

Combining Alkali and Peroxide for Pretreatment of Sugarcane Wastes, Bagasse and Trash, for Bioethanol Synthesis

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ABSTRACT

The use of agricultural waste material in the production of bioethanol can provide an alternative to fossil fuels that is renewable and readily available. This research sought to use a combination of chemical pretreatments to make available reducing sugars from sugarcane bagasse and trash for fermentation into bioethanol. A combination of alkali and peroxide was used. These results indicate that pretreatment with alkali peroxide is quite effective in reducing the levels of hemicellulose sugars and lignin in particular. The slight changes in cellulose quantities are an indication that alkali peroxide pretreatment conserves the quantities of cellulose and does not result in a loss in cellulose as compared to other pretreatment processes such as acid hydrolysis. The drawback is that this can also mean that the crystalline structure of the cellulose was not disturbed, which could slow down downstream processes of enzyme hydrolysis and subsequent fermentation. The pretreated bagasse contained significantly higher levels cellulose of 48 % and 63 % after pretreatment with 3 % and 5 % alkali peroxide, respectively. This represents a 28 % increase and a 70 % increase, respectively, in available exposed cellulose for use in further processes of enzyme hydrolysis and fermentation.

KEYWORDS

Pretreatment, bagasse, renewable energy, alkali peroxide.

1. Introduction

Research into application of renewable resources for the generation of biofuels continues to grow. Production of first generation bioethanol from sugar and starch based food crops is a well established process.¹ It is the production from materials rich in lignocellulose such as agricultural wastes, sugarcane bagasse and sugarcane leaves and tops, of second-generation ethanol that is yet to take off. The use of materials made of lignocellulose for raw materials in the production of bioethanol makes available an inexpensive raw material that is readily available.² Sugar mills produce a lot of waste in the form of sugarcane bagasse of up to 270 kg per tonne of harvested sugarcane.² Lignocellulosic materials provide an inexpensive raw material that has high cellulose and hemicellulose quantities which can be utilized in producing biofuels such as bioethanol and other value added products.^{3,4} The main challenge facing second-generation bioethanol production is the absence of cost-effective, efficient and environmentally conscious methods for industrial production pretreatment.⁵ Lignocellulose consists of cellulose, hemicellulose and lignin.⁶ The cellulose component consists of β -1,4-glycosidic interlinked molecules of glucose making the polymer. Hemicellulose consists of a matrix of sugars such as glucose, galactose, mannose, arabinose and xylose which are also linked to lignin.⁶ Lignin is made up of interlinked phenolic compounds.⁷ These methods need also to make the cellulose in the lignocellulose–cellulose–hemicellulose matrix readily available to downstream enzymatic digestion and yeast fermentation processes.

2. Methods

2.1. Materials

The reagents and chemicals for this research were all of analytical grade and purchased from Sigma-Aldrich (USA), Merck (Germany) and some purchased through Total Laboratory (S.A.) suppliers. The main substrate, the agricultural residues, that is the sugarcane bagasse and sugarcane trash (material) were supplied by TSB Malalane Mill at Mhlathi Farm, Malelane, in South Africa.⁸ The material was cut using a blender into fine particle sizes of mesh size less than 40 mm after it was oven-dried at a temperature of 60 °C. After further drying the material was then stored in sealed plastic bags so as to avoid absorption of moisture in preparation for experimental analyses and assays.

2.2. Alkali Peroxide Pretreatment and Hydrolysis Methods

In alkali-peroxide pretreatment sodium hydroxide (NaOH) was combined with hydrogen peroxide. High yields of glucose from 7 % hydrogen peroxide pretreatment of 691 mg g⁻¹ of bagasse have been obtained.² Thus in an effort to use the minimal amounts of chemical possible this research focused on the alkali peroxide solutions of sodium hydroxide of concentrations of 3 % and 5 % hydrogen peroxide. The solutions of hydrogen peroxide were prepared and sodium hydroxide was added to raise the pH to 11.5.² For each concentration 2 g of the oven-dried material was weighed and placed into 250 mL Erlenmeyer flasks. To each of the conical flasks was then added 50 mL of the required solution to make a 4 % (m/v) loading. Each of the experiments was done in triplicate for each one of the concentrations for both the bagasse and trash. A deionized water control

