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수직형 미생물 고정층 반응기에서 S. Cerevisiae를 이용한 에탄올 발효

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Ethanol Fermentation by S. Cerevisiae in a Bioreactor Packed Vertically with Ceramic Rods

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요 약

불규칙하게 충진된 미생물 고정층 반응기는 생산성을 줄이고 반응기가 막히거나 편류를 일으키는 단점이 있기 때문에, 이러한 단점들을 극복하기 위하여 새로운 수직형 미생물 고정층 반응기를 개발 사용했다. 글루코를 에탄올로 발효시키는 포화상수나 겉보기 최대 비생성속도와 같은 이 반응기의 반응속도 변수들을 결정하고, 에탄올에 대한 적응력을 조사하였다. 이 반응기의 반응속도 및 적응력을 연속교반조의 것들과 비교하였다.

ABSTRACT

A conventional randomly packed bed reactor of immobilized cells often has several problems which reduce productivity, including plugging and channelling. A new fixed bioflim reactor has been developed to overcome these disadvantages and to increase overall productivity. Kinetic parameters, such as the saturation constast and the apparent maximum specific production rate for the fermentation of glucose, have been been determined, and the ethanol tolerance has been examined. The kinetics of the fixed biofilm reactor were compared with those of the continuous stirred tank reactor.

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Ⅰ. INTRODUCTION

After the oil crises of the 1970's, many countries sought new sources of energy and chemical feed stocks to reduce their dependence on petroleum. One of the many ways explored was the biological conversion of unused agricultural residues to ethanol. Biological reactions are generally slow, thus the selection of the most efficient reactor is very importent. Many different types of reactors have been investigated for this reason.[1]

In a batch reactor, it is possible to maintain the maximum reaction rate only for a short period since the microbial environment is constantly changing. The uevelopment of culture techniques such as a continuous stirred tank reactor (CSTR) eliminates this restriction by providing an essentially unchanging microbial environment. However, the outflow rate of the CSTR is limited because the dilution rate must be less than the maximum specific growth rate.

To overcome this restraint, obtain higher cell density and increase the reaction rate, various fixed biofilm reactors have been developed[2,3,4], most of which are randomly packed columns with various packings. A major disadvantage of the randomly packed bed reactor is that the cell growth into the void volume and the accumulation of gas bubbles during fermentation can result in severe channelling and plugging.

A new fixed biofilm reactor in which a bundle of long, thin ceramic rods is vertically packed has been developed in order to reduce the channelling and to minimize the hold up of gas(mainly carbon dioxide). The purpose of this research is to examine the characteri stics and ethanol tolerance of this new reactor, and to compare them with those of the CSTR.

Ⅱ. MATERIALS AND METHODS

Materials and Analytical Methods Apparatus and packings

A fixed biofilm reactor (FBR) was composed of a 50cm long pyrex tube with a diameter of 3 cm covered with a water jacket for temperature control. Glass beads of 4 mm diameter were packed at the inlet (bottom) of the reactor column to evenly distribute the medium velocities. Ceramic tubes of 3.7 mm outside diameter and 45 cm length were coated on the outer surface with gelatin cross-linked by glutaraldehyde, and then vertically packed into the column of the reactor on top of the glass beads as shown in *Fig. 1*. Details of the coating technique are reported elsewhere.[5] The reactor was an upflow mode. The total reactor volume was 360 ml and the total packing volume was 240 ml, thus leaving a total void volume of 120 ml.

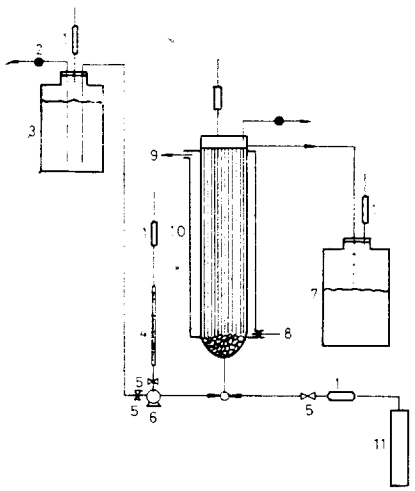
Microorganism and medium

Sacchromyces cerevisiae ATCC 24858 was used in all the experiments. The medium used consisted of various components shown in *Table 1*. All substances were sterilized at 120°c and 15 psig for 45 minutes

