Research Journal of Chemical Sciences _____ ISSN 2231-606X
Vol. **3(3),** 58-64, March **(2013)** *Res. J. Chem. Sci.*

Biological Production of Xylitol from Corn Husk and Switchgrass by *Pichia stiptis*

Chaudhary Neeru*, Balomajumder Chandrajit and Jagati Vidyasagar
Department of Chemical Engineering, Indian Institute of Technology Roorkee, Roorkee, INDIA

Available online at: www.isca.in
Received 24th January 2013, revised 4th February 2013, accepted 25th February 2013

Abstract

Xylitol is a naturally occurring sugar substitute and widely used sweetener. In the present study, xylitol was produced through biological reduction pathway using yeast strain Pichia stiptis CBS 5773 by extracting xylose from agricultural residue like corn husk and switchgrass hemicelluloses as substrate. Acid treatment with sulphuric acid followed by detoxification with 2% $Ca(OH)_2$ was done to reduce the inhibiting factors like acetic, furfuryl and tannic acids and phenolic compounds. A comparative study of xylitol production using corn husk, switchgrass, their mixture (corn husk and switchgrass) and pure xylose was done. Yield of xylitol after 72 h of fermentation at 32°C and pH 5.7 using corn husk, switchgrass, their mixture and pure xylose was found to be 0.62 g, 0.48 g, 0.59 g and 0.73 g respectively per g of xylose initially present. Xylose consumption efficiency was 63.4%, 52%, 60% and 73% in corn husk, switchgrass, their mixture and pure xylose hydrolysates respectively under optimal condition. Therefore production was efficient using pure xylose and corn husk in comparison to switchgrass and mixture of corn husk and switchgrass under optimal condition.

Keywords: Corn husk, detoxification, D-xylose, hemicelluloses, *Pichia stipis,* switchgrass, xylitol.

Introduction

The increased health problem and rising health concern in modern world has promoted various medicinal and natural products. Xylitol is one of the sugar substitutes commercially used in different healthcare sectors, pharmaceuticals, animal nutrition, chemical production and especially as an alternative sweetener for diabetic patients. It is receiving attention not only because of its high sweetening power but also due to its anti carcinogenic property. It is also used in preparation of oral hygiene products, like toothpastes, mouthwashes, in the manufacture of sugarless chocolates, chewing gums, and other confectioneries for diabetics and in clinical applications for prevention of acute otitis, osteoporosis and ear infections[1]. Industrially, xylitol is produced through chemical reduction of xylose derived from different types of hemicelluloses hydrolysates like sugarcane bagasse, hardwood, fruits, vegetables, agriculture residues etc. This commercial production of xylitol is performed in the presence of a catalyst like Ni/Al_2O_3. However, high temperature and pressure requirements, usage of catalyst, intensive separation and purification procedures and a low yield make such an important and useful product relatively expensive, as it costs about $7 per kg using birchwood as raw material[2,3].

Alternatively, bioconversion method has been employed in recent years owing to its cost effectiveness. Biological pathway requires good selection of hemicellulosic source as well as choice of microorganism which is capable of utilising the xylose. Hemicelluloses, comprising of hexosans (in the form of xylose polymer) and pentosans, are abundant in nature as they are found in plant cell wall[4]. Therefore many studies have been done on the xylitol production from agricultural by-product like sugarcane bagasse[5], rice straw[6], hardwood[7] and barley bran[8].

Various fungi (*Fusarium sp., Penicillium sp., Aspergillus sp.)*[9], yeast (*Candida sp., Pichia sp., Debaryomyces sp., Pachysolen sp.)*[10] and bacteria (*Enterobacter sp., Corynebacterium sp., Mycobacterium sp.)*[11,12] have been used for the utilisation of xylose. Most of the studies have been performed using yeast strains and its metabolism has been reported by different authors[9,13].

One of the important steps in xylose conversion from agricultural residue is its pretreatment that alter structural integrity, remove lignin, increase surface area and extract the xylose and convert it into fermentable sugars[14]. Pretreatment can be done by physical, chemical or biological methods such as steam pretreatment, freeze explosion, acid treatments (hydrochloric acid, phosphoric acid, sulfuric acid), alkaline treatment (sodium hydroxide, ammonia) or treatment with organic solvents (ethanol, ethylene glycol)[15,16]. However, pretreatment results in the production of certain compounds like furans, hydroxymethylfurfurals acetate, hydroxybenzaldehyde (HBA), siringaldedyde (SGA), vanillin and other phenolic toxic substances, which proves inhibitory to microorganisms[17]. Some of the methods to remove these inhibitors are use of activated charcoal[18], ion ex-change resins[19], $Ca(OH)_2$[20], laccase enzyme[21], recombinant strains[22] and adaptation of the microbial strains[23], CaO treatment[24] and overliming[20].

