KOREAN J. FOOD SCI. TECHNOL.
Vol. 17, No. 1 (1985)

A Study on Coimmobilized Glucose Oxidase-Catalase System

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Glucose Oxidase-Catalase동시 고정화 효소계의 반응

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Abstract

The reactor performance of a coimmobilized glucose oxidase and catalase enzyme system was investigated. In the determination of efficiencies of glucose oxidase and catalase of dual, mixed and soluble systems, the dual type immobilized one was superior to either the soluble or to the mixed system. In the continuous plugflow bed reactor system of glucose oxidase and catalase, k_d, deactivation rare constant of glucose oxidase only and catalase/glucose oxidase = 10 were 1.12×10^{-2} and 2.17×10^{-2} hr^{-1}, respectively. In the effect of τ, space time, the point of O_2 limitation is 5.5 g·hr/*l* in both catalase/glucose oxidase = 1 and 10. In the effect of O_2 concentration to reduce the O_2 diffusion limitation, it appeared that τ = 8.3 g·r/*l* is the maximum point of O_2 concentration in both catalase/glucose oxidase = 1 and 10.

Introduction

Glucose is oxidized to gluconolactone and hydrogen peroxide by glucose oxidase (GOD, E.C. 1.1.3.4.) and catalase (CAT, E.C. 1.11.1.6.) catalyzes the decomposition of hydrogen peroxide to molecular oxygen and water. Previously[1], we coimmobilized GOD and CAT on microbial cells and characterized their kinetics.

Using this enzyme system in this study, we determined how ratio of two enzymes affect efficiencies of the catalytic particles. Also dual enzyme system was compared to a mixed immobilized system. Reactor perfomance was studied by consecuting the O_2 conversion of the two immobilized systems with varying activity ratio of two enzymes and O_2 concentration.

Materials and Methods

Immobilization of glucose oxidase and catalase

Three kinds of the immobilized enzymes were prepared as described in the previous work,[1] where a sole type indicates the immobilization of single enzyme of GOD or CAT, a dual type represents the immobilization of both GOD and CAT on the same cell matrix, and a mixed type illustrates a mixture of the two kinds of sole type immobilized enzymes, GOD and CAT.

Enzyme activity assay

The activities of both soluble and immobilized GOD and CAT were assayed by determining O_2 consumption or production in the GOD or CAT assay system by a Clark-type electrode (Gilson Medical Electronics, K-ICT-C Oxygraph) at 25°C as described previously.[1]

Relative efficiencies of glucose oxidase and catalase

For the mixed and dual immobilized enzymes, typically the immobilized enzymes containing three different activity ratio of CAT/GOD were used and compared the relative efficiencies. They were calculated by the method of Bouin *et al.*[2] and Lee *et al.*[1] as follows:

$$\% \text{ catalase efficiency} = \frac{K_{GOD} - (K_{GOD\text{-}overall})}{0.5\ K_{GOD}} \times 100$$

where K_{GOD} is the pseudo first-order rate constant of GOD reaction, $K_{GOD\text{-}overall}$ is that of the GOD initiated

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overall reaction and denominator equals to the rate of the GOD reaction in the presence of excess CAT, and

$$\% \text{ glucose oxidase efficiency} = \frac{K_{CAT} - (K_{CAT\text{-}overall})}{K_{CAT}} \times 100$$

where K_{CAT} is the first-order rate constant of the CAT reaction and $K_{CAT\text{-}overall}$ is that of the overall reaction. One advantage of studying a cyclic enzyme system, is that either enzyme can be used as the enzyme to catalyze the first reaction in the sequence depending on the substrate used. In either case, the first enzyme produces a product, P_1, which is then acted on by the second enzyme to produce the final product of the reaction, P_2. The efficiency of the system is defined as how well the second enzyme in the overall reaction sequence utilizes the product of the first reaction compared to utilization by an excess amount of soluble second enzyme. Hence three series of immobilized enzymes were prepared as follows: firstly, the activity ratio of CAT/GOD was kept constant where their absolute activities varied, secondly, GOD activity was kept constant while CAT activity varied, and finally, CAT activity was kept constant and GOD activity varied.

