한국농화학회지 제 39권 제 1호(1996)
Agricultural Chemistry and Biotechnology
Vol. 39, No. 1, pp. 1~8(1996)

The Roles of Lipid Supplements in Ethanol Production Using a Continuous Immobilized and Suspended Cell Bioreactor

Gwang-Hoon Gil*

[1]R&D Center, Cheil Foods & Chemicals Inc., 522-1 Tokpyong-ri, Majang-myon, Ichon-gun. Kyonggi-do, Korea

Abstract : A one-stage, continuous-flow bioreactor with both immobilized and suspended cells was used to investigate the roles of lipid supplements in ethanol production by *Saccharomyces cerevisiae*. The reactor performance and the level of alcohol dehydrogenase(ADH) activities of the suspended cells, grown under various conditions, were measured. When ergosterol and/or oleic acid were added with surfactants to the yeast culture grown under non-aerated conditions, remarkable increases in ethanol production and cell growth was achieved, but specific ADH activities were not affected. Especially, no difference of specific ADH activities of the suspended cells grown under aerated and non-aerated condition was observed. The addition of the surfactant as a supplement also resulted in significant increases in ethanol production, cell growth, and specific ADH activity. When ergosterol and oleic acid were added to the yeast culture exposed to higher ethanol concentration(>40 g/*l*) level, ethanol production, cell growth, and specific ADH activity were increased, but the addition of surfactant was as effective as at lower ethanol concentration level. The results indicated that lipid supplements were more effective at higher ethanol concentration level than at lower ethanol concentration level during ethanol production. ADH isozyme patterns of the yeast cultures grown under various conditions on starch gel electrophoresis showed only one major band, probably ADH I. The migrating distance of the major isozyme, however, varied slightly according to the culture conditions of the cells. No apparent correlation was found between specific ADH activity and amount of ethanol produced. Cell mass was more important factor for ethanol production than specific ADH activity of the cells(Received November 10, 1995; accepted January 3, 1996).

Introduction

Various attempts to improve ethanol productivity have been tried from studies that supplemented fermentation media with nutrients such as ergosterol and vegetable oil,[1-4] short and long chain fatty acids,[5] protein extracts,[6-8] and a proteolipid supplement from Koji mold,[9,10] to those studies which cultivated the yeast in the presence of oxygen.[11-16] Very interesting results were obtained from these studies but the extract mechanisms of action are still not well understood. It is widely accepted that the alteration in the yeast membrane lipid composition (ergosterol, unsaturated fatty acids) via controlled nutrient and fermentation processes is the primary factor that controls ethanol productivity and levels of ethanol tolerance.[17-19] *S. cerevisiae* is known as a facultative anaerobe but oxygen is required for the cell to maintain adequate levels of ergosterol and unsaturated fatty acyl components in plasma the membrane.[20-28] The increase in the membrane unsaturated/saturated fatty acids ratio is widely recognized as a factor that leads to ethanol tolerance. Additionally, ethanol tolerance appears to be related to a high ratio of sterol/phospholipids in the membranes of many microbial systems.[29-33]

There are many membrane-bound enzymes of yeast cell whose activities are affected or modulated by the lipid environment in yeast membrane.[34] Ergosterol is one of those lipid components to affect enzyme activity such as phosphatidyl inositol kinase.[35] Although the ethanol tolerance of yeast cells is relevant to the cellular lipid composition, the ethanol tolerance of various yeast strains has been also shown to be related to alcohol dehydrogenase (ADH) content, with tolerant strains having 1.4 to 2.0 times higher amounts of this enzyme.[36] Furthermore, it was proposed that ergosterol could play a role in regulating the activity of the enzyme, affecting the transfer of electrons to NAD^+ during fermentation.[33]

The roles of lipid supplements on alcohol production were studied in an one-stage, immobilized and suspended cells, continuous-flow bioreactor. This study was conducted on the basis of the proposal by Bloch[37] that ergosterol, one of major sterol components in yeast cell

Key words : *Saccharomyces cerevisiae*, ethanol production, continuous bioreactor, alcohol dehydrogenase, ergosterol, oleic acid
*Corresponding author

membrane, might have metabolic regulatory function. The experiment were designed to identify if a relationship exist between ergosterol levels, alcohol dehydrogenase (ADH) activity, and the bulk function that was proposed to modulate membrane fluidity. Oleic acid was also tested in this experiment because of its proposed requirement for alcohol production, membrane integrity, and tolerance to alcohol.

Materials and Methods

Strain

Several strains of the yeast *Saccharomyces cerevisiae* were isolated from an industrial ethanol fermentation facility (Usina Acucareira Ester) located in Cosmopolis, Brazil. The yeast strain FZ used in this study was selected based on its high tolerance to both ethanol and sucrose which was determined by measuring both viability and growth in respective concentration gradients. Yeast cultures were maintained on an agar medium containing 20 g/*l* sucrose, 10 g/*l* yeast extract (BBL, Cockeysville, MD, USA), and 15 g/*l* Bacto-agar (Difco Labs, Detroit, MI, USA).

