

두 고정화 효소 충전 반응탑에 대한 준 최적화 방법

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Suboptimal Policies for Two-Immobilized Enzyme Packed Bed Reactor

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요 약

두 개의 고정화 효소를 사용한 충전 반응로에서 때때로 최적 방법으로 충전하는 것보다 준 최적 방법으로 충전하는 것이 실제 유리할 때가 있다. 이런 일은 최적방법이 실험적으로 불가능하고 이론적으로만 가능할 때 흔히 일어난다. 최적 pH가 4.5인 고정화당화효소와 최적 pH가 7.0인 고정화 이성화효소를 두 효소의 안정도와 활성도를 감소시키지 않고서는 한 반응탑에 사용될 수가 없다. 그러나 단속충전방법 (bang-bang or bang-bang-bang)을 사용하면 두 효소에 따로 따로 최적조건을 맞춰줄 수 있어 안정성이나 활성도에 지장이 없게 되고 수율도 최적조건이 이론적으로 추측한 값에 못미치나 상당히 근사한 값에 이르게 된다. 본 연구에서는 위의 준 최적반응중의 일부를 효소 불활성화를 고려치 않은 조건에서 실험과 이론으로 시험했고, 그 외의 충전방법에 대해서는 이론적으로 효소의 불활성을 고려하여 조사한 결과 과당시럽의 최적생산은 단속충전방법이 최선의 방법중의 하나임을 알아내었다.

Abstract

A properly chosen suboptimal policy for catalyst distribution in a two-enzyme packed bed reactor is sometimes better than optimal policy itself in actual cases. This often occurs when optimal policy is only theoretically possible and experimentally infeasible. Immobilized glucoamylase (=G.

A.) with optimum pH 4.5 and immobilized glucose isomerase (=G.I.) with optimum pH 7.0 cannot be mixed without sacrificing the activities and the stabilities of two immobilized enzymes. However utilizing suboptimal policies such as bang-bang policy (maximization of product) and bang-bang-bang policy (minimization of reactant) would permit two immobilized enzymes in separate reactors where optimum environments for G.A. and G.I. could be provided. The partial experimental investigation of the above suboptimal policies was conducted using immobilized G.A. and immobilized G.I. at the temperature of 40°C and pH 6.5 where no appreciable decay in the activities of both enzymes could take place. The simulated results of suboptimal policies in a reactor undergoing decay of enzyme activity showed that the predicted conversions using bang-bang policy were comparable to the theoretical conversions of the optimal bed policy and it should be one of the best policy for the maximum production of fructose syrup in practice.

1. Introduction

It will be quite useful to employ two enzyme systems especially when an initial reactant cannot be fully converted to an intermediate product or a final product due to the equilibrium limitation of the first system. This happens in the production of sweet fructose syrup from liquefied starch via glucose. The high concentrations of glucose formed from starch by glucoamylase is catalyzed to form high saccharides, mainly maltose and isomaltose. These reversion products (especially isomaltose) once formed are very difficult to be converted to other more useful products like glucose and fructose.

In order to minimize the formation of undesirable reversion products efforts have been made to understand that immobilized glucoamylase produces more reversion products than free glucoamylase¹⁾. It was suggested that this was caused by the concentrations of glucose being higher in the pores of the enzyme carrier than in the bulk solution. This higher glucose concentration in the pores could allow reversion reactions (presumably second order reaction) using glucose as a reactant to occur at a greater extent than with the soluble enzyme¹⁾. For this reason mixed enzyme system of immobilized glucoamy-

lase-glucose isomerase was introduced in order that glucose concentration in the reactor may be kept sufficiently low enough to prevent the formation of reversion products during the course of saccharification²⁾. On account of this Chang and Reilly³⁾ performed a theoretical study to find optimal catalyst profiles considering the effect of pore diffusion, initial reactant concentration, residence time and catalyst loading. Later it was shown that the maximization of final product or minimization of initial reactant was feasible experimentally in systems of immobilized glucoamylase-glucose isomerase with a maltose feed and of immobilized glucose isomerase-glucose oxidase with a fructose feed⁴⁾. Furthermore, the introduction of two or three differential reactors in sequence made it easier to conduct experiments in two enzyme systems.

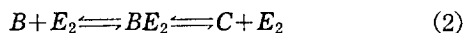
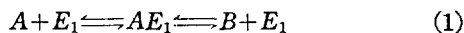
When mixing two enzymes with different characteristics such as optimal pH, temperature, stabilities and so on, a number of parameters should be optimized to obtain the maximum possible yield of a desirable product. In case of G.A. and G.I., pH 6.5 was frequently chosen for the operating condition since at this pH G.A. with the optimum pH 4.5 shows about 75% of the maximum activity and G.I. with the optimum pH 7.0 shows 80% of the activity at pH 7.0. Further difficulties arise due to the

lower stabilities of both enzymes at this pH than at their optimum pH's. Even though there are substantial advantages of mixing two enzymes together to improve the product quality, it would be economically infeasible to operate a mixed-bed enzyme reactor under these circumstances.

