Bioengineering of Xylan Production

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Abstract: ***Xylan has a wide application in different types of fields specifically in coating of pipelines and drug delivery systems. Xylan was extracted from cheap agricultural wastes such as sugarcane bagasse, corncobs, ricehulls, wheat husks by alkaline method. The presence of xylan was confirmed by identifying the functional groups by Fourier Transform Infrared Spectroscopy. The concentration of glucose was analyzed quantitatively by phenol sulphuric acid assay. It was found tha tminimum concentration of glucose was observed as present in the commercial xylan. The extracted xylan was characterized using Scanning Electron Microscopy to determine the morphology. The aim of the study is to compare the efficiency of extraction among various agro- wastes. This study reveals that maximum production of xylan was obtained in sugarcane bagasse when compared with corncobs, rice hulls and wheat husks. The maximum xylan recovery of 67.5% was found from sugarcane bagasse.***

Keywords: Xylan, Fourier Transform Infrared Spectroscopy, Scanning Electron microscopy

I. INTRODUCTION

Polymers are versatile materials with its wide application in various fields, like textile, engineering, packaging, automobile and biomedical. Considerably, synthetic and natural have been enormously used with numerous applications for the production and development of valuable products (Acarilia et al., 2012; Anna et al., 1999). Polymers are mostly used for the coating purposes and one of the most commonly used polymers is xylan (Erinc et al., 2013; Motta et al., 2013). Xylans, a green biopolymer extracted from agro-wastes are widely used in the coating of pipeline materials and drug delivery systems (Bijendar et al., 2012).

Xylan is the polypentose molecule present in the cell wall regions of the plant and displaying a broad area of molecular sizes, compositions and structures addressing itssource (Ebringerova et al., 1999; Burgees et al., 2004). It is the most common hemicelluloses; represent more than 60% of the polysaccharides. It consists of a main chain of D- xylopyranose (xylose) units linked by β-glucosidic bonds (Ebringerova and Heinze, 2000). Therefore, there are a great variety of xylans with the different degrees of polymerization of the poly D-xylopyranose main chain, degrees of substitution, side residues and the side chain length. Identification of xylan produced was confirmed by Fourier Transform Infrared spectroscopy and the characterization was performed using scanning electron microscopy.

II. MATERIALS AND METHODS

Xylan extraction

Collection ofsamples

The agricultural wastes such as corncobs,sugarcane bagasse, rice hulls, wheat husks were collected. The samples were air-dried for the removal of moisture content. The dried samples were milled into powder and fractions were passing through 300µm mesh screens but retained on 150µm mesh. After grinding, the powdered samples were checked for moisture content for every one hour.

Pretreatment

The dried samples were dissolved in an aqueous environment and kept for overnight stirring to avoid flocculation process (Silva *et al*., 2007). After water stirring, the samples were allowed to treat with sodium hypochlorite for the removal of impurities. This sodium hypochlorite serves as a disinfectant.

Alkali treatment

The milled lignocellulosic powder samples were treated with 4% (w/v) sodium hydroxide solution. It is then kept in shaker for 1 hour at 37°C for complete mixing of alkali with the sample. The alkali treated extract was neutralized with acetic acid until the pH becomes 5 (Garcia *et al*.,2000).

Precipitation

The methanol was added to the extract for precipitation. Methanol was added to enhance the precipitation of xylan and isopropanol was used for washing procedures. Subsequently, the samples were processed for filtration and dried at 60°C.

Fourier transform infrared spectroscopy

The infrared spectroscopy was shown to be able to identify the main and side chains of xylan.

Analysis of xylan residues reveals the shape, position, intensity and peaks in the spectrum reveals details about the molecular weight of the sample.

