



Degradation mechanism of polysaccharides on irradiated sugarcane bagasse

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ABSTRACT

Sugarcane bagasse is composed of cellulose, hemicelluloses, lignin, and a minor amount of protein and inorganic materials. Cellulose consists of linear macromolecular chains of glucose, linked by β -1,4-glucosidic bonds between the number one and the number four carbon atoms of the adjacent glucose units. Hemicelluloses are heterogeneous polymers, unlike cellulose, and are usually composed of 50–200 monomer units of pentose such as xylose and arabinose. Lignin is a complex polymer of *p*-hydroxyphenylpropanoid units connected by C–C and C–O–C links. Radiation-induced reactions in the macromolecules of the cellulose materials are known to be initiated through fast distribution of the absorbed energy within the molecules to produce long- and short-lived radicals. The present study was carried out using sugarcane bagasse samples irradiated by a Radiation Dynamics electron beam accelerator with 1.5 MeV and 37 kW, with the objective to evaluate the cleavage of the polysaccharides and the by-products formed as a result of the absorbed dose. The electron beam processing in 30 kGy of absorbed dose changed the sugarcane bagasse structure and composition, causing some cellulose and hemicelluloses cleavage. These cleavages were partial, forming oligosaccharides and liberating the sugars glucose and arabinose. The main by-product was acetic acid, originated from the de-acetylating of hemicelluloses.

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1. Introduction

Lignocellulosic biomass is a heterogeneous complex of carbohydrate polymers and lignin, a complex polymer of phenylpropanoid units. Sugarcane bagasse generally contains up to 40% glucose polymer cellulose, much of which is in a crystalline structure, about 30% hemicelluloses, an amorphous polymer usually composed of xylose, arabinose, galactose, and mannose and 18% lignin, which cannot be easily separated into readily utilizable components due to their recalcitrant nature. The remainder is mineral, wax, protein and other compounds (Sun et al., 2004). Cellulose is highly crystalline with comparatively rigid linear chains, essentially free of side branching. The hydroxyl groups attached to the chains provide strong intermolecular bonding. Cellulose is a linear polymer of cellobiose repeating unit, and the degree of polymerization is normally 10–100 times greater than that of hemicelluloses. Lignin is a complex, variable, hydrophobic, cross-linked, three-dimensional aromatic polymer of *p*-hydroxyphenylpropanoid units connected by C–C and C–O–C links. Lignin and hemicelluloses molecules are linked through ester linkages formed by the carboxyl groups in the lignin (Glasser and Kelly, 1987).

The radiation-induced reactions in the macromolecules of the cellulose materials are known to be initiated through rapid

localization of the absorbed energy within the molecules to produce long- and short-lived radicals. High-energy radiation causes a decrease in the degree of polymerization and an increase in the carbonyl content of cotton cellulose due to the chain scission reaction within the cellulose molecules (Han et al., 1983; Khan et al., 2006; McLaren, 1978; Smith et al., 1985).

Pretreatment is an important tool for practical cellulose conversion processes, and is required to modify the structure of cellulosic biomass to make cellulose more accessible to the enzymes that convert the carbohydrate polymers into fermentable sugars (Mosier et al., 2005). This study reports the effect of ionizing radiation from an industrial electron beam accelerator for absorbed doses lower than 150 kGy on the cleavage of polysaccharides from sugarcane bagasse. Understanding the chemical and physical mechanisms that occur during pretreatment along with an improved comprehension of the relationship between the chemical composition and physicochemical structure of lignocelluloses on the enzymatic digestibility of cellulose and hemicelluloses is fundamental for the generation of effective pretreatment models.

This knowledge is important for further use of this technology by itself or in combination with other pretreatments for the enzymatic hydrolysis of cellulose, since the development of advanced pretreatment technologies that control mechanisms and minimize costs is still needed. The main obstacle of cellulose hydrolysis by irradiation is the destruction of the product, so absorbed doses as low as necessary were applied to get some break in the lignin, but avoiding the loss of sugars due to uncontrolled degradation of cellulose and hemicelluloses.

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2. Experimental

Sugarcane bagasse was obtained from sugar and ethanol factory in Piracicaba, SP. Three samples were collected: the first one was collected in the rainfalls (Assay A), and presented 55.7% moisture. The second and the third ones (Assays B and C) were collected just after the milling, and presented 44.2% and 42.4% moisture, respectively.

