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Effect of Moisture Content of Biocatalyst on the Gas Phase Continuous Bioreaction

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생촉매의 수분함유량이 기상의 연속반응에 미치는 영향

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ABSTRACT

The effect of moisture content of biocatalyst on the performance of a gas phase continuous bioreactor was investigated along with study on the mass transfer limitation. The biocatalysts whose moisture contents are 46.2% and 37.2%, respectively were prepared by immobilization of alcohol oxidase on Amberlite IRA-400, following by slow dehydration method, and packed into a column. Relative production rate (RPR), acetaldehyde composition (X_P) and conversion (X) of biocatalysts (37.2%) are better than those of biocatalysts (46.2%), and it was considered that these are attributed to the mass transfer enhancement in the gas phase compared with the aqueous phase.

INTRODUCTION

Since the discovery that enzyme in the dry state can react on gaseous materials (1), it has been known that enzymes require a small amount of moisture to be active catalytically (2), It is known that enzymatic reaction rate in the gas phase depends on water activity (3) or there exists an optimum total/bound water content for the immobilized enzyme reaction in the organic phase (4). Although a few articles on the gas phase enzymatic reaction were published since 1969 (5, 6), it appears that only one research group (7, 8) ran the continuous bioreacctor in the gas phase using immobilized enzyme. However, they did not investigate further the effect of variables on the performance of a continuous bioreactor. Recently, the effect of water activity of enzyme powder on the performance of a gas phase continuous bioreactor was reported by the same group (9). However, this enzyme powder is not suitable for the fixed bed reactor because enzyme powders cause problems as packing materials. Furthermore, the biocatalyst lost its activity during the dehydration process (10) and this result was confirmed by our study (11). In order

to overcome the difficulties arising when preparing the biocatalyst (10, 11), we developed a new biocatalyst; alcohol oxidase enzymes which are immobilized on Amberlite IRA-400 (spherical bead) and investigated the variable effects; temperature, flow rate of gaseous stream and ethanol vapor concentration (12) on the performance of a continuous bioreactor in the gas phase. This biocatalyst offered a new chapter of biocatalysis on gaseous materials and did make it possible to scale up a fixed bed reactor.

In this paper, we report the effect of the moisture content of biocatalyst on the performance of a gas phase continuous bioreactor.

REACTION KINETICS

The stoichiometry of the reaction from ethanol to acetaldehyde is as follows :

$$C_2H_5OH(g)+O_2(g) \xrightarrow{\text{Alcohol Oxidase}} CH_3CHO(g)+H_2O_2(g)$$

In this study, we used new terminologies : *composition of product* and relative production rate (RPR). The composition of product, acetaldehyde X_P is expressed as follows :

$$X_P=\frac{C_P}{C_A+C_P}$$

where RPR is

$$RPR=\frac{\text{continuous production rate } [\mu\text{mol/min} \cdot \text{mg enzyme}]}{\text{batch production rate } [\mu\text{mol/min} \cdot \text{mg enzyme}]}$$

The derivations and the reasons why these are used are well explained elsewhere (12).

MATERIALS AND METHODS

Materials

Alcohol oxidase (E. C. 1.1.2.13 : initial activity =20 to 30 U/mg) and Amberlite IRA-400 were purchased from Sigma Chemical Co. (St. Louis, MO). Ethanol was purchased from Florida Distillers Company. All other chemicals were of reagent grade and obtained from Fisher Scientific.

Preparation of Biocatalyst

Five mL of sodium phosphate buffer solution (pH 7.5, 0.001M) containing 2mg/ml of alcohol oxidase was added to two grams of Amberlite in a plastic bottle and shaken at 150 rpm by a vibrax (Model VXR S-1, Janke & Kunkel GmbH u. CoKG) for 3 hours at room temperature. The mixture was gravity filtered using Whatman filter paper (No. 4), the treated support was dried for 5 hours in a dessicator at room temperature, and its moisture content was adjusted to constant slowly using a saturated sodium chloride solution. The filtrate was diluted and its absorbance was measured spectrophotometrically by a B & L Spectronic 2000 at 280 nm and its alcohol oxidase concentration was determined. The amount of immobilized alcohol oxidase was determined from that of alcohol oxidase in filtrate subtracted from the total alcohol oxidase.