Table 1. Media Component Concentrations

Component	Composition for 1 liter medium
D(+)Glucose	* g
Yeast extract	8.5g
Ammonium chloride	1.3g
Magnesium sulfate	0.1g
Calcium chloride	0.06g
Antifoam	33μl

* changed for different experiments



1. Filter
2. Sampler
3. Feed Tank
4. Pippet
5. Valve
6. Peristaltic Pump
7. Reservoir
8. Warm WaterInlet
9. Warm Water Outlet
10. Fixed-Biofilm Reactor
11. Nitrogen Cylinder

Fig. 1. Apparatus for Fixed Biofilm Reactor System

Analytical methods

The ethanol concentration was measured by gas chromatography (Hewlett packard 5840 A) using a chromosorb 101 colum (80 to 100 mesh of packing material, 1/8 inch outer diameter and 6 ft long stainless steel tube) with a flame ionization detector. Temperatures of the detector, injector and column oven were 300°C, 200°C and 150°C respectively. Helium was used as a carrier gas.

Glucose was analyzed using the Dinitrosalicylic acid (DNS) method by Summer and Somers.[6] The cell mass was determined from measurements of the optical density of the cell suspension at 525 nm with a spectrophotometer (Beckman Model 35).

Experimental Methods

Continuous fermentation in the CSTR

Continuous experiments were carried out in NBS C-30 with a working volume of 700 ml to examine the productivity and tolerance of ethanol in the CSTR. Pure ethanol was added to the medium for different feed concentrations in the studies of product inhibition on specific growth and production rates. The fermentor and tubes were sterilized at 120°c and 15 psig for 20 minutes. The fermentor was inoculated with 100 ml of the culture and the feed pump was switched on when the cell reached the logarithmic growth phase.

Continuous fermentation in the FBR

The FBR was filled with coated ceramic rods, sterilized with alcohol and then inoculated with 100 ml of the culture. After 4 hours of batchwise culture, fresh medium was continuously fed to reactor. The feeding rate was adjusted for the desired dilution rate. The temperature of the reactor was kept at 25°c and the pH of the feed was adjusted to 4.0. After three turn-overs of the working volume, a steady sate was reached. Samples were taken for analysis.

Experiments were carried out with different concentrations of ethanol in the medium and with different concentrations of glucose to study the product inhibition and productivity at different dilution rates.

The cell mass attached to the packing material was collected with a knife and assessed by measuring the optical density of the cell suspension.

Ⅲ. RESUL AND DISCUSSION

Continus Fermentation in the CSTR

Effect of the dilution rate on continuo-

us fermentation

The experiments were carried out at a fixed feed concentration of glucose of 30 g/l to investigate the effects of the dilution rate on continuous fermentation. *Fig. 2* shows that cell concentratien decreases and the glucose concentration increases as the dilution rate increases. Cell concentration diminishes to zero at the washout rate of about rate of about 0.25 hr^{-1}. This represents a major limitation in the CSTR. The rate of ethanol production increases until the maximum productivity reaches 1.82 g/l/hr at the dilution rate of 0.185 hr^{-1} and decreases rapidly after this point.

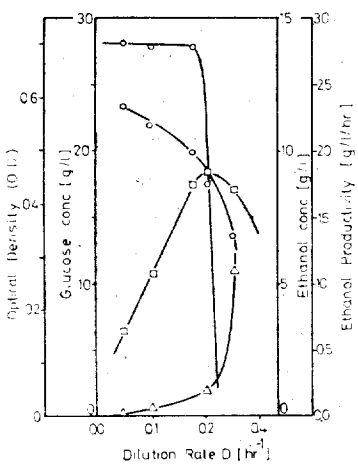


Fig. 2. Steady State Relationships in the CSTR. Symols: o, ethanol concentration; △, glucose concentration; • cell concentration (O.D.); ethanol productivity

Effect of ethanol on kinetics in the CSTR

To examine the effect of ethanol on fermentation, pure ethanol was added to the medium. The feed glucose concentration was fixed at 30 g/l and the feed concentration of ethanol, P_0, was changed from 0 to 50 g/l.

