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ELECTRON BEAM PROCESSING OF SUGARCANE BAGASSE TO CELLULOSE HYDROLYSIS

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ABSTRACT

Sugarcane bagasse has been considered as a substrate for single cell protein, animal feed, and renewable energy production. Sugarcane bagasse generally contain up to 45% glucose polymer cellulose, 40% hemicelluloses, and 20% lignin. Pure cellulose is readily depolymerised by radiation, but in biomass, the cellulose is intimately bonded with lignin, that protect it from radiation effects. The objective of this study is the evaluation of the electron beam irradiation as a pre-treatment to enzymatic hydrolysis of cellulose in order to facilitate its fermentation and improves the production of ethanol biofuel. Samples of sugarcane bagasse were obtained in sugar/ethanol Iracema Mill sited in Piracicaba, Brazil, and were irradiated using Radiation Dynamics Electron Beam Accelerator with 1.5 MeV energy and 37kW, in batch systems. The applied absorbed doses of the fist sampling, Bagasse A, were 20 kGy, 50 kGy, 100 kGy and 200 kGy. After the evaluation the preliminary obtained results, it was applied lower absorbed doses in the second assay: 5 kGy, 10 kGy, 20 kGy, 30 kGy, 50 kGy, 70 kGy, 100 kGy and 150 kGy. The electron beam processing took to changes in the sugarcane bagasse structure and composition, lignin and cellulose cleavage. The yield of enzymatic hydrolyzes of cellulose increase about 40 % with 30 kGy of absorbed dose.

1. INTRODUCTION

Sugarcane bagasse generally contain up to 45% glucose polymer cellulose, much of which is in a crystalline structure, 40% hemicelluloses, an amorphous polymer usually composed of xylose, arabinose, galactose, glucose, and mannose and 20% lignin, which cannot be easily separated into readily utilizable components due to their recalcitrant nature. The remainder is lesser amounts of mineral, wax, and other compounds [1]. 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 to 100 times greater than that of hemicelluloses. The lignin and hemicelluloses molecules are linked through ester linkages formed by the carboxyl groups in the lignin [2, 3].

The process to convert the sugarcane bagasse to ethanol biofuel consist to three steps, those are the delignification (liberation of cellulose and hemicelluloses from lignin named pretreatment), depolimerization of the carbohydrate polymer to produce free sugar (hydrolysis) and fermentation of mixed hexose and pentose sugars to produce ethanol. The Sugarcane bagasse pretreatments are physical and chemical process that reduce the crystallinity, disrupt the hydrogen bonding of cellulose to more accessibility to hydrolytic depolymerization reactions. Enzymatic hydrolysis of cellulose is a reaction carried out by cellulase enzymes, which are highly specific [4].

Several studies have shown that the irradiation of cotton cellulose deteriorated the mechanical parameters due to the chain scission reaction within the cellulose molecules. Literature studies have reported that the high-energy radiation cause a decrease in the degree of polymerization and an increase in the carbonyl content of cotton cellulose. Others studies have showed that, as a pre-treatment method, ionizing irradiation is equivalent in terms of increasing the digestibility to that of NaOH treatment, one of the most commonly used method in upgrading foliage digestibility [5, 6, 7, 8, 9].

The cleavage of polysaccharides from sugarcane bagasse using ionizing radiation from an industrial electron beam accelerator was studied, in order to facilitate enzymatic hydrolysis of cellulose. The main obstacle of cellulose hydrolysis by irradiation is destruction of the product, in this way it were applied absorbed doses as low as necessary to get cleavage in the lignin, but to avoid loss of glucose due to indiscriminatory degradation of cellulose and hemicellulose.

2. EXPERIMENTAL

Two sugarcane bagasse samples from Iracema Mill (Piracicaba-SP) were collected. The first one, called “assay 1” were collected in the rainfalls and the bagasse was 15 days old and presented an average humidity of 50% and density 0.14 g/dm^3; the second one, called “assay 2” was collected just after the milling with a humidity average of 60% and density 0.16 g/dm^3.