2.1. Radiation processing

The electron beam irradiation was carried out with 1.5 MeV of electrons energy, provided by the IPENs Electron Beam Facility (Dynamitron type from Radiation Dynamics Inc., USA). The irradiation parameters were 4.0 mm sample width, 112 cm (94.1%) scan and 6.72 m/min conveyor stream velocity. All the irradiation was performed in a batch system and the delivered irradiation absorbed doses were 5 kGy, 10 kGy, 20 kGy, 30 kGy, 50 kGy, 70 kGy, 100 kGy, and 150 kGy. The bagasse was irradiated in triplicate, and 81 samples were obtained in this way.

2.2. Dosimetry

The dosimeters research group calibrates this system routinely with the Fricke dosimeter to determine the absorbed dose rate. The thickness of samples for each assay to obtain the desirable dose was calculated according to the bagasse densities, and calibrated using a cellulose triacetate, CTA, dosimeter. Ten dosimeters were distributed over and below the sugarcane bagasse, on the corners and on the center of the Pyrex. The coefficients of the variation ranges from 9.3% for 10 kGy to 12.5% for 100 kGy.

2.3. Bagasse compositional analysis

The moisture content of the substrate was determined by measuring the loss in weight after drying at 105 °C until a constant weight was reached. The samples were dried in an oven for 24 h at 60 °C; the lignin, the hot extraction fraction, and the cellulose were analyzed using gravimetric methods adapted from the literature (ASTM, 1977, 1995; Fengel and Wegener, 1989; Khan et al., 2006; Liu et al., 2006; Sun et al., 2004). Residual lignin content was determined as Klason lignin. In this case, 3.0 mL of H₂SO₄ and 85 mL of distilled water were added to 0.3 g sample, and then refluxed for 1 h at 60 °C. The precipitate was dried in an oven for 24 h at 100 °C. For the determination of degraded cellulose, 2.0 g of sample was added to 100 mL of NaOH 1%(p/v). The sample was filtered and the precipitate was dried for 24 h. Total free sugars were determined using 2.0 g of sample added to 100 mL of distilled water and the mixture was refluxed for 3 h at 100 °C. The sample was filtered and the precipitate was dried for 24 h. For total cellulose analysis, 5.0 g of sugarcane was added to a mixture of 80% acetic acid and 70% nitric acid in a proportion of 10:1 v/v, and the mixture was refluxed for 20 min at 60 °C. The precipitate was filtered and dried for 24 h.

2.4. Sugar analysis

The characterization and quantification of the sugars were made by HPLC with an Evaporative Light Scattering Detector (ELSD), model 17A from Shimadzu Co. The column temperature was 80 °C; pump pressure was 22 kg cm⁻³; mobile flow 0.8 mL min⁻¹; detector temperature 50 °C; detector gain 10; and detector pressure 350 kPa. The calibration curve of the sugars was obtained using analytical grade standard of D-glucose (99.5%), D-xyllose (> 99%), D-galactose (99%), L-arabinose (99%), D-mannose (99.9%) and cellobiose (98%) from Sigma Aldrich Brazil Ltd.

The curves regression coefficient was 0.999, and the obtained experimental variability ($N=10$), expressed as standard deviation, was about 10% for all sugars.

2.5. By-products analysis

The by-products formed after irradiation were analyzed by a Shimadzu, model GC-MS QP-5000 with a DB5 column (30 m × 0.25 mm I.D.). The column temperature was 50 °C at 0 min, then 150 °C at 10 °C/min held for 0 min, then 300 °C at 15 °C/min held for 5 min. The injector temperature was 200 °C. The mass detector operation was in electron impact mode (EI), using 1.50 kV of ionizing voltage and temperature of 250 °C, operated in continuous mode (SCAN). The solvent extractors were acetonitrile 1:1 v/v and hexane/dichloromethane 1:1 v/v.

3. Results and discussion

After sugarcane bagasse irradiation, the moisture was determined for all absorbed doses and no changes were detected even in the samples irradiated with 150 kGy. This moisture maintenance is important for further enzymatic hydrolysis. The total cellulose in the sugarcane bagasse from Assays A, B, and C was respectively, $42.9 \pm 0.6\%$, $41.8 \pm 0.8\%$, and $42.4 \pm 0.6\%$.

3.1. Effect of electron beam processing on structure and composition of sugarcane bagasse

The radiation effect on lignin in the three assays shows a slight trend of decreasing with the applied absorbed dose as can be seen in Fig. 1. It can be considered that there was some break in the structure of lignin, but not enough to degrade it. The opposite happened with cellulose, which presented a total degradation with 50 kGy of absorbed dose in all assays, as shown in Fig. 2.