Moisture Content Studies

The prepared biocatalysts were placed in a column (i.d.×L=0.9cm×15cm ; Spectrum). The gaseous reactant flow rates were controlled using metering valves connected to air cylinder lines. Each air stream was mixed with ethanol and water vapors and these streams were mixed together in the mixing chamber. Reaction was initiated by introducing the ethanol/air stream to the bioreactor. At 15minute intervals, a sample of the outlet stream gas (100 $\mu\ell$) was withdrawn and gas composition was determined chromatographically using a Perkin-Elmer Model 3920 Gas Chromatograph equipped with a Hewlett-Packard Model 3393A integrator. Continuous production rates were calculated by multiplying acetaldehyde concentration by flow rates of gaseous stream. Relative humidity and gas flow rate at the outlet were also measured using a thermohygrometer (Model 3309-60, Cole-Parmer) and a digital flow meter (Optiflow 520, Humonics), respectively. Its configurations are shown in Fig. 1.

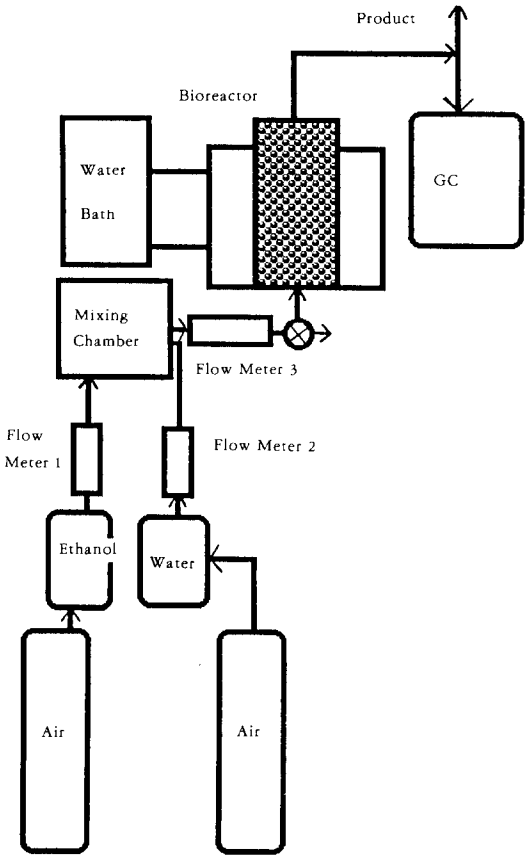

Fig. 1. Flow diagram of continuous flow biocatalytic reactor.

In order to calculate the batch production rate (biocatalyst activity), approximately 0.03g of biocatalyst was placed in a 4-dram vial (15ml) and shaken at 1,000 rpm by the vibrax. Their activities were measured by introducing ethanol vapor-containing air to vials. At designated intervals, 100 $\mu\ell$ of headspace gas was withdrawn and gas composition was determined chromatographically. From the acetaldehyde concentration against reaction time, the reaction rate was determined, and three experiments were repeated to calculate the average batch production rate.

RESULTS AND DISCUSSION

Moisture Content Effects

Biocatalyst (immobilized enzyme powder) activity in a batch bioreactor depends on its water activity (equilibrium relative humidity) showing maximum at 0.75 water activity (3) or there is an optimum moisture content of immobilized enzyme powder with respect to the reaction rate in an organic solvent (4). Water activity is defined as the ratio of the vapor pressure of water in the sample to that of pure water at the same temperature. In practice, it is very difficult to measure water activity without losing the sample prepared. When there is not enough sample, moisture is lost from sample to the surrounding air in the chamber where the electrode of humidity meter (hygrometer) is inserted. This is equal to "drying process" of sample and causes the greatest error in measurement. This phenomenon led us to employ an indirect method to measure the moisture content of biocatalysts.

Instead of measuring the equilibrium relative humidity (water activity), we used the weight difference method before and after treatment. Because the dehydrated enzyme loses its activity (10, 11) when exposed to direct oxygen tension in air, a new method for preparing biocatalyst was designed and developed in this study instead of using the method adopted by Barzana et al. (3). In order to prevent the biocatalyst from losing its activity by the drying process, alcohol oxidases were immobilized on Amberlite, were partially dehydrated instead of complete dehydration and their moisture content was adjusted slowly and held constant.

