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FERMENTATION OF D-XYLOSE BY YEASTS USING GLUCOSE ISOMERASE IN THE MEDIUM TO CONVERT D-XYLOSE TO D-XYLULOSE

P.Y. Wang, B.F. Johnson and H. Schneider*

Division of Biological Sciences

National Research Council of Canada

Ottawa, Canada K1A 0R6

SUMMARY

A method to obtain the fermentative conversion by yeasts of D-xylose to ethanol is described. The method depends on a combination of two factors; (1) the ability of glucose isomerase to isomerise D-xylose to D-xylulose and (2) the ability of a number of yeasts to ferment D-xylulose.

INTRODUCTION

The conversion of saccharified biomass polysaccharides to ethanol by fermentation is of interest as a means of obtaining liquid fuel from a renewable resource. Yeasts are limited in such conversions by being able to ferment hexoses but not aldopentoses. Large amounts of aldopentoses occur in hemicellulose, which can comprise 25-35% of some cellulosic solids (Dunning and Lathrop, 1945). The most common aldopentose is probably D-xylose.

The recent finding (Wang, Shopsis and Schneider, 1980) that several yeasts can ferment D-xylulose, a ketopentose, to produce ethanol prompted investigation of methods to convert D-xylose to D-xylulose in a fermenter. One possibility was to include in the medium glucose isomerase, an enzyme which

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can also isomerise D-xylose to D-xylulose (Bucke, 1977). The present paper demonstrates the feasibility of this approach and delineates some of the factors involved.

MATERIALS AND METHODS

Glucose isomerase. Most of the experiments were carried out with a liquid preparation of glucose isomerase, Maxazyme GI (Gist Brocades N.V. Delft, Holland). This material was dialysed against 0.1% KH_2PO_4 before use to eliminate a material interfering with the subsequent determination of ethanol by gas chromatography. Two commercial preparations of immobilized glucose isomerase were used as well; Maxazyme GI Immob., (Gist Brocades) and Taka Sweet (Miles Laboratories, Elkhardt, Indiana). In addition, experiments were carried out using the clear supernatant from sonicates of cells containing D-xylose isomerase; Lactobacillus plantarum NRCC 13004, Lactobacillus brevis NRCC 13041 and Streptomyces albus NRCC 42006. All of these organisms were grown on media containing D-xylose.

Organisms and growth. Data are shown only for Schizosaccharomyces pombe NCYC 132, an organism which does not assimilate D-xylose, but is one of the better fermenters of D-xylulose examined thus far. Similar results were obtained with Kluyveromyces lactis NRCC 202029.

Inocula were grown and fermentation carried out at 29°C as described previously (Wang, Shopsis and Schneider, 1980). All solutions were sterile filtered. Media using immobilised glucose isomerase contained chloramphenicol, 100 µg/ml, and tetracycline, 25 µg/ml, to inhibit bacterial growth.

Glucose isomerase activity. Xylose isomerase activity

was 11 nanomoles/min/ml in experiments with Maxazyme GI (0.6 mls plus 2.4 mls of medium) and three times as high with Maxazyme GI Immob. (0.7 g plus 4 mls medium). The build up of D-xylulose concentration was used to follow activity (Ashwell, 1957).

Chemicals. The D-xylose was NRC grade (Pfanstiehl, Waukegan, Ill., U.S.A.). Thin layer chromatography showed that it contained only a single component. The melting point and optical rotation agreed with literature values. Bovine serum albumin (cryst.) was supplied by Sigma (St. Louis, Mo., U.S.A.).

RESULTS

Alcohol production using 5% xylose, Schiz. pombe, and Maxazyme GI is shown in Figure 1. The medium also contained 1% of a soluble protein, bovine serum albumin. The soluble protein was obligatory. Only traces of ethanol were produced in its absence. When the soluble protein was present omission of either glucose isomerase, D-xylose or cells resulted in the absence of ethanol formation. The soluble protein is thought to be necessary to prevent destruction of the glucose isomerase by proteolytic enzymes produced by the yeast. Evidence for the presence of proteolytic enzymes was obtained by acrylamide gel electrophoresis, and in the development of a precipitate after 7-10 days.

The highest rate and final amount of alcohol production was found using Maxazyme GI (Figure 1). Of all of the other enzyme preparations used, only the L. brevis sonicate could approach the Maxazyme GI results. The immobilized enzymes could produce alcohol in the absence of soluble protein in