The cellulose degradation presented an exponential trend. The degradation rate reaches 99% with an absorbed dose of 50 kGy. It is important to point out that the changes observed in the cellulose suggest some effects on the lignin structure, since the cellulose was protected by lignin and hemicelluloses.

The results of hot extraction of the bagasse, which is represented mainly by monomers of sugars, are presented in Fig. 3. A slight increase in the free sugar of about 10% was observed when an absorbed dose higher than 100 kGy was applied to the sugarcane bagasse. These results suggest that, although the cellulose was totally decomposed with 50 kGy, it was not fully cleaved in

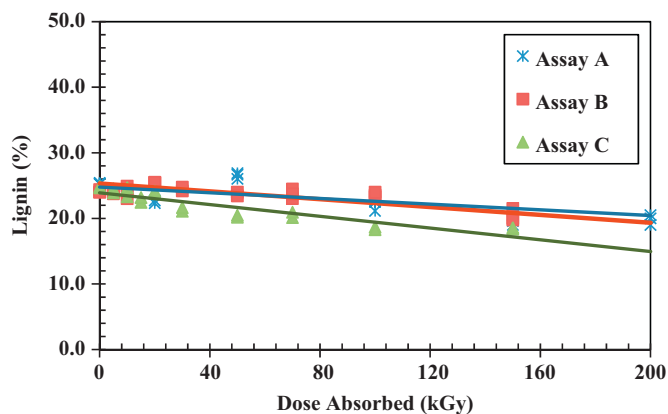


Fig. 1. Total lignin decreasing in sugarcane bagasse samples from Assays A (×), B (■) and C (▲), as a function of the absorbed doses.

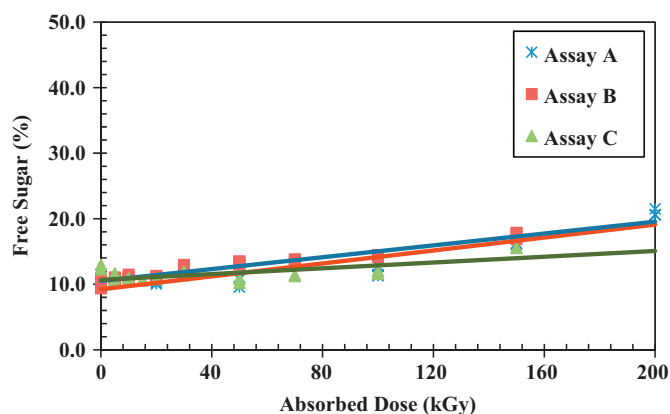


Fig. 2. Cellulose decomposition in sugarcane bagasse samples from Assays A (x), B (■) and C (▲), as a function of the absorbed doses.

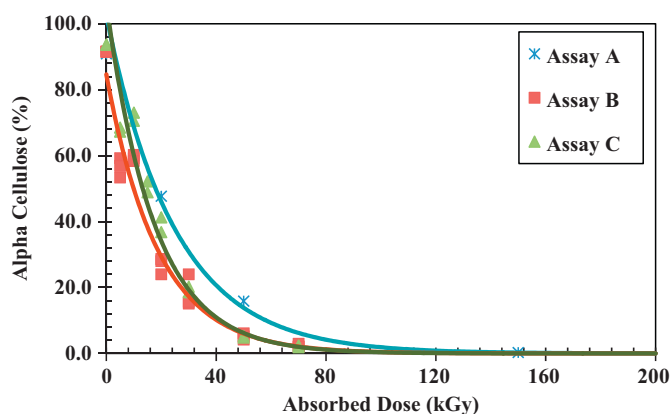


Fig. 3. Free sugar rate (%) in sugarcane bagasse samples from Assays A (x), B (■) and C (▲), as a function of the absorbed doses.

free sugar; however the characterization of these sugars can determine if they come from cellulose or hemicelluloses.

3.2. Characterization of free sugars after electron beam processing of sugarcane bagasse

In Fig. 4 a chromatogram of the hot extracted portion of samples from Assay C is shown. The main sugars identified after irradiation were glucose and arabinose, followed by xylose. A standard with 200 mg/L of glucose, cellobiose, xylose, arabinose, mannose, and galactose was plotted with the irradiated sample in Fig. 4 as a reference.