Phenol sulphuric acid assay

It is the biochemical assay for the quantification of carbohydrates present in the sample. It is considered to be the best methods to estimate total carbohydrate present in the sample. The glucose stock solution with the concentration of 1mg/ml was prepared. Aliquots were made in the range of 40, 80, 120,160 and 200 µg per 200 µl by transferring respective amount of glucose from the

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glucose standard solution (1mg/ml) and adjusted it to a total volume of 200 µl by adding distilled water. Then 0.2 ml of 5% phenol solution was added to the aliquots and 1ml of concentrated sulphuric acid was added and mixed well. After 10 minutes, the tubes were placed in water bath at the temperature of 30°C for 20 minutes. The yellow-orange color 1was absorbed. The absorbance of the characteristic yellow- orange color was measured at 490 nm for hexose monosaccharide and 480 nm for pentose monosaccharide and uronic acid. The amount of polysaccharides were determined and expressed as amounts of hexose and pentose sugars by using constructed standard curves of each standard sugar (Dubois *et al*.,1956).

Scanning electron microscopy

The morphology of powdered xylan was examined under scanning electron microscopy. A scanning electron microscope scans a focused electron beam over a surface to create an image. The electrons in the beam interact with the sample, producing various signals that can be used to obtain information about the surface topography and composition.

III. RESULTS

Chemical treatment

The raw materials were milled and sieved. The powdered samples were dried and checked for moisture content. The moisture content analysis is shown in the table .1

Table 1. Moisture Content analysis

Weight (g)Time (mins)	Rice hulls	Wheat husks	Corncobs	Sugarcane bagasse
0	26.1	23	30.7	17.5
60	25.9	22.9	30.3	17
120	23.7	21.8	29	15.9
180	20.2	19.7	26.4	14.3
240	20.1	19.6	24.5	14.3
300	20.1	19.6	24.5	14.3

2g of each raw material were taken and dispersedin 50ml of water and treated with 4% NaOH. It is then neutralized using acetic acid and subjected to precipitation with 25ml of methanol. In corncobs sugarcane bagasse, rice hulls and heat husks, the weight of the extracts obtained was 1.22g, 1.35g, 0.96g and 0.8grespectively

Fourier transform infrared spectroscopy

The functional groups of xylan have been identified by performing infrared spectroscopy. The FT-IR analysis was performed for the commercially available xylan and the xylan infrared spectrum was obtained. The infrared spectrum for commercial xylan has been taken as the reference for the further analysis. The FTIR results confirms the presence of functional groups in the compound. The main absorption band of FT-IR spectra of the commercial xylan and the extracted xylan are shown in Fig. 1.

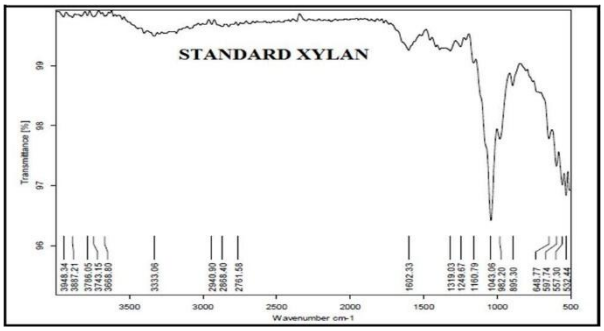


Fig. 1.FT-IR spectrum for commercial xylan

The FT-IR analysis was performed for the corncob extract and the spectrum was given in the Fig. 2. The absorption bands were compared for both the reference spectrum and the spectrum obtained for the extract

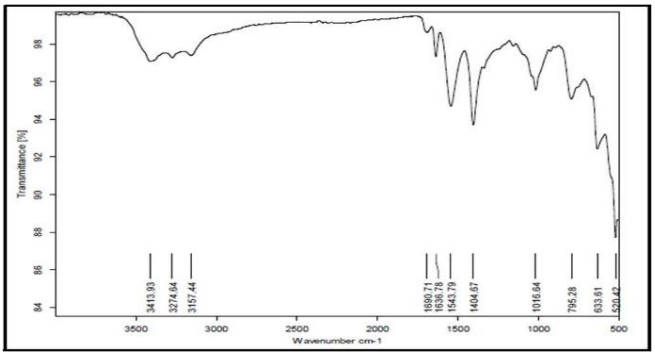


Fig.2.FT-IR spectrum for corncobs extracts

The FT-IR analysis was performed for the sugarcane bagasse extract and the spectrum was given in the Fig.3. The absorption bands were compared for both the reference spectrum and the spectrum

obtained for the extracts.

