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EFFECT OF INTERNAL DIFFUSION ON THE APPARENT STABILITY OF NONUNIFORMLY DISTRIBUTED BIOCATALYSTS

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Abstract—Theoretical analysis was carried out for the stability of enzymes with pseudo first order kinetics immobilized in three different structures. The stabilities with respect to time and temperature were in the order of core-structured, uniform and shell-structured immobilization methods in contrast to the effectiveness factor order of shell-structured, uniform and core-structured. The shell-structured immobilized enzyme was much more efficient in terms of effectivensss factor than the uniform type, but there was not much difference between time stabilities of the two systems. The core-structured system had the lowest effectiveness factor among the three, but it showed improved stability compared with the two especially when initial Thiele modulus was high.

INTRODUCTION

Immobilized enzymes, in general, are superior to chemical catalysts in selectivity and their favorable reaction environments of low temperature and pressure. But because of the short half-life their industrial applications are limited in comparison with chemical catalysts. Immobilized enzymes lose much of their activity in the course of immobilization and furthermore the intrinsic activities are masked by internal and external diffusion limitations. However, the stabilities are improved and it is more economical because of their reuse than soluble enzymes. Thus immobilization is favorably considered for industrial application.

Ollis et al. [1] explained theoretically the temperature and time stability of immobilized enzymes in terms of internal diffusion resistance. Korus and O'Driscoll [2] calculated numerically the half-life of immobilized enzymes in the region where pseudo first order kinetics does not hold. Naik and Karanth [3] showed theoretically that the stability improves with the increase of diffusional resistances. Chang and Joo [4] predicted the effect of internal diffusional resistance on the pH and temperature profiles of immobilized enzymes and compared them with experimental results. Thus the diffusional resistance has an masking effect on the deactivation of immobilized enzymes. The enzyme stabilities appear greater than they should be in the absence of diffusional resistances and/or the apparent deactivation is smaller compared to that of soluble enzymes.

Horvath and Engasser [5] showed that the immobilized enzyme of a pellicular type was of advantage to the effective use of its activity and confirmed it by the experiment [6]. Park et al. [7] theoretically compared the effectiveness of six different types of nonuniform distribution of immobilized enzymes including the cases of substrate inhibition and product inhibition and proposed a proper design criteria. Despite that the stability is as important as the acitivity for its industrial applications, few study has yet been undertaken to connect the apparent activity and the stability of nonuniformly distributed biocatalyt. The purpose of present investigation is to analyze the stabilities of shell-structured and core-structed enzymes and to compare them with that of uniformly distributed enzyme.

THEORETICAL BACKGROUND

Figure 1 shows a schematic representation of uniformly distributed, shell-structed and core-structured immobilized enzymes. Here the amount of enzymes used and the outer diameter of the supports are the same.

Mass Balance Equations

The kinetics of enzymes immobilized in a spherical support are governed by

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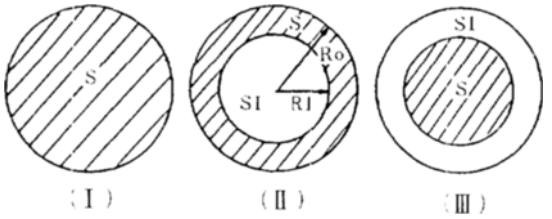

Fig. 1. Design of three types of immobilized enzyme; (Ⅰ) uniformly distributed, (Ⅱ) shell-structured, (Ⅲ) core-structured.

$$\frac{D}{r^2}\frac{d}{dr}\left(r^2\frac{dS}{dr}\right)=v=\frac{v_m S}{K_m+S} \tag{1}$$