Bioreactor system and operation

Bioreactor system consisted of a one-stage, immobilized and suspended bioreactor system with continuous aeration, zoned liquid recycle flow, and continuous feed introduced in an upward flow. The reactor was water-jacketed glass column (5.5×43 cm) of 540 m*l* total volume. A ceramic-like matrix material for cell attachment was inserted into the reactor and occupied approximately 30% of the total reactor volume. The immobilization matrix was an experimental aluminum silicate material with mean porosity of 21.7 μm that was supplied by Manville Corp (Denver, CO, USA). The matrix was arranged in a vertical cellular channel configuration. More detailed description of the system and its operational parameter has been reported in the previous studies.[16,28] Yeast cells were immobilized by circulating yeast cell suspension (approximately 58 g-dry cell weight/*l*) through the sterile bioreactor for 10 h at a flow rate of 1 *l*/h. After the initial period of cell immobilization, the continuous fermentation was started by delivery of synthetic sugar-cane medium consisting of sucrose, 100 g; $(NH_4)_2SO_4$, 1.8 g; KH_2PO_4, 1.2 g; $MgSO_4 \cdot 7H_2O$, 0.4 g; $CaCl_2 \cdot 2H_2O$, 0.04 g; $MnSO_4 \cdot H_2O$, 7.5 mg; $ZnSO_4 \cdot H_2O$, 7.5 mg; and yeast extract (BBL), 5.0 g, per liter, pH 5.6. Varying amounts of ergosterol and oleic acid were added with surfactant to a synthetic sugar-cane medium. Stock solution of ergosterol, 1.25 g/*l* in a mixture of tyloxapol (a surfactant of alkyl aryl polyether alcohol type)-ethanol (1 : 3, by volume), and oleic acid, 30 g/*l* in a mixture of tergitol (a surfactant of polyglycol ether type)-ethanol (1 : 1, by volume), were prepared. Ergosterol was added to the feed in a concentration range from 0.5 to 5 mg/*l*; the oleic acid was added from 5 to 50 mg/*l*. When ergosterol and/or oleic acid were added to the feed, no aeration was provided and nitrogen gas was supplied at a flow rate of 10 m*l*/min. The control experiments were conducted under aerated (air 30 m*l*/min) and non-aerated (nitrogen 10 m*l*/min) conditions without ergosterol or oleic acid supplementation. To test the effect of lipid supplements at higher ethanol concentration, absolute ethanol was added to the medium to give an initial ethanol concentration of 40 g/*l*. The effects of the dilution rate was tested in a range from 0.125 to 0.25 h^{-1}. Suspended cells were collected after the reactor reached steady-state under each experimental condition. The collected cells were centrifuged, washed twice with a physiological saline solution and after recentrifugation, stored at −70℃. Ergosterol, oleic acid, tergitol and tyloxapol were purchased from Sigma Chemical Co. (St. Louis, MO, USA).

Cell-free extract preparation

Frozen cells were slowly thawed at room temperature, and resuspended in 50 mM Tris-HCl (pH 7.5) buffer solution to obtain approximately 20 g dry cell weight/*l*. The resulting cell suspension was passed twice through a French press (8000 psi). The lysate was centrifuged at 10,000×g for 20 min to remove cell debris. The supernatant was recovered and used for enzyme analysis and gel electrophoresis. The protein content of the cell-free extract was measured with Sigma protein kit (Sigma Chemical Co., St. Louis, MO) against crystalline serum albumin.

Alcohol dehydrogenase assay

Alcohol dehydrogenase activity was assayed by the method of Gokhale *et al.*[36] with minor modifications. This method is based on the absorption of NADH at 340 nm at 25℃. The assay mixture contained Tris-HCl (20 mM, pH 8.6), NAD (1 mM) in a total volume of 3.0 m*l*. The reaction was initiated by the addition of cell-free extract (0.5~1.0 m*l*) followed by ethanol (0.56 M). The change in absorbance at 340 nm was monitored every 15 sec for 2 min. The increase in absorbance between the 15 sec and 45 sec readings multiplied by two was taken as enzyme activity per minute. Therefore, one unit of enzyme activity was defined as the change in absorbance per minute. Specific enzyme activity was expressed as unit of enzyme activity per mg of protein.

Gel electrophoresis

Electrophoresis of the cell-free extract was performed

using the vertical starch gel method as described by Lutstorf and Megnet.[38] The 11% hydrolyzed starch gel (14×13.5×0.3 cm, Sigma Chemical Co., St. Louis, MO, USA) was prepared in Tris-citrate buffer (Tris, 9.2 g and citric acid, 1.5 g per liter. pH 8.4). The electrode buffer was the same as that used for the gel preparation. The same amount of protein (160 μg) from each sample was loaded with 0.1% bromothymol blue loading buffer (pH 8.4). Separation, using vertical slab gel unit (Model SE 400, Hoeffer Scientific Instruments, S.F., CA, USA), was routinely carried out at 4℃ for about 20 h with a constant voltage of 84 V. Under these conditions, the fastest moving band migrated approximately 8 cm toward the anode. Alcohol dehydrogenase activity was visualized by incubating the starch gel at 37℃ for 1 h in the dark with the following solution: 20.0 mg nitrotetrazolium blue (NTB), 50 mg NAD, 1.25 mg phenazine methosulfate (all from Sigma Chemical Co., St. Louis, MO, USA), and 1.46 m*l* ethanol dissolved in 50 m*l* of 60 mM Tris-HCl buffer (pH 8.6). Protein bands were visualized by the modified method of Smithies.[39] The gel was stained overnight with 0.25% coomasie blue in 10% TCA solution, and subsequently destained in 7% acetic acid solution for 24 h.