Xylitol production is affected by several factors like culture conditions, strain, inoculums age, cell line, fermentation type,

medium composition, initial xylose concentration and presence of inhibitors in the hemicellulosic hydrolysates.

In the present investigation, two substrates corn husk and switchgrass were used. Corn husk is agricultural by-product present in significant amount but has low commercial value[25] which is utilised mainly as animal feed. It is rich in pentose sugars consisting of mainly xylose in the form of arabin-o-xylan[26]. Switchgrass is a perennial warm season grass and is not employed in large applications. Xylitol production using corn husk, switchgrass and their mixture using *P. stiptis* has not been yet reported. The aim of present study is to show the comparison of xylitol production using corn husk, switchgrass, their mixture and pure xylose by utilising the ability of *P. stiptis.*

Material and Methods

Preparation of hemicellulosic hydrolysates: Corn husk was collected from local market, Roorkee, India and switchgrass was collected near the bank of river Ganga, Roorkee, India. Both were washed thoroughly with distilled water and oven dried at 55°C for 24 h, then crushed, grounded and sieved to particle size of 2 mm. Acid hydrolysis was done using diluted H_2SO_4 acid. For this, 6 ml of 2% H_2SO_4 was added to 300 mg of each substrate. The mixture was allowed to stand for 1 h at 30°C. 168 ml of distilled water was then added and mixture was autoclaved at 125°C for 1 h. Prehydrolysates were filtered to remove the unhydrolysed residue and washed with warm water (60°C).

Detoxification of hemicellulosic hydrolysates: Hemicellulosic hydrolysates were heated to 150°C to reduce the concentration of volatile components and were held at that temperature for 15 minutes. Overliming of prehydrolysates were done with 2% $Ca(OH)_2$ until it reached pH of 10. Precipitation of prehydrolysates was done with 0.1% sodium sulphite. Precipitate was removed by centrifugation at 5000 rpm for 10 minutes and filtrate was re acidified to pH 6.0 with 1N H_2SO_4. 1% powdered charcoal was added and solution was stirred at 100 rpm and 30°C for 45 min. The liquor was recovered by filtration and was characterised for xylose concentration.

Microorganism and inoculums preparation: *Pichia stiptis CBS 5773* used for the study was supplied by Microbial Type Culture Collection, IMTECH Chandigarh. Lyophilized cultures were revived in 50 ml YMP growth medium containing 3.0 g/l Malt extract, 3.0 g/l Yeast extract, 5.0 g/l Peptone and 10.0 g/l Glucose. Cultures were stored on agar slants at 4°C till further use and were sub cultured after 30 days.

Fermentation conditions: The hydrolysates were supplemented with 2.0 g/l peptone, 3.0 g/l yeast extract, 1.0 g/l $(NH_4)_2SO_4$, 2.0 g/l KH_2PO_4 and 1.0 g/l $MgSO_4$.7H2O. Batch fermentation was conducted in 250 ml Erlenmayer flasks with a working volume of 50 ml. The fermentation medium was inoculated with 24 h old, 2% v/v inoculum. The fermentation was carried out at 32^0C, pH 5.7 and 250 rpm for 72 h. Samples were taken at regular time intervals to determine the concentrations of xylitol and remaining xylose in hydrolysates.

Analytical method: Cellulose content was estimated using Semimicro Determination Method of Updegraff[27]. Pentose sugar analysis was carried out by a modification of the method initially proposed by some authors[28]. Initial lignin content was determined by Klason Lignin method ASTM D-1106 and ash content was measured by ASTM D2584, D5630, ISO 3451 A2LA Accredited method. The concentration of xylose and xylitol was determined using high-performance liquid chromatography (HPLC WATERS 2414) with BioRad Aminex HPX-87H column and RI detector using acetonitrile-water (70:30) as mobile phase at 0.6 ml/min flowrate and 45°C temperature.

Results and Discussion

Corn husk and switchgrass were used as hydrolysates for the production of xylitol by *Pichia stiptis.* Figure-1 and figure-2 shows the scanning electron micrograph (Leo electron microscopy Ltd.) of the untreated strand of corn husk and switchgrass respectively. The strand of hemicelluloses substrate shows the irregular surfaces due to the presence of encrusting substances like lignin, pectin etc. which form thick outer layer to protect the cellulose. These impurities affect the appearance and processing of the fibres, therefore, these must be reduced in the substrates for efficient utilisation the hemicelluloses.