2.1 Radiation Processing

The electron beam irradiation was carried out using Radiation Dynamics Inc. USA Electron Beam Accelerator with 1.5 MeV energy and 37 kW, in batch systems. The irradiation parameters used were 2.8 cm sample width for assay 1 and 2.4 cm width for assay 2, 112 cm (94.1%) scan, and 6.72 m/min conveyor stream velocity. The preliminary applied absorbed doses were 20 kGy, 50 kGy, 10 0 kGy and 200 kGy. After obtained results evaluation, lower absorbed doses were applied in the second assay, such as 5 kGy, 10 kGy, 20 kGy, 30 kGy, 50 kGy, 70 kGy, 100 kGy, and 150 kGy.

2.2 Chemical Analysis

After irradiation, sugarcane bagasse was separated by fibber size using sieves of 1.00 mm and 0.42 mm, this separation was important to remove the solids residues present due to sugarcane harvest process. After humidity rate determination, the bagasse was dried in an oven for 24 h at 60 ^oC and characterized by analysis of lignin, free sugar, and cellulose, using chemical methods adapted from literature [1, 5, 10].
Lignin was determinated by Klason method. To 0.3 g of sample it was added 3.0 mL of H_2SO_4, and 85 mL of destilled water and reflux for 1 h to 60^oC. The preciptate was dried in an oven for 24 h at 100 ^oC, and the lignin was determinated gravimetrically.

The phenol content was analyzed after extractions with acetonitrile, using an ultrasonic system per 30 minutes, and determined by gas chromatography in association with mass spectrometry, Shimadzu, model GC-MS QP-5000Klason Lignin were determinated by extraction of polissacarides with strong acids H_2SO_4 and determination of lignin gravimetricaly. The phenol content was analyzed after extraction with hexane/dichloromethane 1:1 v/v solvent, using an ultrasonic system per 30 minutes and determined by gas chromatography, Shimadzu, model GC-FID 17-A.

The low molecular weight carbohydrates (hemicellulose and degraded cellulose) were determinate using the standard method (ASTM D1109, 1984). To 2.0 g of sample it was added 100 mL of NaOH 1% (p/v). The sample was filtered and the precipitate was dried for 24 h and determinate gravimetrically.

Hot solubility that represents the total free sugars was determinate by standard method (ASTM D1110, 1984). To 2.00 g of sample it was added 100 mL of distilled water and reflux for 3 h to 100 ^{o}C. The sample was filtered and the precipitate was dried for 24 h and determinate gravimetrically.

To cellulose total determination it was adopted the method described by Fengel and Wegener, 1989. To 5.0 g it was added a mixture of acetic acid 80% and nitric acid 70% in proportion 10:1, v/v, and reflux for 20 minutes at 60^{o}C. The precipitate was filtered with the aid of suction and determinate gravimetrically.

Alpha and beta cellulose must be determinate on an extractive-free, lignin-free sugarcane bagasse sample, and were determinate using standard methods (ASTM D1103 and NBR14032). Alpha-Cellulose represents high molecular mass cellulose and beta-cellulose represents the degraded cellulose and hemicellulose [1, 5, 10].

2.2 Enzymatic hydrolyze

The enzymatic hydrolysis of the non irradiated and irradiated bagasse was done at the CTC laboratories using samples from the Assay 2, for 24 and 48 h. It was used 50 of total mass in the reactor with 8% of solids charge (dry base). The enzyme was the Celuclast from Novozymes Co., with 5FPU/g-cellulose and Beta-glycosidase 0.5% (p/p).

3. RESULTS AND DISCUSSION

After the irradiation the humidity rate were measured and the difference among the non irradiated and the irradiated samples in all doses were not significative, that is an advantage because the maintenance of humidity is very important to the enzymatic process.

3.1. Lignin

The results of lignin concentration related to applied radiation dose for Assay 1 and 2 are presented in the Fig.1. These results show that, considering the Klason method, the variations of the lignin concentration do not change with the applied absorbed doses in both assay. But the results of phenol, that is liberated by cleavage of lignin molecule, presented in the Figure 2 showed a increasing in phenol concentration related to absorbed doses until 100 kGy. After that it was observed a decreasing, that may be caused by the competition between the formation and degradation of phenol by radiation. The important point is that, even in absorbed doses lower than 50 kGy, there was a atach in the lignin structure.

