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Ethanol Fermentation of Hemicellulose Hydrolyzate Using High-Level Inocula of a *Pachysolen tannophilus* NRRL Y-2460

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*Pachysolen tannophilus*의 고농도 Inocula를 이용한 Hemicellulose hydrolyzate의 알코올 발효

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초 록

*Hemicellulose hydrolyzate*를 이용한 알코올 발효 과정에서 기질의 inhibition 문제를 해결하기 위해 고농도의 inoculum을 사용하는 방법이 연구되었다. Inoculum의 농도가 25g dry cells/liter에 도달해서야 발효가 진척이 되었으며, 이 경우에 기질은 24시간 이내에 완전히 소모되었다. Furfural은 발효과정중에 *Pachysolen tannophilus*에 의해서 흡수가 되어 furfuryl alcohol로 대사되는 것이 확인 되었으며 furfural 성분에 의한 알콜 생성에 관한 inhibition 영향도는 non-furfural 성분에 의한 것 보다 적은 것으로 판명되었다. Hemicellulose hydrolyzate의 알콜 발효 과정에서 비알콜 생성율은 1*l*당 41.8g xylose와 2.3g의 furfural을 함유한 배지에서의 비알코올 생성율의 14%이었다.

Introduction

A great deal of research has taken place with regard to the hemicellulose utilization. The significant portion of this research has been devoted to microbial conversion of hemicellulose hydrolyzate to ethanol[1~6]. While progress has been made, several important problems still remain unresolved in the area of fermentation of hemicellulose hydrolyzates. Especially notable is the problem of microbial inhibition. In addition to sugars, the hydrolyzate contains a large number of components liberated from wood during the hydrolysis process.

Among those components, furfural has been treated as a potential inhibitor of fermentation[7~11]. However, within the context of interactive inhibitory effects of non-furfural components and fermentation of actual hydrolyzates, published information is scarce, even though ethanol and yeast fermentations of wood sugar have been practised on pilot and commercial scales. This study was undertaken with the intent of seeking an efficient way to ferment hemicellulose hydrolyzate without pretreatment. Of particular interest was the evaluation of high inocula fermentation as a means of alleviating the inhibition problem.

Materials and Methods

1. Preparation of hemicellulose hydrolyzate

Prehydrolysis of Southern Red Oak(Hardwood;

40~100 mesh) was conducted in a 3 L-autoclave (Parr Instrument) for 40 mins under the conditions of 160°C and 1 : 5 solid-water ratio. The prehydrolyzate filtrate was concentrated under vacuum and hydrolyzed again in the autoclave for 1 hr under the conditions of 120°C and pH 1.5.

The hydrolyzate thus prepared contained on the average 41.8g xylose and 2.3g furfural per liter together with unidentified inhibitory compounds.

2. Fermentation

Pachysolen tannophilus NRRL Y-2460 was used throughout this study. Inocula were prepared by growing cells to exponential phase in a medium containing 100g xylose and 6.7g yeast nitrogen base per liter.

A simulated medium contained 41.8g xylose, 2.3g furfural, and 6.7g yeast nitrogen base per liter. The raw hemicellulose hydrolyzate was neutralized to pH 5.5 with lime and filtered. The hydrolyzate was supplimented with 6.7g yeast nitrogen base per liter and filter-sterilized. Thus the hemicellulose hydrolyzate media were used for shaker flask fermentation, together with simulated media.

Fermentations were conducted in 250ml of Erlenmyer flasks with Moton closures, at 32°C, initial pH of 5.5, and at 100 rpm of agitation in a rotary shaker bath.

3. Analytical methods

The cell concentration was determined by dry cell weight. Xylose, xylitol, and furfuryl alcohol contents were measured by an HPLC equipped with RI detector (Waters Associate), using a column packed with ion exchange resin(Bio-Rad, Q-15S). Ethanol concentration was determined by a GC equipped with FID (Varian 3700), using a column packed with Chromosorb 101.

Furfural content was determined by the method described elsewhere (12).