Analytical methods

Ethanol was quantified using a Varian 3700 gas chromatograph equipped with a flame ionization detector (FID) and a 2 m glass column (4 mm I.D.) packed with Porapak Q (80/100 mesh). The detector was operated at 220℃ and the column temperature at 170℃. Injection temperature was 210℃. Helium was used as carrier gas at a flow rate of 20 m*l*/min. n-Butanol was used as an internal standard. Results were quantified with a Vista series 402 integrator. Carbohydrates were analyzed using a Hewlett Packard 1090 high performance liquid chromatograph equipped with an Econosphere Amino column (Alltech Associates, Inc., Deerfield, IL, USA) and a Hewlett Packard 1037A refractive index detector. The mobile phase was acetonitrile/water (3 : 1, by volume, HPLC grades) at a flow rate of 1 m*l*/min. The dry cell weight of yeast culture was determined gravimetrically after drying at 105℃.

Results

Effect of ergosterol and oleic acid

Respiratory competent cells were maintained with air supplied at a flow rate of 30 m*l*/min in aerated system. To show the effects of ergosterol and oleic acid, the lipids were added to the synthetic sugar-cane medium, and the air was replaced with 10 m*l* of nitrogen/min. Table 1 and Fig. 1 show the effect of ergosterol and oleic acid on specific ADH activity, cell growth, ethanol production and total sugar consumption during continuous ethanol production. In the control experiment with no lipid supplement, there was no difference in the specific ADH activity between the cells grown under non-aerated and aerated conditions, but the protein content of the cells grown under the non-aerated condition was two times greater than that of the aerated system. The highest cell growth (42.2 g-dcw/*l*) of the suspended cells was observed in aerated condition, but it was only 1.6 g-dcw/*l* in non-aerated condition. Ethanol production (48.4 g/*l*) was also much higher in the aerated condition than the non-aerated condition (17.7 g/*l*). When a small amount of ergosterol (0.05 mg/*l*) and oleic acid (5 mg/*l*) was added, the specific ADH activity was not affected

Table 1. Effect ergosterol and oleic acid on continuous ethanol production.

Condition[a]	Specific ADH activity (U/mg-protein)	Protein content (%)	Cell mass (g-dcw/*l*)	Actual ethanol production (g/*l*)	Final ethanol conc. (g/*l*)	% sugar consumption	Ethanol yield (g-ethanol/g-sugar)
Non-aerated condition	2.130	37.1	1.6	17.7	17.7 (±0.2)	43.3	0.409
Oleic acid (50 mg/*l*) plus ergosterol (5 mg/*l*) supplement	2.272	16.4	21.0	41.1	44.1 (±1.4)	89.0	0.462
Ergosterol (5 mg/*l*) supplement	1.722	20.1	21.6	38.4	41.4 (±1.1)	86.5	0.442
Oleic acid (50 mg/*l*) supplement	2.452	17.9	17.7	36.0	39.0 (±1.7)	76.1	0.477
Aerated condition	2.196	18.4	42.2	48.4	48.4 (±1.0)	97.8	0.494

Results are steady-state determinations. Value in the parenthesis of final ethanol concentration is standard deviation of the mean. Values in specific ADH activity and protein content are average values from three independent measurements, respectively. One unit of ADH activity was defined as the4 change os absorbance per minute. Ethanol yield was calculated based on amount of sugar consumed. Cell mass was determined as gram dry cell weight/liter. Actual ethanol production was obtained from final ethanol concentration by subtracting initial ethanol concentration. Bioreactor operation conditions: 100 g/*l* sucrose feed, 0.25 h^{-1} dilution rate. [a]Nitrogen was suppload to the reactor at a flow rate of 10 m*l*/minm except aerated condition. Air was supplied to the reactor at a flow rate of 30 m*l*/min under aerated condition.

and its level remained almost the same as that of the cells grown under the non-aerated condition. The specific ADH activity, however, dropped significantly with the addition of ergosterol (0.1 mg/*l*) and oleic acid (10 mg/*l*) and thereafter, it increased proportionally to the amounts of ergosterol and oleic acid added to the synthetic sugarcane medium. Ethanol concentration, cell growth and sugar consumption also increased proportionally, but protein content and ethanol yield remained relatively constant, regardless of the amounts of ergosterol and oleic acid added (Fig. 1). Maximum ethanol production (41.1 g/*l*), which was comparable to ethanol production (48.4 g/*l*) in aerated condition, was obtained with the addition of relatively large amounts of ergosterol (5 mg/*l*) and oleic acid (50 mg/*l*). When only ergosterol (5 mg/*l*) was added (no oleic acid), the specific ADH activity decreased slightly as compared to that with the addition of both lipids, but when only oleic acid (50 mg/*l*) was added (no ergosterol), the specific ADH activity increased slightly. Nevertheless, the ethanol concentrations in both conditions remained approximately the same (Table 1).