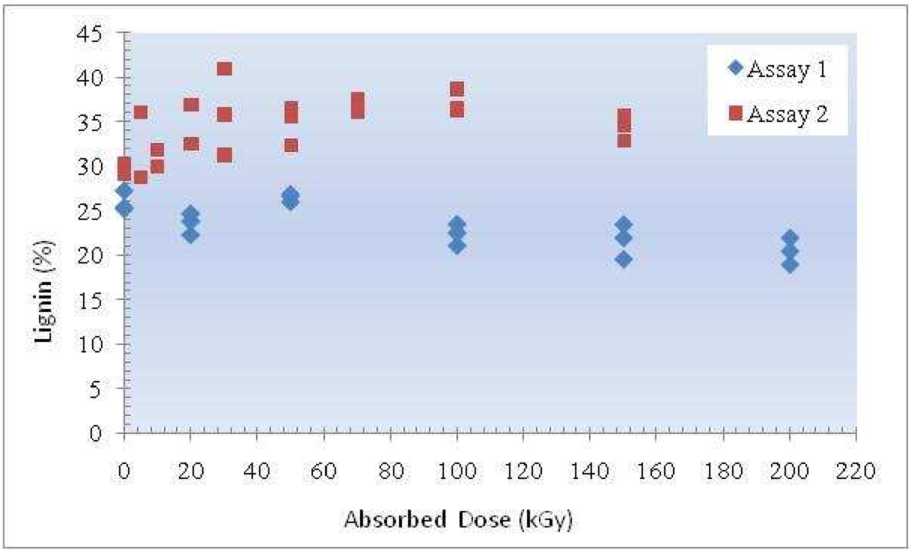

Figure 1. Degradation of lignin from sugarcane bagasse after irradiation in various absorbed doses evaluated by the Lignin concentration

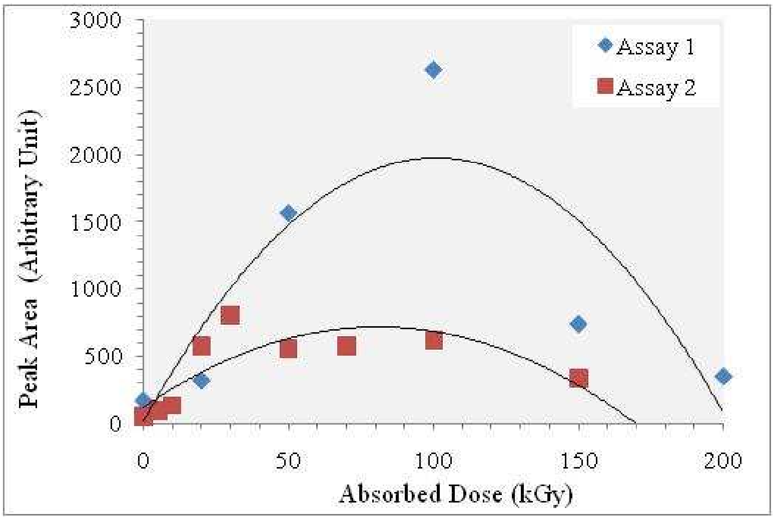

Figure 2. Phenol liberation after irradiation of sugarcane bagasse in various absorbed doses

3.2. Hemicellulose

The results of low molecular mass carbohydrates (hemicellulose) related to absorbed dose are showed in the Figure 3. It can be observed an increasing of these polyssacharide with the applied absorbed, about 20% after irradiation with 100 kGy for samples from both assay (Fig. 3), but even with the low doses (5 kGy and 10 kGy) a light increasing can be noticed in samples from the second assay.

