SIMULTANEOUS BIOCONVERSION OF GLUCOSE AND XYLOSE IN LIGNOCELLULOSE HYDROLYZATE TO ETHANOL

By

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Thesis submitted

in fulfilment of the requirements of the degree of

Doctor of Philosophy

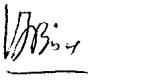
to the

INDIAN INSTITUTE OF TECHNOLOGY, DELHI

MARCH 1999

CERTIFICATE

This is to certify that the thesis entitled "Simultaneous bioconversion of glucose and xylose in lignocellulose hydrolysate to ethanol", being submitted by Ms. Priya Chandrakant to the Indian Institute of Technology, New Delhi, for the award of the degree of "Doctor of Philosophy", is a record of the bonafide research carried out by her, which has been prepared under my supervision in conformity with rules and regulations of the "Indian Institute of Technology, Delhi". The research reports and results presented in the thesis have not been submitted for any degree or diploma in any other University or Institute.



Prof. V. S. Bisaria

ACKNOWLEDGEMENT

First and foremost, I would like to express my deep sense of gratitude to my Ph.D supervisor Prof. V.S. Bisaria. I don't think I could have got a better guide than him. He possesses many qualities which I feel make him a perfect guide, a few of which are : his calm and composed manner in which he works, his soft spokenness, patience, dedication, seriousness, professional acumen and competence and a fine sense of perfection in any given task. Prof. Bisaria took keen interest in my research giving timely advice and valuable suggestions at every step thus leading to the successful and timely completion of the objectives of my project. Working under him was a great pleasure for me and I fall short of words to express how much respect and admiration I hold for him.

I would like to specially thank my SRC members: Dr.Saroj Mishra, Prof.S.N.Mukhopadhyay and Dr.P.S.Pandey for putting forth many useful suggestions regarding my project.

I would like to thank all the members of the DBEB family: faculty members, the office staff (Mr Kalra, Mr Anand, Mrs. Sunita, Babu Lal), members of BTIS, Mrs Neera (Doc. unit), Didar Mal (storekeeper), members of WTL, BPL, EEL, r-DNA Lab., Pilot Plant, M. Tech Lab., Biochem Eng. Lab., who have helped me at some time or the other in my research work.

I sincerely acknowledge the technical assistance rendered by the BRL staff : Mr.V.K. Ghosh, Mukeshji, Kishenji, Ram Gopal and Meherchand.

My friends (research scholars and project staff) of BRL have been instrumental in creating a lovely lab. atmosphere conducive to work and I thank each of them profusely.

I am also very grateful to Santosh (DST) and Samsheer Dagar (Friends Computers) for their expertise in thesis formulation.

Last but not the least, I will be ever grateful to my parents for their constant encouragement, moral support and sacrifice throughout my research work.

Priya Chandrakant

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ABSTRACT

Xylose is the major pentose sugar obtained on hydrolysis of hemicellulose. Whereas the fermentation of glucose (the main constituent of cellulose hydrolyzate) is a well-developed technology, the bioconversion of xylose to ethanol presents a biochemical challenge, especially if present along with glucose.

Saccharomyces cerevisiae can ferment glucose to high concentration of ethanol at an optimum pH 5.0 and temperature 30°C. It cannot utilise xylose, although it can ferment its ketoisomer xylulose to ethanol. *Candida boidinii* is an yeast that produces xylose isomerase with an optimum activity at pH 4.5 - 5.0 and temperature 30 - 40°C.

The approach was, therefore, to use xylose isomerase and *S. cerevisiae* in a glucose-xylose mixture such that xylose isomerase converted xylose to xylulose and *S.cerevisiae* fermented xylulose and glucose to ethanol.