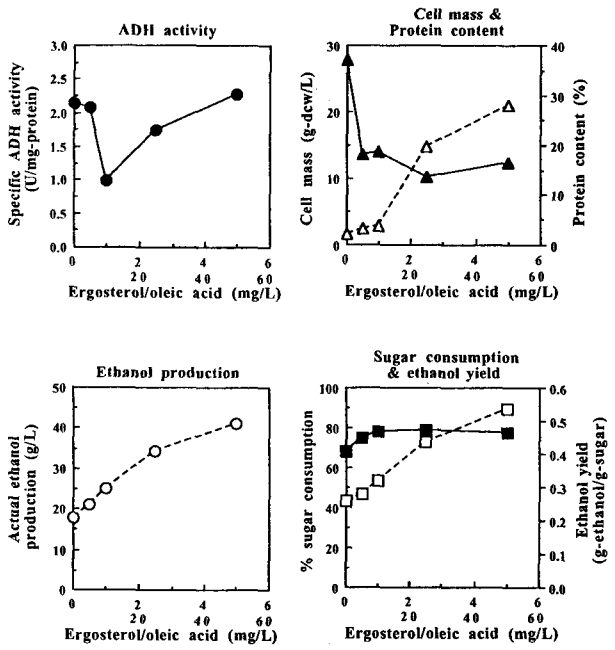

Fig. 1. Effect of ergosterol and oleic acid supplement on continuous ethanol production. ●—●, specific ADH activity; ○—○, actual ethanol production; ▲—▲, protein content; △—△, cell mass; ■—■, ethanol yield; □—□, % sugar consumption. Refer to Table 1 for details.

Effect of surfactant

The effects of surfactants (tergitol and tyloxapol) were also measured. The results are presented in Table 2. When only surfactants [4 m*l* of tyloxapol-ethanol mixture (1 : 3, by volume) and 1.68 m*l* of tergitol-ethanol mixture (1 : 1, by volume)] were added, the specific ADH activity of the cells increased from 2.130 to 3.639 U/mg-protein. Ethanol production also increased from 17.7 to 30.3 g/*l*. However, when the amounts of the surfactants added were doubled, the specific ADH activity decreased significantly from 3.639 to 1.903 U/mg-protein and the actual

Table 2. Effect of surfactant on continuous ethanol production.

Condition[a]	Specific ADH activity (U/mg-protein)	Protein content (%)	Cell mass (g-dcw/*l*)	Actual ethanol production (g/*l*)	Final ethanol conc. (g/*l*)	% sugar consumption	Ethanol yield (g-ethanol/g-sugar)
No surfactant supplement	2.130	37.1	1.6	17.7	17.7 (± 0.2)	43.3	0.409
Surfactant (1X) supplement[b]	3.369	25.9	11.1	30.3	33.0 (± 0.8)	66.6	0.455
Surfactant (2X) supplement[c]	1.930	18.1	8.8	26.8	32.8 (± 1.6)	64.3	0.410

Results are steady-state determinations. Bioreactor operation conditions: 100 g/*l* sucrose feed, 0.25 h^{-1} dilution rate. [a]Nitrogen was supplied to the reactor at a flow rate of 10 m*l*/minm except aerated condition. [b]Same amounts of surfacants (tergitol and tyloxapol) were added as in oleic acid (50 mg/*l*) plus ergosterol (5 mg/*l*) supplement, and then doubled[c]. Refer to Table 1 for details.

Table 3. Effect of ergosterol, oleic acid and surfactant on continuous ethanol production at higher ethanol concentration level.[a]

Condition[b]	Specific ADH activity (U/mg-protein)	Protein content (%)	Cell mass (g-dcw/*l*)	Actual ethanol production (g/*l*)	Final ethanol conc. (g/*l*)	% sugar consumption	Ethanol yield (g-ethanol/g-sugar)
Non-aerated condition	1.350	21.0	1.0	5.5	45.5 (± 1.5)	21.2	0.259
Oleic acid (50 mg/*l* plus Ergosterol (5 mg/*l*) supplement	2.432	19.2	8.9	21.7	64.7 (± 1.1)	44.9	0.530
Surfactant (1X)[c]	0.826	14.0	4.4	7.7	50.7 (± 1.2)	25.9	0.298
Aerated condition	2.507	21.1	32.9	34.5	74.5 (± 1.7)	79.7	0.433

Results are steady-state determinations. Bioreactor operation conditions: 100 g/*l* sucrose feed, 40 g/*l* initial ethanol concentration[a], 0.25 h^{-1} dilution rate. [b]Nitrogen was supplied to the reactor at a flow rate of 10 m*l*/minm except aerated condition. Air was supplied to the reactor at a flow rate of 30 m*l*/min under aerated condition. [c]Same amounts of surfacants (tergitol and tyloxapol) were added as in oleic acid (50 mg/*l*) plus ergosterol (5 mg/*l*) supplement, and then doubled. Refer to Table 1 for details.

ethanol production also decreased from 30.3 to 26.8 g/*l*, even though the final ethanol concentration was not changed. It was reported that the surfactant interacts with the hydrophobic portion of the membrane, affects membrane permeability and possibly improves sugar and other nutrients transports.[40] That might be the reason why cell growth and ethanol production were increased in this study. However, it is not clear why the specific ADH activity was increased significantly with the addition of surfactants in this study.

