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에탄올 발효에서 초산 및 아세트알데히드 첨가에 의한 에탄올 수율의 증진

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Improvement of Ethanol Yield by Addition of Acetic Acid and Acetaldehyde in Ethanol Fermentation

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ABSTRACT

The major by-products in ethanol fermentation by Saccharomyces cerevisiae were glycerol, acetaldehyde, acetic acid, lactic acid, and formic acid. The effects of these by-products on the cell growth and ethanol production were studied. By adding acetaldehyde or acetic acid in the fermentation broth, the cell growth decreased while the ethanol production increased. But glycerol and lactic acid had nearly no effects on the cell growth and the ethanol production. Acetic acid and acetaldehyde inhibited the cell growth by diminishing the growth rate as well as by prolonging the lag phase. The ethanol yield increased with the elevation of concentrations of acetic acid and acetaldehyde in the fermentation broth. The maximum ethanol yield was obtained for 3g/ℓ acetic acid and 2g/ℓ acetaldehyde, respectively.

INTRODUCTION

Up to now, many researches have been performed to produce ethanol from biomass. In those fermentatios, yeasts play a key role as an ethanol producer. *S.cerevisiae* converts substrates not only to ethanol, but also to by-products such as acetic acid, acetaldehyde, lactic acid, glycerol, and formic acid[1].

Among those by-products excreted during the ethanol fermentation, acetic acid is well known for its toxicity, being used as an anti-microbial food additive. In the case of yeasts, acetic acid inhibits the cell growth[2], and even induces the death[3]. In recent years, much efforts have been concentrated on the effect of various by-products with a relation to ethanol toxicity. Ramos and Madeira-Lopes showed that acetic acid at concentrations up to 1%(v/v) depressed the tolerance of *S.cerevisiae* to added ethanol and simultaneously shifted the temperature profile of

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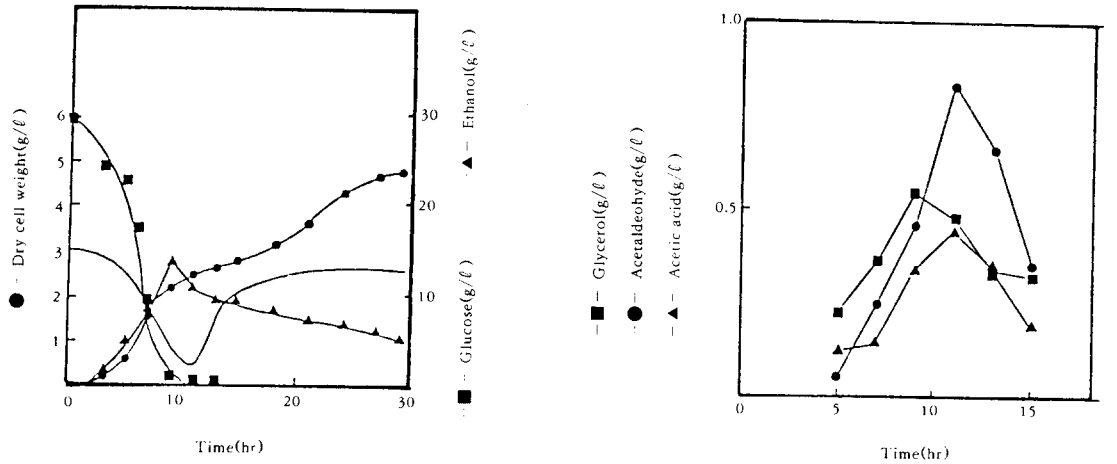

Fig. 1. Time courses of the ethanol fermentation(initial glucose concentration = 30g/ℓ).

growth from 3-42℃ to 19-26℃[4].

Acetaldehyde has recently been shown to be the potential inhibitor of cell growth of *S. cerevisiae* and is currently expected to give a clue for elucidating the ethanol toxicity in yeast alcohol fermentations[5]. Although some researches have been conducted on the effect of by-products on the performance of ethanol fermentation, only limited informations are currently available for the subject.