In the absence of external diffusion the boundary conditions are

$$\text{at}\quad r=R_o \quad S=S_o \tag{2}$$

$$r=0 \quad \frac{dS}{dr}=0 \tag{3}$$

When S is much smaller than Km, Eq. (1) is reduced to a first-order kinetics. Introducing a number dimensionless variables, we have

$$\frac{1}{x^2}\frac{d}{dx}\left(x^2\frac{dc}{dx}\right)=\phi^2 c \tag{4}$$

$$\text{at}\quad x=1 \quad c=1$$

$$\text{at}\quad x=0 \quad \frac{dc}{dx}=0 \tag{5}$$

where

$$x=\frac{r}{R_o},\quad c=\frac{S}{S_o},\quad \phi=R_o\sqrt{\frac{v_m}{DK_m}} \tag{6}$$

For the shell-structured and core-structured systems it is assumed that the acitive and inert regions are equally divided in volume and the same amount of immobilized enzyme is concentrated in the active region. Then, the governing equation for the shell-structured system becomes.

$$x_i \leqq x \leqq 1$$

$$\frac{1}{x^2}\frac{d}{dx}\left(x^2\frac{dC}{dx}\right)=2\phi^2 C \tag{7}$$

$$0 \leqq x \leqq x_i$$

$$\frac{1}{x^2}\frac{d}{dx}\left(x^2\frac{dC_I}{dx}\right)=0 \tag{8}$$

$$\text{at}\quad x=1 \quad C=1 \tag{9}$$

$$\text{at}\quad x=x_i \quad \frac{dC}{dx}=\frac{dC_I}{dx} \tag{10}$$

$$C=C_I \tag{11}$$

$$\text{at}\quad x=0 \quad \frac{dC_I}{dx}=0 \tag{12}$$

For the core-structured type Euations (7) and (8) are reversed and the same boundary conditions are used. C_I refers to the substrate concentration in the inert region and x_i refers to the boundary between the active and inert regions and has a value of 0.794 in this system.

Effectiveness factors

The effectiveness factors for uniform, shell structured and core-structured types are obtained from equations (4)-(12):

$$\eta_I=\frac{3}{\phi^2}\frac{dC}{dx}\Big|_{x=1}=\frac{3}{\phi}\frac{(\phi\coth\phi-1)}{\phi} \tag{13}$$

$$\eta_{II}=\frac{3}{\phi^2}\left(\frac{dC}{dx}\Big|_{x=1}-0.794^2\frac{dC}{dx}\Big|_{x=0.794}\right)$$

$$=\frac{3}{\phi^2}\{f_1(\phi)(\sqrt{2}\phi-1)e^{\sqrt{2}\phi}$$

$$-f_2(\phi)(\sqrt{2}\phi+1)e^{-\sqrt{2}\phi}\} \tag{14}$$

where

$$f_1(\phi)=\frac{1}{e^{\sqrt{2}\phi}+\dfrac{(1.123\phi-1)}{(1.123\phi-1)}e^{0.831\phi}}$$

$$f_2(\phi)=\frac{1}{e^{-\sqrt{2}\phi}+\dfrac{(1.123\phi+1)}{(1.123\phi-1)}e^{-0.831\phi}}$$

$$\eta_{III}=\frac{3}{\phi^2}0.794^2\frac{dC}{dx}\Big|_{x=0.794}$$

$$=\frac{3}{\phi^2}\{g_1(\phi)(1.123\phi-1)e^{1.123\phi}$$

$$-g_2(\phi)(1.123\phi+1)e^{-1.123\phi}\} \tag{15}$$

where

$$g_1(\phi)=\frac{1}{(0.291\phi+1)e^{1.123\phi}+(0.291\phi-1)e^{-1.123\phi}}$$

$$g_2(\phi)=-g_1(\phi)$$

The validity of the above solutions can be checked by letting Thiele modulus approach zero.