Two kinds of biocatalysts with different moisture content, 46.2% and 37.2%, were prepared. The first ones (46.2%) were wet, unrollable and were very difficult to be packed into the column which was used as a fixed bed bioreactor. The content of moisture filling the pores of Amberlite is known to be about 46.0% of total weight by Sigma Chemical Co.. So the pores of biocatalysts of high moisture content (46.2%) were considered to be filled with water fully (aqueous phase). It was considered that ethanol vapor and oxygen molecules diffuse from the bulk gas phase

to the biocatalyst surface and contact with alcohol oxidase in the aqueous phase. Thus, most reaction occurs in the aqueous phase. Most acetaldehyde molecules move through aqueous phase and diffuse into the gas phase while a few diffuse into the gas phase directly. Hence it was considered that there is mass transfer limitation compared with the gas phase reaction.

The second ones (37.2% moisture) were rollable as beads and seemed to be dry to the touch and visual inspection. In the case where biocatalysts have 37.2% moisture content, pores inside biocatalysts were partially filled with water and it was considered that there is less water at outlayer of biocatalyst pellet compared with the inside because of slow dehydration. This provides the biocatalyst (immobilized alcohol oxidase) with less water compared with the former case (46.2% moisture content). In immobilization step, dynamic contacting method (agitation) was used, thus most alcohol oxidases were immobilized near the outlayer of biocatalyst pellets (13). Unlike aqueous phase diffusion, ethanol vapor molecules and oxygen molecules diffuse through the pores of biocatalyst from the bulk gas phase, bind with alcohol oxidase molecules, acetaldehyde and hydrogen peroxide molecules are produced in the gas phase, and diffuse into the bulk gas phase. When the biocatalysts contain less water, either reactants or products diffuse mostly in the gas phase. This enhancement of mass transfer rate makes the system produce more acetaldehyde.

In this study, volumetric flow rate was 5.18ml/min for biocatalyst of 46.2% moisture content and 5.08 ml/min for biocatalyst of 37.2% moisture content and temperature was 35℃. This minor difference in flow rates had no effect on the performance of a continuous bioreactor. The relative humidity of inlet gas stream was controlled at 40% to 50% and, from the results, it was known that the relative humidity effect of reactant on the performance was very limited.

The results are shown in Fig. 2-4. In RPR (relative production rate) profile, the biocatalysts of 46.2% moisture content produce less acetadehyde

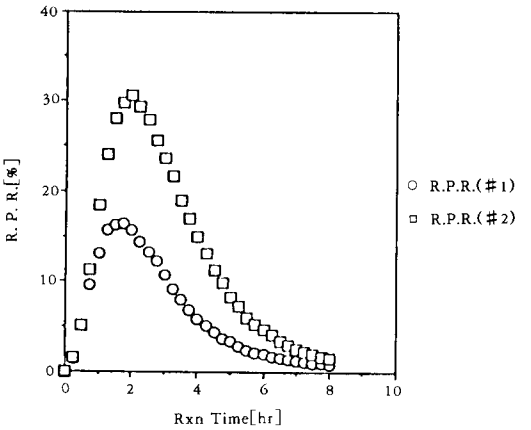

Fig. 2. Continuous relative produciton rate as a function of reaction time: Run 1=moisture content 46.2% at 35℃ and 5.18 ml/min, Run 2 = moisture content 37.2% at 35℃ and 5.08ml/min.

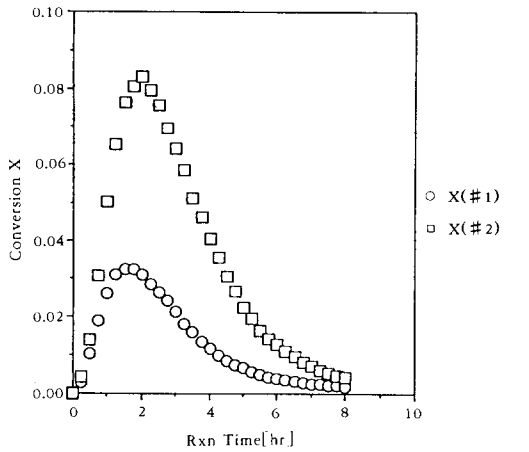

Fig. 3. Continuous relative conversion as a function of reaction time: Run 1=moisture content 46.2% at 35℃ and 5.18 ml/min, Run 2=moisture content 37.2% at 35℃ and 5.08ml/min.

