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EFFECT OF SURFACTANTS ON ETHANOL FERMENTATION USING GLUCOSE AND CELLULOSIC HYDROLYZATES

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Abstract

The effect of the non-ionic surfactants on the ethanol fermentation was greatly dependent on the surfactant added. While Tween 20 and Tween 80 slightly enhanced ethanol fermentation, Triton X-100 which exhibited the highest increase in the enzymatic saccharification had a negative effect on the ethanol fermentation. The negative effect of Triton X-100 on ethanol production was the most pronounced when the cellulosic hydrolyzates were used. Tween 80 showed the best performance for the ethanol production from steam exploded wood hydrolyzate.

INTRODUCTION

The selected surfactants were reported to have the enhancement effect on the hydrolysis of cellulose (Ooshima et al., 1986; Park et al., 1992; Helle et al., 1993). The hydrolysis of steam exploded wood was increased by 67 % in the presence of sopholipid (Helle et al., 1993). Park et al. found that Triton series surfactants showed the highest enhancement on the hydrolysis of cellulose among five different kinds of nonionic surfactants examined (Park et al., 1992).

The surfactant added in the hydrolysis step should remain in the hydrolyzate and may have some effect on the ethanol fermentation. Only a few works have dealt with the effect of surfactants remained in the medium on the ethanol fermentation (Janssens et al., 1983; Ohta and Hayashida, 1983; Ballesteros et al., 1994). All works only investigated the effect of the various lipid mixtures with a fraction of Tween 80 on the ethanol fermentation. Janssens et al. reported that the fermentation ability was improved dramatically by the addition of unsaturated fatty acid and ergosterol to the medium (Janssens et al., 1983). The improvement was mainly due to the protective effect by lipid supplementation on the maintenance of cell viability. But the effect of only surfactants on the ethanol fermentation was not determined. The effect of surfactants on the phytase and fumaric acid production has been investigated (Asheh and Duvnjak, 1994; Goldberg and Stieglitz, 1985) Asheh and Duvnjak reported that the biomass growth and phytase production were higher in the presence of Tween 80 and sodium oleate than in the control medium which was not supplemented with the surfactants (Asheh

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and Duvnjak, 1994). But the addition of Triton X-100 had a negative effect on the studied process. These authors suggested that the surfactants altered the cell permeability which resulted in a higher release of the enzyme. They did not give the reason why lower enzyme activity was obtained with the addition of Triton X-100. To our knowledge, no one had determined the effect of surfactants on the ethanol fermentation which would be added to enhance the enzymatic hydrolysis of lignocellulosic biomass.

To optimize the ethanol production process from lignocellulosic biomass, not only the effect of surfactants on the hydrolysis but that on the ethanol fermentation should be determined. In this work, the effect of the surfactants, having enhancement effects on the enzymatic saccharification, on the ethanol fermentation using glucose and cellulosic hydrolyzates were investigated.

MATERIALS AND METHODS

Microorganism

Saccharomyces cerevisiae H1-7 obtained from Suwon Universuty was used in this work. The organism was maintained on agar slants containing 0.3% yeast extract, 0.5% peptone, 2% glucose (all % are w/v).

Materials

Cellulose and wood hydrolysates were prepared by enzymatic hydrolysis of pure cellulose (Sigma Chemical Co., USA) and steam exploded wood respectively. Steam exploded wood was prepared by the procedures described elsewhere (Lee et al., 1995). Cellulases (Celluclast and Novozym, Novo Inc., Dennmark) were added to produce the cellulose and wood hydrolyzates of 72 g/l and 52 g/l glucose concentrations respectively. Tween 20, Tween 80 and Triton 100 were chosen as surfactants to supplement the fermentation. The surfactants were all purchased from Merck Chemical Co. (USA). All other chemicals used in the work were reagent grade.

