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Alcohol fermentation of enzymatic hydrolysate of exploded rice straw by *Pichia stipitis*

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The xylose in an enzymatic hydrolysate of steam-exploded rice straw was not consumed by *Pichia stipitis* until the glucose was almost exhausted. A diauxic lag of 2 to 3 h in both cell growth and ethanol production occurred as metabolism switched from glucose to xylose utilization. Ethanol production was maximal [6 g ethano/l from 15 g reducing sugars/l (78% theoretical yield)] at an aeration rate of 0.2 vol/vol. min.

Key words: Alcohol fermentation, *Pichia stipitis*, rice straw, saccharification, steam explosion.

The importance of lignocellulose as a renewable energy resource has increased with the depletion of fossil fuels. However, lignocellulose is difficult to decompose by direct biological means. Recently, decomposition involving the coupled chemical and physical processes of auto-hydrolysis and explosion has been considered (Jurasek 1979; Nakamura *et al.* 1991). The sugars produced by enzymatic saccharification of steam-exploded material are mainly glucose and xylose. The efficient fermentation of both of these sugars to ethanol is therefore essential if the process is to be cost-effective.

Although the discovery of xylose-fermenting yeasts has enhanced interest in microbial conversion of biomass to ethanol, there are various problems in the development of an efficient fermentation. The main problem is that ethanol productivities from xylose are generally low compared with those obtained from glucose with *Saccharomyces cerevisiae* (Hahn-Hagerdal *et al.* 1994). However, Du Preez *et al.* (1986) achieved a maximum volumetric ethanol productivity of about 0.9 g/l.h and yields of 85% to 90% of the theoretical maximum using *P. stipitis* to ferment xylose under optimized pH and temperature. Several other experiments have also been carried out to try and improve ethanol productivity from xylose, using either *P. stipitis* or another xylose-fermenting yeast (Du Preez 1994; Jeffries & Kurtzman 1994). As O_2 supply is one of the keys of xylose catabolism in these yeasts, the efficiency of xylose fermentation, should be improved by optimizing the availability of O_2 to the test culture.

The present study was of ethanol production, from an enzymatic hydrolysate of steam-exploded rice straw, by *P. stipitis*. Different aeration conditions were examined in order to maximize the ethanol production from mixed sugars. *Saccharomyces cerevisiae* was also of interest here because of its traditional role in glucose fermentation.

Materials and Methods

Substrate Preparation

Air-dried rice straw was chopped into 10-cm pieces and steam-exploded in a 1.2-l reactor at 3.53 MPa and 275°C for 2 min. Samples were then freeze-dried. Subsequent saccharification was performed on 2 g sample in 100 ml 0.5 M phosphate buffer, pH 5.0, in a 300-ml conical flask agitated at 100 rev/min and held at 50°C for 120 h. Five IU of Cytolase 300™ (a commercial cellulase preparation from Genencor, San Francisco, CA), 108 locust-bean-gum viscosity units of Cytolase M103S™ (a commercial hemicellulase-rich preparation from Genencor) or 28.4 cellobiase units of Novozyme 188™ (a commercial cellobiase preparation from Novo Laboratories, Wilton, CT) were then added to each gram of dry substrate. Saccharification was stopped by boiling the sample flasks for 15 min.