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was also used for both the bagasse and the trash and the experiments were also carried out in triplicate. The reacting mixture was then placed in a boiling water bath for 1 h. The hydrolysates were filtered and thrice rinsed with deionized water to wash off dirt and other undesired water solubles.⁸

2.3. Cellulase Enzyme Hydrolysis

Commercial cellulase enzyme of cellulolysin[®] type was used in the hydrolysis of alkali peroxide pretreatment hydrolysates enabling the generation of reducing sugars. The IUPAC (International Union of Pure and Applied Chemistry) method for determining reducing sugar quantities present in hydrolysates by using dinitrosalicylic (DNS) acid.⁹ All enzyme hydrolyses were at optimum conditions of temperature and pH for cellulase enzymes of 45–50 °C.^{8,9} The pH was maintained at pH 4.8 by using a citrate buffer. Application of enzyme hydrolysis offers an advantage over acid hydrolysis in that equipment does not need to be expensive corrosion resistant material.¹⁰ The amount of cellulase in filter paper units administered in this research was at 10 filter paper units g⁻¹ which has been cited to achieve high levels of glucose in an acceptable time of 48–72 h.^{9,11}

3. Characterization

Raw and pretreated sugarcane leaves and tops-trash as well as the bagasse were characterized using the Chesson method for percentage composition comparisons before and after pretreatment. Reducing sugar estimates after pretreatment were obtained by following International Union of Applied Physicists and Chemists (IUPAC) method, the dinitrosalicylic-acid (DNS) method, as it is laid out by T.K. Ghose.⁹ To each sample 3.0 mL of the dinitrosalicylic-acid (DNS) solution is added to the test tubes followed by boiling the mixture for 5 min in vigorously boiling water. All samples, glucose standards and enzyme blanks are treated the same and a cold water bath is used to cool down the samples. To each test tube is added 20 mL of deionized water followed by converting the test tube a few times to ensure the contents are well mixed. Upon settling, the colour produced as then measured against the spectro zero at a wavelength of 540 nm. A graph plotted on semi-log paper for glucose standards is used to estimate the amount of reducing sugars produced.

4. Results and Discussion

4.1. Material Percentage Compositions

Lignocellulosic bagasse and trash were pretreated with alkali peroxide. The raw untreated material as well as the pretreated material's compositions were characterized for percentage composition by using Chesson method.¹ Results of the experimental procedures undertaken to quantify the percentage compositions of the raw trash and pretreated trash as well as the raw bagasse and pretreated bagasse are indicated in Table 1. Each experiment was done in a minimum of duplicates and the results averaged and recorded.

The calculations for the respective percentage compositions of lignin, cellulose and hemicellulose were carried out using the following equations:

$$\text{Hemicellulose} = \frac{b-c}{a} \times 100\%$$

$$\text{Cellulose} = \frac{c-d}{a} \times 100\% \text{ and}$$

$$\text{Lignin} = \frac{d-e}{a} \times 100\%$$

The equations above were used to convert the results into the percentage composition results of the raw untreated and treated sugarcane leaves and tops (trash) as well as the compositions of the bagasse. These results are indicated in Table 2.

Results in Table 2 indicate the raw trash before pretreatment had been effected consisted of 31 % of hemicellulose, 37 % of cellulose and 13 % of lignin content. These percentages changed considerably after pretreatment with 3 % and 5 % alkali peroxide concentrations. Pretreatment with 3 % and 5 % alkali peroxide resulted in the hemicellulose content being reduced to 14 % and 9 % of the pretreatment residue, with hemicellulose being also reduced to 0.06 % and 0.04 % of the pretreatment residue, respectively. Cellulose content on the other hand increased slightly after 5 % alkali peroxide pretreatment to 42 % of the pretreatment residue indicating a 13.5 % increase. However there was a 1 % decrease in cellulose fraction after pretreatment with 3 % alkali peroxide. Pretreatment of trash with 3 % alkali peroxide resulted in lower changes than when compared to pretreatment with 5 % alkali peroxide. Delignification of sugarcane bagasse was also higher for pretreatment with 5 % hydrogen peroxide than pretreatment with 3 % hydrogen peroxide.