Effect of ergosterol, oleic acid and surfactant supplement at higher ethanol concentration level.

The previous studies were performed in a one-stage reactor system at relatively low ethanol concentration. To test the effects of lipid supplements on cells exposed to higher ethanol levels, absolute ethanol was added to the synthetic sugar-cane medium to give 40 g/*l* of initial ethanol concentration level. As shown in Table 3, the final ethanol ethanol concentration was 45.5 g/*l* at 0.25 h^{-1} dilution rate under the non-aerated condition without lipid supplement. Under these conditions, the actual ethanol production from the fermentation process was only 5.5 g/*l*. Additional increases in the actual ethanol production as well as the cell growth were observed when ergosterol and oleic acid were supplemented to the synthetic sugar-cane medium, although the increases were much smaller than those at lower ethanol concentration. The surfactant supplement alone didn't show any effect on actual ethanol production and the result was the same as the control. Thus, the response of the cells to the surfactant occurred only at lower ethanol concentration level.

The specific ADH activity of the cells grown under aerated condition at higher ethanol concentration level was 2.507 U/mg-protein, which was higher than 2.190 U/mg-protein of the cells grown under aerated condition without the initial ethanol addition. The specific ADH activity of non-aerated condition, however, decreased to 1.305 U/mg-protein. Moreover, when only surfactants were supplemented, it dropped to 0.826 U/mg-protein, but when ergosterol (5 mg/*l*) and oleic acid (30 mg/*l*) were added, it was maintained as 2.342 U/mg-protein, which was similar to the level of the specificity ADH activity of the aerated condition. These results suggested that ergosterol and oleic acid supplements were more effective at higher ethanol concentration level than at lower ethanol concentration level for ethanol production.

Gel electrophoresis of alcohol dehydrogenase

In order to find out if ADH isozyme patterns of the yeast cells grown under the various experimental conditions, starch gel electrophoresis analyses of cell extracts were conducted. As shown in Fig. 2.a, each sample had only one major band indicating the absence of isozymes. It was previously reported that at least three isozymes (ADH I, II, III) existed in *S cerevisiae*.[38,41] ADH I, however, is a classical fermentative enzyme responsible for ethanol formation during anaerobic condition whereas ADH II and III are concerned with ethanol metabolism in aerobic condition. Accordingly, the one major band found (Fig. 2 a-b) was tentatively identified as ADH I isozyme because of the anaerobic conditions under which the cells were grown. When ergosterol and oleic acid were added to the culture, the major ADH isozyme band migrated farther toward the anode from origin than those from cells not receiving the lipid supplement. The

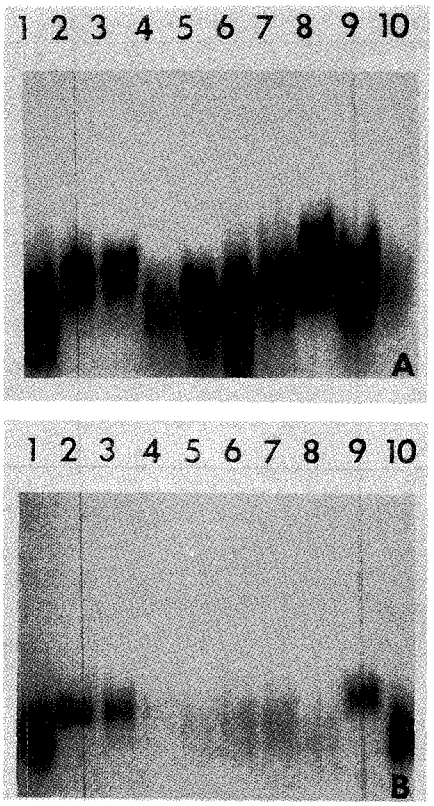

Fig. 2. Starch gel electrophoresis for measuring alcohol dehydrogenase activity. Details are in Materials and Methods. (a) 1, ADH standard; 2, non-aerated condition; 3, aerated condition; 4, ergosterol plus oleic acid supplement; 5, only surfactant supplement; 6, ADH standard; 7, non-aerated condition (40 g/*l* initial ethanol concentration); 8, aerateed condition '(40 g/*l* initial ethanol cencentration); 9, ergosterol plus oleic acid supplement (40 g/*l* initial ethanol cencentration); 10, only surfactane supplement (40 g/*l* initial ethanol cencentration). (b) 1, ADH standard; 2, non-aerated condition; 3, ergosterol (0.5 mg/*l*) plus oleic acid (5 mg/*l*) supplement; 4, ergosterol (1 mg/*l*) plus oleic acid (10 mg/*l*) supplement; 5, ergosterol (2.5 mg/*l*) plus oleic acid (25 mg/*l*) supplement; 6, ergosterol (5 mg/*l*) plus oleic acid (50 mg/*l*); 7, only oleic acid (50 mg/*l*) supplement; 8, only ergosterol (5 mg/*l*) supplement; 9, aerated condition; 10, ADH standard.

results suggest that the surfactant molecules interact with the ADH I protein, changing its electrophoretic properties. The ADH isozyme patterns from the cells treated with varing amounts of ergosterol and oleic acid are given in Fig. 2.b. The trend for an increase in intensity of the ADH activity on the gel was consistent with that of the total ADH activity shown in Fig. 1. When the amount of ergosterol and oleic acid supplement was increased gradually, the ADH isozyme patterns changed only slightly.