Composition of the substrate were determined by standard methods and noted in table-1. Switchgrass showed high lignin and ash content than corn husk so it was expected to give lower production than corn husk as lignin and ash pose inhibition to the process. Xylitol is the product of bioconversion of xylan (xylose polymer), therefore, xylan content should have direct influence on the production of xylitol. Initial xylose concentration was found experimentally to be high in corn husk than switchgrass as reported in table-2. As the initial xylose content was too low to be utilised by *P.stiptis,* acid treatment and overliming was done to concentrate hemicelluloses and reduce inhibitor which was successfully performed as result shown in table-2.

Table-1
Composition of substrate (% dry weight basis)

Hydrolysates	Celluloses	Hemicelluloses	Lignin	Ash
Corn husk	45.7	33.5	14.1	6.7
Switchgrass	41.3	28.6	21.6	8.5

Table-2
Effect of detoxification

Hydrolysates	Xylose before detoxification (g/l)	Xylose after detoxification (g/l)
Corn husk	32	52
Switchgrass	19	35

Figure-3 and figure-4 shows the pattern of xylose uptake and xylitol production by *P. stiptis* with fermentation time using corn husk and switchgrass respectively. It was noted through graph that xylose uptake was not so efficient using switchgrass as compared to corn husk. The reason for low efficiency was due to low xylan content and higher lignin content in switchgrass. Pure xylose and mixture of switchgrass and corn husk were also used as the separate hydrolysates in experiment and result reported in figure-5 and figure-6. By using mixture of switchgrass and corn husk, an attempt has been made to show the influence of presence of one type of hydrolysate over other type of hydrolysate. Pure xylose was used to observe the ability of *P. stiptis* towards xylitol formation. Comparison of the yield of different hydrolysates (corn husk, switchgrass, their mixture and pure xylose) are shown in figure-7, which showed that yield of the mixed hydrolysate lied between corn husk and switchgrass, however it was closer to corn husk. It was concluded that mixing the low xylan containing hydrolysate with hydrolysate containing high xylan proves to be beneficial. For pure xylose, yield was about 0.73 g/g of xylose in 72 h of fermentation time. It was comparable with other yeasts like *C. tropicalis* (0.82 g/g)[29] and *C. guilliermondii* (0.79 g/g)[30], which shows the capability of *P. stiptis* for xylitol production. Yield was highest in pure xylose among all the hydrolysates but, use of pure xylose is not economical since it is very costly and therefore alternative substrates like switchgass and corn husk have been used.