Reactor performance of immobilized enzymes

Effects of flow rate on GOD and CAT were examined with varying CAT/GOD ratio in a plug-flow reactor system. Releative O_2 concentration were determined by changing flow rate from 5 to 80 *ml/min* in a column (1.5 × 5 *cm*). The column was packed with 1 *g* of immobilized enzyme with 0.5 *M* aerated glucose solution. Effects of O_2 concentrations on the two immobilized systems were determined in the continuous reaction system. Different levels of O_2 concentration were obtained in the feed by cintinuous sparging feed tank, controlling air flow with air flow meter at 0.8 VVM. Mixtures of O_2 from cylinder and compressed air was used as the sources of air that generatres the different O_2 levels. Saturation points of various O_2 concentration were determined with different ratio of O_2 and compressed air after equilibrium reached.

Results and Discussion

Relative efficiencies of glucose oxidase and catalase

As shown in Table 1, in an increase of the activity ratio of CAT/GOD, efficiency of CAT increased and led to lowering the efficiency of GOD. When the GOD activity was constant and the CAT activity varied (Table 1, sample 2 and 3), the GOD efficiency decreased as the CAT activity increased. The most likely reason for this is that GOD can be utilized fully with the small CAT activity. It was true for the case of *vice versa* (Table 1, sample 1 and 2). In the meanwhile, when the ratio of both enzymes remained constant but their absolute enzyme activities were different, their efficiencies appeared almost the same. As the activity ratio of CAT/GOD, however, is 15 or more, the GOD efficiency was almost negligible and resulted in almost 100% CAT efficiency. Small differences of % efficiency according to the ratio of CAT/GOD were observed in the reported value[2] and our data; as example when the ratio was 0.4, the reported ones of GOD and CAT were 81 and 24%, respectively, while our data showed 100 and 57.4%, respectively. This seemed due to differences in particle size of the matrices used in each experiment. Bouin *et al.*[2] already observed that smaller particle size yielded better catalase efficiency.

In addition, the comparison of the dual, mixed and soluble enzyme systems of GOD and CAT suggested that the dual immobilized enzyme system was superior to either the soluble or to the mixed system. The result was shown in Table 2. In all three systems, the catalase efficiency increased as the ratio of CAT/GOD increased. The absolute values, however, were not the same. GOD efficiencies in the CAT-initiated overall reaction were af-

Table 1. Efficiencies of the dual type immobilized system

Sample Number	Enzyme activity (U/*g* matrix)		Activity ratio	Efficiency (%)	
	CAT	GOD	(CAT/GOD)	CAT	GOD
1	0.4	0.8	0.5	57.4	100.0
2	2.0	2.5	0.8	63.2	94.0
3	14.3	2.2	6.5	74.6	28.9
4	17.7	1.7	10.4	91.8	10.8
5	17.2	1.2	15.0	100.0	5.6

Table 2. The comparison of efficiencies of the dual, mixed and soluble systems of glucose oxidase and catalase

Activity ratio (CAT/GOD)	% Catalase efficiency			% Glucose oxidase efficiency		
	Dual	Mixed	Soluble	Dual	Mixed	Soluble
0.8	63.2	3.2	25.8	94.0	12.3	85.5
6.5	74.6	23.3	66.7	28.9	8.0	11.4
15.0	100.0	80.1	81.5	5.6	0	6.9

fected in an analogous manner by a change in ratio of enzymatic activities in the three systems.