Discussion

Ergosterol, oleic acid and surfactant stimulated cell growth under non-aerated condition, but their effects wasn't enough to be equal to the aeration effect. The possible explanation is that the lipids and surfactants increased membrane permeability, enhanced nutrient transports including sugar uptake, and especially with ergosterol, stabilized the membrane structure.[40] From the data of the experiments to measure the relationship between lipid supplements and ADH activity, there was no significant difference in the specific ADH activity of the cells cultivated under the aerated and non-aerated condition, or under non-aerated condition with ergosterol and oleic acid supplement at relatively lower ethanol concentration. At higher ethanol concentration (>40 g/l), there was a difference in the specific ADH activity of the cells grown under the aerated and the non-aerated condition. The specific ADH activity of the cells grown with the supplementation of ergosterol and oleic acid was almost as high as that of the aerated condition. The specific ADH activity of the cells supplemented with only surfactant dropped remarkably at higher ethanol concentration. Thus, a complex array of interactions seems to occur between membrane components that affect transports, metabolic activities and membrane stability.

ADH might be one of many enzymes whose activities are effected by the lipid environment of plasma membrane. From the results of this study, it can only be suggested that ergosterol and oleic acid stabilize the membrane structure of yeast cells and maintain the ADH activity at high ethanol concentration level. However, according to results on the starch gel electrophoresis, ergosterol showed no evidence for the regulatory roles to control the ADH isozyme patterns and activity during ethanol production. The most possible function of ergosterol might be related to the bulk function to control the physical state of the yeast membrane. According to the results presented in this study, the high ADH activity of yeast cells wasn't necessarily translated to high ethanol production. The cell growth was apparently a more important factor for ethanol production. This relationship is clearly expressed in Fig. 3 a-b.

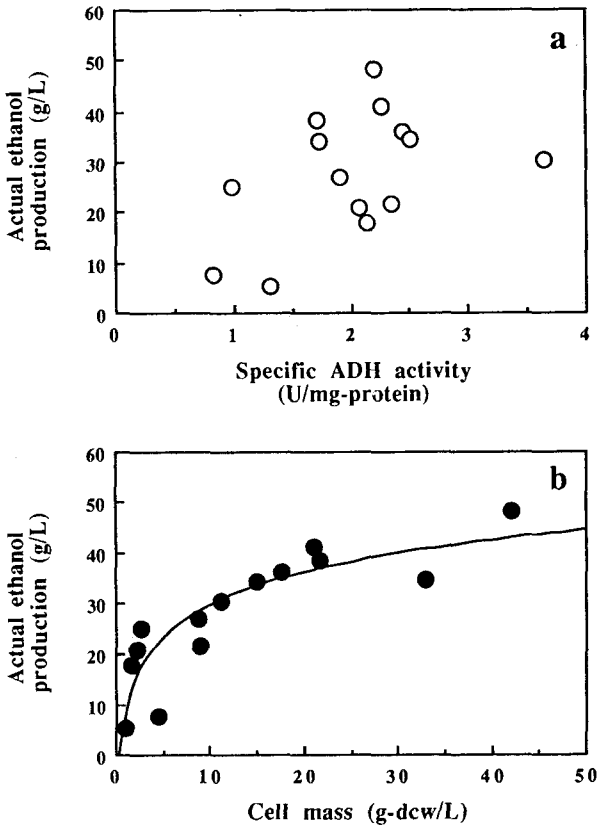

Fig. 3. Relationship of specific ADH activity of yeast cells and cell mass of the bioreactor to ethanol production. (a) specific ADH activity versus ethanol procuction, (b) cell mass versus ethanol production.

Acknowledgements

Author gratefully thanks Dr. Thomas G. Tornabene, School of Biology, Georgia Institute of Technology, Atlanta for critically reviewing the manuscript.

References

1. Hayashida, S. and K. Ohta (1980) Effect of phosphatidyl choline or ergosterol oleate on physiological properties of *Saccharomyces sake*. *Agr. Biol. Chem.* **44**, 2561-2567.
2. Traverso-Reuda, S. and R. E. Kunkee (1982) The roles of sterols on growth and fermentation of wine yeasts under vinification conditions. *Dev. Ind. Microbiol.* **23**, 131-143.
3. Ohta, K. and S. Hayashida (1983) Role of tween 80 and monoolein in a lipid-sterol-protein complex which enhances ethanol tolerance of sake yeast. *Appl. Environ. Microbiol.* **46**, 821-825.
4. Saigal, D. and L. Viswanathan (1984) Effect of oil and fatty acids on molasses fermentation by distillers' yeast. *Enz. Microb. Technol.* **6**, 78-80.
5. Watson, K. (1982) Unsaturated fatty acid but not ergosterol is essential for high ethanol production in *Saccharomyces*