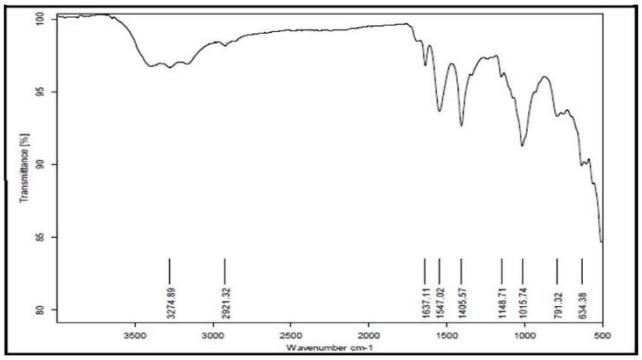


Fig. 3. FT-IR spectrum for sugarcane bagasse extracts

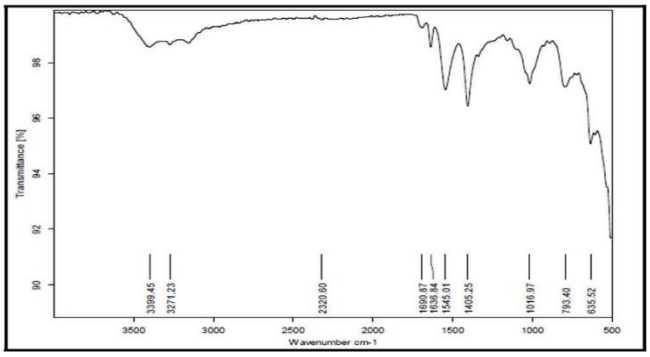


Fig. 4 FT-IR spectrum for wheat husks extracts

The FT-IR analysis was performed for the Rice hulls extract and the spectrum was given in the Fig. 4. The absorption bands were compared for both the reference spectrum and the spectrum obtained for the extracts.

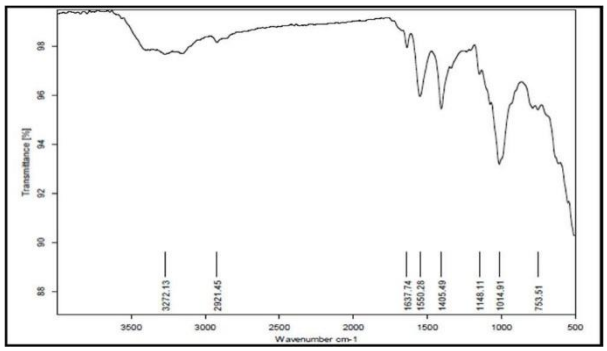


Fig. 5.FT-IR spectrum for rice hulls extracts

The FT-IR analysis was performed for the Wheat husks extract and the spectrum was given in the Fig. 5. The absorption bands were compared for both the reference spectrum and the spectrum obtained for the extracts.

From these results, we identify the major functional groups present in the xylan, thereby we inferred that xylan is present in our extracts. Also there might be some glucose content; it was identified by phenol sulphuric acid assay.

FT-IR spectrum peaks of xylan, corncobs, sugarcane bagasse, ricehulls and wheat husks clearly depicts a sharp and a wide band that contributes hydroxyl group stretch linked to groups in polar environment bonded through intra and intermolecular modes of hydrogen molecular bonding. The appropriate band for glycosidic groups was detected which shows the CH stretch vibrations due to methylene and methyl groups. Prominent band at specific absorption was detected as a HOH stretch vibration. Similarly, broad adsorption bands are detected due to the CH bending stretch in chemical structures of hemicellulose and cellulose. Finally, β-glycosidic linkages are detected as prominent bands between the sugar units present in hemicelluloses molecules.