compared with those of lower moisture content (37.2%). This phenomenon is the same either in the case of acetaldehyde composition X_P or conversion X. In all cases, these average maximum values obtained with high moisture content (46.2%) biocatalysts are about one-half of those obtained with low moisture content biocatalysts

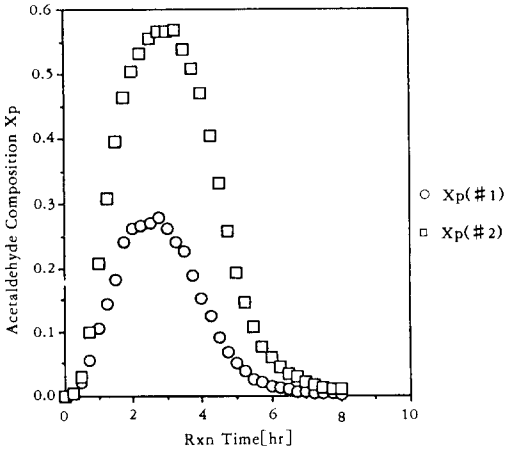

Fig. 4. Continuous acetaldehyde composition as a function of reaction time: Run 1 = moisture content 46.2% at 35℃ and 5.18 ml/min, Run 2 = moisture content 37.2% at 35℃ and 5.08ml/min.

Table 1. Average maximum RPR, X_P and X by different moisture content of biocatalyst.

Moisture Contnt[%]	RPR	X_P	X
0.0	0.0	0.0	0.0
37.2	0.299	0.567	0.081
46.2	0.163	0.276	0.032

(37.2%). In a continuous bioreactor, ethanol and oxygen concentration on the biocatalyst surface of lower moisture content (37.2%) are higher because of higher mass transfer rate compared with those on the biocatalyst surface, which have higher moisture content (46.2%). This leads to a higher acetaldehyde production as shown in Table 1. Based on this result, in other cases where the effects of temperature, flow rate of gaseous reactants, ethanol vapor concentration are investigated, this moisture content (37.2%) was used in order to prepare the biocatalyst (12).

Toxicity of Hydrogen Peroxide on the Gas Phase Reaction

In a continuous bioreactor, the acetaldehyde molecules are swept by gas flow continuously

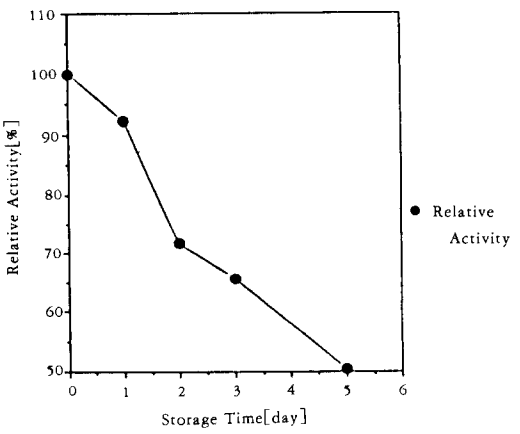

Fig. 5. Relative activity of biocatalyst (alcohol oxidase on Amberlite) as a function of storage time at room temperature.

and their build up in the bulk gas phase are unlikely. This prolongs the operations of the bioreactor. Hence, the decrease of acetaldehyde production rate within a few hours was attributed to the decrease of biocatalyst activity by hydrogen peroxide, one of products (6) considering that the biocatalyst half life without reaction is 5 days (Fig. 5). Hydrogen peroxide has a high boiling point, 158℃. Thus, the hydrogen peroxide is considered to stay inside the biocatalyst particle, and this would eventually lead to a hydrogen peroxide build-up resulting in the deactivation of the biocatalyst.

In the future study, it is suggested that chemical functional group which has the capabilities to decompose hydrogen peroxide into water and oxygen should be tried and attached to the surface of the support materials, thus creating a lot of active sites next to alcohol oxidase and enabling hydrogen peroxide molecules to be decomposed without deactivation of biocatalyst. In example, the use of support materials with hydrogen peroxide decomposing materials like TiO_2 or MnO_2 or supplementation with inorganic catalysts like platinum is suggested by Barzana (14). In the case of using whole cells (15), the operation period was more than 30 days. This longevity of bio-

Table 2. Parameters used in the calculation of the observable modulus.

