OXIDATION OF GLUCOSE BY GLUCOSE OXIDASE ENTRAPPED IN HOLLOW FIBER MEMBRANE

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Introduction

Several works have been done to utilize glucose oxidase and catalase immobilized by adsorption or covalent linkage to various type of supports (Herring *et al.*, 1972, Sada *et al.*, 1983, Schachinger *et al.*, 1985, Tsukamoto *et al.*, 1982, Weibel *et al.*, 1973, Wen-Hsiung *et al.*, 1980). The kinetic properties of enzymes immobilized by these method differ from those of free enzymes.

On the other hand, immobilization by entrapment of free enzymes by a semipermiable membrane offers an advantage, as the kinetic properties of the enzyme remain unchanged. Moreover, this method can be easily applied to various types of enzymatic reactions.

Hashimoto *et al.* (1983) have investigated a membrane reactor for glucose oxidation using glucose oxidase and catalase entrapped in a hollow fiber membrane. They have operated the membrane reactor only in the region where mass transfer of oxygen controls the over-all reaction rate.

We have presented a simple method of analysis applicable for a membrane reactor where the overall reaction rate depends on both the intrinsic rate of enzymatic reaction and the mass transfer rate of substrates. Experimental results on the overall glucose oxidation rate using glucose oxidase and excess catalase entrapped in the fiber bore were compared with the prediction by the present method.

1. Experimental Apparatus and Procedure

1.1 Intrinsic kinetic constants for enzymatic oxidation of glucose

The initial rate of enzymatic oxidation of glucose was determined by measuring the decreasing rate of dissolved oxygen concentration in a buffered solution (pH = 5.5) containing glucose (Yoneyama Yakuhin Kogyou), glucose oxidase (Merck 24586) and catalase (Merck 5186). These enzymes were used without further purification. The concentration of dissolved oxygen was measured by an oxygen probe (OxyGuard Handy MKII).

Experimental conditions for the measurement of the initial rate of glucose oxidation are summarized in **Table 1.**

1.2 Oxidation of glucose by glucose oxidase and catalase entrapped in the bore of hollow fiber module

A schematic diagram of the experimental apparatus is shown in **Fig.1.** For the hollow fiber membrane reactor, a commercial hollow fiber module for ultra-filtration (Molecular weight cut-off 30000, FB-02 FUS-0382 supplied by Daicel Chemical Industry) was used. This module consists of 380 hollow fibers with length of 270 mm, inner diameter of 0.8 mm, and outer diameter of 1.3 mm, and the bundle of fibers is sealed in an outer case with diameter of 40 mm and length of 325 mm.

After the buffered solution of glucose oxidase and catalase was circulated through the reservoir tank of enzyme solution and the bore of the hollow fibers, the valves at the inlet and outlet of the stream of the fiber bore were closed to entrap buffered solution of glucose oxidase and catalase in the bore of the hollow fiber membranes,whose volume, V_F^i,is $7.80\times10^{-5}m^3$. Buffered solution of glucose was circulated through the reservoir tank of glucose solution and the shell-side of the hollow fiber module. N_2-O_2 mixture gas was fed into the stream of glucose solution and ascended in the shell-side of the hollow fiber module as a bubble swarm. When 10 minutes elapsed after circulation of glucose solution was started, 1 cm^3 of glucose solution was sampled from the reservoir of glucose solution at time interval of 30 minutes. The concentration of glucose in the sampled solution was measured by HPLC (Shodex KS-801) after enzymes were separated by centrifugal filtration. Concentration of glucose oxidase in the bore of the hollow fiber was determined by the initial rate of glucose oxidation measured by the above mentioned method.

Experimental conditions are summarized in **Table 2.**

2. Experimental Results and Discussion

2.1 Intrinsic kinetic constants for enzymatic oxidation of glucose

In the presence of excess catalase, the rate equation of enzymatic oxidation of glucose is the well known equation expressed by Eq.(1) (Linek *et al.*, 1980).