Phenol sulphuric acid assay

It is a biochemical assay to determine the concentration of glucose present in the sample. The phenol sulphuric acid assay procedure has been carried out for glucose as mentioned in Table 3.3 and the standard graph was made. Also, the same procedure has been carried out for the obtained extracts as mentioned in Table 2. and the concentration of glucose present in the extracts was found

Sugar concentration can be calculated using the following formula:

Sugar concentration in test sample = Concentration of unknown ‘X’ in µg/200µl.

In corncob xylan, the concentration of glucose was found to be 0.0303 mg. In sugarcane bagasse xylan, the concentration was 0.088 mg. In rice hull xylan, the concentration was 0.044 mg. In wheat husk xylan, the concentration was 0.101 mg.

From this experimental assay, we inferred that the concentration of glucose present in our extracts is in a minor amount. So, we concluded that predominantly xylan is present in our extracts.

Scanning electron microscopy

The scanning electron microscope was done for the commercial xylan from birchwood (Himedia, Mumbai, India) and for the four extracts obtained from corn cob, sugarcane bagasse, rice hull and wheat husk shown in figures 3.4.1 and 3.4.2 (A, B, C and D).

Table 2. Phenol sulphuric acid assay for test sample

Tube no	Rice hulls xylan	Corn cob xylan	Sugarcane Bagasse xylan	Wheathusks xylan
Conc.of glucose (µg)	40	40	40	40
Volume of glucose stock taken (µl)	40	40	40	40
Volume of 5% phenol solution added (ml)	0.2ml	0.2ml	0.2ml	0.2ml
Volume of conc, sulphuric acid added (ml)	1ml	1ml	1ml	1ml
Keep at room temperature for 10 min, Mix well and place in a waterbath at 25-30°C for 20 minutes				
Absorbance at 490 nm	0.0787	02288	0.144	0.2626

The SEM images of the commercial xylan are found to have several morphologies and predominantly are spherical shapes with

irregularities and when was compared with SEM images of sample extracts many of the extracts were similar to that of the standard xylan. This similarity in the physical appearance points up that commercial and our sample extracts with similar morphology.

The scanning electron microscope was performed for our extracts also to compare with the SEM image of the commercial xylan

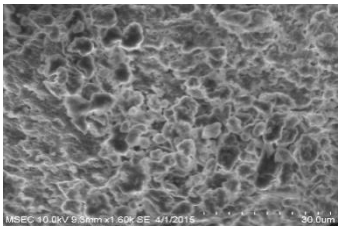
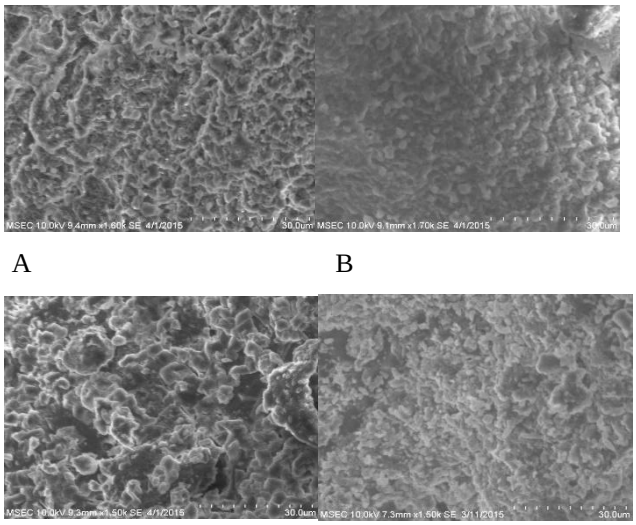


Fig. 5. SEM image for commercial xylan



A B C D

Fig. 6.SEM image of (A) corncob xylan, (B) ricehull xylan, (C) sugarcane bagasse xylan, and (D) wheathusk xylan