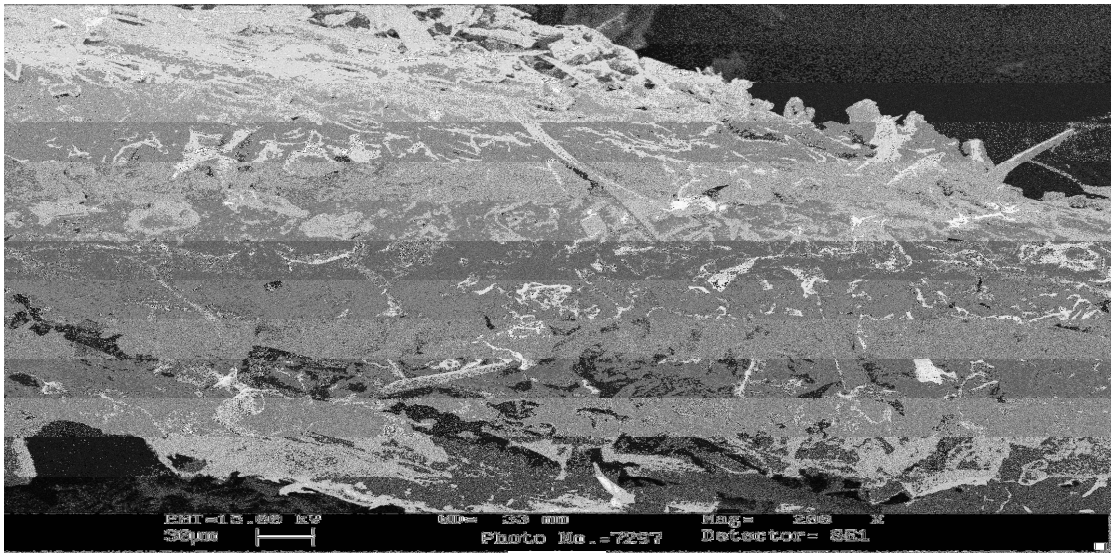

Figure-1
SEM of untreated corn husk strand

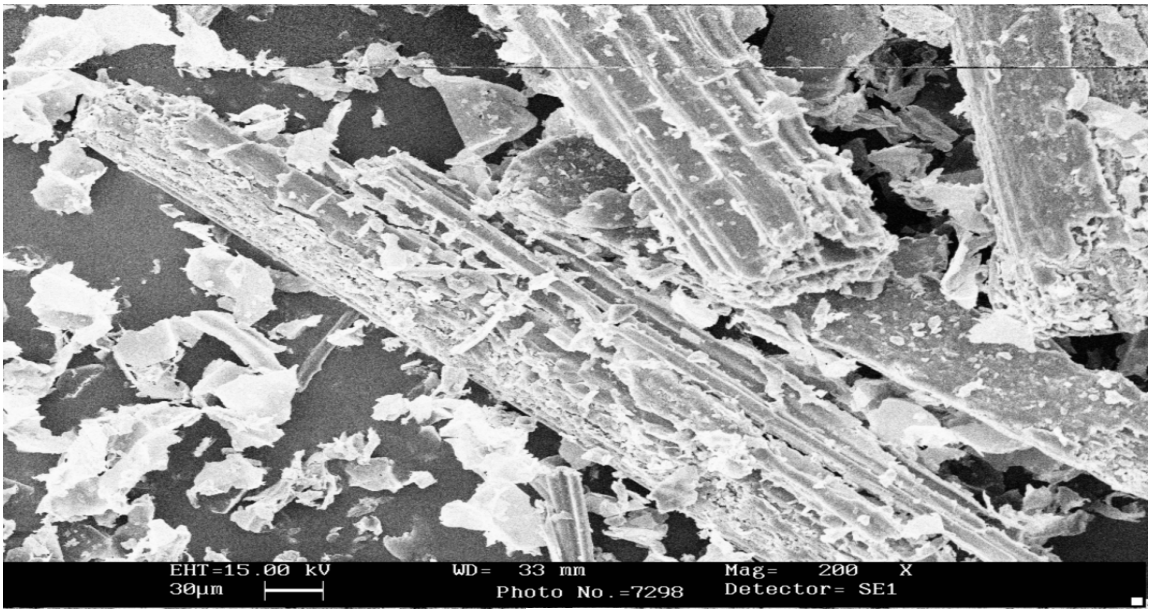

Figure-2
SEM of untreated switchgrass strand

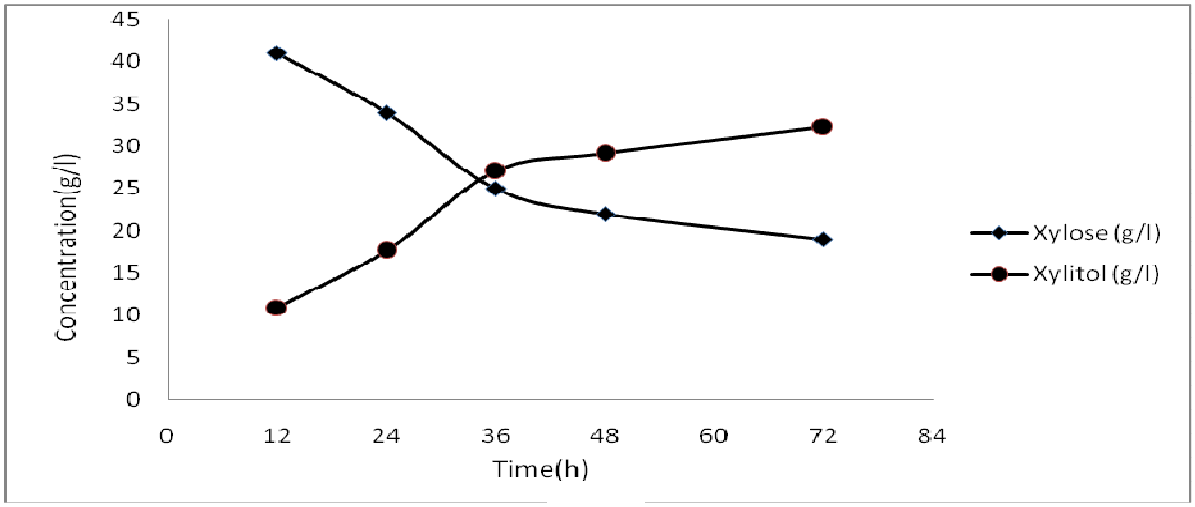

Figure-3
Xylose uptake and xylitol production by *P. stiptis* using Corn husk