Temperature stability

According to the reversible denaturation model of Ollis [1] the reaction rate of soluble enzyme is given

$$v_s=\frac{v_m}{K_m}S=\frac{\dfrac{A}{K_m}\exp(-E/RT)S}{1+\exp(-\Delta G_d/RT)}$$

$$=\frac{\alpha\exp(-E/RT)}{1+\exp(-\Delta G_d/RT)} \tag{16}$$

where $\alpha=AS/K_m$

Thiele modulus ϕ is given by

$$\phi=\beta\sqrt{\frac{\exp(-E/RT)}{1+\exp(-\Delta G_d/RT)}}$$

where $\beta = R_o\sqrt{\frac{A}{DK_m}}$

The reaction rate of the immobilized enzyme is

$$v_i = \eta v_S = \alpha\,\eta\,\frac{\phi^2}{\beta^2} \tag{18}$$

Since $\eta = \eta(\phi)$, $\phi = \phi(T)$ from eq. (18), the reaction rate becomes a function of temperature. Letting the reaction rates of uniform, shell-structured and core-structured types be V_I, V_{II} and V_{III}, respectively and the Thiele modulus at the maximum reaction rate be ϕ_m, the relative reaction rates are

$$\frac{v_I}{v_{I,\max}} = \frac{\eta_I}{\eta_{I,\max}}\left(\frac{\phi}{\phi_m}\right)^2 \tag{19}$$

$$\frac{v_{II}}{v_{II,\max}} = \frac{\eta_{II}}{\eta_{II,\max}}\left(\frac{\phi}{\phi_m}\right)^2 \tag{20}$$

$$\frac{v_{III}}{v_{III,\max}} = \frac{\eta_{III}}{\eta_{III,\max}}\left(\frac{\phi}{\phi_m}\right)^2 \tag{21}$$

where η_I, η_{II} and η_{III} are obtained from equations (13), (14) and (15), respectively.

Time stability

Assuming that the enzyme activity decays exponentially (8), the reaction rate of the soluble enzyme is given as

$$v_S = \frac{v_m}{K_m}S = \frac{v_{m,t=0}}{K_m}S\exp\left(-\frac{t}{\tau}\right) \tag{22}$$

Then the Thiele modulus ϕ is given as a function of time (1)

$$\phi = \phi_o\,\exp\left(-\frac{t}{2\tau}\right) \tag{23}$$

where ϕ_o represents Thiele modulus at time t = 0. Thus the three types of immobilized enzymes have the following relative reaction rates as functions of Thiele moduli

$$\frac{v_I}{v_{I(t=0)}} = \frac{\eta_I}{\eta_{I(t=0)}}\left(\frac{\phi}{\phi_o}\right)^2 \tag{24}$$

$$\frac{v_{II}}{v_{II(t=0)}} = \frac{\eta_{II}}{\eta_{II(t=0)}}\left(\frac{\phi}{\phi_o}\right)^2 \tag{25}$$

$$\frac{v_{III}}{v_{III(t=0)}} = \frac{\eta_{III}}{\eta_{III(t=0)}}\left(\frac{\phi}{\phi_o}\right)^2 \tag{26}$$

Letting the value of Thiele modulus be $\phi_{1/2}$ when the values of the left hand sides in the equations (24)-(26) become 1/2, the relative half life, the ratio of activity of immobilized enzyme to that of soluble enzyme is given as

$$\frac{t_{1/2}(\text{imm.})}{t_{1/2}(\text{sol.})} = 2.886\,\ln\frac{\phi_o}{\phi_{1/2}} \tag{27}$$

RESULTS AND DISCUSSION

Fig. 2 shows the effectiveness factors of the three im-

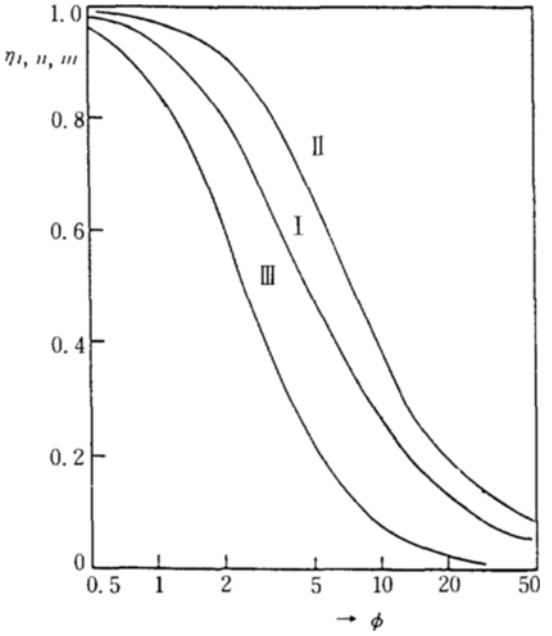

Fig. 2. Effectiveness factor vs. Thiele modulus of three types of immobilized enzyme; (I) uniformly distributed, (II) shell-structured, (III) core-structured.