Ethanol fermentation

The organism was precultured for 24 hour at 30 °C in the culture media with the same compostion described above except agar. Ethanol fermentations were carried out in 500 ml Erlenmeyer flasks, each containing 200 ml of fermentation medium with initial glucose concentrations of 76.2 and 152.4 g/l. The flasks were inoculated 5 %(v/v) of yeast cultures. Surfactants were added to the medium before autoclaving. The concentrations of surfactants in the fermentation media were 1 g/l and 10 g/l. Additional nutrients were added to the basal medium. This broth consisted of (%, w/v): yeast extract, 0.15; KH_2PO_4, 0.25; $MgSO_47H_2O$, 0.05; and glucose 15. The inoculated flasks were incubated at 30 °C and 150 rpm. Initial pH was adjusted at 4.5.

Assay

Glucose concentration was determined with the glucose oxidase-peroxidase method. Ethanol was measured by gas chromatography, using a Hewlett-Packard 5890A gas chromatograph with automatic sampler 7671A(Hewlett-Packard Inc., USA). A capillary column(HP-1, Hewlett-Packard Inc., USA) was used with a flame ionization detector. The injector and detector temperatures were 190 °C and 220 °C, respectively, and the column oven operated isothermally at 35 °C. Iso-propanol was used as internal standard. Enumeration of cell populations during growth and fermentation was carried out with a hemacytometer(AO Inc., USA).

RESULTS AND DISCUSSION

Fermentation tests were conducted to identify the effect of surfactants on the ethanol fermentation using synthetic media containing glucose and cellulosic hydrolyzates. Two different concentrations, 0.1 % and 1 %, of Tween 20 (T-20), Tween 80 (T-80), and Triton

X-100 (TX-100) were added to the cultures, based on the fact that they are the most effective surfactants for the saccharification enhancement (Ooshima et al. 1986; Park et al., 1992; Helle et al., 1993). A set of typical results of the experimental works are shown in Figrue 1. The presence of Tweens improved initial growth as well as the fermentation

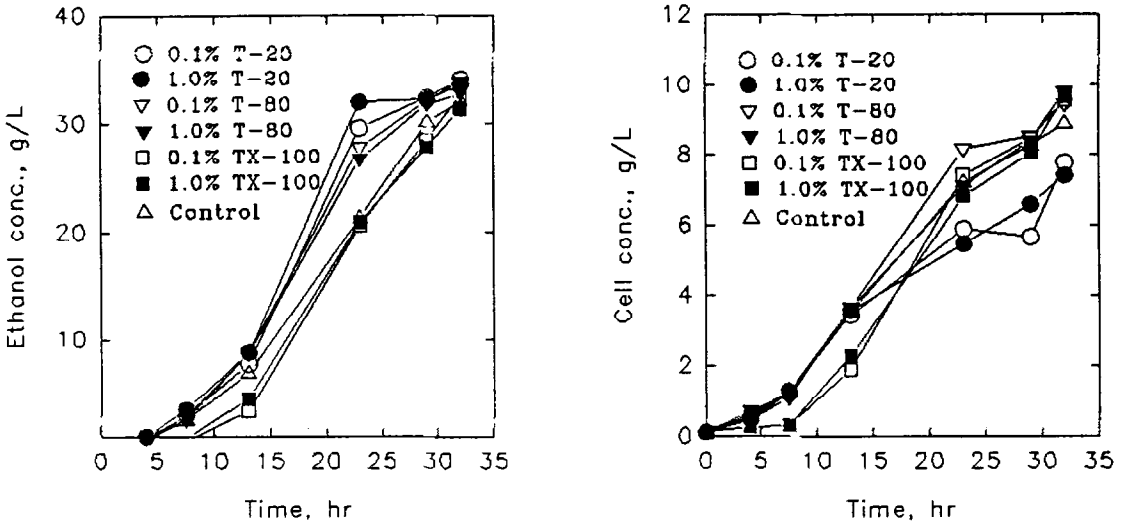

Figure 1. Effect of surfactants on ethanol fermentation using glucose of 76.2 g/l.

activity of the yeast. While Tween 20 was the most efficient for the ethanol fermentation, the cell growth rate was the highest in the presence of Tween 80. Addition of 1 % Tweens had slightly higher enhancement effects than 0.1 % had. Triton X-100 had a negative effect on the fermentation. The negative effect of Triton X-100 was also observed during the phytase production by *Aspergillus carbonarius* (Asheh and Duvnjak, 1994).

