



## Value Addition to Bagasse: An Agricultural Waste for Production of Xylitol by Fermentation Process Using *Aspergillus niger*†

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Xylitol is a five-carbon sugar alcohol that has attracted great attention because of its potential uses as a natural sweetener, in dental care and as a sugar substitute in diet. In current scenario agricultural wastes are being undervalued, this study focuses on increasing the value addition and usage of agricultural waste. The present study aims to bring about a value addition to bagasse by converting it to a five sugar alcohol (xylitol). In present work, the bioconversion of crude xylose from plant biomass (bagasse) with help of *Aspergillus niger* is performed. The obtained biomass was first autoclaved for 20 min then subjected to acid hydrolysis. Following this, using *Aspergillus niger* fermentation process for 145 h was carried out. After every 24 h, fermented sample was tested for the presence of xylitol and confirmed by thin layer chromatography. It was found that during first 24 h, the production of xylitol was null. The next 24 h showed some presence of sugar alcohol. On testing the sample after 72 h, the presence of xylitol is observed.

**Keywords:** Xylitol, Bagasse, *Aspergillus niger*, Fermentation, Hydrolysis.

### INTRODUCTION

Xylitol is natural occurring five carbon alcohol sugar having a high value product, due to its anticarcinogenic and other pharmacological and food nutritional properties. Xylitol has a low caloric sweetener and insulin free property in comparison to sucrose. It is widely used in sweet consumable products chewing gums, candy, soft drinks, ice creams and oral hygiene products. It also finds application in animal nutrition, chemical production [1-4].

Xylitol can be synthesized by catalytic hydrogenation and hydrolysis of hemicellulose of D-glucose from the raw material [2]. This process requires as a nickel catalyst and high temperature and pressure conditions [5]. This process faces the drawbacks like high cost of manufacturing, requires pure xylose sugar, high labour intensive, high energy consumption and also needs expensive and sophisticated refining processes [6]. Therefore as an alternative strategy hydrolysis of biomass followed by microbial fermentation is adopted [3,7]. In biotechnological production of xylitol from agricultural wastes like rice husk, wheat straw, corn cobs, bagasse and many fruits [3,8] are used along with several yeast strains, including *Pachysolen tannophilus*, *Candida* sp. *Kluyveromyces fragilis*,

*Debaryomyces hansenii*, various bacteria and mycelial fungi [2,3].

India is one of the largest consumers and producers of sugar in the world and is the world's second largest producer next to Brazil of the sugarcane. Bagasse is the leftover of the sugarcane after crushing and is burnt as a fuel in the boiler of sugar mill. Sugar cane production of India for 2014-2015 is estimated at 80.79 million tonnes [9]. Bagasse production for the year 2014-2015 is estimated to be 26.5 million tonnes. Typical chemical characteristics of bagasse include cellulose 42 %, hemicellulose 25 %, lignin 20 % with ash and waxes making up the remaining [10].

Bagasse cogeneration has been practiced in sugar mills since long to meet sugar mills own energy needs competing technology grid connectivity, due to financial constraints [10]. Paper mills has not yet been used as a feedstock for a commercial pulp mill. Environmentally sustainable pulp mill [6] has so far proven to be an unsuccessful investment due to high fluctuation in the market and currency value [11]. Alternate value added products like xylitol, xylooligo saccharides, etc. [12,13].

*Aspergillus niger* is a fungus and one of the most common species of the genus *Aspergillus*. Many useful enzymes are

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produced using industrial fermentation of *A. niger* like *A. niger glucoamylase*, etc. The major advantage of using *Aspergillus niger* is that it provides higher yield [14].

In this work, we considered the production of xylitol by biochemical method using *Aspergillus niger*. The main objective of present work is to give bagasse a value addition. Bagasse being an agricultural waste is undervalued, by this process we aim to give it an greater economic value and increase its use for more practical causes [15,16].

### EXPERIMENTAL

Bagasse was procured from the local sugarcane juice vendor. The raw material was cleaned, dried and sieved. Sulphuric acid (98.9 %),  $K_2HPO_4$ , CaOH,  $NaNO_3$ , KCl,  $MgSO_4 \cdot 7H_2O$ ,  $FeSO_4 \cdot 7H_2O$ , 2-propanol, ethyl acetate, yeast Extract from S.D Fine Chemicals India. Sucrose and Agar from HiMedia Lab. Pvt. Ltd. Methyl orange from Spectrum India. Auto clave and pH meter were according to lab standards. All the chemicals used were analytical grade.

*Aspergillus niger* was first cultured from the mother vial and then sub cultures were grown. The hydrolyzed bagasse was then fermented using *Aspergillus niger*. The process was monitored on daily basis and a small portion of the sample was extracted to monitor the progress of the fermentation, using paper chromatography.

### RESULTS AND DISCUSSION

The procured bagasse was washed with water and sun-dried for 3 days. The washed and dried bagasse was grinded and then subjected to sieve analysis using mesh number 36. In three different conical flasks, 25 g of bagasse (overflow and underflow mesh number 36) was added along with 350 mL of distilled water followed by autoclave process for 20 min. Autoclaved sample were filtered which made upto 250 mL. To the obtained samples by weight percentage conc.  $H_2SO_4$  was added and autoclaved. The weight percentages of acid used were 1, 3 and 5 %. pH of the samples is maintained between 5 and 6 [17] by adding CaOH. *Aspergillus niger* growth culture was prepared by using  $K_2HPO_4$ , yeast extract, sucrose,  $NaNO_3$ , KCl,  $MgSO_4 \cdot 7H_2O$  and  $FeSO_4 \cdot 7H_2O$ . The growth was examined for 170 h [18,19].

Inoculation of the cultured *Aspergillus niger* was followed by acid hydrolysis. The samples were agitated using a rotary shaker for 145 h. 20 mL of sample was extracted on daily basis to check the conversion. The fermented samples are subjected to paper chromatography [9] to confirm the presence of xylitol. 0.1  $\mu$ m of the sample was diluted to 100 mL using distilled water. 2-Propanol, ethyl acetate and water are mixed in the ratio of 7:1:2 to make the mobile solution of 25 mL. A small portion on Whatman No. 1 was taken and sample was put on it. After 25 min the paper was dipped in the mobile phase and kept for 30 min.

The paper was then dried and methyl red indicator was sprayed on the paper and then dipped in an iodine solution. The change in the colour of paper concludes the presence of xylitol. The colour change of paper from red to yellow is the parameter that helps in confirming the process of fermentation and the presence of sugar alcohol. On the first 24 h of the testing, a little intensity of colour change is observed. Test after 48 h gave us a better intensity of colour change. The intensity was evident on checking the sample for 72 h. The test on the sample after 96 h gave a strong colour intensity change and on 120 h the intensity was the highest. Hence, it is said that the maximum amount of fermented sugar was produced by the end of 120 h.

### Conclusion

Bagasse as an industrial waste of sugar refineries is used in small portion for paper making and power generation, by this process we can conclude that the agricultural waste obtained can be further processed and used in the production of xylitol, hence by doing so we are able to add value to a material of negligible value.

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