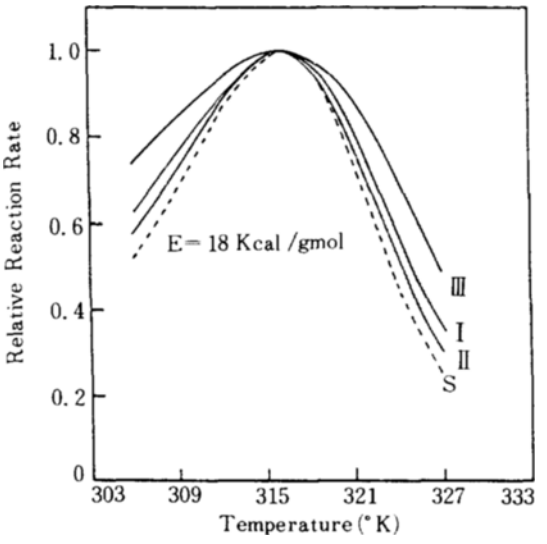

Fig. 3. Relative reaction rate vs. temperature of soluble and three types of immobilized enzyme; (···, S) soluble enzyme; (--) immobilized enzyme, (I) uniformly-distributed, (II) shell-structured, (III) core-structured.

mobilized enzyme structures as a function of Thiele modulus. In terms of the effectiveness factor the shell-structured system is the most efficient as expected and the core-structured type is the least efficient due to its diffusional resistance. The temperature stability is shown in Fig. 3. All the data used in this literature are given by the following values and those are from the literature (1)

$Vm = 1.1 \times 10^{-4}$ moles/liter/min
$Km = 4.5 \times 10^{-3}$ moles/liter
$D = 10^{-5}$ cm^2/sec
$L = 0.1$ cm
$\Delta Gd = 68000 - 213T$

The stabilities of the three types were superior to that of soluble enzyme and especially the core-structured type has the broadest curve. The optimum temperatures of the three types were the same as the soluble one, which was in agreement with the work of Naik and Karanth [3] who showed that the optimum temperature of an immobilized enzyme did not change in the existence of diffusional resistance. Thus the shift of optimum temperature observed in many immobilized enzyme systems was not due to the diffusional resistances, but due to the other intrinsic factors [1].

Fig. 4 shows the activity decay with time when the initial Thiele moduli were 2 and 10. The activity change in the soluble enzyme occurred very sharply, and the stabilities of the immobilized systems were in the order of core-structured, uniform and shell-structured. But for

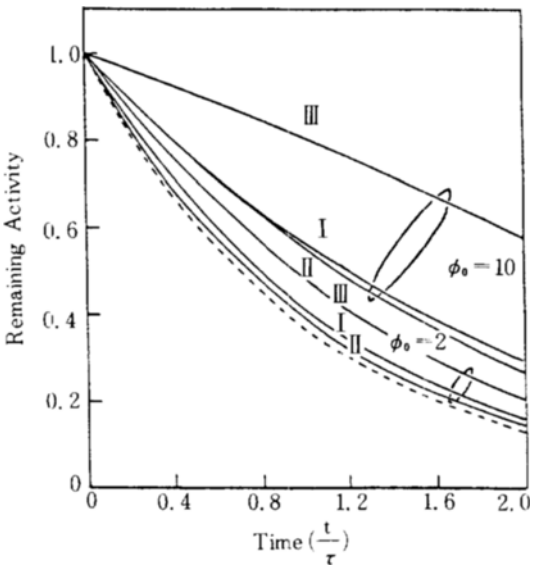

Fig. 4. Activity decay curve with respect to time; (···) soluble enzyme; (—) immobilized enzyme, (Ⅰ) uniformly distributed, (Ⅱ) shell-structured, (Ⅲ) core-structured.

