DIRECT BIO-CONVERSION OF HEMICELLULOSE SUGARS TO ETHANOL

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CERTIFICATE

This is to certify that the thesis entitled "Direct Bio-conversion of Hemicellulose Sugars to Ethanol" submitted by Maitreyee Banerjee, has been prepared under our supervision in conformity with the rules and regulations of the Indian Institute of Technology, Delhi. The research report and results prepared in the thesis have not been submitted for any degree in any other university.

(K.S. Gopalkrishnan)

(T.K. Ghose)

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SYNOPSIS

Agricultural residues like rice husks, peanut shells, corn-cobs, wheat straws etc. constitute today a major substrate for ethyl alcohol production all over the world. But a considerable portion (about 30-45%) of the residues available in the form of pentoses, are not converted into ethanol by the microorganisms used in the present day fermentation technology. The present thesis deals with studies on microbial conversion of pentose sugars of agricultural residues to ethanol.

Two pentose fermenting bacteria were isolated from the local soil samples. These were classified as Klebsiella pneumoniae and Citrobacter freundii, both belonging to the fm. Enterobacteriaceae. The isolates were compared with the other existing pentose fermenting microorganisms like Pachysolen tannophilus, Candida sp. XF 217 etc. for their growth rate, ethanol yield, fermentation time, ethanol productivity, ethanol yield based on cell mass etc. The comparative analyses of the fermentation parameters led to the conclusion that the new isolates K. pneumoniae was better placed for fermentation of pentose to ethanol. It was also found that K. pneumoniae had superior characteristics vis-a-vis the C. freundii and therefore was chosen for further study.

The different aspects of pentose fermentation by K. pneumoniae which were investigated included (i) identification and optimization of the critical parameters which had direct relation with fermentation of pentoses to ethanol. These parameters included temperature, pH, medium composition, effect of aeration, agitation and reducing agents in the fermentation broth, carbon - nitrogen ratio and the level of phosphate and of substrate concentrations in the fermentation broth (ii) studies on kinetic parameters of the isolate (iii) estimation of end products of fermentation to obtain a carbon balance of pentose substrate and also to postulate a metabolic pathway which may be involved in the bioconversion of pentose sugars to ethanol by K. pneumoniae. The original isolate of K. pneumoniae was also subjected to mutagenesis to improve the ethanol yield and also to increase the level of tolerance to ethanol. Since all the above studies were conducted using D-xylose (the primary pentose carbon in hemicelluloses) as the sole carbon source, the ability of the original isolate and the mutants to ferment the other hemicellulose sugars was also assessed by using hemicellulose hydrolysates, D-glucose and mixtures of D-glucose and D-xylose and also D-glucose, D-xylose and D-cellobiose.

The results of the study showed that the maximum achievable ethanol yield was obtained by the original strain of K. pneumoniae in a chemically defined medium with pH 8.0

and temperature 25-28°C. It was further seen that fermentation of D-xylose to ethanol by K. pneumoniae was completed in only 10 h and occurred anaerobically. The end products of fermentation consisted besides ethanol, of acetic acid and carbon dioxide . A considerable portion of substrate carbon was converted into pyruvate which indicated the involvement of glycolysis as the major pathway for ethanol production from D-xylose.

The mutation studies resulted into two mutants, MB-16 and MB-16-1048. Comparison of kinetic parameters of the two mutants and the original strain showed that the ethanol yield of the second generation mutant MB-16-1048 improved over the first generation MB-16 and the original strain by 30% and 82% respectively and also exhibited higher level of tolerance to ethanol.

The original strain and the mutant MB-16-1048 fermented hemicellulose hydrolysate D-glucose and mixtures of pentoses and hexoses including D-cellobiose. The ethanol yield obtained from D-glucose was same as that from D-xylose whereas the ethanol yield from mixed sugars was higher than that obtained either from D-glucose or D-xylose. The study therefore proved the efficiency of the isolate in fermenting hemicelluloses as well as celluloses to ethanol.

It was concluded that the fermentation of pentoses to ethanol based on the soil isolate K. pneumoniae would result in an efficient process for pentose sugar conversion to ethanol. Directions of future work in this area are also suggested in the thesis.

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