cerevisiae. Biotechnol. Letts. **4**, 397-402.
6. Viegas, C. A., I.Sa-Correia and J. M. Novais (1985) Nutrient-enhanced production of remarkably high concentrations of ethanol by *Saccharomyces bayanus* through soy flour supplementation. *Appl. Environ. Microbiol.* **50**, 1333-1335.
7. Bajpai, P., A. Sharma, N. Raghuram and P. K. Bajpai (1988) Rapid production of ethanol in high concentration by immobilized cells of *Saccharomyces cerevisiae* through soy flour supplementation. *Biotechnol. Letts.* **10**, 217-220.
8. Patil, S. G., D. V. Gokhale and B. G. Patil (1989) Novel supplements enhance the ethanol production in cane molasses fermentation by recycling yeast cells. *Biotechnol. Letts.* **11**, 213-216.
9. Hayashida, S., D. D. Feng and M. Hongo (1975) Physiological properties of yeast cells grown in the proteolipid-supplemented media. *Agr. Biol. Chem.* **39**, 1025-1031.
10. Hayashida, S. and K. Ohta (1978) Cell structure of yeast grown anaerobically in *Aspergillus oryzae* -proteolipid-supplemented media. *Agr. Biol. Chem.* **42**, 1139-1145.
11. Ryu, D. D. Y., Y. J. Kim and J. H. Kim (1984) Effect of air supplement on the performance of continuous ethanol fermentation system. *Biotechnol. Bioeng.* **26**, 12-16.
12. Hamamci, H. and D. D. Y. Ryu (1988) Effects of oxygen on ethanol fermentation in packed-bed tapered-column reactor. *Appl. Microbiol. Biotechnol.* **28**, 515-519.
13. Lisyuk, G. M., and L. V. Permyakova (1988) Sterol formation on yeast *Saccharomyces cerevisiae* in dependence on continuous of aeration. *Priklandnaya Biokhimia i Mikrobiologiya* **24**, 660-664.
14. Farleigh, S., H. R. Bungay and L. S. Clesceri (1990) Aerobic formation of ethanol by *Saccharomyces cerevisiae* in a computerized pH auxostat. *J. Biotechnol.* **13**, 61-72.
15. Ryu, Y. W. and H. W. Jang (1991) Effect of oxygen and unsaturated fatty acids on the ethanol tolerance of yeast strains. *J. Microbiol. Biotechnol.* **1**, 6-11.
16. Gil, G. H, W. J. Jones and T. G. Tornabene (1991) Continuous ethanol production in a two-stage immobilized/suspended-cell bioreactor. *Enzyme Microb. Technol.* **13**, 390-399.
17. Hossack, J. A., and A. H Rose (1976) Fragility of plasma membranes in *Saccharomyces cerevisiae* enriched with different sterols. *J. Bacteriol.* **127**, 67-75.
18. Thomas, D. S., H. A. Hossack and A. H. Rose (1978) Plasma-membrane lipid composition and ethanol tolerance in *Saccharomyces cerevisiae. Arch. Microbiol.* **117**, 239-245.
19. Thomas, D. S. and A. H. Rose (1979) Inhibitory effect of ethanol on growth and solute accumulation by *Saccharomyces cerevisiae* as affected by plasma-membrane lipid composition. *Arch. Microbiol.* **122**, 49-55.
20. Andreasen, A. A. and T. J. B. Stier (1953) Anaerobic nutrition of *Saccharomyces cerevisiae.* I. Ergosterol requirement for growth in a defined medium. *J. Cellular and Comp. Physiol.* **41**, 23-36.
21. Andreasen, A. A. and T. J. B. Stier (1954) Anaerobic nutrition of *Saccharomyces cerevisiae.* II. Unsaturated fatty acid requirement for growth in a defined medium. *J. Cellular and Comp. Physiol.* **43**, 271-281.
22. Bloomfield, D. K. and K. Bloch (1960) The formation of Δ^9-unsaturated fatty acids. *J. Biol. Chem.* **235**, 337-345.
23. Katsuki, H. and K. Bloch (1967) Studies on the biosynthesis of ergosterol in yeast. *J. Biol. Chem.* **242**, 222-227.
24. Thompson, E. D., P. R. Starr and L. W. Parks (1971) Sterol accumulation in a mutant of *Saccharomyces cerevisiae* defective in ergosterol production. *Biochem. Biophys. Res. Commun.* **43**, 1304-1309.
25. David, M. H. and B. H. Kirsop (1973) A correlation between oxygen requirements and the products of sterol synthesis in strains of *Saccharomyces cerevisiae. J. Gen. Microbiol.* **77**, 529-531.
26. Rogers, P. J., S. B. Yue and P. R. Stewart (1974) Effects of oxygen tension and glucose repression on mitochondrial protein synthesis in continuous cultures of *Saccharomyces cerevisiae. J. Bacteriol.* **118**, 523-533.
27. Lisyuk, G. M., G. V. Shaburova and L. V. Permyakova (1989) Intensity of glycolysis in *Saccharomyces carlsbergensis* in relationship to the supply of oxygen. *Priklandnaya Biokhimia i Mikrobiologiya* **25**, 664-669.
28. Gil, G. H., W. J. Jones and T. G. Tornabene (1990) Relationship of alcohol production to lipid composition of yeast in a continuous bioreactor. *Biochem.Cell Biol.* **68**, 661-668.
29. Hunter, K. and A. H. Rose (1972) Lipid composition of *Saccharomyces cerevisiae* as influenced by growth temperature. *Biochim. Biophys. Acta* **260**, 639-653.
30. Uchida, K. (1975) Alteration of the unsaturated to saturated ratio of fatty acids in bacterial lipids by alcohol. *Agr. Biol. Chem.* **39**, 1515-1516.
31. Beaven, M. J., C. Charpentier and A. H. Rose (1982) Production and tolerance of ethanol in relation to phospholipid fatty acyl composition in *Saccharomyces cerevisiae* NCYC 431. *J. Gen. Microbiol.* **128**, 1447-1455.
32. Nagar-legmann, R. and P. Margalith (1987) A comparative study of the lipid composition of yeast with different fermentative capacities. *Appl. Microbiol. Biotechnol.* **26**, 49-54.
33. Jones, R. P. (1989) Biological principles for the effects of ethanol. *Enz. Microb. Technol.* **11**, 130-158.
34. Prasad, R. (1985) Lipids in the structure and function of yeast membrane. *Adv. Lipid Res.* **21**, 187-241.
35. Dahl, J. S. and C. E. Dahl (1985) Stimulation of cell proliferation and phosphoinositide metabolism in *S. cerevisiae. Biochem. Biophys. Res. Commun.* **282**, 3-9.
36. Gokhale, D. V., B. S. Rao and S. Sivaramakrishnan (1986) Alcohol dehydrogenase and invertase activities in ethanol tolerant yeasts. *Enz. Microb. Technol.* **8**, 623-626.
37. Bloch, K. (1988) Sterol control of phospholipid biosynthesis in yeast. In Biological membranes: Aberrations in Membrane structure and Function. M. L. Karnovsky, A. Leaf and L. C. Bolis, editors. pp.3-9, Alan R. Liss, Inc.
38. Lutstorf, U. and R. Megnet (1968) Multiple forms of alcohol dehydrogenase in *Saccharomyces cerevisiae. Arch. Biochem. Biophys.* **126**, 933-944.
39. Smithies, O. (1959) An improved procedure for starch-gel electrophoresis: Further variations in the serum proteins of normal individuals. *Biochem. J.* **71**, 585-587.
40. King, A. T., M. R. Davey, I. R. Mellor, B. J. Mulligan and K. C. Lowe (1991) Surfactant effects on yeast cells. *Enz.*