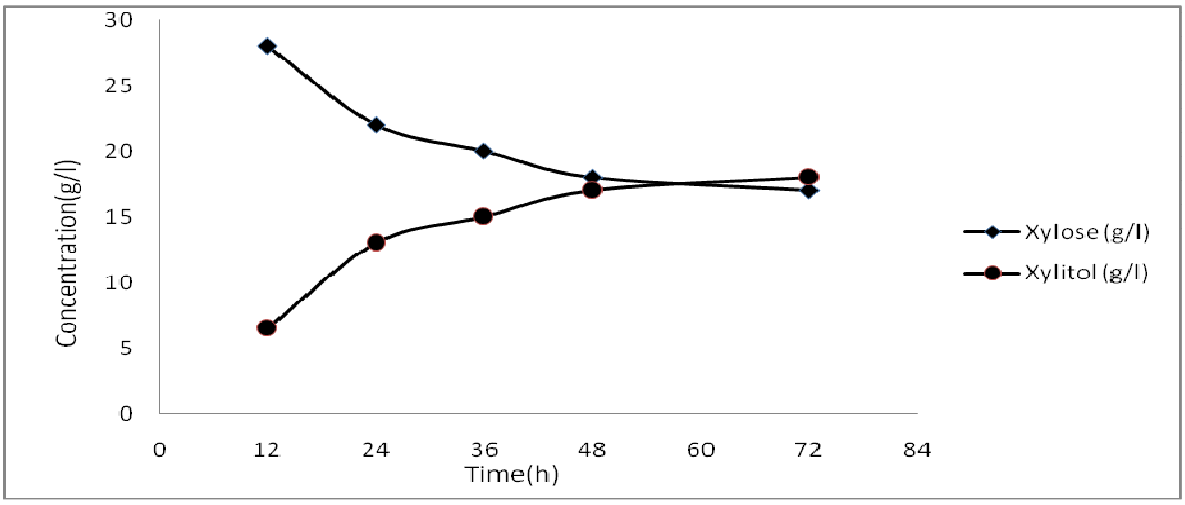

Figure-4
Xylose uptake and xylitol production by *P. stiptis* using Switchgrass

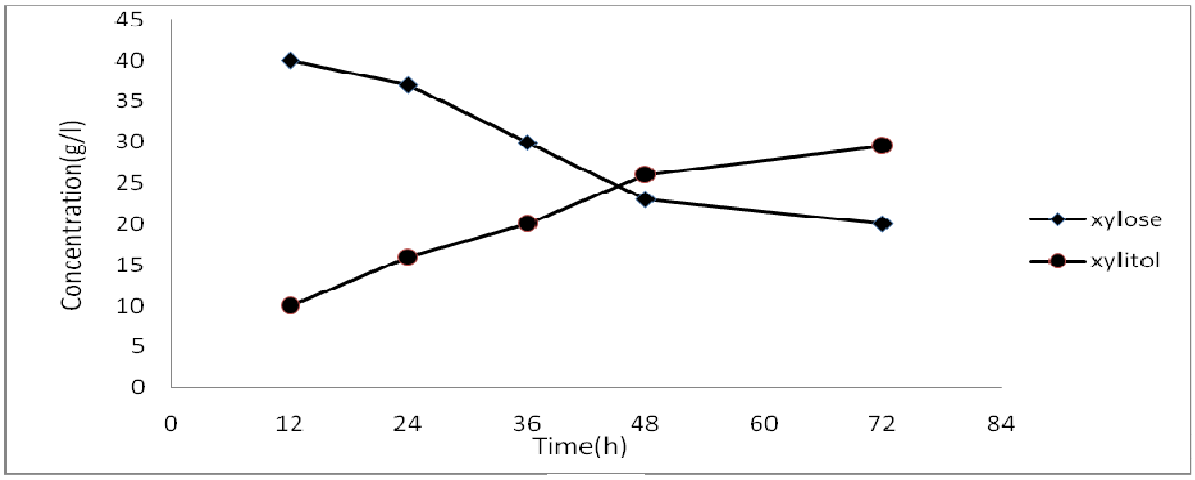

Figure-5
Xylose uptake and xylitol production using mixture of corn husk and switchgrass and *P. stiptis*