One of the possible ways to get around these problems is to find enzymes of the same function with identical or close optimum temperatures, pH's and stability characteristics. Another way is to immobilize enzymes to such supports where their microenvironments would look optimal to the immobilized enzymes like in examples of shifted pH after immobilization. Up to now none of the above efforts look successful so that these two enzymes can be used in an optimal configuration of catalyst distribution industrially. This study aims at finding the best suboptimal policies of two enzyme systems without losing activities at all or minimizing the loss of activities, stabilities in obtaining products closest possible to those of the optimal mixed bed system.

2. Theoretical Considerations

The kinetics of starch degradation by glucoamylase to glucose is known to be very complicated in reality⁵⁾, however simple reversible Michaelis Menton equation is employed here in order to account for the formation of reversion products. Then the general reversible two-step reactions considered here are as follows.



Let A, B, and C represent concentrations of starch, glucose, and fructose while E_1 and E_2 refer to those of immobilized glucoamylase and glucose isomerase, respectively. If X_1 and X_2 denote amounts converted from A and B to B

and C respectively, the concentrations of A, B and C at any time during the reaction will be

$$A = A_0 - X_1 \quad (3)$$

$$B = B_0 + X_1 - X_2 \quad (4)$$

$$C = C_0 + X_2 \quad (5)$$

When reactions (1) and (2) are allowed to reach equilibrium

$$X_{1\max} = \frac{K_1(K_2 + 1.0)A_0 - B_0 - C_0}{K_1K_2 + K_1 + 1} \quad (6)$$

$$X_{2\max} = \frac{K_1K_2(A_0 + B_0) - (K_1 + 1)C_0}{K_1K_2 + K_1 + 1} \quad (7)$$

Since $X_{1\max}$ and $X_{2\max}$ are maximum values of X_1 and X_2 , the maximum of dimensionless conversions of X_1 and X_2 will be 1.0 with the application of any catalyst profile policy.

In maximizing X_2 maximum conversion at the exit of a packed bed reactor X_{1f} and X_{2f} by bang-bang policy is given as

$$X_{1f} = \frac{A_0K_1 - B_0}{1 + K_1} \quad (8)$$

$$X_{2f} = \frac{B_0K_2(1 + K_1) + A_0K_1 - B_0 - C_0}{(1 + K_1)(1 + K_2)} \quad (9)$$

In maximizing X_1 maximum X_{1f} by bang-bang policy is given by

$$X_{1f} = \frac{A_0K_1 - B_0 + X_{2f}}{(1 + K_1)} \quad (10)$$

and X_{2f} will be the same as X_{2f} given in equation (9). Here bang-bang or bang-bang-bang policy refers to packing of immobilized enzyme 1 or enzyme 2 in sequence one after another in a packed bed.

Table 1. shows the maximum conversions of suboptimal policies such as bang-bang and bang-bang-bang policies. The other suboptimal policy often compared to the previous two policies is uniform bed policy which refers to the mixing of two catalyst particles in the same bed at a uniform ratio. The optimal bed policy in this system consists of bang-inter mediate bang control. The intermediate control is a mixed bed policy generally with the varying ratio of the two particles along the reactor length. When-

Table 1. Conversion as a fraction of maximum conversion for the bang-bang-bang (X_{1f}^* maximized) and the bang-bang profile (X_{2f}^* maximized) when the reactions are carried to equilibrium. $A_0=1.0$, $B_0=0$, $C_0=0$

K_1	K_2	X_{1f}^* Maximized		X_{2f}^* Maximized	
		X_{1f}^*	X_{2f}^*	X_{1f}^*	X_{2f}^*
0.1	0.1	0.993	0.917	0.917	0.917
0.1	1	0.793	0.545	0.545	0.545
0.1	∞	0.174	0.091	0.091	0.091
1	1	0.937	0.750	0.750	0.750
10	1	0.998	0.954	0.954	0.954
10	10	0.993	0.917	0.917	0.917
∞	∞	1.000	1.000	1.000	1.000

ever mixed bed of two catalyst particles is employed in a uniform bed or an optimal bed and sufficient time for the system to reach equilibrium is given, the conversion will become essentially the maximum of 1.0.