Microb. Technol. **13**, 148-153.
41. Wenger, J. and C. Bernofsky (1971) Mitochondrial alcohol dehydrogenase from *Saccharomyces cerevisiae*. *Biochim. Biophys. Acta* **227**, 479-490.

연속식 고정화 및 현탁 세포 생물 반응기에 의한 에탄올 생성중 지질 첨가 영향

길광훈* (제일제당주식회사)

초록 : *Saccharomyces cerevisiae*의 고정화 및 현탁 세포로 구성된 연속식 생물반응기에서 에탄올 생성시 지질 첨가 영향을 연구하였다. 여러가지 배양조건하에서 에탄올 생산량 및 현탁 세포의 알코올 탈수소효소(alcohol dehydrogenase, ADH)의 비활성도를 측정하였다. 무통기 조건하에서 ergosterol과 oleic acid를 세포 배양액에 첨가하였을때, 에탄올 생산량과 균체 생육이 현저히 증가하였으나, 알코올 탈수소효소의 비활성도는 영향을 받지 않았다. 특히 무통기 조건 및 통기 조건하에서 얻어진 현탁 세포간의 알코올 탈수소효소의 비활성도는 차이가 없었다. 계면활성제 첨가시에도 에탄올 생성, 균체 생육, 알코올 탈수소효소의 비활성도가 크게 증가하였다. 고농도(40 g/*l* 이상) 에탄올에 노출된 세포배양액에 ergosterol과 oletic acid 첨가시에도 에탄올 생성량, 균체 생육, 알코올 탈수소효소의 비활성도가 증가하였으나, 계면활성제 첨가시에는 효과가 없었다. 따라서, 지질 첨가효과는 저농도 에탄올 조건에 비해 고농도 에탄올 존재시 크게 작용하였다. 여러가지 배양조건에서 얻어진 현탁 세포의 알코올 탈수소효소의 isozyme patteren을 전기영동법에 의해 조사한 결과 ADH I으로 추정되는 한개의 isozyme만이 확인되었으며, isozyme의 이동거리는 세포의 배양조건에 따라 약간의 차이가 있었다. 에탄올 생성량과 알코올 탈수소효소의 비활성도사이의 상관관계는 성립되지 않았으며, 알코올 탈수소효소의 비활성도보다는 균체량이 에탄올 생성에 더 중요한 인자로 작용하였다.

*연락저자