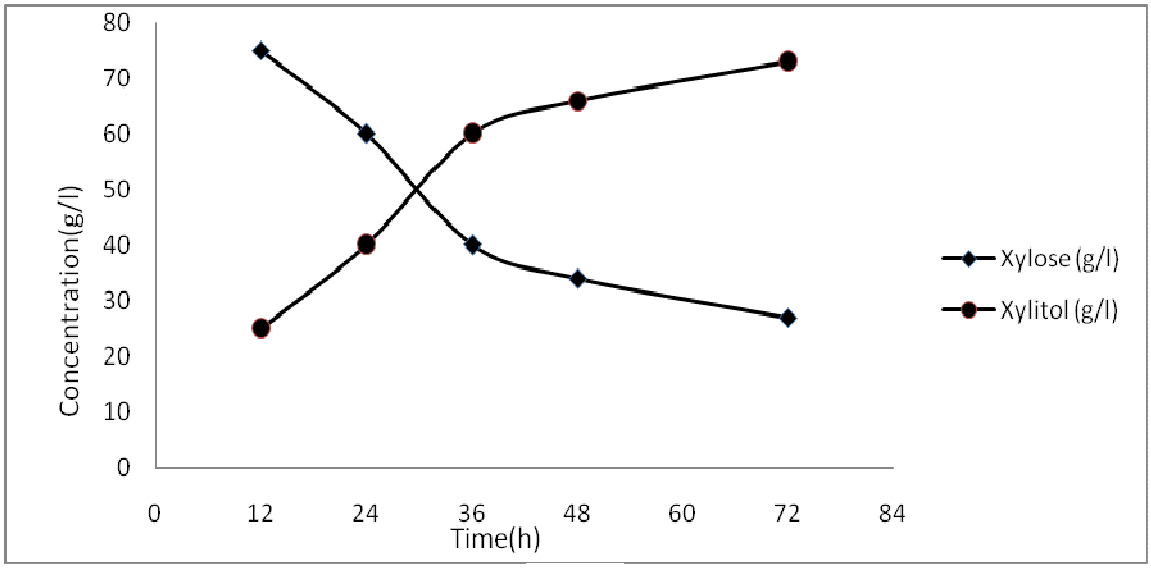

Figure-6
Xylose uptake and xylitol production by *P. stiptis* using Pure xylose

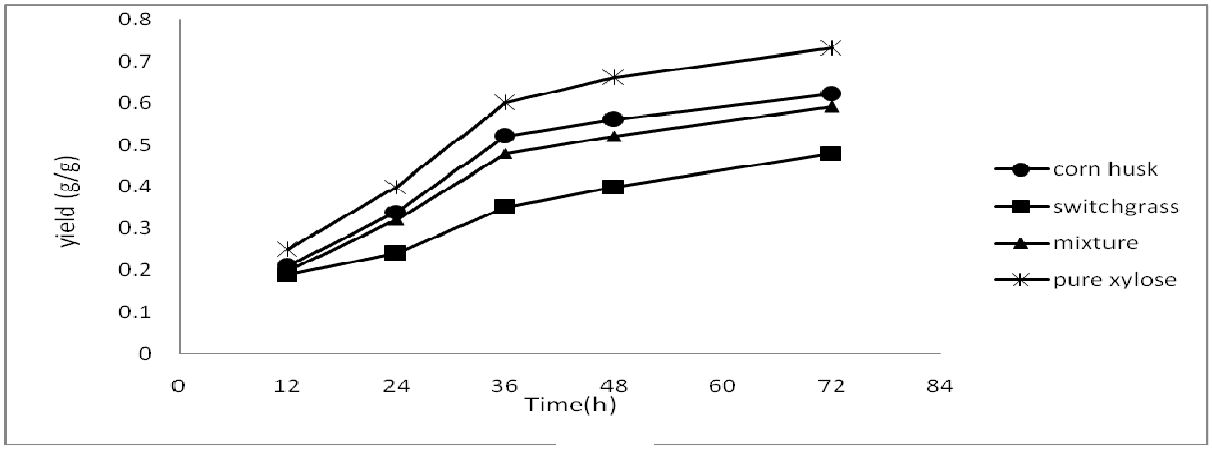

Figure-7
Yield using different hydrolysates

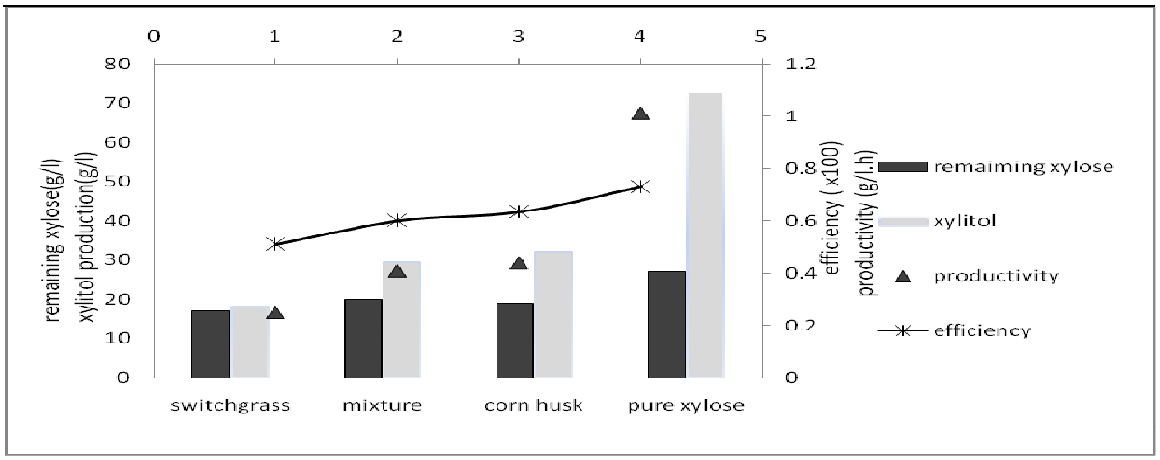

Figure-8
Profile of hydrolysates after 72 h of fermentation time

From the figure, it was also found that initially production was low and almost same for all cases. During the first few hours, microorganism takes time to adapt to media, therefore low yield was expected. Also the presence of inhibitors which was not completely removed during detoxification affects the growth of organism during fermentation. Steepness in curve was observed between 24 h to 36 h and afterwards the increase in production was not so appreciable. From the analysis, it was found that, in the first 12 h, 20 % of xylose by all the hydrolysates was utilised. Xylitol production in 36 h, as yield of xylitol was 0.52, 0.35, 0.48 and 0.6 g per g of initial xylose using corn husk, switchgrass, their mixture and pure xylose respectively. Results obtained from different hydrolysates after 72 h of fermentation time are summarised in figure-8 which shows their efficiency, productivity, remaining xylose concentration and xylitol concentration. Xylose consumption efficiency was 63.4%, 52%, 60% and 73% and productivity was 0.44, 0.25, 0.409, 1.013 g/l.h using corn husk, switchgrass, mixture and pure xylose respectively under optimal condition.

Conclusion

Xylitol can be seen as important constituent in food and pharmaceutical industries, thus its price should be lower and production should be higher for better access to population. Bioconversion pathway proves to good alternative. Xylitol production by biological method can be performed by proper selection of hemicellulosic hydrolysates with sufficient concentration of xylose in it, selection of capable microbes, maintaining optimum condition for its growth and fermentation. Biological production using yeasts are most common but stringent anaerobic conditions are necessary to maintain. Therefore, maintenance of proper growth condition is crucial for production.