As can be seen in Table 1, the application of bang-bang or bang-bang-bang policy depending on the chosen maximizations will not be particularly inferior to those of uniform bed or optimal bed policy in terms of equilibrium conversions. The equilibrium constants of starch-glucose-fructose system are approximately 10 for starch to glucose and 1 for glucose to fructose. Therefore if these two policies are employed, theoretically 99.8% and 95.4% of maximum conversions will be obtained, respectively. Other systems with equilibrium constants such as $K_1=1$ and $K_2=1$ will yield better maximum conversions with the application of several bang-bang policies.

The biggest advantage of bang-bang or bang-bang-bang policies will be that optimum environments such as temperature and optimum pH can be provided for a particular enzyme since only one enzyme is present in the bed. Thereby immobilized enzyme will retain higher activities and stabilities than at compromised PH's and temperatures in the case of uniform bed or optimal bed policies.

3. Experimental

1) Materials

Glucoamylase from *A. oryzae* with the activity of 1200-1300 Sigma units/gr was purchased from Sigma Chemical Company, U.S.A.. Glucose isomerase from *Streptomyces* species and controlled pore alkylamine silica of 30-45 mesh with $400\text{\AA} \pm 10\%$ pore diameter from Corning Glass Works, U.S.A. were used. Dextrin (DE36) and dextrose were purchased from Young-Il Chemicals, Korea. Other chemicals used in preparing buffer solution were from Wako Chemicals, Japan.

2) Immobilization of Enzymes

Glucoamylase and glucose isomerase were immobilized to porous silica essentially in the same manner reported previously⁴⁾. This time 5 gr of glucoamylase was bound to 30gm of the porous silica and 30gm of partially purified glucose isomerase to 30 gm of the porous silica, respectively.

3) Methods

Experiments were performed essentially in the same way as in the previous report⁴⁾. Moleate buffer of 0.02M containing 10^{-4}M of CoCl_2 and 0.01M of MgCl_2 was used throughout the experiment. PH was adjusted by adding 0.2M of NaOH or 0.2M of HCl. No pH change was observed during the reaction when the hydrolysis of dextrans is done at pH 4.5. Fructose was determined by the L-cysteine method of Messineo and Musarra⁶⁾ and glucose using Worthington's glucostat⁷⁾.

4) Parameters for Simulation

The parameters needed for the calculation of

catalyst profiles are obtained from the activity measurements. Optimal and suboptimal policies are obtained by the method described elsewhere³⁾. The parameters at the temperatures of 55°C and 60°C are estimated from the experimental values at 50°C using activation and deactivation energies reported previously¹⁾ (Table 5.).

4. Results and Discussions

Table 2. shows the results of bang-bang policy and uniform bed policy with a feed of 18 gr/100ml of dextrin solution at the reaction temperature of 50°C. The experimental results are in good agreements with the predicted ones. Also it is expected that enzyme decay is not important since the reaction time (4 hours) is much shorter than the half lives (order of several hundred hours) of the two immobilized enzymes at 50°C and pH 6.5. As expected bang-bang policy at their optimum pH's (pH 4.5 for G. A., pH 7.0 for G. I.) gave higher yield of glucose and fructose than the other bang-bang policy and uniform policy at the compromised pH 6.5. This proves that the reactions run at their optimum pH's gave higher yield and will provide better stabilities than at any other conditions.

1) Suboptimal policies in glucoamylase-glucose isomerase system

The experiment in accordance with the theoretical predictions showed that bang-bang policy operated at the optimum pH's of glucoamylase and glucose isomerase was better than uniform bed policy or bang-bang policy operated at pH 6.5. Bang-bang policy may become optimal policy itself when optimal catalyst distribution are sought for short residence time or when two sequential systems are essentially irreversible

as in the case considered by Choi and Perlmutter⁸⁾, however optimal control will contain intermediate control in general.

The problem is that it is impossible or very difficult to operate uniform or optimum bed policies when the two immobilized enzymes possess two different optimum conditions. Table 3. shows the experimental feasibility of system I (operating reactors at the optimum pH's of glucoamylase and glucose isomerase) and system II (at the compromised pH of glucoamylase and glucose isomerase). In system I bang-bang and bang-bang-bang policies are only feasible policies, whereas all the policies are possible in system II. Table 4. shows the theoretical performance of systems I and II. In maximizing X_1 bang-bang-bang policy of system I would yield $X_{1f}^*=0.995$ which is quite comparable to $X_{1f}^*=0.998$ of the optimal bed policy. Bang-bang policy would give only $X_{2f}^*=0.948$ which is slightly inferior to $X_{2f}^*=0.995$ of the optimum bed. This is due to the equilibrium limitation of bang-bang policy in the first system of which dimensionless maximum conversion is 0.954. Also it is clear that system II is inferior to system I although they are all experimentally feasible.