In the present study, we investigated the effects of various by-products on the cell growth of *S.cerevisiae* and the ethanol production.

MATERIALS AND METHODS

The microorganism used was *S.cerevisiae* ATCC 24858. The growth medium was composed of 20g/ℓ glucose, 5g/ℓ bactopeptone, 3g/ℓ malt extract, and 3g/ℓ yeast extract. The production medium consisted of 100g/ℓ glucose, 5g/ℓ yeast extract, 5g/ℓ $(NH_4)_2SO_4$, 5g/ℓ KH_2PO_4, and 1g/ℓ $MgSO_4 \cdot 7H_2O$. When glucose was used at different concentrations, concentrations of the other components of the medium were changed in proportion to the concentration of glucose.

The 100ml broth in 250ml flask was used as an inoculum after growing in a shaking incubator for 24 hours at 30℃ and 150rpm. Batch cultivation was carried out in a 5ℓ bioreactor(Marubishi, Model MD300) with a working volume of 2ℓ. Air supply was 1 vvm and pH was controlled at 4 during the growth phase. The bioreactor was operated at 30℃ and 400 rpm.

he cell concentration was estimated by measuring optical density at 525nm. Concentrations of glucose, ethanol, glycerol, acetaldehyde, acetic acid, and other by-products were measured by high performance liquid chromatography(HPLC : Waters Associates Co.). The HPLC analyses were performed with a Fermentation Monitoring Column (Bio-Rad Laboratories) with refractive index detector.

RESULTS AND DISCUSSION

Fermentation Broth Analysis

Table 1 shows the results of the analysis of the fermentation broth during the exponential growth phase in the batch fermentation. In the fermentation broth, a large amount of glycerol, acetaldehyde, and acetic acid were produced compared to other by-products. Each concentration of the by-products excreted was 0.54g/ℓ of glycerol, 0.45g/ℓ of acetaldehyde, 0.33g/ℓ of acetic acid, 0.04g/ℓ of lactic acid, and 0.03g/ℓ of formic acid. In general,

Table 1. The amount of the by-products produced after 9hr of the ethanol fermentation by *S.cerevisiae*.

Products	Conc.(g/ℓ)	Product/Glucose(%)
Ethanol	13.70	45.67
Glycerol	0.54	1.80
Acetaldehyde	0.45	1.50
Acetic acid	0.33	1.10
Lactic acid	0.04	0.13
Formic acid	0.03	0.10

Table 2. Effect of the initial concentration of added by-products on the cell growth and the ethanol production.

By-product	Maximum dry cell weight (g/ℓ)	Maximum ethanol conc. (g/ℓ)
Control	4.6	34
Acetic acid	3.8	41
Acetaldehyde	4.0	39
Formic acid	n	n
Lactic acid	4.5	33
Glycerol	4.6	35

* initial concentration of by-product = 2.0g/ℓ
initial glucose concentration = 100g/ ℓ
* n: negligible

side reactions are known to occur in ethanol fermentations which may consume up to 4~5% of the total substrate used. If these reactions could be eliminated, an additional 2.7% of ethanol yield on carbohydrates would be obtained[6].

Fig. 1 shows the time courses of cell growth, ethanol production, glucose consumption, and by-products formation during the batch fermentation. After 9 hours, glucose was completely consumed, and the consumption of by-products excreted was commenced. Ethanol and glycerol began to be consumed first, and later acetic acid and acetaldehyde were consumed. The amount of by-products produced depends generally on the raw materials used and the fermentation condition in ethanol fermentations[7].

