0.85	0.33
(B)	1.55	0.046	0.35	2.68	0.27

$$J_D = 1.625\,(N_{Re})^{-0.507} \qquad (16)$$

where

$$J_D = \frac{k_L}{u}\left(\frac{\mu}{\rho D_{Ae}}\right)^{2/3}$$

$$N_{Re} = \frac{\rho u d}{\mu}\ ;\quad N_{Re} < 120 \qquad (17)$$

From Eq. (17), k_L was calculated to be 0.48 cm/hr for a small particle and 0.30 cm/hr for a large particle. These values of k_L obtained from Eq. (17) agree well with the value predicted from the theoretical analysis of the effectiveness factor.

Estimation of deactivation constant, k_f A continuous stirred tank reactor of immobilized cells was employed for the determination of the deactivation constant, since for a long-time experiment the preheated cells were adsorbed to the membrane of the Amicon reactor. The effectiveness factor η_D was calculated from the ratio of the experimental reaction rate to the ideal rate obtained using the kinetics of the preheated cells. Figure 11 shows the effect of particle size on the change of effectiveness factor with process time. It can be observed that the reaction rate of small particles decreases more rapidly than that of large particles. Now, refer back to Fig. 4, where η_D is plotted against $k_f t$ with the

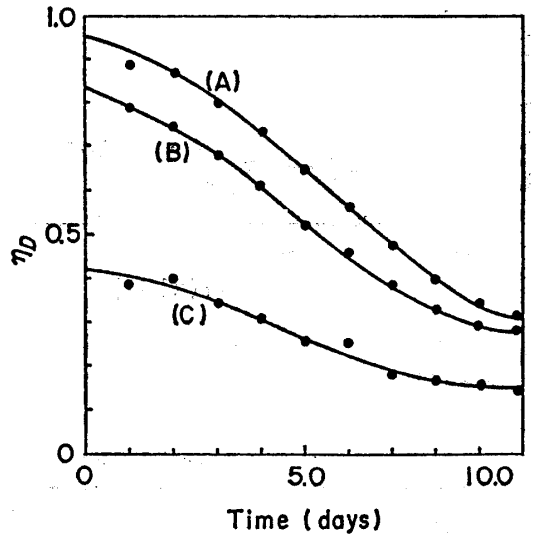

Fig. 11. Effect of particle size on enzyme deactivation. Temperature=70°C, flow rate=27.8 ml/hr, weight of enzyme=0.3 g, glucose concentration=0.5 M.
(A) $R=0.018$ cm, (B) $R=0.046$ cm, (C) $R=0.125$ cm.

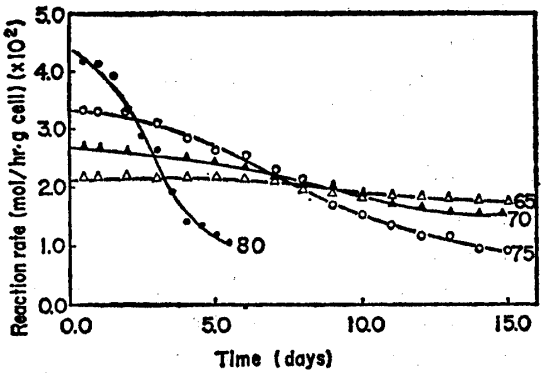

Fig. 12. Effect of temperature on the overall reaction rate of immobilized enzyme subject to deactivation. Weight of enzyme=0.3 g, R=0.046 cm, flow rate =27.8 ml/hr, glucose concentration=0.5 M. Numbers in figure indicates temperature (°C).

radius as a parameter; the deactivation constant, k_f, could be estimated by fitting the curve of η_D corresponding to the experimental reaction rate (Fig. 11) to the theoretical η_D curve for the specified particle radius (Fig. 4) by means of the least square method. Figure 12 shows the effect of temperature on the deactivation of the immobilized enzyme. A high temperature gives a high reaction rate initially, however, it also gives a high deactivation rate of the enzyme. Similarly, the deactivation constant at various temperatures was estimated. The temperature dependence of the deactivation constant is shown in Fig. 13 as an Arrhenius type plot.

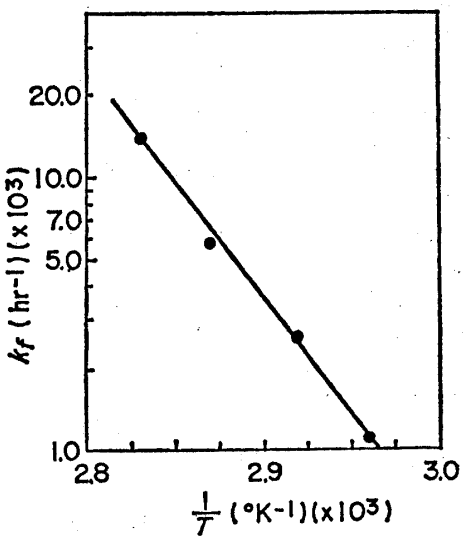

Fig. 13. Arrhenius type plot of the deactivation constant of enzymes.