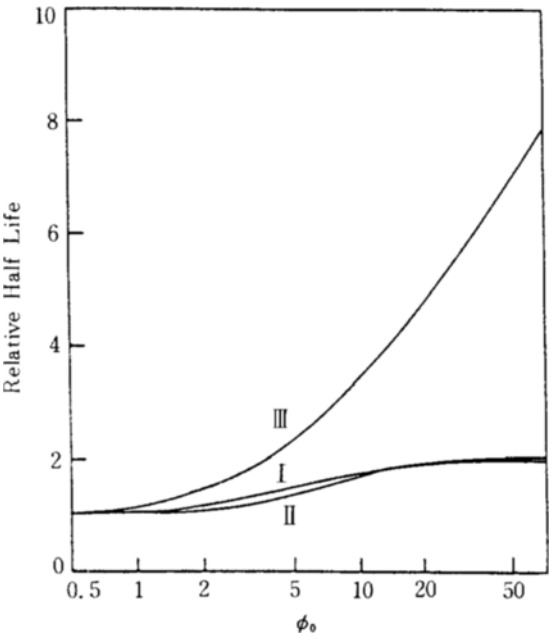

Fig. 5. Relative half life vs. initial Thiele modulus of three types of immobilized enzyme; (Ⅰ) uniformly distributed, (Ⅱ) shell-structured, (Ⅲ) core-structured.

the relative half-life the shell-structured type was slightly better than the uniform type when ϕ_0 was greater than 15 (Fig. 5). While the shell-structured type was superior to the uniform type in effectiveness factor, the time stability was very much similar. The core-structured type was the least efficient in the effectiveness, but the stability was best and became better with the increase of the Thiele modulus.

The theoretical analyses in this study were limited to shell-structured and core-structured types, but each immobilized enzyme system should have its optimum design depending on its kinetics and stability. Shell-structured type was made by Horvath [6], but the core-structured type was never realized. But this type of enzyme could be prepared by the coating technique which surrounds the surface of the immobilized enzyme with the porous inert material such as polymers, agarose gels etc. Thus core-structured type is worth trying since this configuration has better stability characteristics than the other two.

CONCLUSIONS

The design criteria of immobilized enzyme systems should consider the stability as well as effectiveness factors. The shell-structured type of immobilized enzymes

is of advantage to the efficient use of enzymes, but the stability consideration selects the core-structured type which is the least efficient in the effectiveness.

NOMENCLATURE

A : Frequency factor
C : Dimensionless substrate concentration S/S_o
C_I : Dimensionless substrate concentration in the inert region S_I/S_o
D : Diffusion coefficient
E : Activation energy
ΔGd : Change in free energy
K_m : Michaelis constant of reaction
r : Radial distant
R : Universal gas constant
R_o, R_I : Radius of catalyst particle and inert core, respectively
S : Substrate concentration
S_o, S_I : Substrate concentration in the bulk and inert region, respectively
t : time
$t_{1/2}$: Half-life
T : Temperature
v : Reaction rate
v_m : Maximum reaction rate
v_s : Reaction rate of soluble enzyme
v_I, v_{II}, v_{III} : Reaction rate of uniformly, shell structured and core structured biocatalyst, respectively
x : Dimensionless radial distant
x_I : Dimensionless radial distant of inert core
ϕ : Thiele modulus $R_o\sqrt{\frac{v_m}{DK_m}}$
$\phi_o, \phi_{1/2}, \phi_m$: Thiele modulus at $t = 0$, $t = t_{1/2}$, and $T = T_m$, respectively
η : Effectiveness factor
$\eta_I, \eta_{II}, \eta_{III}$: Effectiveness factor of uniformly, shell structured and core structured biocatalyst, respectively
α : AS/K_m
β : $R_o\sqrt{\frac{A}{DK_m}}$
τ : Time constant of decay

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