Parameters	Values	
C_{As}	$0.013X10^{-3}$	[M]
D_P	0.048	[cm]
D_{so}	0.117	[cm²/sec]
K_P/K_r	1	
V	$1.27X10^{-6}$	[M/min]
ε_p	0.506	
τ	4	

catalyst was considered due to catalase in peroxisomes of whole cells, thus suggesting the use of catalase.

Internal Mass Transfer Limitation

Based on the above results, internal and external mass transfer limitations were investigated. Assuming that the reactant concentration at the external surface of the biocatalyst pellet is equal to the reactant concentration of the bulk gas phase, the observable modulus was calculated in order to investigate whether there is internal mass transfer limitation or not.

For 0th order irreversible reaction (16), the observable modulus is

$$\Phi = \frac{V(V_P/A_P)^2}{2D_e C_{As}}$$

where D_e is

$$D_e = D_{so}\frac{\varepsilon_P}{\tau}\frac{K_P}{K_r}$$

The data used in this calculation are shown in Table 2.

The calculated value of observable modulus was in the order of 10^{-6}, which is 5 order of magnitude less than the criterion of 0.3 for internal mass transfer limitation (17). Thus, this indicates that there is no internal mass transfer limitation in the gas phase.

External Mass Transfer Limitation

In order to investigate the external mass transfer limitation on the reaction rate in the gas phase, a batch bioreactor (volume=15ml) was shaken by increasing rpm of a vibrax in order to increase the mixing extent. The reaction rates of ethanol oxidation in the gas phase were shown as a function of a vibrax's rpm (revolution per minute) (Fig. 6). It showed that there is no effect of rpm on the reaction rate suggesting no external mass transfer limitation above 1,000 rpm. The decrease in reaction rate at higher rpm might be attributed to the severe relative motion of biocatalyst particles which causes partial enzyme loss by particle-particle abrasion. Although the effect of flow rates (superficial velocity) on the performance of a continuous bioreactor was investigated under the different residence time (12), it is suggested that the effect of flow rates on the performance of a continuous bioreactor should be investigated under the same residence time in order to investigate the external mass transfer limitation of a continuous bioreactor

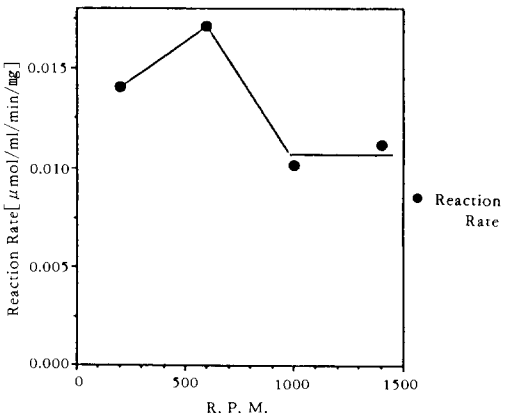

Fig. 6. Reaction rates of biocatalyst (alcohol oxidase on Amberlite) as a function of rpm (revolution per minute).

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NOMENCLATURE

A_P : surface area of biocatalyst; $4\pi D_P^2$[cm²]

C_A : ethanol concentration [μmol/ml]
C_{As} : ethanol concentration at the surface of biocatalyst [μmol/ml]
C_P : acetaldehyde concentration [μmol/ml]
D_e : effective diffusivity of ethanol vapor in air [㎠/sec]
D_{so} : diffusivity of ethanol vapor in air [㎠/sec]
D_P : particle size [㎝]
K_p : equilibrium partition coefficient
K_r : reduction factor of restricted diffusion
V : reaction velocity [M/min]
V_P : volume of biocatalyst particle; $(\pi/6)D_P^3$ [㎝³]
X : reaction conversion
X_P : acetaldehyde composition
ε_P : porosity
τ : tortuosity factor
Φ : observable modulus

요 약

생촉매의 수분함유량이 기상에서의 연속반응기의 성능에 미치는 영향을 물질전달제한에 대한 연구와 함께 조사하였다. 각각 46.2%와 37.2%의 수분을 함유한 생촉매가 알코올 옥시데이즈 효소의 앰버라이트 IRA-400에로의 고정화 및 저속의 탈수화에 따라 준비되어져, 컬럼에 채워졌다. 연속식 기상반응에서의 상대생산속도(RPR)와 아세트알데하이드 조성(X_P) 및 전환율(X)이 비교되었고 37.2%의 경우가 46.2%의 경우보다 우수하였는데 이는 기상에서의 물질전달 향상에 따른 것으로 판단되었다.

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