Deactivation of coimmobilized enzymes

As it has been proved by Krishnaswamy *et al.*[3], we found that the primary glucose conversion and the deactivation reactions were first order. According to this model, the system can be described as

$$\ln [1/(1-X)] = \ln (K\tau) - k_d t \quad \text{.....Eq.(1)}$$

where τ, space time, is defined on the weight basis of the catalyst, K the apparent first-order reaction rate constant (l/g.hr), k_d the first-order deactivation rate constant (hr^{-1}), t the clock time (hr), and X the fractional conversion. From this model, comparative deactivation characteristics of the sole and dual type immobilized enzymes were studied. Fig. 1 shows how the conversion dropped with time as the sole type and dual type immobilized enzymes were deactivated during the time span of the experiment and Table 3 shows the comparison of deactivation characteristics of the dual and mixed type immobilized enzymes, respectively. These experimental data were well fitted to the deactivation model presented in Eq. (1). From Eq. (1), k_d (hr^{-1}), deactivation rate constants of the dual type and the sole type immobilized enzymes were 2.17×10^{-3} hr^{-1} and 1.12×10^{-2} hr^{-1}, respectively. This difference of k_d indicates that the deactivation of GOD mainly due to hydrogen peroxide produced in the two enzyme system. Between the dual and mixed type of immobilized enzymes, however, there was no difference in deactivation characteristics (Table 3).

Table 3. The comparison of deactivation characteristics of the dual and mixed type immobilized enzyme systems

Type	% Oxygen converstion		
	0 hr	20 hr	40 hr
Dual	80	76	73
Mixed	80	77	70

Reactor performance of coimmobilized glucose oxidase and catalase system

Effects of space time were examined in plug-flow reactor system, using two cases of CAT/GOD, 1 and 10 as typical experiments. The reaction conditions were O_2: air saturated, glucose: 0.5M in 0.1M citrate-phosphate buffer, pH 5.5, reaction temperature: 25°C. Fig. 2 shows the effect of space time on O_2 consumption of the dual type immobilized enzyme. Regardless of CAT/GOD ratio changed, no difference in O_2 consumption was observed at the space time below 5.55 g·hr/l and this space time appeared as the point of O_2 limitation in both 1 and 10 of CAT/GOD. However, at the spacer time over 5.55, higher CAT/GOD required larger amount of O_2 consumption. This indicates that the influence of external mass transfer would be pronounced at the space time over 5.55, where O_2 limitation occurred for the reaction. This difficulty of external mass transfer seems more significant

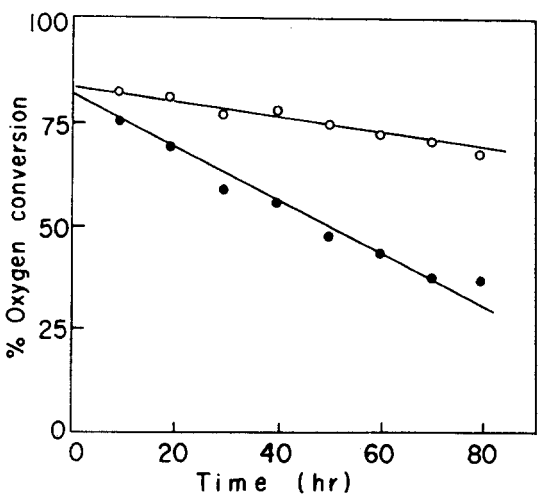

Fig. 1. Deactivation characteristics of the sole and dual type immobilized enzymes

●: Sole type immobilized glucose oxidase, o: dual type immobilized glucose oxidase and catalase (CAT/GOD = 10), and reaction condition: 0.2 mM O_2 in feed, 3.85 g·hr/l of space time, and 1.0×10 cm of column.

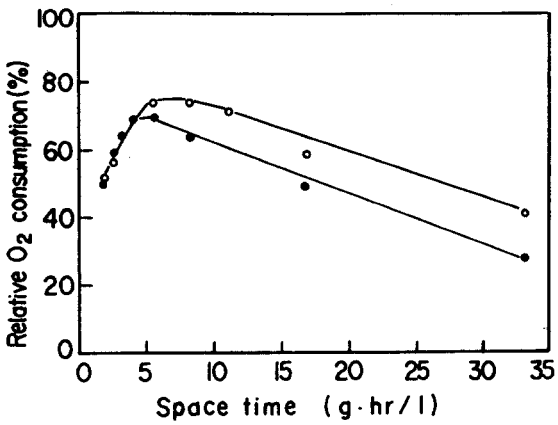

Fig. 2. Effect of space time on O_2 consumption of the dual type immobilized enzymes