The peaks identified as A and B could be water-soluble cello-oligosaccharides, formed by the partial degradation of cellulose and hemicelluloses, and they showed maximum concentration at 50 kGy and a total degradation with 100 kGy of absorbed dose (Fig. 4). However these compounds were not hydrolyzed to a significant extent during the radiation pretreatment, because a significant increase of glucose or xylose was not observed, as shown in Fig. 5. The characterization of these products is very important for posterior enzymatic hydrolysis, and can help to define the best enzymatic complex to be used.

In Fig. 6 the concentrations of glucose and arabinose obtained from sugarcane bagasse as a function of the absorbed doses are illustrated. The maximum liberation of glucose was reached with the application of 20 kGy, and then decreased for higher doses. Arabinose, which comes from hemicelluloses hydrolysis, increased with doses up to 70 kGy and then decreased. These

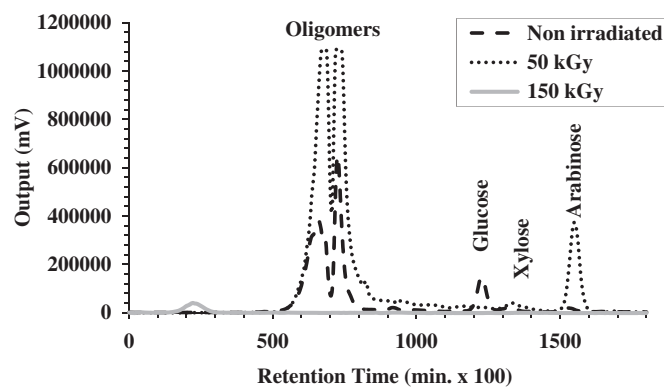


Fig. 4. Main sugar and unidentified compounds A and B in sugarcane bagasse irradiated with various absorbed doses. The standard 200 mg/L of sugars is a reference.

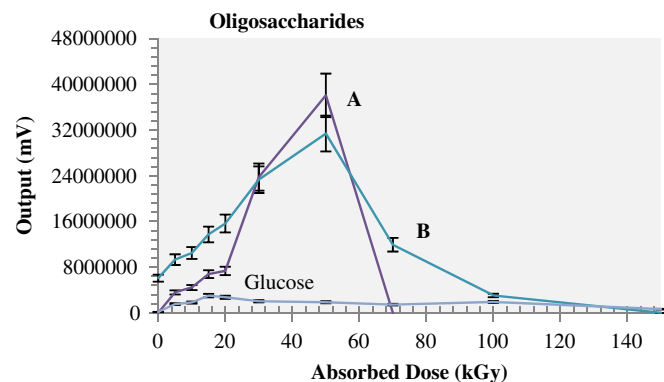


Fig. 5. Formation and degradation of compounds A and B, and the sugar glucose and xylose as a function of absorbed doses.

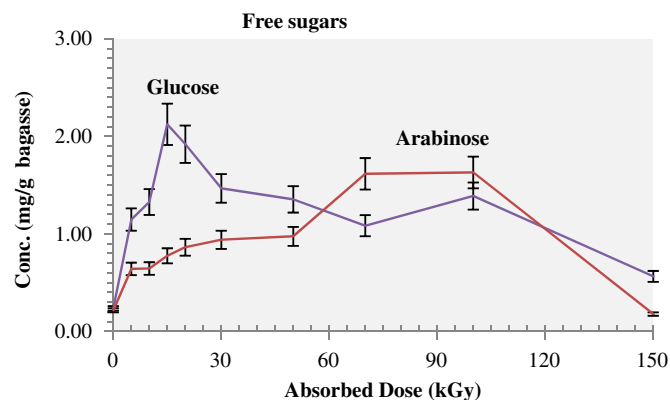


Fig. 6. Concentration of the main sugars and acetic acid in sugarcane bagasse, as a function of absorbed doses.

results show that the radiation interacted with the surface of the hemicelluloses liberating the arabinose, but did not act on the xylose polymers, probably because of the location of xylose in the backbone of arabinoxylan, while arabinose is located in the branches of the macromolecules, where the glycosidic bonds are easier to hydrolyze.

3.3. Acid hydrolysis of free sugar

To confirm if the peaks identified as A and B are water-soluble cello-oligosaccharides, an acid hydrolysis of the hot extracted portion from bagasse irradiated with 50 kGy was carried out.