Xylose isomerase from *C. boidinii* was extracted and partially purified, to give a specific activity of 0.1 IU (mg protein)$^{-1}$. The enzyme was then immobilized onto various supports among which hen egg shell was found to be ideal. The enzyme retained 47.5% of its activity and was stable for 3 consecutive fermentation runs. Xylose isomerase loading of 4.5 IU (g initial xylose)$^{-1}$ was found to be optimum for fermentation of xylose and glucose-xylose mixture to ethanol by *S. cerevisiae.* Xylose isomerase preparation and *S. cerevisiae* were used to carry out simultaneous isomerization and fermentation (SIF) of xylose to

ethanol. The fermentation of 50 gl^{-1} xylose gave an ethanol concentration of 7.5 gl^{-1}, xylose utilization efficiency 42%, metabolic yield 0.36 g (g xylose consumed)$^{-1}$ and overall productivity 0.13 $gl^{-1}h^{-1}$. The fermentation led to the formation of byproducts, viz., xylitol, arabitol, glycerol and acetic acid. Simultaneous isomerization and cofermentation (SICF) of glucose and xylose was carried out in the following mixtures of glucose and xylose: 80 gl^{-1} glucose + 20 gl^{-1} xylose; 50 gl^{-1} glucose + 50 gl^{-1} xylose; 30 gl^{-1} glucose + 70 gl^{-1} xylose. SICF of 30 gl^{-1} glucose and 70 gl^{-1} xylose gave an ethanol concentration of 22.3 gl^{-1}, xylose utilisation efficiency of 62.7%, metabolic yield of 0.36 g (g sugar consumed)$^{-1}$ and overall productivity 0.40 gl^{-1} h^{-1}.

The presence of borate favoured the formation of more xylulose from xylose and markedly enhanced the performance of SIF and SICF. SIF of 50 gl^{-1} xylose was carried out in the presence of borate present in an optimum xylose : borate molar ratio of 6.6. The fermentation parameters improved to give ethanol concentration 13.4 gl^{-1}, xylose utilisation efficiency 65.6%, metabolic yield 0.4 g (g xylose consumed)$^{-1}$ and overall productivity 0.24 $gl^{-1}h^{-1}$. The SICF of 30 gl^{-1} glucose and 70 gl^{-1} xylose in the presence of borate improved the fermentation parameters to give ethanol concentration 32 gl^{-1}, sugar utilisation efficiency 74.2%, metabolic yield 0.43 g (g sugar consumed)$^{-1}$, and overall productivity 0.57 $gl^{-1}h^{-1}$.

Owing to optimal fermentation of xylulose at 35°C, further improvement was observed in the fermentation parameters when the temperature of fermentation was increased from 30°C to 35°C at 40 h.

The effect of media components and fermentation byproducts on fermentation was studied. Among the products of fermentation formed, glycerol found to inhibit the activity of xylose isomerase. The nature of inhibition was non competitive. The growth of yeast was inhibited by the byproducts arabitol and acetic acid. The study was extended to peanut shell hydrolyzate. The peanut shells were subjected to acid hydrolysis. The hydrolysate containing 50 gl^{-1} xylose was fermented to give an ethanol concentration of 6 gl^{-1} and sugar utilization efficiency of 36% and metabolic yield 0.33 g (g sugar consumed)$^{-1}$. A booster dose of xylose isomerase at 48 h led to an increase in the fermentation parameters. An ethanol concentration of 7.5 gl^{-1}, sugar utilization efficiency 40.8% and metabolic yield 0.36 was obtained. The acid hydrolysate was further treated with cellulase which resulted in the release of glucose and xylose. The hydrolysate containing a glucose-xylose mixture of 65 gl^{-1} glucose and 50 gl^{-1} xylose on fermentation gave an ethanol concentration of 32.5 gl^{-1}, sugar utilization efficiency of 74.7% and metabolic yield of 0.43 g (g sugar consumed)$^{-1}$. The pretreatment of peanut shells using acid led to the release of furfural which at concentrations as low as 0.25 gl^{-1} inhibited the enzyme xylose isomerase irreversibly.

The approach used in this study can be used for the bioconversion of sugars in lignocellulose hydrolysate as seen in simulated glucose-xylose mixture and peanut shell hydrolystate. The presence of borate and temperature modulation can further enhance the utilization efficiency of sugar giving improved ethanol concentration and yield.

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