2) Suboptimal policies of the glucoamylase-glucose isomerase system with enzyme decay

Up to now only optimization based on the activities of glucoamylase and glucose isomerase without enzyme decay were considered. In order to operate enzyme reactors in an optimal condition for a considerable period of time decay of enzyme activities should be taken into account. To cope with this decay of enzyme activities a couple of strategies should be employed. One of these is to slow down the flow rate so that residence time is sufficiently long enough for

Table 2. Experimental Results of Two Enzyme Systems (Glucoamylase-glucose isomerase systems)

	Bang-Bang	G. A. pH4.5 G. I. pH7.0	Bang-Bang	G. A. pH6.5 G. I. pH6.5	Uniform	G. A. pH6.5 G. I. pH6.5
	Predic.	Exp.	Predic.	Exp.	Predic.	Exp.
Glucose	51.2	57.5	41.3	48.3	45.2	40.2
Fructose	23.1	24.0	17.3	23.4	12.7	17.1
	$\alpha_1=1.05, \alpha_2=4.52$ $\beta_{10}=1.00, \beta_{20}=3.20$ $\beta_{11}=0.0145, \beta_{21}=0.88$ $t_s=0.46$		$\alpha_1=0.795, \alpha_2=3.61$ $\beta_{10}=1.00, \beta_{20}=3.20$ $\beta_{11}=0.0145, \beta_{21}=0.88$ $t_s=0.45$		$\alpha_1=0.795, \alpha_2=3.61$ $\beta_{10}=1.00, \beta_{20}=3.20$ $\beta_{11}=0.0145, \beta_{21}=0.88$ $U=0.44$	

$K_1=10, K_2=1, A_0=172.5\text{g/l}, B_0=7.5\text{g/l}, C_0=0.0, X_{1\max}=163.9\text{g/l}, X_{2\max}=85.7\text{g/l}, \theta_R=4\text{hrs.}$

Table 3. Experimental Feasibility of Two Enzyme Systems

	Minimization of Dextrins			Maximization of Fructose Yield		
	B-B-B	Uniform	Optimal	B-B	Uniform	Optimal
System I G. A. pH 4.5 G. I. pH 7.0	0	X	X	0	X	X
System II G. A. pH 6.5 G. I. pH 6.5	0	0	0	0	0	0

0 experimentally feasible

X experimentally infeasible

Table 4. Comparisons among Suboptimal Policies of Systems I & II

	MAX X_1			MAX X_2		
	B-B-B	Uniform	Optimal	B-B	Uniform	Optimal
System I G. A. pH=4.5 G. I. pH=7.0	$X_{1f}=0.995^*$ $X_{2f}=0.907$	$X_{1f}=0.994$ $X_{2f}=0.892$	$X_{1f}=0.998$ $X_{2f}=0.918$	$X_{1f}=0.948^*$ $X_{2f}=0.929$	$X_{1f}=0.994$ $X_{2f}=0.893$	$X_{1f}=0.995$ $X_{2f}=0.961$
System II G. A. pH=6.5 G. I. pH=6.5	$X_{1f}=0.988^*$ $X_{2f}=0.763$	$X_{1f}=0.989^*$ $X_{2f}=0.787$	$X_{1f}=0.995^*$ $X_{2f}=0.856$	$X_{1f}=0.942^*$ $X_{2f}=0.880$	$X_{1f}=0.988^*$ $X_{2f}=0.787$	$X_{1f}=0.985^*$ $X_{2f}=0.900$

Temp. = 50°C, * experimentally feasible

System I : $\alpha_1=5.25, \beta_{10}=1.00, \beta_{11}=0.0145, \alpha_2=22.6, \beta_{20}=3.20, \beta_{21}=0.88$ System II : $\alpha_1=3.94, \beta_{10}=1.00, \beta_{11}=0.0145, \alpha_2=18.1, \beta_{20}=3.20, \beta_{21}=0.88$ $K_{eq1}=10, K_{eq2}=1, A_0=172.5\text{g/l}, B_0=7.5\text{g/l}, C_0=0.0$ $X_{1\max}=163.9, X_{2\max}=85.7, \theta_R=20\text{hrs}$

the desired conversion. The other alternative is raising the reaction temperature, which would yield higher activities even though it would

accelerate enzyme decay⁹⁾. Although the first strategy is a common industrial practice to obtain a desired conversion of fructose syrup