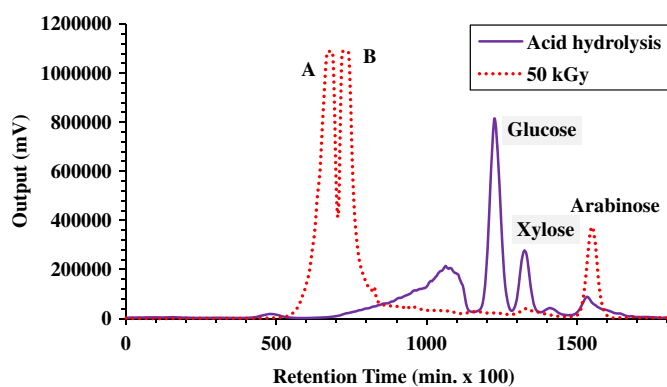


Fig. 7. Acid hydrolysis of the hot extraction portion of sugarcane bagasse irradiated at 50 kGy, degradation of compounds B and C and liberation of glucose and xylose.

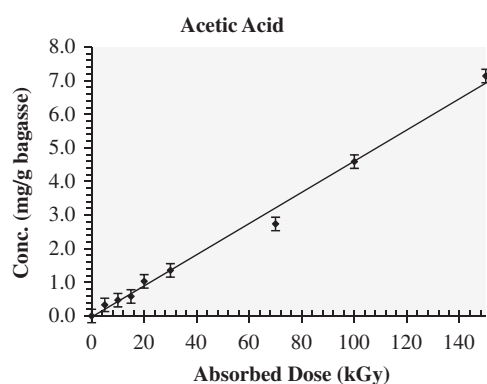


Fig. 8. Acetic acid in sugarcane bagasse irradiated at different absorbed doses.

Through the results of the HPLC analysis a degradation of the compounds A and B and an increase of glucose and xylose can be observed, as shown in Fig. 7. The increase of the sugars glucose and xylose confirm that the oligosaccharides are formed not only by glucanases, but also from xylanases.

3.4. By-products

Applying the gas chromatography in association with mass spectrometry, the identification of the main organic by-products present in the samples was completed. The only by-product identified after irradiation was acetic acid, which is formed due to the de-acetylation of hemicelluloses. In Fig. 8 the linear increase of acetic acid as a function of absorbed dose, reaching 7.00 mg/g of bagasse at 150 kGy, is shown. The absence of formic acid, furfural, and hydroxymethylfurfural confirms that the radiation has not degraded the xylose monomer.

Using the conversion factors (Gouveia et al., 2009) of hemicelluloses ($0.72[\text{acetic acid mass}] + 0.88[\text{arabinose mass}]$), and cellulose ($0.90[\text{glucose mass}]$), the total conversion of these polysaccharides was obtained and the results are shown in Fig. 9. The maximum cellulose conversion was 0.5% with 20 kGy, and hemicelluloses conversion was 2% with 150 kGy.

4. Conclusions

Structure and composition modifications in sugarcane bagasse were demonstrated with absorbed doses from 5 to 150 kGy. Lignin was not degraded completely, but cellulose was cleaved, forming oligosaccharides. The acid hydrolysis of the hot extract showed

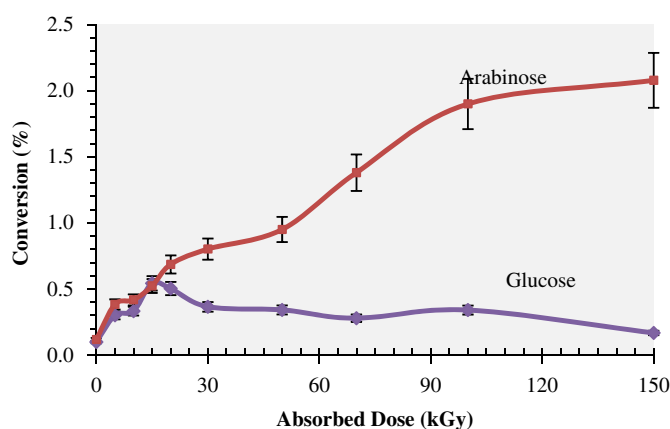


Fig. 9. Conversion rate of cellulose to glucose and hemicelluloses to arabinose in irradiated sugarcane bagasse.

that the partial degradation of cellulose and hemicelluloses formed cello-oligosaccharides from glucanases and xylanases.

The radiation processing converts 0.5% of cellulose at 20 kGy and 2% of hemicelluloses at 70 kGy, the major part remaining as oligosaccharides. About 99% of cellulose and hemicelluloses are converted to oligosaccharides with 70 kGy. It is important to point out the absence of by-products such as furfural and hydroxymethylfurfural, because for pretreatment technologies it is important to preserve the pentose fractions, decreasing the formation of by-products which inhibit the enzymes and the growth of fermentative microorganism.

Acknowledgments

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