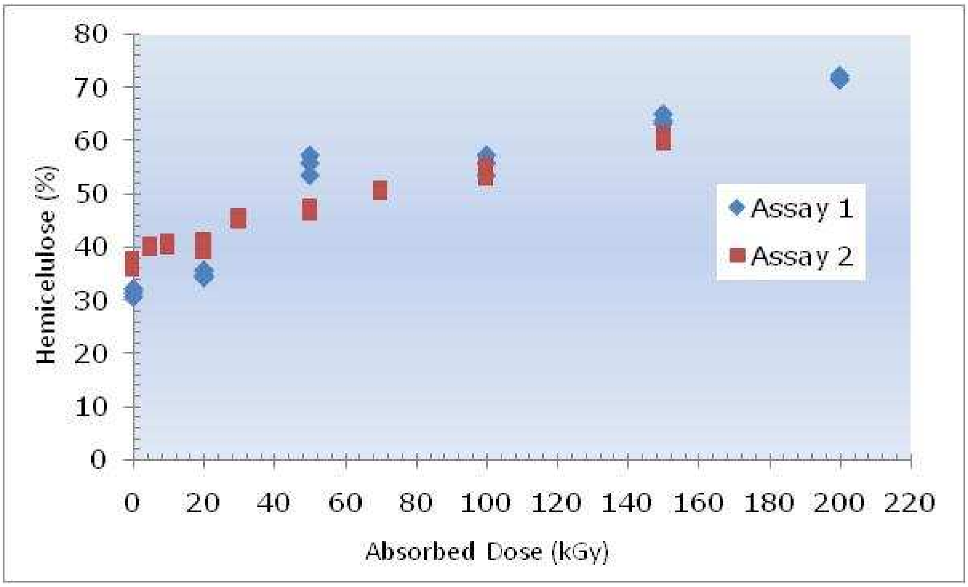

Figure 3. Low molecular weight carbohydrates (hemicellulose and degraded cellulose) from sugarcane bagasse after irradiation in various absorbed doses

3.3. Sugars

In the Figure 4 is showed the results of extraction with hot water, that represents the total free sugars in the bagasse samples. After irradiation an increasing of the free sugar can be observed when absorbed doses higher than 100 kGy were applied in samples from both assay. These sugars are liberated by the total cleavage of cellulose and hemicellulose and are represented mainly by glicose and xylose, respectively.

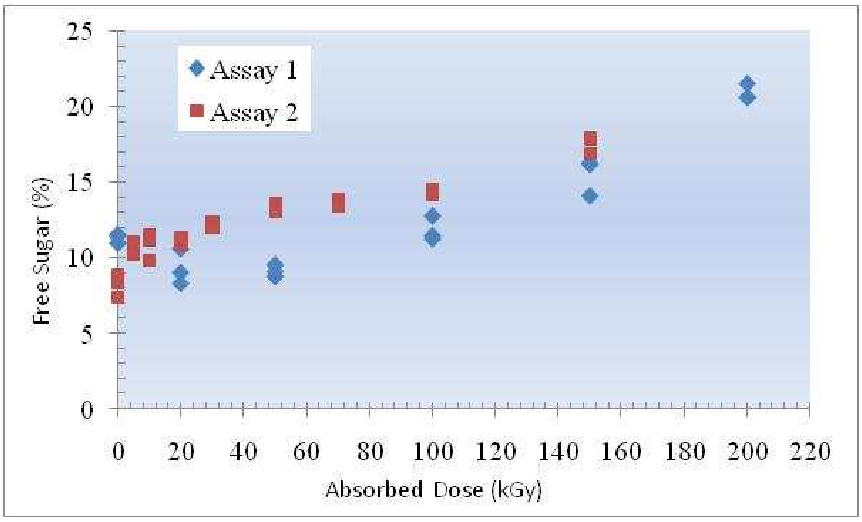

Figure 4. Liberated sugar from sugarcane bagasse after irradiation in various absorbed doses

3.4. Cellulose

The sugarcane bagasse from Assay 1 presented 46.9±0.6% of total cellulose and the bagasse from Assay 2 presented of 49.8±0.8%. In the Figure 5 is presented the results the alpha-Cellulose, that is the portion insoluble in NaOH 17.5% and represent high molecular mass cellulose, before and after irradiation. It is well known that cellulose show a higher sensibility to radiation than the other lignocelluloses; this can be observed by decreasing of concentration with the applied absorbed doses, even in lower than 20 kGy for bagasse samples from the assay 1 and 2. The reductions were very significative, about 99% with 100 kGy of absorbed doses.