the simulation of bang-bang and uniform bed policy for a given residence time was chosen in this study. Therefore as time goes on the productivity will decrease. Although this may not correspond to the yield of the first strategy of slowing down the flow rate in an integral reactor one to one basis, this simulation would predict the trend of productivity during the operation of enzyme reactors undergoing decay of the enzyme activity. Bang-bang reactor will be operating at 50°C, pH 4.5 for glucoamylase and at 60°C, pH 7.0 for glucose isomerase. Uniform bed reactor will be operating at 55°C, pH 6.5 for both G.A. and G.I.. As shown in Table 5. enzyme decay constants can be made equal by choosing the proper operating temperatures. This will be quite efficient when we need to slow down the flow rate in the integral reactor. Fig. 1 shows that relative activities of glucoamylase and glucose isomerase at various temperatures and pHs with time. Operating glucoamylase and glucose isomerase at 55°C pH 6.5 is obviously not good at all compared to bang-bang policy. When two enzymes decay at two

different rates, this will cause quite a problem in the operation of integral reactors.

Optimizations in two differential reactor system would yield dimensionless conversions of X_1 and X_2 shown in Fig. 2. Up to 1200 hours of operation time bang-bang policy would yield about twice for X_1 and 2.5 times for X_2 than uniform bed policy.

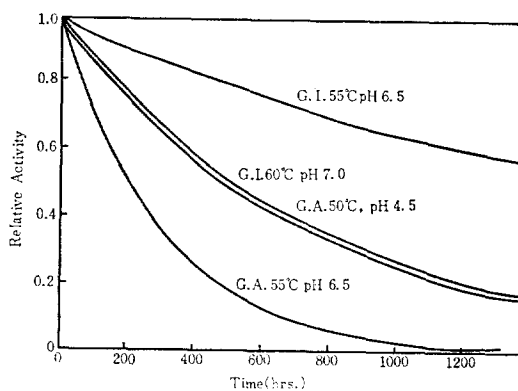


Figure 1. Relative activities of G. A. and G. I. undergoing decay.

Table 5. Parameters for the Simulation of Enzyme Reactors

Relative activities	50°C	55°C	60°C
G. A. pH 4.5	1	1.13	1.28
G. I. pH 7.0	1	1.52	2.28
G. A. pH 6.5	0.75	0.85	0.96
G. I. pH 6.5	0.80	1.22	1.82

Activation energies

G. A. 5.26×10^3 cal/g-mole

G. I. 1.78×10^4 cal/g-mole

Enzyme decay constants

$k_d = 1.327 \times 10^{-3} \text{ hr}^{-1}$ at 50°C for G. A.

$k_d = 1.300 \times 10^{-3} \text{ hr}^{-1}$ at 60°C for G. I.

Deactivation energies

$E_d = 4.2 \times 10^4$ cal/g-mole for G. A.

$E_d = 4.9 \times 10^4$ cal/g-mole for G. I.

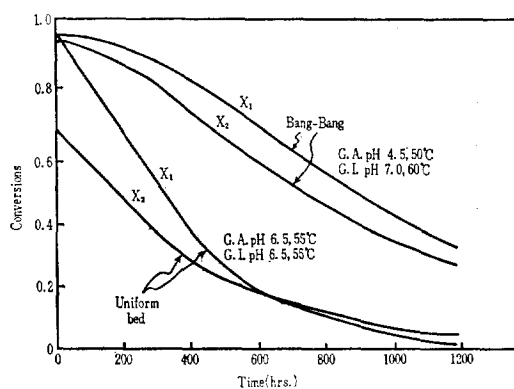


Figure 2. Dimensionless conversions of X_1 and X_2 with time.

Nomenclature

A, B, C bulk concentrations of components A, B, and C (g/liter)

A_0, B_0, C_0 initial concentrations of A, B, and C

	(g/liter)
E_i	quasi-homogeneous catalyst concentrations averaged over particle volume (g/liter)
K_i	equilibrium constants, dimensionless
k_1, k_{-2}, k_3, k_{-4}	rate constants (liter/(g·hr))
k_{-1}, k_2, k_{-3}, k_4	rate constants (hr ⁻¹)
k_d	enzyme decay constant (hr ⁻¹)
t_s	switching time of catalytic enzyme bed, dimensionless
U	volume of particles that contain E_i divided by total particle volume, dimensionless
X_i	conversion (g/liter)
X_i^*	dimensionless conversion
$X_{i\max}$	maximum values of X_i (g/liter)
X_{if}	values of X_i at the end of reactor (g/liter)

Greek Symbols

α_i	dimensionless rate terms
β_{0i}, β_{i1}	dimensionless terms related to Michaelis-Menton constants
θ_R	total residence time (hr)

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