Table 1 Results for alkali peroxide pretreatment.

Acid concentration	Concentration					
	Trash			Bagasse		
	Raw	3 % H ₂ O ₂	5 % H ₂ O ₂	Raw	3 % H ₂ O ₂	5 % H ₂ O ₂
Biomass-a/g	1.00	1.00	1.00	1.00	1.00	1.00
Residue-b/g	1.00	0.56	0.56	0.93	0.65	0.70
Residue-c/g	0.82	0.47	0.42	0.64	0.56	0.68
Residue-d/g	0.51	0.05	0.06	0.27	0.08	0.05
Ash-e/g	0.14	0.009	0.002	0.025	0.005	0.002

Table 2 Percentage composition of alkali peroxide pretreated trash and bagasse.

Composition	Concentration					
	Trash			Bagasse		
	Raw	3 % H ₂ O ₂	5 % H ₂ O ₂	Raw	3 % H ₂ O ₂	5 % H ₂ O ₂
Hemicellulose %	31	14	9	29	9	2
Cellulose %	37	36	42	37	48	63
Lignin %	13	0.06	0.04	25	0.08	0.05

Results for raw untreated bagasse indicate that there were almost similar fractions of hemicellulose and cellulose in bagasse as in the trash of 29 %, 37 % but with a higher fraction of lignin of 25 %, respectively. The pretreated bagasse contained significantly higher levels cellulose of 48 % and 63 % after pretreatment with 3 % and 5 % alkali peroxide, respectively. This represents a 28 % increase and a 70 % increase, respectively, in available exposed cellulose for use in further processes. As the amount of lignin decreased, this resulted in higher percentages of cellulose available therefore indicating the more effective the pretreatment method. The same results of biomass composition are indicated in the form a bar graph in Fig. 1.

These results indicate that pretreatment with alkali peroxide is quite effective in reducing the levels of hemicellulose sugars and lignin in particular. The slight changes in cellulose quantities are an indication that alkali peroxide pretreatment conserves the quantities of cellulose and does not result in a loss in cellulose as compared to other pretreatment processes such as acid hydrolysis. The drawback is that this can also mean that the crystalline structure of the cellulose was not disturbed, which could slow down downstream processes of enzyme hydrolysis and subsequent fermentation.

4.2. FTIR Results of Peroxide Pretreatment

FTIR was also used to analyze the results of peroxide treatment and these are shown in the following figures. The following peak assignments in Table 3 are linked to corresponding infrared absorption at corresponding frequencies¹² and as such were used to interpret the resultant spectra.

FTIR analysis results for bagasse that was pretreated with hydrogen peroxide are indicated in Fig. 2.

FTIR spectra for the pretreatment of bagasse and trash pretreatment results are given in Fig. 2 and Fig. 3, respectively. There was a gradual decrease in the –C–O–vibrations typical of glucose at 1035 cm⁻¹ to 1080 cm⁻¹ with increase in pretreatment concentration from raw untreated material, to 3 % and 7 % peroxide pretreatment. This was coupled with an increase in the –CH₃– vibrational stretch at 1440 cm⁻¹ which was more pronounced after pretreatment with 5 % alkali peroxide. This is due to the decrease in the quantities of hemicellulose present after pretreatment.