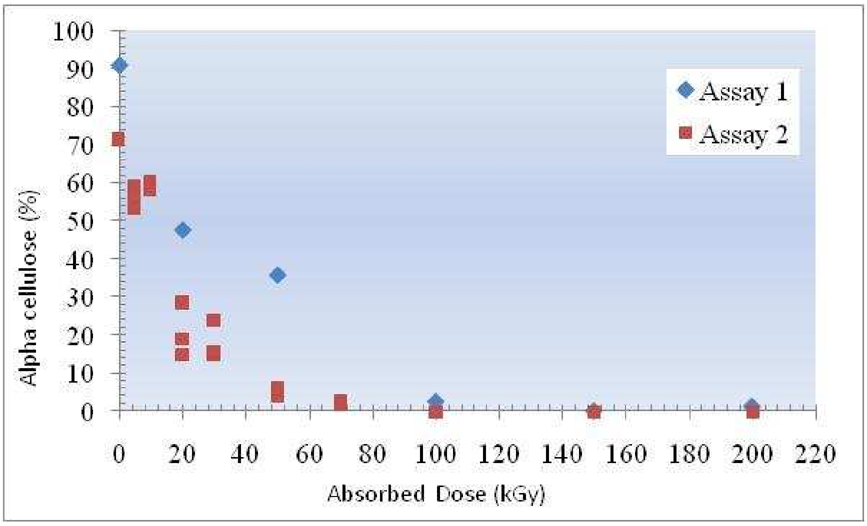

Figure 5. Alpha cellulose (high molecular mass cellulose) from sugarcane bagasse after irradiation in various absorbed doses

The radiation effect on the beta cellulose that is the solubilizated in NAOH 17.5% and precipitated in acid and represents the degraded cellulose and hemicellulose are presented in the Fig. 6. The behaviour of beta cellulose were similar in the two assays, showing a increasing of the degradation with dose until 100 kGy, and a decreasing after that.

These is an inexpected result, but it could be explained by the comparision with the picture of the preciptate from Assay 1 presented in the Fig. 7, where can be observed the form of the particles that became coarser until 100 kGy, and then became fine again. The preciptate obtained with 50 kGy is similar to the obtained for 200 kGy, suggesting that in higher absorved doses the cellulose can repolimerize.

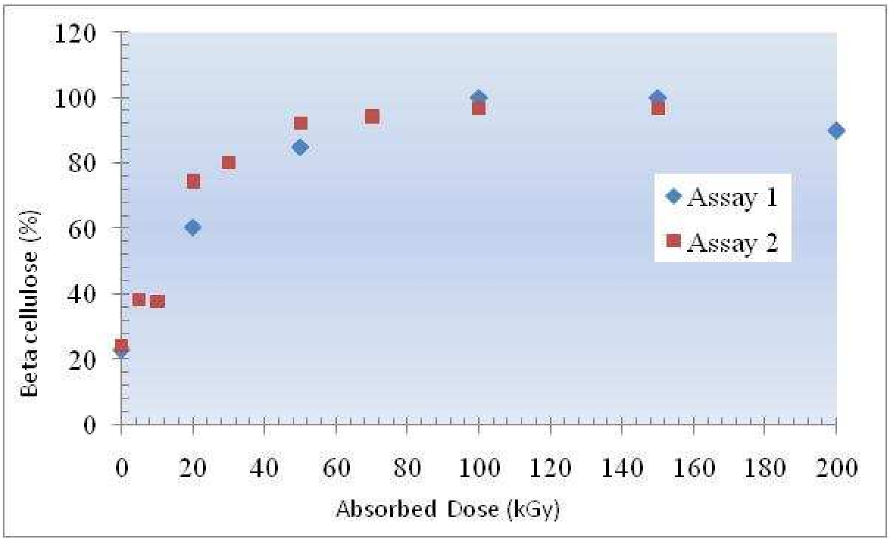

Figure 6. Beta cellulose (degraded cellulose) from sugarcane bagasse after irradiation in various absorbed doses.

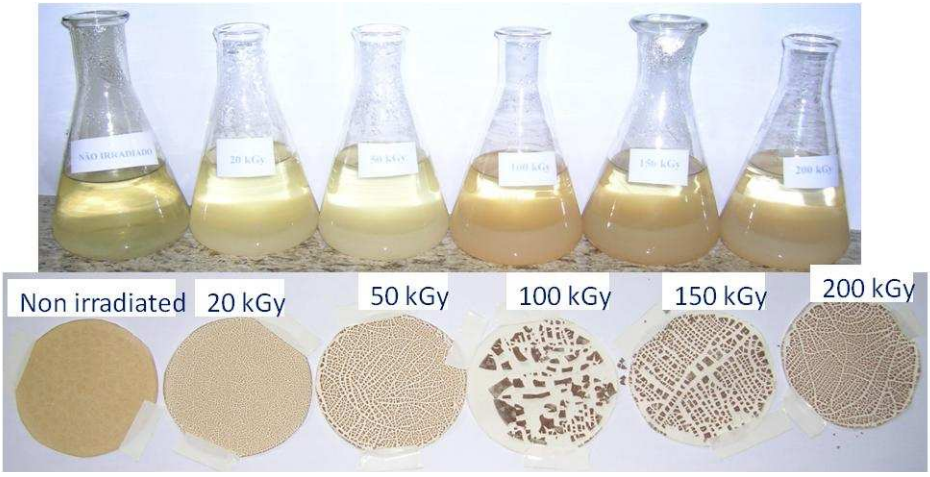

Figure 7. Precipitate of degraded cellulose from sugarcane bagasse after irradiation in various absorbed doses.