Organisms and Cultivation Methods

Pichia stipitis NRRL Y-7124 and *Saccharomyces cerevisiae* ATCC

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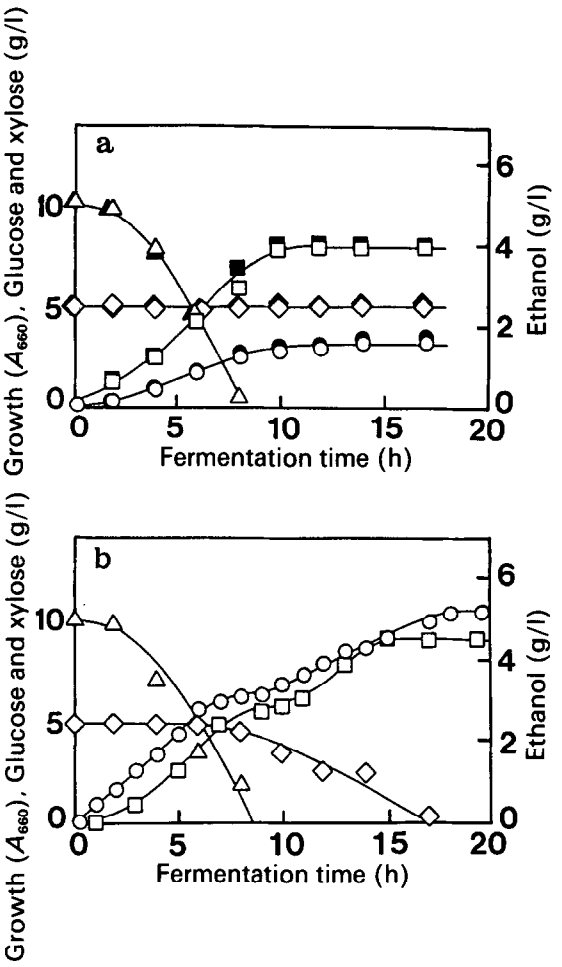

Figure 1. Cell growth (●, ○) and glucose (▲, △), xylose (◆, ◇), and ethanol (■, □) concentrations during fermentation by *S. cerevisiae* (a) or *P. stipitis* (b) of an enzymatic hydrolysate of steam-exploded rice straw (open symbols) or a medium containing reagent-grade glucose and xylose at the same concentrations as the hydrolysate (closed symbols; shown for *S. cerevisiae* only).

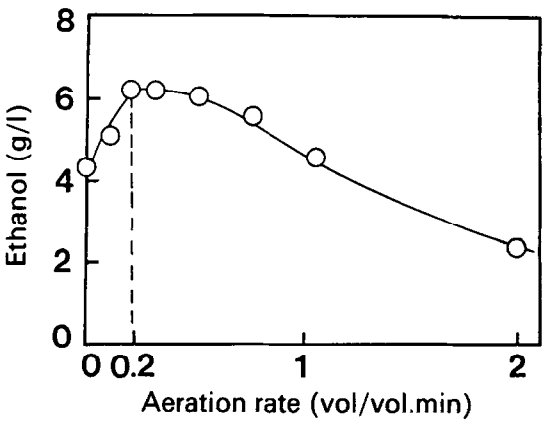

Figure 2. Effect of aeration rate on maximum ethanol production by *P. stipitis* from the rice hydrolysate.

26603 were grown at 30°C on medium containing (g/l): KH_2PO_4, 13; K_2HPO_4, 0.7; NH_4Cl, 2.0; $MgSO_4.7H_2O$, 0.1; yeast extract, 1; and glucose, 10; at pH 5.0. Glucose was autoclaved separately. Cultures were grown, from a 10% (v/u) inoculum taken from 48-h-old agar slants, in 300-ml Erlenmeyer flasks, each containing 100 ml of this medium, for 18 to 24 h at 100 rev/min. Cells were harvested by centrifugation and resuspended in fermentation (basal) medium to give a final A_{660} of approximately 0.5 (equivalent to about 0.12 mg cell dry wt/ml).

The saccharification hydrolysate liquid (containing glucose and xylose in a ratio of 2:1, w/w) was fermented in 1 l fermentation (basal) medium (containing 10 mg penicillin and 10 mg streptomycin/l) in a 3-1 fermenter, with stirring at 500 rev/min. Aeration, from the bottom of the fermenter, was from 0 to 2 vol/vol.min.

Analyses

Glucose and xylose concentrations were determined by HPLC (Nakamura *et al.* 1991) on a Bio-Rad HPX-87H ion-exclusion column fitted with a RI detector. Cell concentration was determined from A_{660}, after three washes with saline. Ethanol was assayed by GC.