○ CAT/GOD = 10, ●: CAT/GOD = 1, and reaction conditions : air saturated O_2 and 1.5 × 5.0 *cm* of column.

for lower CAT/GOD ratio. The deactivation phenomena can be explained by O_2 and H_2O_2 profile inside the particles, which Prenosil[4] already observed. He found that the hydrogen peroxide formed in the oxidation reaction deactivates CAT first; if an excess of CAT is present, the activation of GOD remains small. As Reuss *et al.*[5] illustrated, the effect appeared as a reduced O_2 limitation of the reaction in the packed bed reactor.

In the effect of O_2 concentration of GOD and CAT system, saturation point at different O_2 levels were obtained by feeding the substrate continuously with O_2 cylinder at first and with compressed air after determination of saturation point of O_2 cylinder. Fig. 3 shows the amount of O_2 consumption. Both cases of 1 and 10 of CAT/GOD revealed increments of O_2 consumption in the range of space time below 8.3 *g*·hr/*l*. In the range of space time over 8.3 for both CAT/GOD = 1 and 10, however, O_2 consumption did not increase. Besides, the case of CAT/GOD = 1 showed a slight decrease of O_2 consumption instead. It seemed that the higher the O_2 level was fed, the better performance became at constant enzyme loading. This data were well agreeable with the results of Prenosil.[4] He found that the rate of reaction is of pseudo-first order and so that it increases faster with oxygen concentration.

Consequently, optimum space tiem = 5.55 *g*·hr/*l* under the steady state is not necessarily the optimum of the productivity as seen in Fig. 3. The true optimum can only be determined by taking into account the deactivation of the enzyme, which in turn is a function of H_2O_2 concentration including CAT/DOG activity ratios.

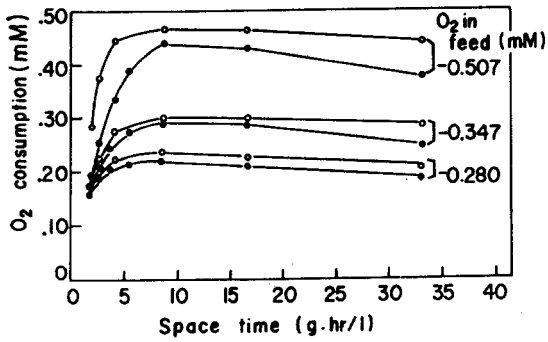

Fig. 3: Effect of space time on consumption according to O_2 concentration

○ : CAT/GOD = 10 and ●: CAT/GOD = 1.

초 록

Glucose oxidase-catalase동시 고정화 효소제에 관한 반응을 연구하였다. 두 가지 효소를 미생물 세포벽에 동시 고정화한 제품과, glucose oxidase만 고정화 시킨 것 그리고 두가지 효소를 따로 고정화시켜 혼합한 제품의 반응성을 각각 조사한 결과 동시 고정화 제품이 가장 우수하였다. 충진식 연속 반응조에서 불활성화 상수 (k_d) 는 glucose oxidase만의 고정화 효소경우 1.12 × 10^{-2} hr^{-1}이었고, 동시 고정화 효소의 경우 catalase/glucose oxidase=10일때 2.17×10^{-3} hr^{-1}이었다. 또한 체장시간(τ)이 5.55*g*·hr/ℓ O_2 일때 catalase/glucose oxidase 1 및 10 모두 반응율이 가장 좋았고 이보다 길어지면 외부 물질 전달의 영향으로 반응율이 오히려 떨어졌다. O_2의 최대 허용치는 체장시간 8.3*g*·hr/ℓ 일때 나타났다. 본 연구 결과로 부터 glucose oxidase와 catalase 동시고정화 효소에서 생산성을 높이기 위해서는 glucose oxidase의 불활성화와 이 효소의 효율이 동시에 고려되도록 두 효소의 비율을 정해야 하는 것을 알았다.

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(Received January 7, 1985)