3.1 Particle size Analyzer

The particle size analysis was performed for both the commercial xylan and the extracted xylan powder. The powdered samples were dispersed in Millipore water and it is sonicated using ultrasonicator for fine dispersion. Considering the commercial xylan,the size distributed in the range of 50 nm to 10000 nm as shown in the Fig.7. A. It has a single sharp peak which rises at the intensity of 9% and also it has a short peak which rises at the 2% o fintensity. On calculating the average distribution of size, the mean particle size of the standard xylan was found to be 374.8 nm. The size of the corncobxylan is distributed in the range of 50nm to 10000 nm as shown in the figure 7. B . It has a sharp peak which rises at the intensity of 9% and also it has a short broad peak which rises at the 2% of intensity.On calculating the average distribution of size, the mean particle size of the standard xylan was found to be 414.7 nm. For the Sugarcane bagasse xylan, the size distributed in the range of 60 nm to 900 nm as shown in the figure 7. C. It has a sharp peak which rises at the intensity of 12%. On calculating the average distribution of size, the mean particle size of the sugarcane bagasse xylan was found to be 393.6 nm. For the Rice hulls xylan, the size distributed in the range of 50 nm to 900 nm as shown in the figure 7. D. It has a sharp peak which rises at the intensity of 18% and also it has a short peak which rises at the 5% of intensity. On calculating the average distribution of size, the mean particle size of the rice hulls xylan was found to be 605.7 nm. For the Wheat husks xylan, the size distributed in the range of 90 nm to 7000 nm as shown in the figure 7. E. It has a sharp peak which rises at the intensity of 7.5% and another peak which rises at the 7% of intensity. On calculating the average distribution of size, the mean particle size of the wheat husks xylan was found to be 367 nm. From the results, we inferred that the particle size of corncobs xylan and wheat husks xylan were equal to the particle size of the commercial xylan. For sugarcane bagasse xylan, it is merely equal to the particle size of commercial xylan and the particle size of the rice hulls xylan is little much higher than that of the commercial xylan.

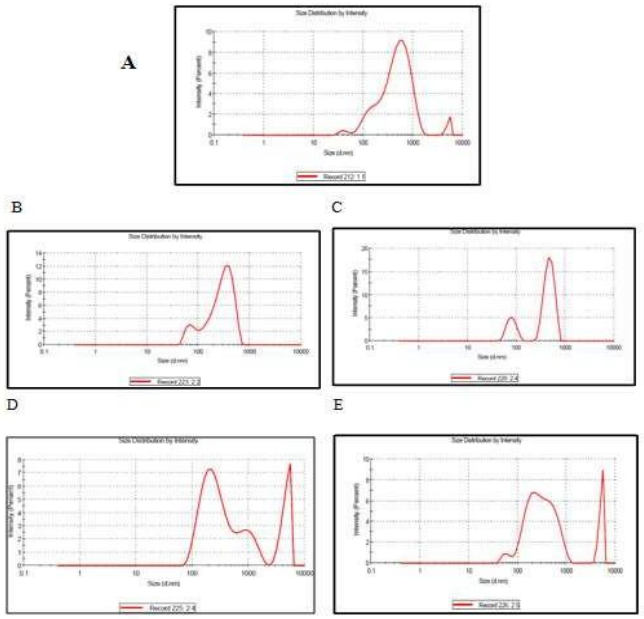


Fig.7. ***Size analysis of (A) commercial xylan (B) corncob xylan (C) sugarcane bagasse xylan (D) rice hulls xylan (E) wheat husk xylan***

4.5 Xylan recovery

The percentage of xylan recovery for Corncob xylan, Sugarcane Bagasse xylan, Ricehull xylan and Wheathusk xylan is 61%, 67.5%, 48% and 40%. Hence we inferred that the percentage of xylan extracted from sugarcane bagasse is 67.5%. So the xylan production is maximum in sugarcane bagasse.

IV CONCLUSION

The main purpose of this work was to obtain, compare and characterize xylan powder from various agro- wastes. Fourier Transform infrared spectroscopy was identified as an appropriate procedure for detecting xylan molecules. The concentration of glucose present in the sample was found from the phenol sulphuric acid assay. The shape and morphology, size analysis of the powder was investigated, resulting in beneficial for pharmaceutical and medical applications of xylan. The yield of xylan from sugarcane bagasse is high when compared to the other wastes. Thus it can be concluded that the sugarcane bagasse is found to be the suitable source for xylan production.

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