Agricultural residues and forest wastes like corn husk and switchgrass have high xylose content and studies should be encouraged on these types of hemicelluloses. These are abundant in nature but not employed in many applications. Thus utilising it for xylitol production could minimise the waste accumulation and production cost of xylitol. Mixing the hydrolysate containing low xylose concentration with hydrolysate containing higher xylose content can improve the overall yield. Inhibitors have to be removed atleast to some extent in pre treatment steps for better efficiency. There are various treatment methods, any one or in combination can be employed. Research on production of xylitol is essential as it is a value-added product with a growing market potential.

Acknowledgement

The authors are thankful to Ministry of Human Resource Development, Government of India and institutes Instrumentation Centre, IITR for extending their financial support for present research work.

References

1. Uhari M., Kontiokari T. and Niemela M., A Novel Use of Xylitol Sugar in Preventing Acute Otitis Media, *Pediatrics,* 879–884 **(1998)**

2. Hyvonen L. and Slotte M., Alternative Sweetening of yoghurt, *J. Food Technol.*, 97-112 (**1983**)

3. Leathers, T.D., Bioconversion of maize residues to value-added coproducts using yeast like fungi, *FEMS Yeast Res*., 133–140 **(2003)**

4. Rao R.S., Jyothi C.P., Prakasham R.S., et al., Xylitol production from corn fiber and sugarcane bagasse hydrolysatess by *Candida tropicalis*, *Bioresource Technol.,* 1974–1978 (**2006)**

5. Roberto I.C., Felipe M.G.A., Lacis L.S., et al., Utilization of Sugar Cane Bagasse Hemicellulosic Hydrolyzate by *Candida guilliermondii* for Xylitol Production, *Bioresource Technol,* 271-275 **(1991)**

6. Roberto I.C., Mancilha I.M., Souza C.A. de, et al., Evaluation Of Rice Straw hemicellulose hydrolysates in the production of xylitol by *Candida guilliermondii, Biotechnol. Lett.,* 1211-1216 **(1994)**

7. Converti A., Perego P. and Domínguez J. M., Xylitol Production from Hardwood Hemicellulose Hydrolysates by *Pachysolen tannophilus, Debaryomyces hansenii* and *Candida guilliermondii. Appl. Biochem. Biotech.,*141-151 **(1999)**

8. Cruz M. J., Dominguez J. M., Dominguez H., et al., Xylitol production from barley bran hydrolysates by continuous fermentation with *Debaromyces hansenii, Biotechnol. Lett.*, 1895–1898 **(2000)**