Since it is desirable to produce high concentrations of ethanol to reduce separation costs,

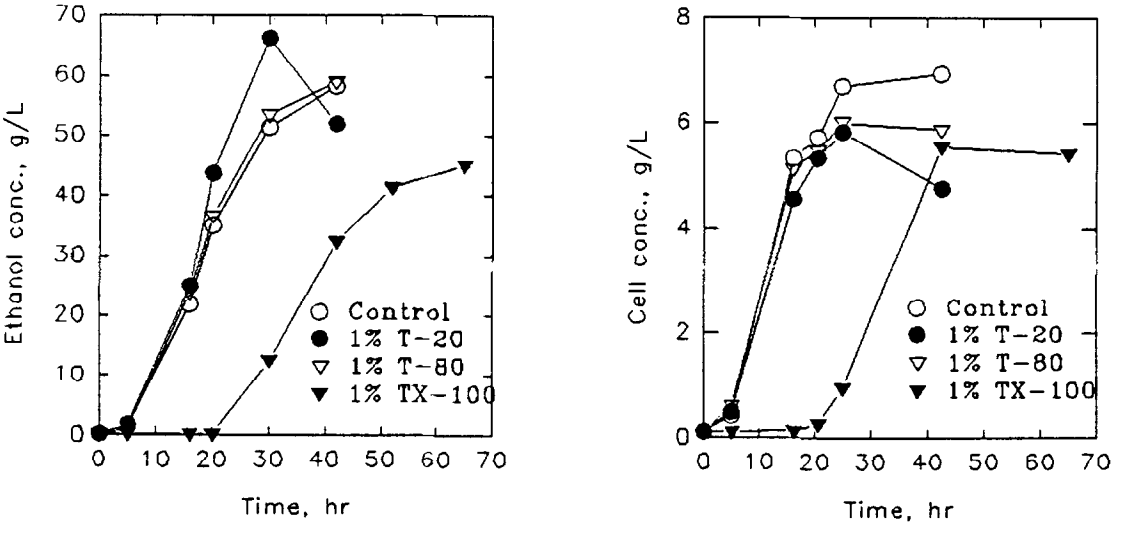

Figure 2. Effect of surfactants on ethanol fermentation using glucose of 152.4 g/l.

the effect of surfactants on the ethanol production was investigated in the media 152.4 g/l glucose. As shown in Figure 2, the general patterns of enhancement by Tweens were similar. The rate of ethanol production was the highest with 1 % Tween 20 media and the highest cell growth rate and final cell concentration were observed in the control. The negative effect of Triton X-100 was more significant as the initial glucose concentration increased. The lag time was also increased to 20 hour with the increase of initial glucose concentration. While the ethanol fermentation was virtually completed by 30 hour with the supplementation of Tween 20, the ethanol fermentation with Triton X-100 was not finished until 65 hour.

The fermentation results are summarized in Table 1. As described before, the ethanol yields of the culture with Tweens were higher than the control. Tween 20 showed the highest yield. While the productivities were the highest with Tween 20, the cell growth rates with Tween 80 were higher than with other surfactants. The improvement of the ethanol productivities by Tween 20 was remarkable. The ethanol productivities in the presence of 0.1 % Tween 20 were 1.8 to 2 times higher than the control. The enhancement of ethanol production by surfactants was also reported by others (Panchal and Stewart, 1980; Buzzi et al., 1993). Panchal and Stewart observed that the addition of surfactants such as Tweens improved ethanol production. They reported that the improvement was mainly due to the

Table 1. Summary of the results on the batch fermentation with surfactants..