4.3. Thermogravimetric Analysis Results for Trash

Thermogravimetric analysis on trash which was pretreated with alkali peroxide also revealed changes in behaviour after pretreatment as indicated in Fig. 4. There was a major drop in percentage weight for material that was pretreated for 48 h between 300 °C and 400 °C more than 24 h pretreatment and raw material which also exhibited a similar drop within the same temperature range. DTG graphs also indicate there were two peaks at 300 °C and 400 °C for the original (O.G.) raw untreated material whereas there appeared to be one major peak for the pretreated materials. The two peaks at 320 °C and 360 °C are attributed to hemicellulose and cellulose, respectively.¹³ The presence of a major single peak after pretreatment is attributed to the presence of cellulose in larger quantities after pretreatment. There is a steady decrease in mass at temperatures higher than 400 °C for the raw untreated material due to the presence of

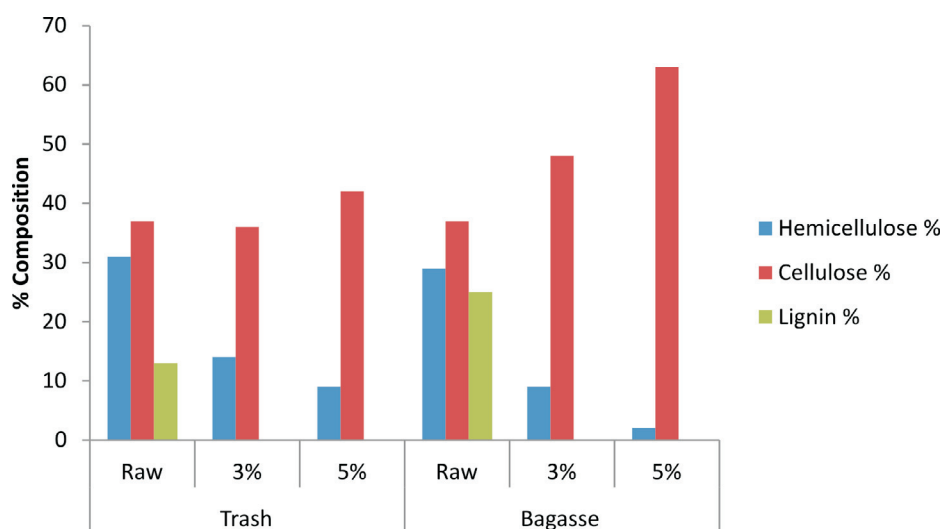


Figure 1 Percentage composition of material after pretreatment with 3 % and 5 % alkali peroxide (H₂O₂).

Table 3 FTIR peak assignments.

Wavenumbers/cm ⁻¹	Peak assignment
4000–2800	A. 3414 cm ⁻¹ O–H stretch, intermolecular hydrogen bonding. B. 2854 and 2925 – C–H stretch
1900–1500	C. 1737 aromatic ring stretch (typical lignin) D. 1619 – very strong aromatic ring stretch, aromatic –C–O stretch. C=C, C=O, C=N, aromatic skeletal vibration
1500–1300	1459 –C–H deformation, 1376 – weak C–O stretching, acetylated hemicelluloses, 1320 – CH ₂
1300–1100	1161 – glycosidic linkage, C–O–C–Hemicellulose ring vibrational stretching, 1717 – cellulose –C–O stretch
1050–1200	E. 1060 – β-polysaccharide, 1045 –C–OH bending, 1035 –C–O, C=C and C–C–O vibrational stretching typical of glucose