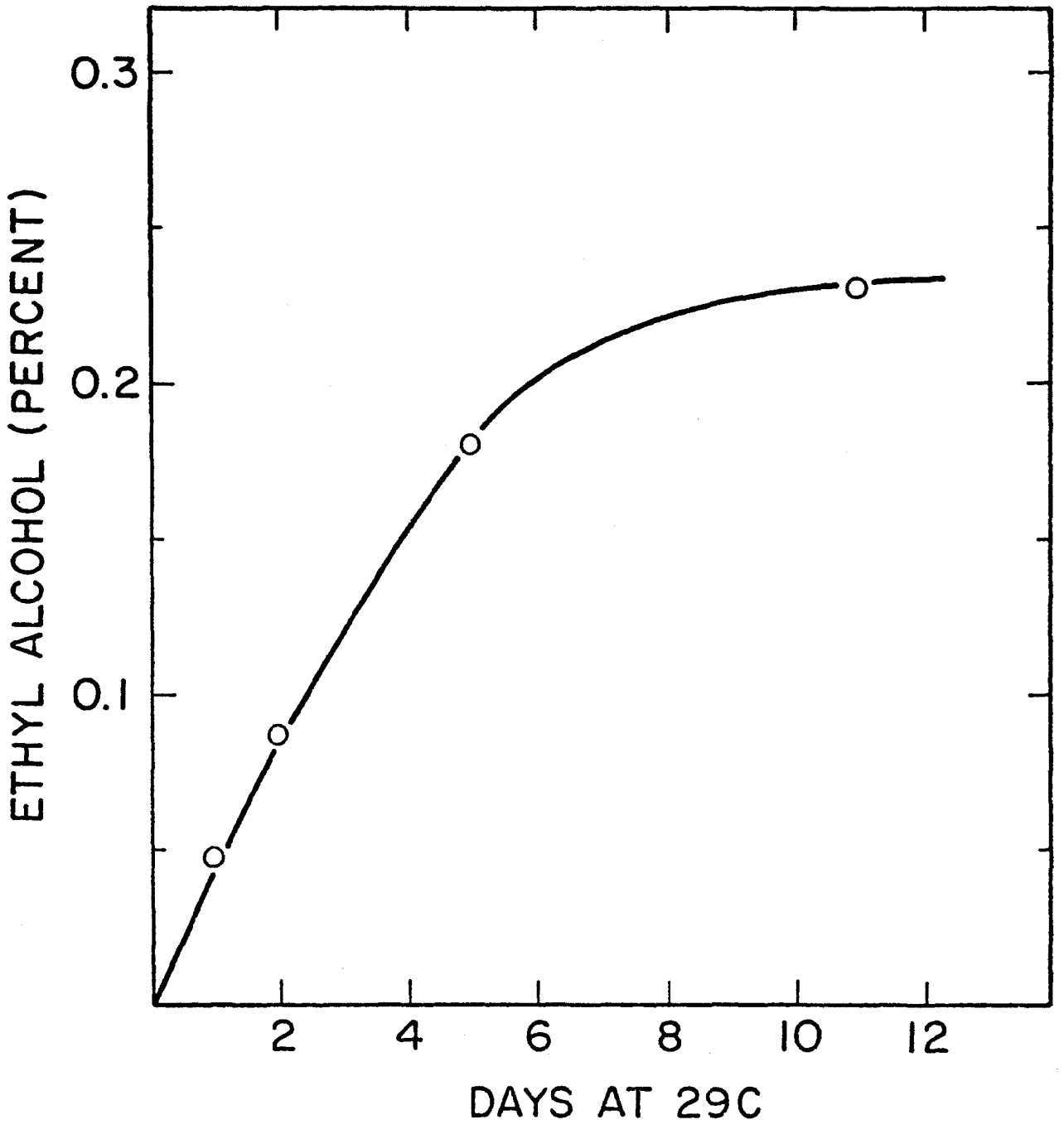

FIGURE 1. Alcohol production at 29°C by Schizosaccharomyces pombe NCYC 132 in 5% D-xylose plus soluble glucose isomerase (Maxazyme GI) and 1% bovine serum albumin. Also present was 0.67% yeast nitrogen base without amino acids (Difco), a synthetic medium containing vitamins, minerals and an inorganic nitrogen source.

the medium, but to about one half of the level when it was present.

The alcohol produced after 15-20 days approximates 10% of the theoretical (Wang, Shopsis and Schneider, 1980). This low yield may involve one or several of the following factors. 1. A drop in cell viability which occurs after 2-3 days. In a typical experiment using Maxazyme GI the viable cell count increased from 2.6×10^6 cells/ml to 4.1×10^7 cells/ml in two days, and then dropped to 10^5 cells/ml in seven days. 2. A build up of proteolytic activity in the medium, which then inactivates glucose isomerase despite the presence of excess soluble protein. 3. A build up of Maillard reaction products (the medium darkens after about ten days) which inhibit yeast growth. 4. Denaturation of soluble glucose isomerase by the continual shaking of the culture.

The rate of alcohol production in 5% xylose plus glucose isomerase was about 1/4 that when pure 1% D-xylulose was used. This difference is due to the relatively low concentration of D-xylulose present in D-xylose-glucose isomerase mixtures. Equilibrium is reached when the concentration of D-xylulose in the medium reaches about 15% (Topper, 1961). Increasing D-xylose concentration to 10-20% with the intent of increasing that of D-xylulose did not lead to more rapid rates of alcohol production, because cell viability dropped off rapidly at these higher D-xylose concentrations.

DISCUSSION

The results indicate how yeasts can be used to ferment an abundant aldopentose, D-xylose, to ethanol. They also

provide suggestions to improve the rate and amount of alcohol production. One suggestion is the use of a yeast, or medium conditions, where viability does not drop off as rapidly as in xylose-xylulose media. Others are the use of a yeast which does not produce proteolytic enzymes which can inactivate glucose isomerase, or the use of an isomerase insensitive to yeast proteases.

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