A Simple Method for D-Xylose Extraction From Jute Stick and Rice Husk

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Abstract: Hydrolysis of agricultural wastes of jute sticks and rice husks were studied to extract D-xylose. When jute sticks were used as substrates, NaOH was found to be suitable for D-xylose extraction at boiling temperature after a period of 1 h. 1N H2SO4 was also found best for D-xylose extraction from rice husks. No cellulose was detected in hydrolysate.

Keywords: D-xylose, jute stick, rice husk

Introduction
Biomass has served as a substrate in microbial processes for the production of alcoholic beverages for thousands of years. Recently that broader applications of this material have been envisaged. Thus biotechnologists are now developing efficient systems for the production of liquid fuels, pharmaceutical, foods and chemical feedstocks from "waste" organic materials (Magee and Kosaric, 1985). Bangladesh is an agricultural based country where jute is one of the major cash crops. Every year there is mass production of various species of jute plants throughout Bangladesh in order to obtain fibers. But the jute sticks are discarded as waste or used as a burning fuel in rural areas. Jute stick is a lignocellulosic material and in nature this is one of the most abundant, continuously renewable organic resources. Their major components, cellulose, hemi-celullose and lignin, vary with plant species. It has great potential as an ecologically advantageous feedstock for the production of valuable products including a number of useful chemicals and liquid fuels. Hemicellulose is a polymer of several different sugars and sugar derivatives. Roughly 75% of the monomers for hemicellulose are pentoses, and the sugar D-xylene is roughly 76% of these sugars. That's why another name for hemicellulose is xylan. Hemicellulose can constitute up to 35% of agricultural residues by dry weight, with the aldopentose D-xylene (usual not less than 96%) (Winkelhausen and Kuzmanova, 1998) forming the main constituent of this fraction when derived from hardwood or agricultural residues (Singh and Mishra, 1995; Kern et al., 1998). Cellulose, the most abundant carbohydrate on the earth, is almost always found associated with hemicellulose and lignin. Most fast-growing woody and annual crops are high in hemicelluloseic sugars such as D-xylene.

In industrial application, one of the desirable property is the ability to utilize readily available cheap resources as starting substrates for bioconversion processes. Sugarcane was the basis for the World's first renewable biofuel program in Brazil. Corn is the basis for the present renewable ethanol fuel industry in the United States. The sucrose produced by sugarcane, sugarcane and sweet sorghum can be fermented directly after squeezing them from the crop. The residues left over after removing fermentable sugars can also be utilized. In some cases they end up as animal feeds, but many agricultural residues can be converted into additional fermentable sugars through saccharification with cellulases and hemicellulases. Numerous waste streams are often inexpensive to obtain and in many instances they have a negative value attributable to current disposal costs. Waste streams with lower lignin contents and smaller particle sizes are easier to deal with than those with higher lignin contents and larger particle sizes. This renewable resource will become more valuable when methods of converting them into chemicals, materials and fuels are developed through research. These multi functional chemicals can be used as raw materials, among numerous other applications and other advances made in a number of technical areas which have contributed to the reduction of the cost of renewable materials. Therefore, this has made the mission of this experiment to extract D-xylose from waste jute sticks and rice husks.

Materials and Methods
Rice husks and jute sticks from different varieties of jute plants (Corchorus capsularis var. C-8987 and var. C-443) and allied fibrous plant kenaf (Hibiscus cannabinus var. C-95) were used in this experiment. Different acid/alkali (H2SO4, HCl and NaOH) were used to hydrolyze of jute stick pieces/powder and rice husks. D-Xylose isomerase was commercially obtained from Novo Industry Co. Ltd., Japan. High performance liquid chromatography (HPLC, Nihonbunko HPLC 880 PU liquid chromatography, Shimadzu RID-6A refractive index detector and Shimadzu C-R6A chromatopac) was done to detect the presence of D-xylose using a Hitachi HPLC column GL-911 and the separation was achieved at 60°C using 10-4 M NaOH at a flow rate of 1.0 ml/min.

Extraction method from jute stick: Jute sticks were cut into small pieces and also crashed mechanically to powder form using grinder. The hydrolysis was done using the following methods:

Method 1: Samples (2.5 g) were boiled for 1 h with 1N HCl (50 ml). The debris was then discarded and the supernatant was neutralized by 1N NaOH with continuous stirring. After neutralization, the supernatant (pH 7.0) was filtered by course filter paper and deionized by passage through Diaion SKSB (H+ form) and Amberlite IRA-411 (CO32- form) ion exchange resins for HPLC analysis.

Method 2: Samples (2.5 g) were boiled in distilled water for 1 h and after filtered through the course filter, supernatant was deionized and analyzed by HPLC analysis.

Method 3: Samples (2.5 g) were boiled for 1 h in 10% NaOH (50 ml) and neutralized with 1N HCl. Then again hydrolysis in 1N HCl for 1 h by boiling which was done then filtered and supernatant was neutralized by 1N NaOH. Finally, supernatant
was deionized and analyzed by HPLC analysis.

Method 4: Samples (2.5 g) were boiled for 1 h in 1N H₂SO₄ (50 ml). After boiling, the sample was filtered and the supernatant was neutralized (pH 7.0) by CaCO₃ and deionized by above mentioned resins for HPLC analysis. In order to determine the suitable boiling time, jute stick (small pieces and powder form) were boiled between 10 min to 3 h where same process was followed as mentioned in method-4. The comparative study of D-xylene extraction from jute stick at 30 min to 3 h was done using the same procedure described in method-4. Different varieties of jute plants (O. gossypium, C. gigantea) and allied fibers plant (kenaf) were also tested to extract D-xylene using the same procedure described in method-4.