Results and Discussion

Enzymatic saccharification of steam-exploded rice straw produced up to 10 g and 5 g xylose/l (data not shown). Figure 1 shows the results of the alcohol fermentation of the hydrolysate. An alcohol fermentation was also performed using the same concentrations of reagent-grade glucose and xylose as in the hydrolysate, to see if the hydrolysate possessed any inhibitory material; Growth-inhibitory substances have been reported in steam-exploded samples (Mes-Hartree *et al.* 1984). There were, however, no significant differences in cell growth or alcohol production between the two fermentations (see Figure 1a). In fermentations using *S. cerevisiae*, only glucose was fermented to ethanol, the maximum ethanol concentration achieved being 4 g/l; xylose was unmetabolized (Figure 1a). However, both glucose and xylose were utilized by *P. stipitis* (Figure 1b), glucose being consumed exclusively in the first arithmetic growth state, before xylose consumption began. There was a diauxic lag of 2 to 3 h in cell growth and ethanol production as the yeast's metabolism switched from glucose to xylose utilization. Preferential utilization of glucose over xylose may reflect selective transport, as proposed by Slininger *et al.* (1987).

Figure 2 shows the effect of aeration rate on ethanol production from the enzymatic hydrolysate by *P. stipitis*. As the rate was increased ethanol concentration initially increased, to a maximum of 6 g/l, at an aeration rate at 0.2 vol/vol.min, and then decreased gradually. Ethanol at 6 g/l is equivalent to 78% of the theoretical maximum yield. The reason for the decrease in ethanol concentration at high aeration rates is probably the re-assimilation of ethanol by the yeast. Ethanol re-assimilation has also been shown to increase with increasing oxygenation in xylose fermentation by *Candida tropicalis* (Lohmeier-Vogel *et al.*

1989). O_2 therefore has a dual effect: low concentrations promote ethanol production whereas high concentrations cause re-assimilation of the ethanol produced. Therefore, in the development of a process for efficient production of ethanol by xylose-fermenting yeasts, emphasis should be given to the optimization of aeration.

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References

Du Preez, J.C. 1994 Process parameters and environmental factors affecting D-xylose fermentation by yeasts. *Enzyme and Microbial Technology* **16**, 944–956.

Du Preez, J.C., Bosch, M. & Prior, B.A. 1986 Xylose fermentation by *Candida shehatae* and *Pichia stipitis*: effects of pH, temperature and substrate concentration. *Enzyme and Microbial Technology* **8**, 360–364.

Hahn-Hagerdal, B., Jeppsson, H., Skoog, K. & Prior, B.A. 1994 Biochemistry and physiology of xylose fermentation by yeasts. *Enzyme and Microbial Technology* **16**, 933–943.

Jeffries, T.W. & Kurtzman, C.P. 1994 Strain selection, taxonomy, and genetics of xylose-fermenting yeasts. *Enzyme and Microbial Technology* **16**, 922–932.

Jurasek, L. 1979 Enzymic hydrolysis of pretreated aspen wood. *Developments in Industrial Microbiology* **20**, 177–183.

Lohmeier-Vogel, E., Skoog, K., Vogel, H. & Hahn-Hagerdal, B. 1989 ^{31}P nuclear magnetic resonance study of the effect of azide on xylose fermentation by *Candida tropicalis*. *Applied and Environmental Microbiology* **55**, 1974–1980.

Mes-Hartree, M., Hogan, C.M. & Saddler, J.N. 1984 The enzymatic hydrolysis and fermentation of agricultural residues to ethanol. *Biotechnology and Bioengineering Symposium* **14**, 397–405.

Nakamura, Y., Moniruzzaman, M., Nagao, M., Sawada, T. & Motoi, M. 1991 Modification of rice straw by steam explosion and enzymatic saccharification of steam-exploded products. *Kagaku Kogaku Ronbunshu* **17**, 504–510.

Slininger, P.J., Bolen, P.L. & Kurtzman, C.P. 1987 *Pachysolen tannophilus*: properties and process considerations for ethanol production from D-xylose. *Enzyme and Microbial Technology* **9**, 5–15.

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