Table 1 Experimental condition for determination of intrinsic kinetic constants for enzymatic oxidation of glucose

Initial concentration of glucose, C_{G0} [mol·dm^{-3}]	0.05 — 0.1
Initial concentration of dissolved oxygen, C_{O0} [mol·dm^{-3}]	3.73×10^{-4} — 1.29×10^{-3}
Concentration of glucose oxidase, C_E [mg·dm^{-3}] (8Units/mg stated activity)	8.87 — 323
Concentration of catalase	7.10×10^{4} — 2.58×10^{6} U·dm^{-3} (stated activity)
Reaction temperature	25 ± 0.5°C
pH	5.5

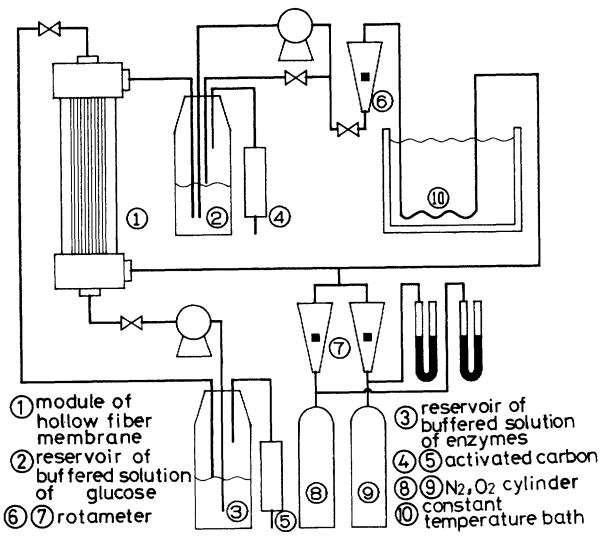

Fig. 1 Schematic diagram of experimental apparatus for hollow fiber membrane reactor

$$r = -dC_G/dt = -2dC_O/dt = k_3C_E/(1+k_1/C_O+k_2/C_G) \quad (1)$$

From the regression line of the reciprocal of the initial oxidation rate, $1/r_0$ against the reciprocal of the initial concentration of glucose, $1/C_{G0}$, k_2/k_3C_E and $(1+k_1/C_{O0})/(k_3C_E)$ were obtained for constant initial concentration of dissolved oxygen and constant concentration of enzymes. From the regression line of $1/r_0$ against $1/C_{O0}$, $k_1/(k_3C_E)$ was obtained for the constant initial concentration of glucose and the constant concentration of enzymes. The reaction rate constants were determined as $k_1 = 3.04\times10^{-4}$mol·dm^{-3}, $k_2 = 2.61\times10^{-2}$ mol·dm^{-3}, and $k_3 = 4.25\times10^{-7}$mol·mg^{-1}·s^{-1}. The obtained value of k_3 is less than the value presented in the previous works using purified enzyme (Linek *et al.*,1980) or high activity enzyme (Fukushima *et al.*,1978).

2.2 Oxidation of glucose by glucose oxidase and catalase entrapped in the bore of hollow fiber module

a) Method of calculation for overall rate of glucose oxidation The concentration of glucose in the reservoir of the glucose solution was assumed to be equal to that in the shell-side of the hollow fiber module, as the enzymatic oxidation rate of glucose was much lower than the circulation rate of glucose solution. The resistance for mass transfer of oxygen and glucose was assumed to be restricted to the mass transfer for membrane permeation.

Based on the above assumptions, mass balance equations for glucose and dissolved oxygen were written as follows.

$$-(V_F^O+V_T)dC_G^O/dt = V_F^i k_3 C_E/(1+k_1C_O^i+k_2C_G^i) \quad (2)$$

$$-(V_F^O+V_T)dC_G^O/dt = k_{LG}A(C_G^O-C_G^i) \quad (3)$$

$$-(1/2)(V_F^O+V_T)dC_G^O/dt = k_{LO}A(C_O^O-C_O^i) \quad (4)$$

$$t=0;\ C_G^O = C_{G0}^O \quad (5)$$

Eqs.(3) and (4) were substituted into Eq.(2) to eliminate the concentration of glucose and dissolved oxygen in the bore of the hollow fiber module, C_G^i,C_O^i. By the foward explicit difference method, glucose concentration in the shell-side of the hollow fiber module was obtained succesively with time difference varied from 10 to 60s.

b) Determination of the membrane permeation coefficient for dissolved oxygen When the concentration of glucose, C_G^O is much higher than that of the dissolved oxygen, C_O^O in the shell-side of the hollow fiber module, dissolved

Table 2 Experimental condition for enzymatic oxidation of glucose in the hollow fiber membrane reactor