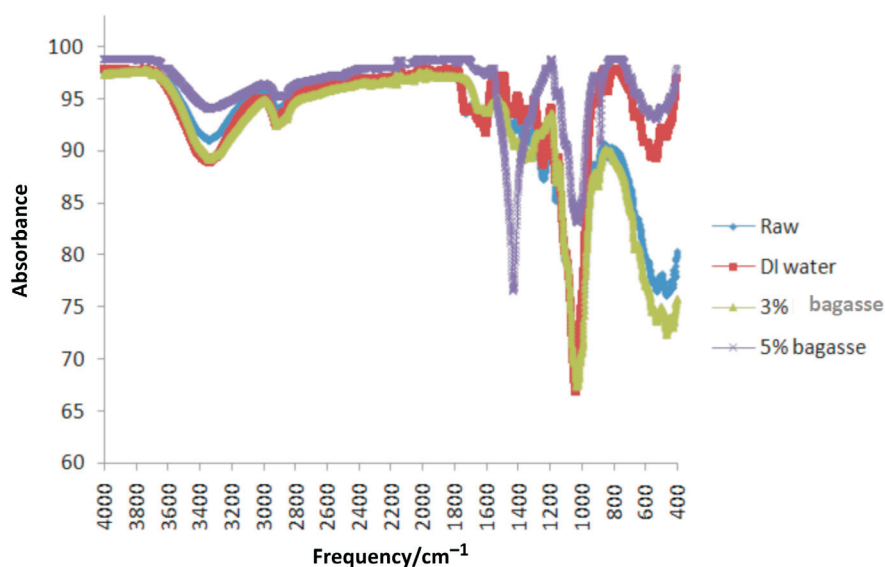


Figure 2 FTIR spectra for bagasse pretreated with alkali peroxide.

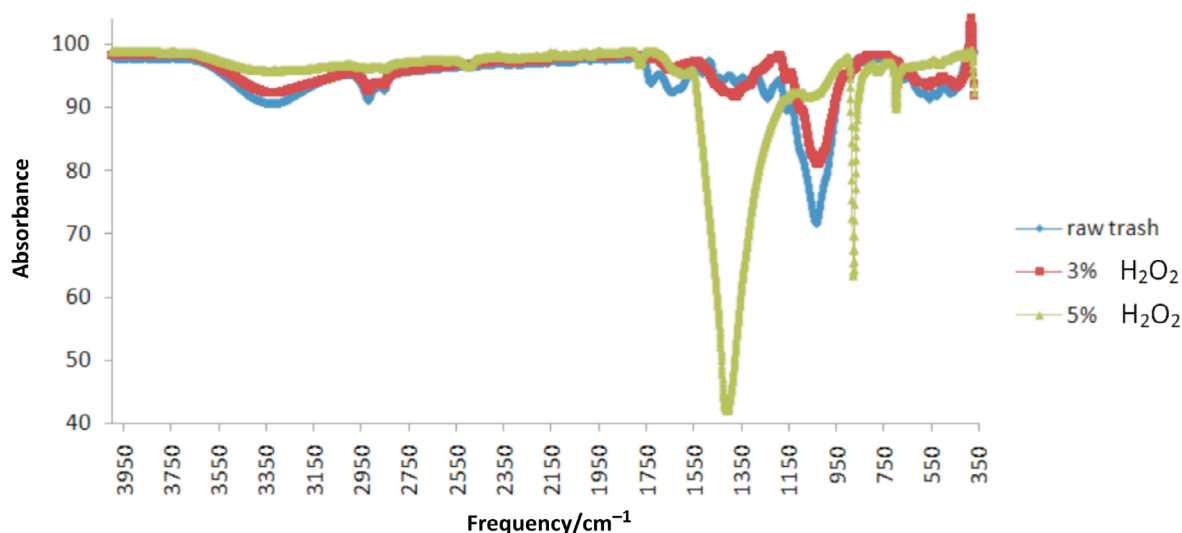


Figure 3 FTIR spectra for alkali peroxide pretreated trash.

lignin and other material which have not been removed yet through pretreatment compared to a rapid drop for the pretreated material.

4.4. SEM of Alkali Peroxide Pretreated Material

Scanning electron microscopy images for bagasse which was pretreated with alkali peroxide indicate there was an

increase in visibility of individual cells with an increase in percentage concentration as shown in Fig. 5. The raw untreated bagasse (C) did not have clearly visible cellular division as compared to 3 % and 5 % alkali peroxide pretreated bagasse (A) and (B), respectively. This increase in cellular outline visibility can be attributed to the swelling of the material upon pretreatment.