Results and Discussion

1. Inhibitory effect of furfural on ethanol production

Shown in the Figure 1 is a typical run of shaker flask fermentation using the simulated media. In the presence of furfural, the ethanol concentration reached 9.0g/l. From the data on ethanol productivity at various levels of inocula (not shown), it became clear that furfural inhibition is less adverse toward ethanol production at high cell concentrations. A possible reason would be that in the fermentation of simulated media using high cell concentration the furfural was taken up by yeast and catabolized to a less

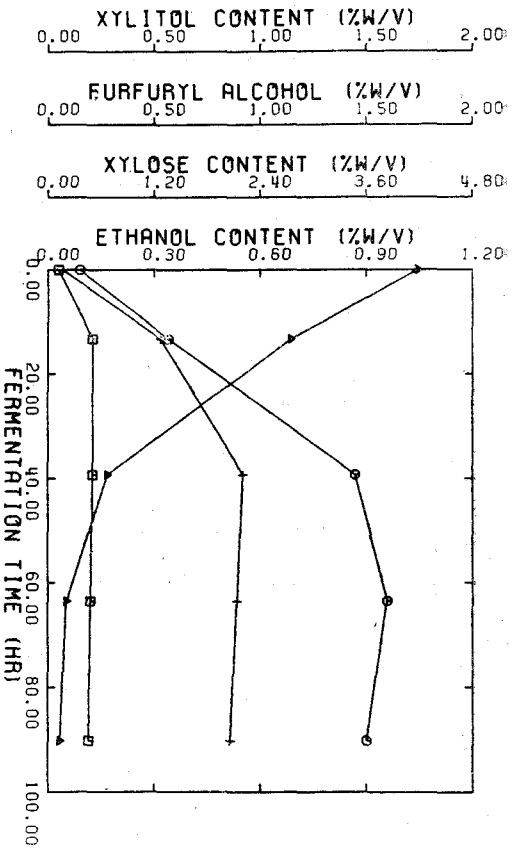

Fig. 1. Xylose fermentation in the presence of 2.3g furfural per liter. Inoculum level, 8.1g dry cells/*l*.
□ : Furfuryl alcohol, ○ : Ethanol,
△ : Xylose, + : Xylitol

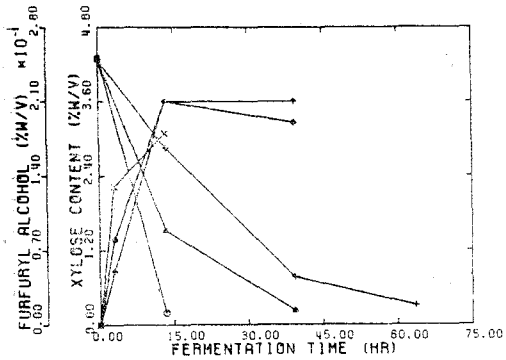

Fig. 2. Furfuryl alcohol formation in the fermentation of xylose containing 2.3g furfural per liter.
+ : Xylose, ↑ : Furfuryl alcohol (run at the level of 8.1g dry cells/liter), △ : Xylose, ◇ : Furfuryl alcohol (run at the level of 17.0g dry cells/liter), ○ : Xylose, × : Furfuryl alcohol (run at the level of 33.9g dry cells/liter)

inhibitory compound. Since yeast takes up furfural, furfuryl alcohol may be formed. Shown in the Figure 2 are the data for the formation of furfuryl alcohol from furfural. One can see that 80% of the furfural was converted to furfuryl alcohol by *P. tannophilus*. This would provide an explanation for the less inhibitory effect of furfural on the ethanol production at high-level yeast inocula.

2. Inhibitory effect of hemicellulose hydrolyzate components on ethanol production

The hemicellulose hydrolyzate contained furfural and nonfurfural components. The toxicity of hemicellulose hydrolyzate was tested in the fermentation of the hydrolyzate using four different levels of *P. tannophilus* (5.7, 13.9, 18, 24g dry cells/liter). In all cases, fermentation proceeded to a certain point and then ceased. From this result, it was clear that hemicellulosed hydrolyzate is inhibitory enough to kill most of the viable cells (below 24g dry cells/liter of cell concentration). The fermentability of hemicellulose hydrolyzate was further examined using 5 different levels of yeast inocula (in excess of 25g dry cells/liter of cell concentration). At this time, fermentation proceeded completely to the end, in all runs, within 24 hours. It appears that the critical cell loading point to make the batch process viable would be 25g dry cells/liter of cell concentration.