Hollow fiber membrane module	: Module manufactured by Daicel Chemical Industry Co. Ltd. was used (FB-02 FUS-0382)
Outer diameter of hollow fiber	: 1.30 mm
Inner diameter of hollow fiber	: 0.80 mm
Number of hollow fibers	: 380
Length of hollow fiber	: 270 mm
Molecular weight cut-off	: 30000
Reaction temperature	25 ± 0.5°C
pH	5.5
Initial concentration of glucose in outside of hollow fiber, $C_G^o{}_0$ [mol·dm^{-3}]	1.05×10^{-3} — 0.182
Concentration of dissolved oxygen in outside of hollow fiber, Coo [mol·dm^{-3}]	3.19×10^{-4} — 1.28×10^{-3}
Concentration of glucose oxidase in insede of hollow fiber, CE [mg·dm^{-3}]	79 — 3000
Rate of circulation of glucose solution	50 — 150 cm^3·min^{-1}

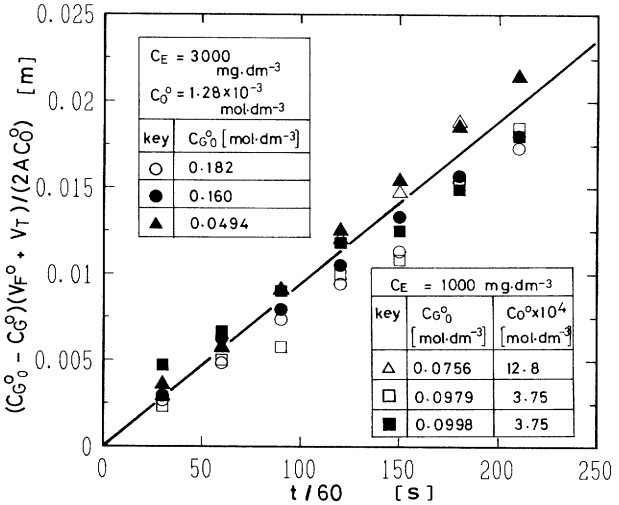

Fig. 2 Determination of membrane permeation coefficient for oxygen

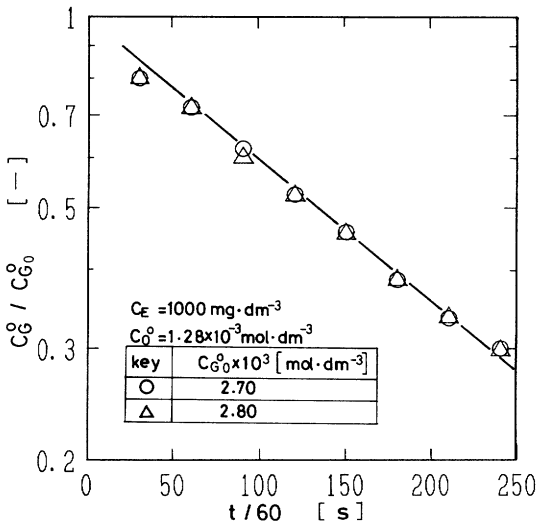

Fig. 3 Determination of membrane permeation coefficient for glucose

oxygen permeated through the membrane would be consumed instantaneously and the concentration of dissolvedoxygen in the bore of hollow fiber, $C_O{}^i$ would approach zero.

In this case, Eq.(4) reduces to Eq.(6).

$$\left(V_F^O + V_T\right)\left(C_{G0}^O - C_G^O\right) / \left(2AC_O^O\right) = k_{LO}t \qquad (6)$$

The left-hand side term of Eq. (6) was plotted against reaction time,t,under the condition where the initial glucose concentration, $C_G{}^O{}_0$ was about 40 times greater than the dissolved oxygen concentration. As shown in **Fig. 2**, a linear relationship was observed between the left-hand side term of Eq. (6) and reaction time, t. Using the values for the volume of the shell-side of the hollow fibers module, $V_F{}^O$, 2.59×10^{-4}m^3, the volume of the reservoir for glucose solution, V_T, 5.00×10^{-4}m^3, the area of the inner surface of hollow fibers, A, 0.258 m^2 and the slope of the regression line in Fig.2, the membrane permeation coefficient for dissolved oxygen was obtained as 1.57×10^{-6} m/s with a