The kinetic data obtained in this study is analyzed by thefollowing model for non-competitve inhibition:

$$V = \frac{V_m S}{(K_m + S + S/K_{si})(1 + P/K_{pi})} \quad (1)$$

The specific production rate, V, is determined experimentally by the following definition:

$$V = D(P - P_o)/X \quad (2)$$

Since the glucose feed concentration of 30 g/l is within the growth limiting concentration rate, substrate inhibition is negligible. Therefore, Eq. (1) is changed into the following:

$$V = \frac{V_m S}{(K_m + S)(1 + P/K_{pi})} = \frac{V_{ma} S}{K_m - S} \quad (3)$$

where V_{ma} is the apparent maximum specific production rate. The reciprocal of Eq. (3) gives

$$\frac{1}{V} = \frac{1}{V_{ma}} + \frac{K_m}{V_{ma}} \frac{1}{S} \quad (4)$$

When $1/S$ is zero, $1/V$ is equal to $1/V_{ma}$ and V_{ma} can be calculated. The specific growth rate, μ, has the following equation which is obtained by the same method used for the specific production rate:

$$\frac{1}{\mu} = \frac{1}{\mu_m}\left(1 + \frac{P}{K_p}\right) + \frac{K_s}{\mu_m}\left(1 + \frac{P}{K_p}\right)$$

$$\frac{1}{S} = \frac{1}{\mu_{ma}} + \frac{K_s}{\mu_{ma}} \frac{1}{S} \quad (5)$$

where μ_{ma} is the apparent maximun specific growth rate. This value is obtained by the same method used for the apparent maximum production rate.

Using Eqs. (2) and (4), a Lineweaver Burk plot is drawn as shown in *Fig. 3*, and the the saturation constant K_m and the apparent maximum specific production rate V_{ma} are obtained. *Fig. 4* shows the Lineweaver Burk plot for the specific growth rate. The values of K_s and μ_{ma} can be determined from from this figure and are listed in *Table 2*. From this table, it is apparent that the maximum specific growth and production rates decrease

as the ethanol concentration in the feeding medium increases. This indicates that cell growth and product formation are inhibited by adding ethanol to the feeding medium.

Table 2. Values of Saturation Constants and Maximum Specific Growth and Production Rates for Different Ethanol Concentration

P_o	K_s(g/l)	K_m(g/l)	μ_{ma}(hr^{-1})	V_{ma}(hr^{-1})
0	1.2	1.73	0.28	0.917
18.4	1.2	1.73	0.24	0.71
39.8	1.2	1.73	0.20	0.57
43.5	1.2	1.73	0.19	0.48

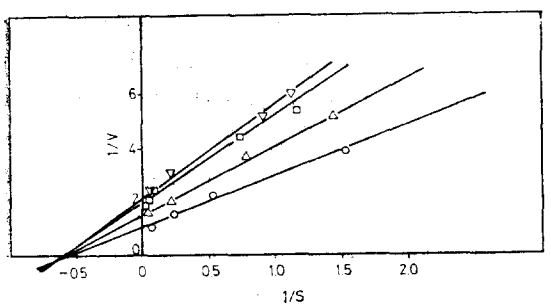


Fig. 3. Lineweaver Burk Plot for Specific Ethanol Production Rates of Various Ethanol Feed Concentration Obtained from CSTR Experiments. Symbols for Ethanol Feed Concentration(g/l):0, 0; △, 18.4; □, 39.8; ▽, 43.5