9. Chiang C. and Knight S.G., Metabolism of D-xylose by moulds, *Nature,*79-81 **(1961)**

10. Barbosa M.F.S., Medeiros M.B. de, Mancilha I.M. de, et al., Screening of yeasts for production of xylitol from D-xylose and some factors which affect xylitol yield in *Candida guilliermondii, J. Indust. Microbiol.,* 241-251 **(1988)**

11. Yoshitake J., Shimamura M. and Imai T., Xylitol production by *Corynebacterium species*, *Agric. Biol Chem.,* 2251-2259 **(1973)**

12. Izumori K. and Tuzaki K., Production of xylitol from D-xylose by *Mycobacterium smegmatis, J. Ferment. Technol.,* 33-36 **(1988)**

13. Parajo J.C., Dominguez H. and Dominguez J.M., Biotechnological production of xylitol Part1: Interest of xylitol and fundamentals of its biosynthesis, *Bioresource Technol*.,191-201 **(1998)**

14. Mussatto S.I. and Roberto I.C., Alternatives for detoxification of diluted acid lignocellulosic hydrolysates

for use in fermentative processes: a review, *Bioresource Technol*., 1-10 **(2004)**

15. Olsson L. and Hagerdal-Hahn B., Fermentation of lignocellulosic hydrolyzates for ethanol production, *Enzyme Microb. Technol.,* 312–331 **(1996)**

16. Vallander L. and Erickson K.E.L., Production of ethanol from lignocellulosic materials: State of the art, *Adv. Biochem. Eng.,* 63–95 **(1990)**

17. Tsao G.T., Cao N. and Gong C.S., Hemicellulose conversion, Encyclopedia of Bioprocess Technology: Fermentation, *Biocatalysis and Bioseperation*, 1391–1400 **(1999)**

18. Pandey A, Soccol C. R., Nigam P., et al., Biotechnological potential of agro-industrial residues. I. Sugarcane bagasse, *Bioresour. Technol.,* 69–80 **(2000)**

19. Mancilha I.M. and Karim M.N., Evaluation of ion exchange resins for removal of inhibitory compounds from corn husk hydrolysates for xylitol fermentation, *Biotechnol. Prog.,* 1837–1841 **(2003)**

20. Dien B.S., Nichols N.N., Bryan P.J.O', et al., Development of new ethanologenic *Escherichia coli* strains for fermentation of lignocellulosic biomass, *Appl. Biochem. Biotechnol.,* 181–196 **(2000)**

21. Cassland P. and Jonsson L.J., Characterization of a gene encoding trametes versicolor laccase A and improved heterologous expression in *Saccharomyces cerevisiae* by decreased cultivation temperature, *Appl. Microbial biotechnol.,* 393–400 **(1999)**

22. Dien B.S., Hespell R.B., Ingram L.O. et al., Conversion of corn milling fibrous co-products into ethanol by recombinant *Escherichia coli* strains K011 and SL40, *World J. Microbiol. Biotechnol.,* 619–625 **(1997)**

23. Rivas B., Torre P., Domfinguez J.M. et al., Carbon material and bioenergestic balances of xylitol production from corncob *by Debaryomyces hansenii, Biotechnol. Prog.*, 706–713 **(2003)**

24. Silva S.S., Maria G.A., Silva B.A. et al., Acid hydrolysis of Eucalyptus grandischips for microbial production of xylitol, *Process Biochem*., 63–67 **(1998)**

25. Kadam K.L. and Mcmillan J.D., Availability of corn husk as a sustainable feedstock for bioethanol production, *Bioresour. Technol.,* 17–25 **(2003)**

26. Torget R., Walter P., Grohmann H.M.K., Dilute acid pretreatment of corn residues and short-rotation woody crops, *Appl. Biochem. Biotech.,* 75–86 **(1991)**

27. Updegraff D.M., Semi-micro determination of cellulose in biological materials, *Anal. Biochem*., 420–424 **(1969)**

28. Roe J.H. and Rice E.W., A photometric method for the determination of free pentoses in animal tissues, *J. Biol. Chem*., 507-512 **(1948)**

29. Yahashi Y., Horitsu H., Kawai K. et al., Production of xylitol from D-xylose by *Candida tropicalis*: the effect of D-glucose feeding, *Journal of Ferment. Bioeng.,* 148–152 **(1996)**

30. Alves L.A., Felipe M.G.A., Almeida J.B., Pretreatment of Sugarcane Bagasse Hemicellulose Hydrolysates for Xylitol Production by *Candida guilliermondii*, *Appl. Biochem. Biotechnol.,* 89-98 **(1998)**