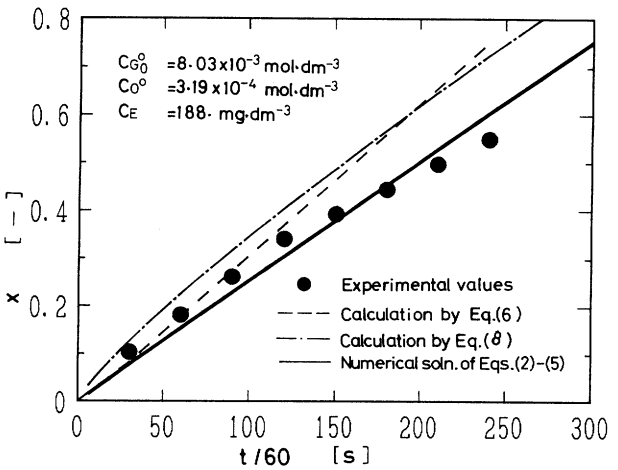

Fig. 4 Comparison of the calculation by the present method with exerimental result on over-all rate of glucose oxidation in hollow fiber membrane reactor

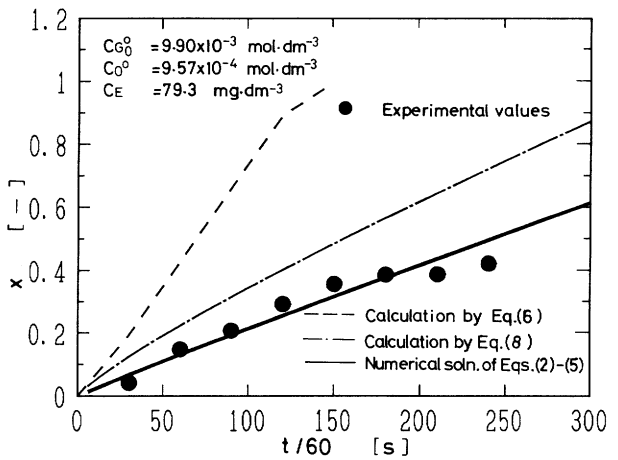

Fig. 5 Comparison of the calculation by the present method with exerimental result on over-all rate of glucose oxidation in hollow fiber membrane reactor

relative error less than 25%.

c) Membrane permeation coefficient for glucose When the concentration of glucose in the shell-side of the hollow fiber module, $C_G{}^O$ is equal to, or smaller than that of the dissolved oxygen, $C_O{}^O$, Eq. (3) could be approximated to Eq. (7) as the permeation coefficient for dissolved oxygen could be expected to be much larger than that for glucose.

$$-\left(V_F^O + V_T\right)dC_G^O / dt = k_{LG}AC_G^O \qquad (7)$$

Integration of Eq.(7) with initial condition, Eq.(5) reduces to Eq.(8).

$$-\ln\left(C_G^O / C_{G0}^O\right) = \left\{k_{LG}A / \left(V_F^O + V_T\right)\right\}t \qquad (8)$$

The values of the left hand-side term of Eq.(8), $ln(C_G{}^O / C_G{}^O{}_0)$ were plotted against reaction time, t. The membrane permeation coefficient for glucose, k_{LG} was obtained as 2.50×10^{-7}m/s, using the slope of the regression line in **Fig.3**.

d) Comparison of experimental values with calculation for enzymatic oxidation rate of glucose in hollow fiber membrane reactor

The overall rate of glucose oxidation obtained by numerical solution of Eqs.(2),(3) and (4) was compared with experimental result in **Figs.4** and **5**.As the concentration of enzymes for the data in these figures was much lower than that for the data in Figs. 2 and 3, the overall oxidation rate obtained numerically by Eqs.(2),(3) and (4) is less than the overall oxidation rate obtained by Eq.(6) or Eq.(8), which corresponds to the limiting cases where the concentration of dissolved oxygen or glucose in the bore of hollow fiber module becomes nearly zero. As shown in Figs.4 and 5, experimental results on the overall glucose oxidation rate for relatively low concentration of enzymes agreed well with the numerical solution of Eqs.(2), (3) and (4).

Conclusion

The overall oxidation rate of glucose in the hollow fiber membrane reactor, where enzymes were entrapped in the bore of hollow fiber modules, was calculated by the present method taking into account the mass transfer of glucose and dissolved oxygen through the membrane and the intrinsic reaction rate of enzymatic oxidation in the bore of hollow fiber module. The calculated values for the overall oxidation rate agreed well with the experimental result.