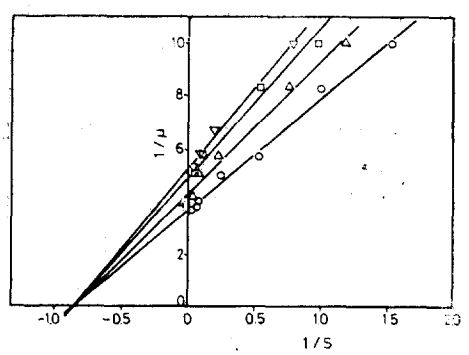


Fig. 4. Linewaver Burk Plot for Specific Growth Rates of Various Ethanol Feed Concentrations Obtained from CSTR Experiments. Symbols for Ethaol Feed Concentration(g/l); 0, 0; △, 18.44; □, 39.8; ▽, 43.5

Continuous Fermentation in the Fixed Biofilm Reactor productivity of ethanol

The productivity of ethanol is defined by

$$\text{productivity} = D'(P' - P'_o) \quad (6)$$

The effect of the flow rate on productivity is shown in *Fig. 5*. Below the dilution rate of 1.5 hr^{-1}, the productivity increases shapely with an inerease in the feed glucose concentration. At a high feed glucose concentration, Maximum productivity is attained with a dilution rate of about 1.5 hr^{-1}. When glucose concentration is 150 g/1 and the dilution rate is 1.58 hr^{-1}, the maximum productivity is 27.5 g/1/hr. When the glucose concentration is 30 g/1, the maximum productivity is 12.5 g/1/hr. This value is much higher than the 7.4 g/1 /hr obtained by Sitton and Gaddy.[5] This increased productivity may be due to the reduction of the carbon dioxide accumulation in FBR. Gas will collect in a system with immobilized cells where gas elimination is slower than gas production. Thus, an excess of gas causes a decrease in liquid void volume, an increase in liquid velocity, and severe channelling.

Effect of ethanol on kinetics in the fixed biofilm reactor

To investigate the product tolerance in the FBR, experiments were peformed with vary-

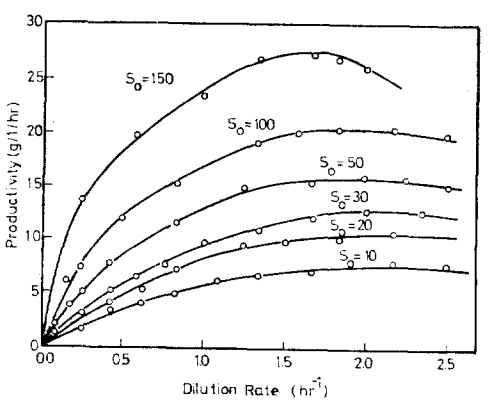


Fig. 5. Effect of Dilution Rate on Ethanol Productivity for Various Glucose Feed Concentrations in the FixedBiofilm Reactor.

ing concentrations of ethanol in the feeding medium from 0 to 64 g/l.

The specific ethanol production rate V' is calculated using the follow ing equation:

$$V' = D'(P' - P'_0)/X' \quad (7)$$

As in CSTR, the inhibiton affected by the ethanol is regarded as a noncompetitive type. The reciprocal equation of specific ethanol production rate is similar to Eq. (4) and derived from the Lineweaver Burk plot of *Fig. 6*:

$$\frac{1}{V'} = \frac{1}{V'_m}\left(1 + \frac{P}{K'_{pi}}\right) + \frac{K'_m}{V'_m}$$

$$\left(1 + \frac{P}{K'_{pi}}\right)\frac{1}{S'} = \frac{1}{V_{ma}} + \frac{K'_m}{V'_{ma}}\frac{1}{S'} \quad (8)$$

The values of K'_m and V'_{ma} are listed in *Table 3*. Since the apparent maximum specific production rate decreases with an increase in the ethanol concentration, it is evident that the product is inhibited.