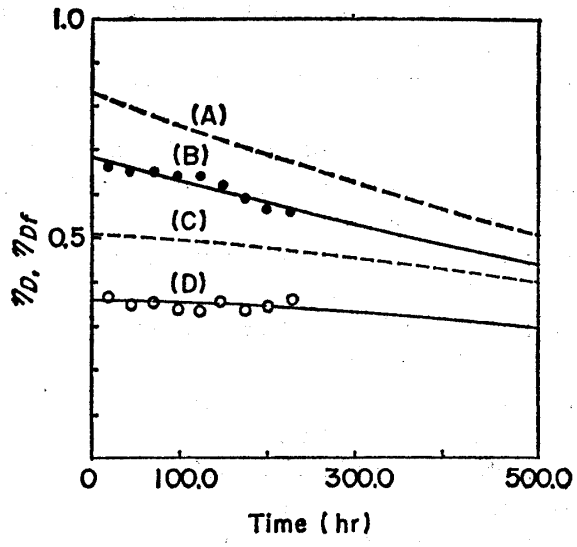

Fig. 14. Time course of η_{Df} in a differential type reactor.
V_m'=2.04 (mol/hr·g cell), K_m=0.099 (mol/ml), enzyme weight=1.28 g, glucose concentration= 5.15 M, flow rate=118.6 ml/hr, temperature= 65°C, R=0.018 cm, R=0.046 cm.

η_D	η_{Df}	ϕ	m_m
(A)	(B)	0.62	0.85
(C)	(D)	1.55	2.68

Simulation

The influence of the pore and film resistances on the overall reaction rate during the operating period was verified by experiments with a high substrate concentration of 5.15 M in a differential type reactor. The variation of η_{Df} calculated from experimental data was in good agreement with the theoretically calculated values, as shown in Fig. 14. η_D, calculated based on the surface concentration, is expressed by the broken line. η_{Df} of the small particles decreases with time more rapidly compared to that of the large particles. A significant difference between η_D and η_{Df} was observed in both cases according to the strong film resistance.

Nomenclature

a : constant defined in Eq. (10), —
C_A : glucose concentration, mol/ml
C_B : fructose concentration, mol/ml
C_{A0} : initial glucose concentration, mol/ml
C_{B0} : initial fructose concentration, mol/ml

C_{Ae} : glucose concentration at equilibrium, mol/ml
C_{Be} : fructose concentration at equilibrium, mol/ml
C_{AS} : glucose concentration in bulk, mol/ml
C_{AR} : glucose concentration at particle surface, mol/ml
C_S : reduced glucose concentration, $C_S=C_A-C_{Ae}$, mol/ml
C_{SS} : reduced bulk concentration, $C_{SS}=C_{AS}-C_{Ae}$, mol/ml
C_{SR} : reduced surface concentration, $C_{SR}=C_{AR}-C_{Ae}$, mol/ml
D_A : effective diffusivity, cm²/hr
D_{Ae} : diffusivity, cm²/hr
d : particle diameter, cm
E_0 : effectiveness factor for zero-order reaction, —
E_1 : effectiveness factor for first-order reaction, —
J_D : $(k_L/u)(\mu/\rho D_{Ae})^{2/3}$, —
K : equilibrium constant, —
K_A : Michaelis constant for forward reaction, mol/ml
K_B : Michaelis constant for backward reaction, mol/ml
K_m : constant defined in Eq. (5), mol/ml
k_f : deactivation constant of enzyme, 1/hr
k_L : mass transfer coefficient in the liquid film, cm/hr
m : constant defined in Eq. (11), —
m_f : film modulus, V_mR/K_mk_L, —
N_{Re} : particle Reynolds number, $\rho ud/\mu$, —
R : particle radius, cm
r'_A : reaction rate based on the weight of preheated cells, mol/hr·g cell
r_A : reaction rate based on the volume of immobilized cells, mol/hr·ml gel
r : radial direction distance, cm
t : time
V_A' : maximum reaction rate for forward reaction based on the weight of preheated cells, mol/hr·g cell
V_B' : maximum reaction rate for backward reaction based on the weight of preheated cells, mol/hr·g cell
V_m' : constant defined in Eq. (5), mol/hr·g cell
V_m : constant defined in Eq. (7), mol/hr·ml gel
u : superficial velocity (flow rate/reactor cross-sectional area), cm /hr

Greek symbols

α_R : constant, K_m/C_{SR}, —
α_S : constant, K_m/C_{SS}, —
β : film factor, defined by Eq. (16), —
γ : constant defined in Eq. (11), —
η_D : effectiveness factor defined by Eq. (10), —
η_{Df} : overall effectiveness factor defined by Eq. (12), —
μ : viscosity, g/cm·hr
ρ : density, g/cm³
ϕ : Thiele modulus, —
ψ : activity coefficient, —

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