Nomenclature

A	=	total area of inside surface of hollow fibers	[m^2]
C_G	=	concentration of glucose	[$mol \cdot dm^{-3}$]
C_G^i	=	concentration of glucose in the bore of hollow fiber module	[$mol \cdot dm^{-3}$]
C_G^O	=	concentration of glucose in the shell-side of hollow fiber module	[$mol \cdot dm^{-3}$]
$C_G^O{}_0$	=	initial concentration of glucose in the shell-side of hollow fiber module	[$mol \cdot dm^{-3}$]
C_O	=	concentration of dissolved oxygen	[$mol \cdot dm^{-3}$]
C_O^i	=	concentration of dissolved oxygen in the bore of hollow fiber module	[$mol \cdot dm^{-3}$]
C_O^O	=	concentration of dissolved oxygen in the shell-side of hollow fiber module	[$mol \cdot dm^{-3}$]
C_E	=	concentration of dissolved glucose oxidase	[$mg \cdot dm^{-3}$]
k_1	=	kinetic constant in Eq.(1)	[$mol \cdot dm^{-3}$]
k_2	=	kinetic constant in Eq.(1)	[$mol \cdot dm^{-3}$]
k_3	=	kinetic constant in Eq.(1)	[$mol \cdot mg^{-1.1}$]
k_{LO}	=	membrane permeation coefficient for oxygen	[$m \cdot s^{-1}$]
k_{LG}	=	membrane permeation coefficient for glucose	[$m \cdot s^{-1}$]
r	=	intrinsic reaction rate of glucose oxidation expressed as decreasing rate of glucose concentration	[$mol \cdot dm^{-3} \cdot s^{-1}$]
r_0	=	initial rate of glucose oxidation	[$mol \cdot dm^{-3} \cdot s^{-1}$]
t	=	reactin time	[s]
V_T	=	volume of reservoir for glucose solution	[m^3]
V_F^O	=	volume of the shell-side of hollow fiber module	[m^3]
V_F^i	=	volume of the bore of hollow fiber module	[m^3]
x	=	conversion of glucose defined as $(C_G^O{}_0 - C_G^O)/C_G^O{}_0$	[-]

Literature cited

1) Fukushima,S., A.Uyama and S.Katayama : "Oxygen Absorption Accompanying Enzymic Reaction-Oxidation of d-Glucose in the Presence of Glucose Oxidase and Catalase," *J.Chem.Eng.Japan*, **11**, 227-233 (1978)
2) Hashimoto,M., T.Tsukamoto, S.Morita and J.Okada : "Application of a Membrane Reactor to Gas-Liquid Two-Phase Enzyme Reactions: Oxidation of Glucose by Soluble Immobilized Glucose Oxidase and Catalase," *Chem. Pharm. Bull.*, **31**, 1-11 (1983)
3) Herring, W.M., R.L.Laurence and J.R.Kittrell: "Immobilization of Glucose Oxidase on Nickel-Silica Alumina," *Biotechnology and Bioengineering*, **14**, 975-984 (1972)
4) Linek,V., P.Beneš, J.Sinkule, O.Heleček and V.Malý : "Oxidation of d-Glucose in the Presence of Glucose Oxidase and Catalase," *Biotechnology and Bioengineering*, **22**, 2515-2527 (1980)
5) Sada,E., S.Katoh, M.Shinozawa and I.Matsui : "Rate of Glucose Oxidation with a Column Reactor Utilizing a Magnetic Field," *Biotechnology and Bioengineering*, **25**, 2285-2292 (1983)
6) Schachinger,L., E.Altman, B.Diepold and H.Kl ter :"Determination of Thermodynamic Data by Microcalorimetry:The Michaellis Constant of Glucose Oxidase Immobilized on Various Carriers," *Thermochimica* Acta, **94**, 169-177 (1985)
7) Tsukamoto,T., S.Morita and J.Okada :"Oxidation of Glucose on Immobilized Glucose Oxidase," *Chem. Pharm. Bull.*, **30**, 782-789 (1982)
8) Weibel, M.K., W.Dritschilo, H.J.Bright and A.E.Humphrey : "Immobilized Enzymes:A Prototype Apparatus for Oxidase Enzymes in Chemical Analysis Utilizing Covalently Bound Glucose Oxidase," *Analytical Biochemistry*, **52**, 402-414 (1973)
9) Wen-Hsiung Liu, Rong-Fong Shen, Fong-Fei Lee and Yuan-Chi Su : "Enzymatic Oxidation of Glucose via Crab Chitin Immobilized Glucose Oxidase and Catalase," *Proc.Natl.Sci.Counc.ROC*, **4**, 338-345 (1980)