

## COMPARISON OF CORN STEEP LIQUOR WITH OTHER NUTRIENTS IN THE FERMENTATION OF D-XYLOSE BY *PICHIA STIPITIS* CBS 6054

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### Summary

*Pichia stipitis* CBS 6054 ferments D-Xylose to ethanol in a medium containing corn steep liquor as the only source of nitrogen, amino acids, vitamins and other nutrients. The ethanol yield and fermentation rate compare favorably to those obtained with media containing more expensive sources of nitrogen, vitamins and amino acids. Corn steep liquor is a good source of nutrients that can support growth and fermentation activity of this xylose fermenting yeast.

### Introduction

The fermentation of xylose to ethanol is of considerable interest to the grain ethanol industry. Xylose is a significant component of angiosperm hemicellulose, and it is particularly abundant in corn hulls, corn fiber and other agricultural residues. Corn hulls are formed as a byproduct of corn starch processing, and their disposal is problematical. The development of a commercially viable xylose fermentation would permit utilization of acid or enzymatic hydrolysates of grain hulls, or energy crops such as napier grass, sugar cane bagasse or corn stover.

Few yeasts are capable of fermenting xylose to ethanol. Among these, *Candida shehatae* and *Pichia stipitis* are the most promising (du Preez and Van der Walt 1983; Bruinenberg et al. 1984; du Preez and Prior 1985; Jeffries 1985; Prior et al. 1989). Components of the fermentation medium, such as nitrogen source, vitamins, amino acids and trace elements influence ethanol production (Jeffries 1983; du Preez et al. 1984; 1986; Dellweg et al. 1984; Tran and Chambers 1986; Lee et al. 1988; Palnitkar and Lachke 1992; Guebel et al. 1992). In the past, expensive yeast and peptone extracts have been used to provide these nutrients. Because the cost of the fermentation medium is one of the principal factors that determines the economic viability of the ethanol production, it is very important that low cost medium components supply all the nutritional requirements for good growth and fermentation activity. Corn steep liquor (CSL) is a major byproduct of corn starch processing and is an inexpensive source of such nutrients. In this paper we show that a fermentation medium containing CSL as the sole source of nitrogen, vitamins and other nutritional requirements compares favorably with several more complex media formulations for the fermentation of xylose to ethanol by *P. stipitis* CBS 6054.

### Materials and Methods

**Microorganism:** *Pichia stipitis* CBS 6054 was used for all studies. Stock cultures were maintained and grown on slants of YPD agar which contained yeast extract, 10 g/L; bacto-peptone, 20 g/L; D-glucose, 20 g/L; D-xylose, 10 g/L and agar, 20 g/L.

**Media:** The media investigated were as follows: A: YNB (Yeast nitrogen base without amino acids or  $(\text{NH}_4)_2\text{SO}_4$ ), 1.7 g/L; supplemented with casamino acids, 5 g/L and urea, 5 g/L; B: Malt extract, 3 g/L; yeast extract, 3 g/L and  $(\text{NH}_4)_2\text{SO}_4$ , 5 g/L; C: Peptone, 3.5 g/L; yeast extract, 3 g/L;  $\text{KH}_2\text{PO}_4$ , 2 g/L,  $\text{MgSO}_4$ , 1 g/L and  $(\text{NH}_4)_2\text{SO}_4$ , 1 g/L; D: Corn steep liquor, 30 g/L; E: Corn steep liquor, 30 g/L;  $\text{MgSO}_4$ , 1 g/L and  $\text{KH}_2\text{PO}_4$ , 2 g/L. Each medium

contained 80 g/L of D-xylose which was autoclaved separately and added aseptically. Apart from medium A which was filter sterilized, all medium components were autoclaved.

**Inoculum preparation and fermentation:** Inoculum was prepared by transferring a loopful of colonies from an agar slant into 50 ml of each of the above media, pH 5.0 in a 125 ml Erlenmeyer flask plugged with a foam stopper. Incubation was at 30°C on a rotary shaker at 150 rpm for 48 hr. Cells were harvested by centrifugation at 5000 × g for 10 min, and washed twice with cold sterile distilled water. About 2 g/L dry weight cell mass was used to inoculate the fermentation medium which was the same as those for the inoculum preparation.

**Analytical methods:** Cell growth was estimated by dry weight. Samples were taken in duplicate, centrifuged, washed twice with distilled water and dried at 110°C until constant weight. Sugars and polyols were determined by HPLC with a BioRad HPXC column at 85°C using a refractive index detector. Distilled water was the mobile phase at a flow rate of 0.5 ml/min. Ethanol was determined by gas chromatography (Jeffries 1982).