3. Batch fermentation of hemicellulose hydrolyzate

Shown in the Figure 3 are the product yield variations with respect to cell concentration. The ethanol yield showed a flat pattern over 30 ~100g dry cells/liter. The xylitol yield, however, increased steadily with the cell concentration. These findings indicate that in the case of higher cell loading, the oxygen supply is the limiting factor (due to cell crowding), rather than toxicity of hemicellulose hydrolyzate. Obviously the higher cell concentration reduces the effect of inhibition; however, the limited oxygen per cell due to increased cell loading, induces reduced ethanol production by a shunt to xylitol formation. Therefore, the benefit of reduced inhibition is by and large offset by the oxygen deficiency under high cell loading. The extent of inhibition was further examined by measurement of the initial specific rate of ethanol production. Shown in the Figure 4 are the profiles of ethanol productivity as related to cell concentration. The figure was constructed in

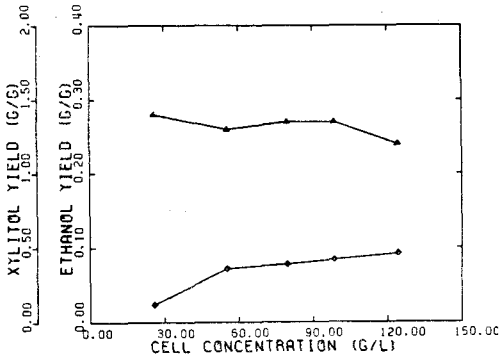

Fig. 3. Product distribution in the fermentation of wood hydrolyzate at high-level inocula.
△ : Ethanol yield, ◇ : Xylitol yield

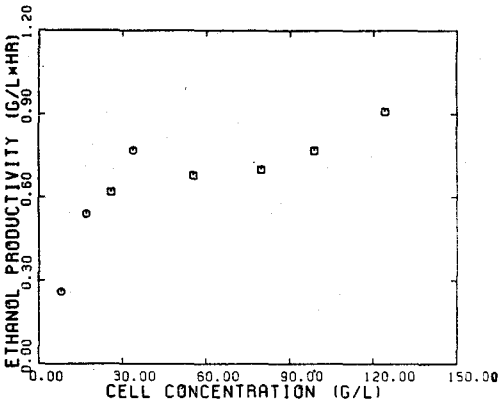

Fig. 4. Ethanol productivity versus cell concentration in the fermentations of wood hydrolyzate and xylose containing furfural

□ : wood hydrolyzate, ○ : xylose media containing furfural

such a way that the slope of the curve would yield a specific ethanol productivity.

The specific rate of ethanol production in the fermentation of hemicellulose hydrolyzate was compared with that for the fermentation of simulated media (Fig. 4). The ratio of the slope estimated from this figure was 0.14 (WHM/SM =0.0027/0.0189=0.14), where WHM=specific ethanol productivity in the fermentation of hemicellulose hydrolyzate and SM=specific ethanol productivity in the fermentation of simulated media.

The extent of total toxicity of non-furfural components was such that the overall fermentation efficiency was reduced by 86%. The implication of this result is that the toxicity of inhibitors in wood hydrolyzate other than furfural is far grater than that of furfural itself.

Abstract

High-level yeast inocula was investigated as a means of overcoming the inhibition problem in ethanol fermentation of hemicellulose hydrolyzate. When the inoculum exceeded 25g dry cells/liter, the fermentation proceeded completely to the end within 24 hours. Furfural was taken up by *Pachysolen tannophilus* and catabolized to furfuryl alcohol. Thus inhibitory effect of furfural component was less adverse toward ethanol production than that of non-furfural components in hemicellulose hydrolyzate. The specific ethanol productivity in the fermentation of hemicellulose hydrolyzate was 14% of that of simulated media containing 41.8g xylose and 2.3g furfural per liter.

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