Initial glucose concentration, g/l	Surfactants added	$Y_{p/s}$	$Y_{x/s}$	Q_s	Q_p
76.2	Control	0.421	0.117	0.267	0.113
	0.1% T-20	0.448	0.102	0.464	0.198
	0.1% T-80	0.445	0.124	0.309	0.130
	0.1% TX-100	0.428	0.125	0.249	0.107
	1% T-20	0.441	0.097	0.399	0.169
	1% T-80	0.430	0.129	0.312	0.13
	1% TX-100	0.411	0.126	0.246	0.101
152.4	Control	0.381	0.045	0.522	0.199
	1% T-20	0.434	0.038	0.877	0.381
	1% T-80	0.387	0.039	0.603	0.233
	1% TX-100	0.306	0.035	0.440	0.134

$Y_{p/s}$ = Yield coefficient of ethanol (g ethanol/g glucose). $Y_{x/s}$ = Yield coefficient of yeast (g cell/g glucose). Q_s = Specific consumption rate of glucose (g glucose/g cell-hr). Q_p = Specific production rate of ethanol (g ethanol/g cell-hr).

enhancement of cell permeability. As the enhancement of cell permeability by surfactants was dependent on the chemical composition of cells like membrane sterol content, the effect of the surfactants can be different according to the cells used. For exmaple, Buzzi et al. reported that the invertase activity secreted by *Neurospora crassa* with Tween 80 was increased to 1.6 times without altering the cell growth rate than in the control (Buzzi et al., 1993). Triton X-100 was reported to be the most efficient for the phosphatase production by *A. ficuum* and increased the enzyme level by 3.9 times while Tween 80 increased only 1.3 times compared

to the control (Han and Gallenger, 1987).

Since the lignocellulosic hydrolyzate, the most economic substrate for ethanol production, contains various toxic substances like phenolic compounds and organic acids, the fermentability of the hydrolyzate is poor (Castro and Hotten, 1994; Chung and Lee, 1985). The effect of the surfactants on the fermentation using cellulosic hydrolyzates was investigated. As presented in Figure 3, the best performance for the ethanol production from cellulose hydrolyzate was obtained in the presence of Tween 80 until 20 hour but the initial ethanol production rate was the highest with Tween 20 up to first 10 hour. Unlike the synthetic media containing glucose, the high membrane permeability with Tween 20 was not helpful for ethanol production from cellulose hydrolyzate. Taking into account that cellulose hydrolyzate