Table 3. Values of Apparent Saturation Constants and Maximum Specific Production Rates for the Fixed Biofilm Reactor

P'_o	K'_m(g/l)	V'_{ma}(hr^{-1})
0	2.36	0.922
12.87	2.36	0.667
53.2	2.36	0.444
64	2.36	0.374

Comparison of results with the FBR and the CSTR

The value of the apparent saturation constant is significantly higher in the FBR than in the CSTR. This phenomenon may be explained by the fact that the barrier of the glucose transfor to the cell due to the production of gas is greater in the FBR than in the CSTR. The FBR has a higher tolerance to the presence of ethanol in the feeding medium than does the CSTR, because the former has higher ethanol production rate for ethanol concentration as shown in *Fig. 7*. When the glucose concentration is 30 g/l, the maximum productivity of 12.5 g/l/hr for the FBR is much greater than that of 1.82 g/l/hr for the CSTR. The FBR can operate at a much higher dilution rate than can the CSTR.

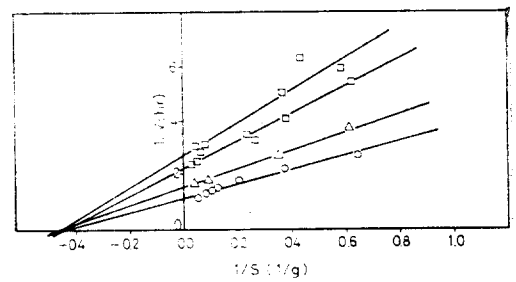


Fig. 6. Lineweaver Burk Plot for Specific Ethanol Production Rates of Various Ethanol Feed Cencentrations Obtained from Fixed Biofilm Reactor Experiments. Symbols for Ethanol Feed Concentration(g/l): 0, 0; △, 12.87; ■, 53.2; □, 64

Ⅳ. CONCLUSION

In a bioreactor with vertically packed ceramic rods, the maximum productivity is 12.5 g/l/hr when the glucose concentration in the feeding medium is 30 g/l. This value is much higher than the 7.4 g/l/hr randomlly packed bioreactor. The greater productivity of the vertically packed bioreactor may be due to the reduction of carbon dioxide builbup. Accordingly, channelling and plugging can also be prevented with use of this reactor. When the glucose concentration is 150 g/l, the optimum productivity can be obtained as 27.5 g/l/hr at the dilution rate of 1.58 hr^{-1}.

The value of the apparent saturation constant (2.36 g/l) is significantly higher in the FBR than in the CSTR (1.73 g/l). This may be causedby the greater barrier of glucose transfer from the liquid bulk to the cell du-

ring the production of gas in the CSTR than in the FBR. A comparison of ethanol tolerance shows that the FBR has better tolerance than the CSTR.

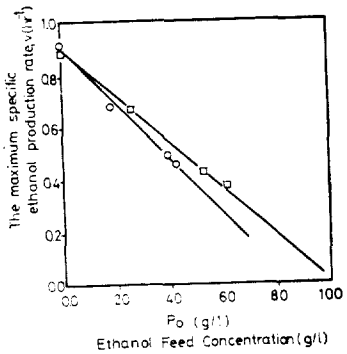


Fig. 7. Correlation Between the Maximum Specific Ethanol Production Rate and Ethanol Feed Concentration for the CSTR and the Fixed Biofilm Reactor. Symbols: 0, CSTR: □, Fixed Biofilm Reactor

Nomenclature

D Dilution rate, (hr^{-1})

K_m Saturation constant for a specific production rate, (g/l)

K_p Product inhibition for a specific growth rate, (g/l)

K_{pi} Product inhibition constant for a specific production rate, (g/l)

K_s Saturation constant for a specific growth rate, (g/l)

K_{si} Substrate inhibition constant for a specific production rate, (g/l)

P_o Ethanol feed concentration, (g/ll)

P Ethanol concentration in the reactor, (g/l)

S_o Feed concenrtation of glucose, (g/l)

S Glucose concentration in the reactor, (g/l)

V Specific ethanol production rate, (hr^{-1})

V_m Maximum specific production rate, (hr^{-1})

V_{ma} Apparent maximun. specific production rate, (hr^{-1})

X Cell concentration, (g/l)

μ Specific growth rate, (hr^{-1})

μ_m Maximum specific growth rate, (hr^{-1})

μ_{ma} Apparent maximum specific growth rate, (hr^{-1})

Superscript standing for the fixed biofilm reactor

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