Effect of By-products

Table 2 shows the effects of added by-products on the cell growth and the ethanol production during the batch fermentation(initial concentration of the by-product was 2g/ℓ). By adding each by-product in the fermentation broth, the cell growth decreased while the ethanol production increased. It was found that acetic acid or acetaldehyde inhibited the cell growth by reducing the growth rate as well as by prolonging the lag phase. The order of the cell inhibition was as follows: formic acid> acetic acid> acetaldehyde> lactic acid> glycerol. The experiment with 2g/ℓ formic acid showed the negligible cell growth and ethanol production for more than 50 hours. Glycerol and lactic aci had nearly no effects on the cell growth and ethanol production. By adding acetic acid and acetaldehyde, the lag phase increased whereas the time to reach the glucose depletion decreased.

Table 3. Effect of initial concentration of acetic acid and acetaldehyde on the ethanol fermentation.

	Initial conc. (g/ℓ)	Maximum cell conc. (g/ℓ)	Maximum ethanol conc. (g/ℓ)
Acetic acid	0	4.6	34
	1	4.2	36
	2	3.8	41
	3	3.1	44
	4	2.3	36
Acetaldehyde	0	4.6	34
	1	4.4	35
	2	4.0	39
	3	3.6	37
	4	2.8	37

* initial glucose concentration = 100g/ℓ

Higher ethanol concentration were obtained with acetic acid and acetaldehyde, respectively. For this reason, the effects of concentrations of acetic acid and acetaldehyde added were further studied. As summarized in Table 3, there are optimal concentrations of acetic acid and acetaldehyde which maximize the ethanol production. Fig. 2 obtained from the culture data shows that higher apparant ethanol yields could be obtained by adding 3g/ℓ acetic acid and 2g/ℓ acetaldehyde,

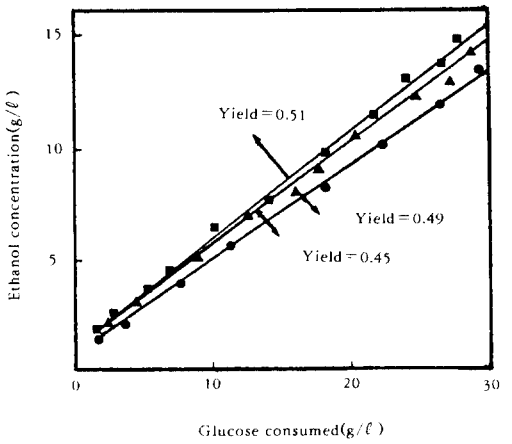

Fig. 2. The apparent ethanol yields in the batch fermentation:
—●—;control, —▲—;acetaldehyde(2g/ℓ), —■—;acetic acid(3g/ℓ)

respectively. In the ethanol production by yeast, the theoretical maximum yield of ethanol is 0.51 [8]. It has been reported that the presence of acetic acid in the fermentation broth might increase the maintenance requirement of the cell and thus repress the cell growth while ethanol production increases to make available sufficient ATP for cell maintenance[8]. Acetaldehyde added seems to alleviate the ethanol toxicity by synergistic effects, even though details are at present unknown [9].

Since acetic acid or acetaldehyde can be easily obtained from the distillation process during the recovery of ethanol, this results can be thus easily applied for industrial ethanol productions.

요 약

*S.cerevisiae*에 의한 에탄올 발효에서 생성되는 부산물인 acetic acid, acetaldehyde, glycerol, lactic acid, formic acid가 세포성장과 에탄올 생성에 미치는 영향을 고찰하였다. Acetic acid와 acetaldehyde를 발효액 내에 투입하였을 때, 세포성장은 저해되었으나, 에탄올 생성은 증가되었다. 한편, glycerol과 lactic acid는 세포성장과 에탄올 생산에 거의 영향이 없었다. Acetic acid와 acetaldehyde는 비성장속도를 줄임과 동시에 정체기를 늘임으로써 세포성장을 저해하였다. 에탄올 수율은 첨가된 acetic acid와 acetaldehyde 농도에 비례하여 증가하였고, acetic acid 3g/ℓ, acetaldehyde 2g/ℓ 일 때, 최대가 되었다.

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