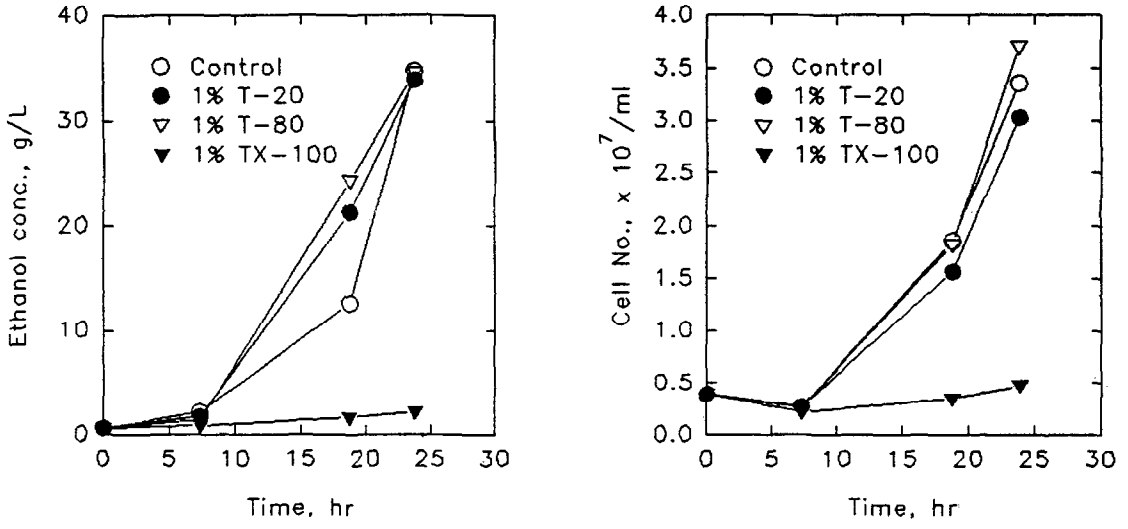

Figure 3. Effect of surfactants on ethanol fermentation using cellulose hydrolyzate, (glucose concentration: 72 g/l).

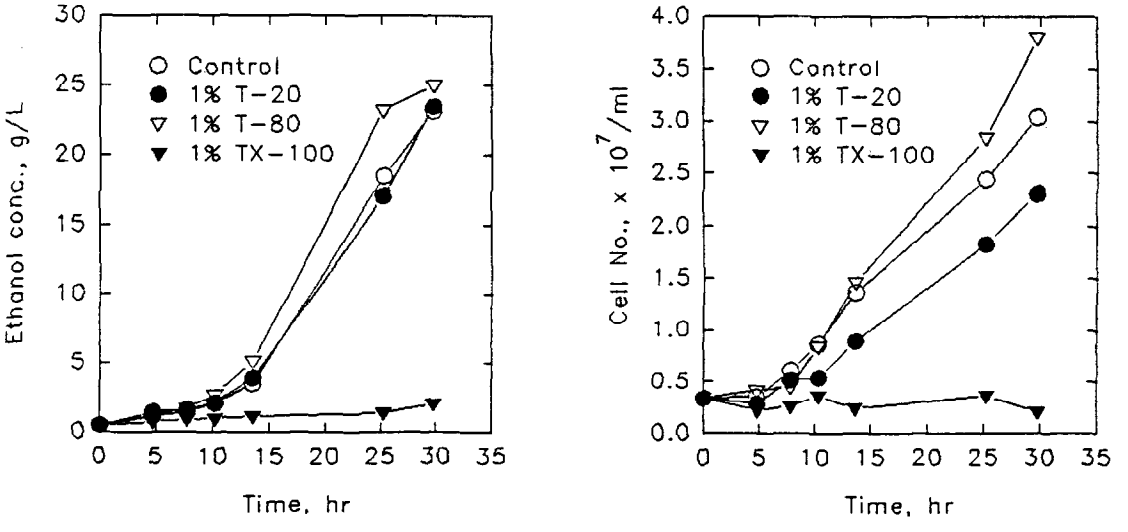

Figure 4. Effect of surfactants on the ethanol fermentation using wood hydrolyzate, (glucose concentration: 52 g/l).

contained various toxic materials, it was logical to expect that the ethanol production would also be influenced by the toxic materials diffused into cells. The negative effect of Triton X-100 was noticeable for the ethanol production using cellulose hydrolyzate. Ethanol production was not done at all with Triton-X 100. The enhancement effect of Tween 80 on the ethanol production was remarkable with steam exploded wood hydrolyzate. The final ethanol and cell concentrations were 10-20 % higher with Tween 80 than control or with Tween 20 in the ethanol production using wood hydrolyzate (Figure 4).

The effects of surfactants on the ethanol production were greatly dependent on the surfactant added. Compared with the control, Tween 20 and Tween 80 increased and Triton X-100, having the highest enhancement for the enzymatic saccharification, decreased the rates of ethanol production but all surfactants had more or less negative effects for the cell growth. Considering the effects of the surfactants on both saccharification and fermentation, the supplementation of Tween 80 is desirable for the enzymatic hydrolysis of lignocellulosic biomass.

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