Extraction method from rice husks: Rice husks (~8.0 g) were hydrolyzed in different concentration (Ni) of H₂SO₄ (1100 ml) ranging from 0.1N to 1N by boiling for 1h. Each sample was neutralized (pH 7.0) by CaCO₃ and the supernatant was collected by filtration. A similar deionization with the resins described previously, HPLC was done for the detection of D-xylene.

Confirmation of the product as D-xylene: The product was confirmed as D-xylene by analysis of the retention time and peak area of HPLC in two different ways. Firstly, an enzymatic reaction of the product with commercially available immobilized D-xylene isomerase was done which was then HPLC in the above mentioned condition. In another way, equal concentration (0.5%) of D-xylene and the product was mixed together and HPLC was carried out in order to know the product authenticity.

Results and Discussion

Considering different chemical treatments on jute stick, it was found that among the four methods, only 1N H₂SO₄ showed the best result (Fig. 1) where only D-xylene was extracted without producing any residual by-product during the reaction. Although D-xylene was found in other methods but separation of D-xylene from the hydrolyzed sample in all the other three methods are difficult which require various column chromatography techniques. Therefore, method-4 was found to be the best among them where there is no need to D-xylene separation. It was also observed in the experiment that 1N hydrolysis with 1N H₂SO₄ seemed to be optimum for the extraction of D-xylene (Fig. 2) and prolonged hydrolysis produced by-product in the hydrolyzed besides D-xylene. In case of D-xylene extraction from small pieces of jute stick and jute stick powder, it was found that small pieces of stick are better than the powder (Fig. 3). Among the various concentration (Ni) of H₂SO₄ tested for D-xylene extraction from rice husks, 1N H₂SO₄ was also found to be better than other concentrations (Fig. 4) where no by-product was produced.

Test was also carried out to know the suitable source of D-xylene among various sticks of different varieties of jute plant and allied fiber. Results shows that jute stick of variety O. gossypium was better source (Fig. 5). From the results of confirmation of the product as D-xylene, it was observed that only one peak was found from the HPLC of the mixture of authentic D-xylene and the product (Fig. 6a). After the enzymatic reaction of the product, another peak was found along with D-xylene which retention time (19.1 min) is same as that of D-xylene (Fig. 6b). So the product extracted from jute sticks and rice husks was confirmed as D-xylene.

Environmental engineers find lots of pentoses in natural environments and in wastes that have plant materials, such as undegraded foods and paper. Knowing something about pentoses helps in understanding waste treatment, bioconversion of cellulosic materials, and degradation of wood (Chiang et al., 1981; Ahmed, 2001; Winkelhausen and Kuzmanova, 1998). The major potential biodegradable agricultural and agro-industrial cellulosic wastes available in Bangladesh are rice straw, rice husk, wheat straw, sugar cane bagasse, saw dust, jute stick, jute mills waste etc. (Tareq, 1985). There are many different biomass feedstocks. They include crops, specifically grown for biomass energy, various agricultural residues, wood residues and waste streams. Their costs and availability vary widely. Collection and transportation costs are often critical. Jute stick and rice husk have been shown as model substrates because of their abundance, high carbohydrate content (64% cellulose and 34% hemicellulose) (Mohindran et al., 1987, Ali et al., 1991), inexpensive and constantly available, although jute grows seasonally. During the past few years both fungal and bacterial cellulases have been explored to develop the saccharification technology but the technology is yet to develop because it is a complicated process, affected by a
number of factors like substrate type, pretreatment, temperature, pH, time, characteristic of enzyme preparation and reuse of enzyme (Mandel and Sternberg, 1976). On the other hand, this report supports a very simple chemical extraction process where this kind of parameters are needed not to be regulated.

The original process for the large scale production of sweeteners from corn starch based primarily on solid acid hydrolysis of starch, resulted in low yields and the production of undesirable by-products through carbohydrate modification (C.C. Taylor, US patent 3,445,972, 1967). In contrast, the current process utilizes simple chemical steps, where only D-xylose can be extracted. It was also observed from HPLC analysis that no cellobiose was produced from these samples. Different aspects of the conversion of D-xylose by pro-and autotrophic microorganisms are considered, including the transport of this pentose into the cells and its metabolism. D-xylose isomerases are a very common enzyme found in various bacteria (Sanchez and Smiley, 1978). The results suggested that the hydrolysis of jute stalk and rice husk has good potential for the production of D-xylose which can be further used for the production of various rare monosaccharides like D-lyxose, L-lyxose (Fig. 7). Moreover, various aldolases (Stankova and Kovanova, 1981; Iwamoto and Tuzuki, 1988) can be obtained from the D-xylose. On the other hand, D-xylose can also be used for diagnosis of intestinal malabsorption in children and also used as a parameter in the so-called "xylose-capacity-limit" test, in order to recognize small intestine and kidney failure (Nayar et al., 1992). Although very small scale where very small amount of D-xylose was extracted from these waste products, but with further study on this ground can pave the way for extraction of D-xylose in large amount. We hope that these observations may contribute to a better understanding of utilization of these waste materials for the production of useful product (Chang et al., 1991; Ahmed, 2001; Winkelhaake and Kuzmanova, 1988; Pronk et al., 1988). Moreover, the production method described here is
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also cost effective.

References