3.5. Enzymatic hydrolyze

The results of enzymatic hydrolyze are showed in the Fig 8, that presented the rate of conversion of cellulose to glucose before and after irradiation. The conversion rate increased with absorbed doses from 5 to 30 kGy, then for higher doses there were a significative decreasing. With 20 kGy the conversion yield of cellulose to glucose increases from 8% to 14%, which represent 75% of efficiency. Even with higher doses the yield after irradiation was higher than the non irradiated sample, and the minimal were 10% for 150 kGy.

The decreasing in the conversion rate after 30 kGy need more studies, but may be explained by the aliphatic acids, e.g. acetic, formic and levulinic acid, furan derivatives, e.g. furfural and 5-hydroxyl-methyl-furfural (HMF), and phenolic compounds that are formed during the saccharification of lignocelluloses polysaccharides. These compounds might seriously inhibit the enzyme decreasing the hydrolyze yield [4, 6]. Another explanation may be the degradation of the glucose when higher doses are applied.

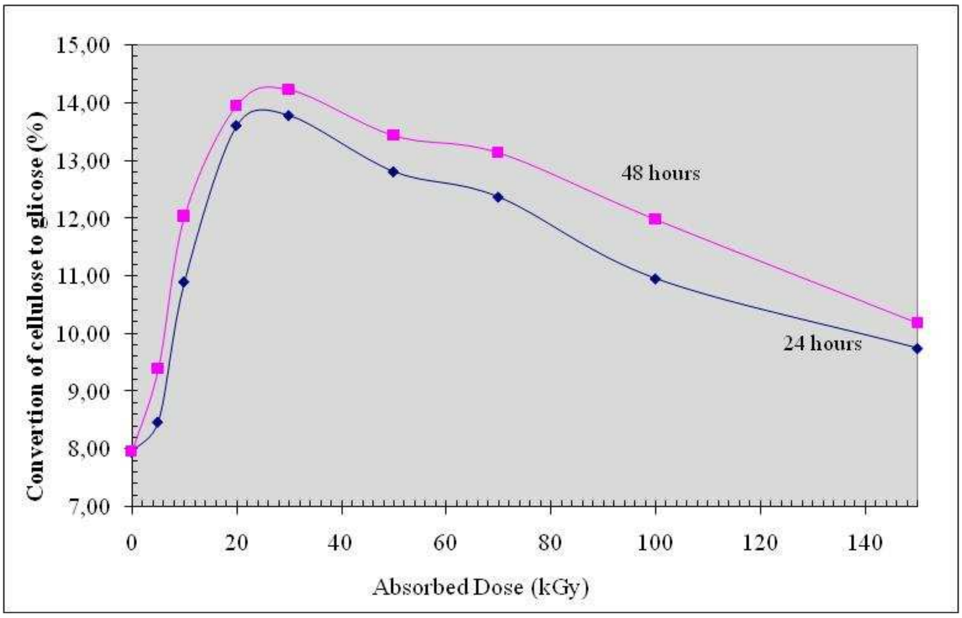

Figure 8. Convertion of cellulose to glicose by the enzymatic complex related to absorbed doses

4. CONCLUSIONS

With the results presented it is possible to conclude that there were a very light degradation of lignin, but a liberation of free sugars have occurred after radiation processing.
The decreasing of alpha-celulose and the increasing of beta-cellulose means that a cleavage of high molecular cellulose were observed in relatively very low absorbed doses (lower than 20 kGy) considering other investigations in the literature.

These results reflect that the ionizing radiation with low doses can cleavage the external structure of sugarcane bagasse without destroy the cellulose or lost the sugar, that is desirable as pretreatment to enzymatic atack. There were observed no significant variations on the results from the two samplings, stocked for 20 days and the just squeezed.

The yield of enzymatic hydrolyzes of cellulose increased about 75 % with 20 kGy of absorbed dose, and the decreasing after that have to be investigated in future studies.

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