## **Results and Discussion**

Results of cell growth, rate of xylose utilization and ethanol production in the different media are presented in Figs. 1a, b, and c, respectively. Growth rates are higher in media containing CSL (media D and E) than in the other media. Medium B, which contained malt and yeast extracts, resulted in the least growth. Addition of MgSO<sub>4</sub> and KH<sub>2</sub>PO<sub>4</sub> to the CSL (E) did not have any effect on growth. The rates of xylose utilization (Fig. 1b) are marginally higher in media D and E than in the other media. In all media, with the exception of medium B, xylose was completely utilized within 48 hours.

The better growth obtained in the media containing CSL was probably due to the presence of certain nutrients that were absent in the other formulations. CSL is a major byproduct of the corn wet milling industry. The composition depends on the method of preparation, but it is generally a rich source of nitrogen, water soluble vitamins, amino acids, minerals and other growth stimulants. It contains approximately 50% solids (Atkinson and Mavutuna 1983; Miller and Churchill 1986). The CSL we used contained dextrose, 1.2%; maltobiose, 0.64%, maltotriose 4.0%, lactic acid, 4.8%, acetic acid 0.06% and glycerol 0.35% which could act as extra sources of carbon for growth. Limited growth was obtained in a CSL medium without xylose (data not shown).

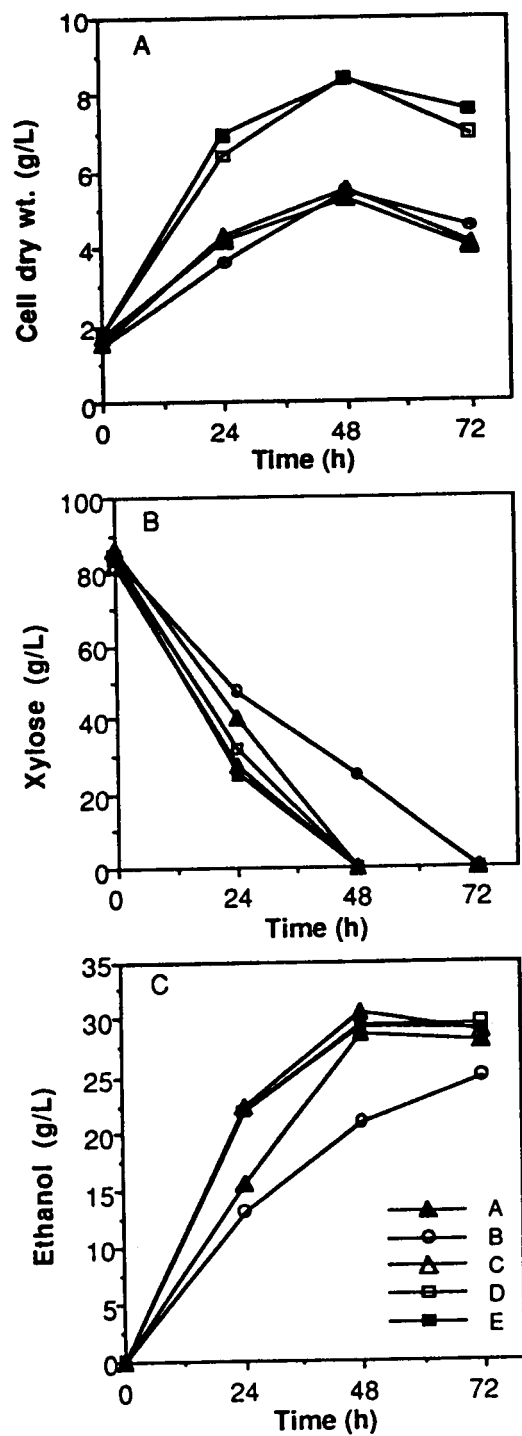
The rate of ethanol production in media C, D, and E were similar and higher than those in media A and B. The maximum ethanol produced in media A, C, D and E were 28.7 g/L, 30.6 g/L, 29.5 g/L and 29.2 g/L respectively. These represents ethanol yields ranging from 0.35 to 0.42 g/g carbon metabolized. The highest yield obtained in medium D. Higher ethanol yields can be obtained by further restricting aeration.

Generally, the highest productivities in all the media were obtained within the first 24 hours of fermentation. The highest volumetric productivity of 0.93 g/L•h. was obtained in media C and D. However the highest specific productivity of 0.22 g/g.h was obtained in medium C and the lowest production of 0.14 g/g•h and 0.13 g/g•h were in media containing CSL (D and E) respectively. This is probably due to the higher growth obtained in these media as a result of the presence of the extra carbon sources in the CSL.

The above discussed results show that CSL can be used as a cheap source of nitrogen and other nutrients for xylose fermentation by *P. stipitis* CBS 6054.

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**Figure 1.** Comparison of cell growth, sugar utilization and ethanol production on xylose with five complex media. A: Yeast nitrogen base with supplements; B: Malt extract, yeast extract and ammonium sulfate; C: Peptone, yeast extract,  $MgSO_4$ , and  $KH_2PO_4$ ; D: 3% corn steep liquor; E: 3% corn steep liquor plus  $MgSO_4$ , and  $KH_2PO_4$ ; see text for details.

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