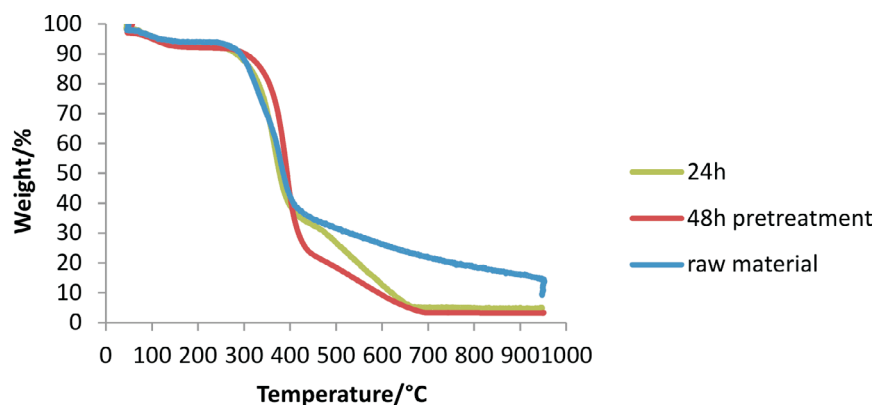


Figure 4 TGA graphs for trash pretreated with alkali peroxide.

4.5. EDS Results for Peroxide Pretreatment

EDS results indicated there was an increase in the amount of available oxygen sites on the surface of the material after pretreatment as indicated in Fig. 6. There was also some residue of the element sodium (Na) present in the material after pretreatment indicating that sodium hydroxide had been adsorbed

on to the material. This explains the increase in mass of material observed after pretreatment.

5. Conclusions

A two-step approach to pretreatment by combining pretreatment methods was successful in disrupting the lignocellulose

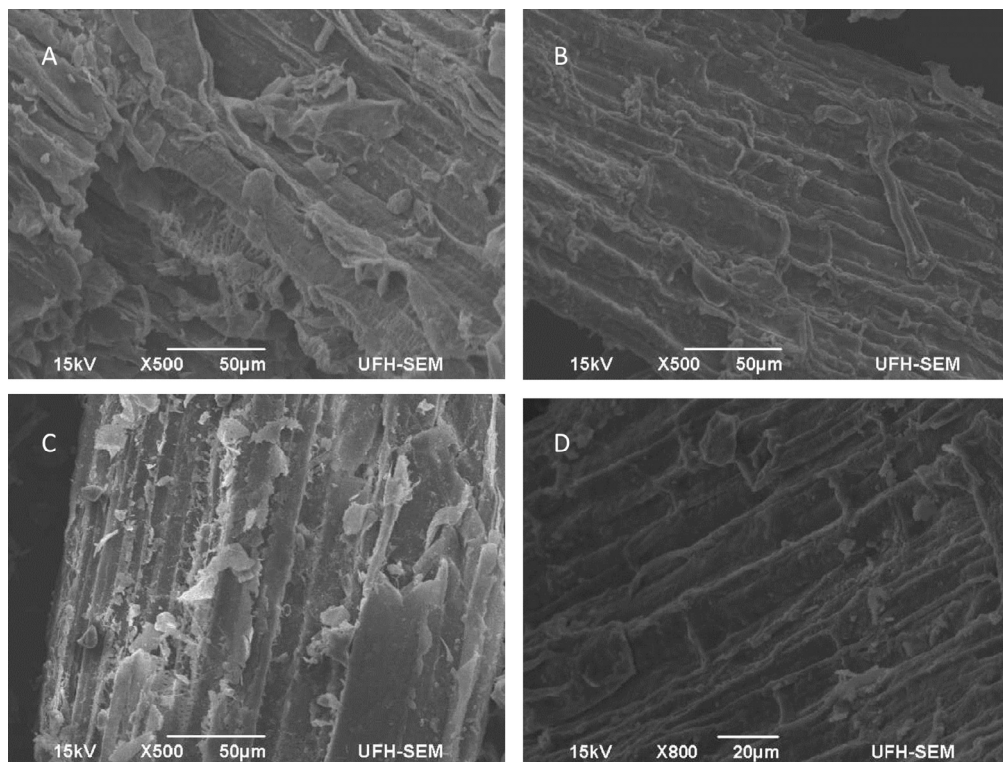


Figure 5 Alkali peroxide pretreated bagasse imagery for (A) 3 %, (B) 5 %, (C) raw bagasse and (D) 5 % at $\times 800$.

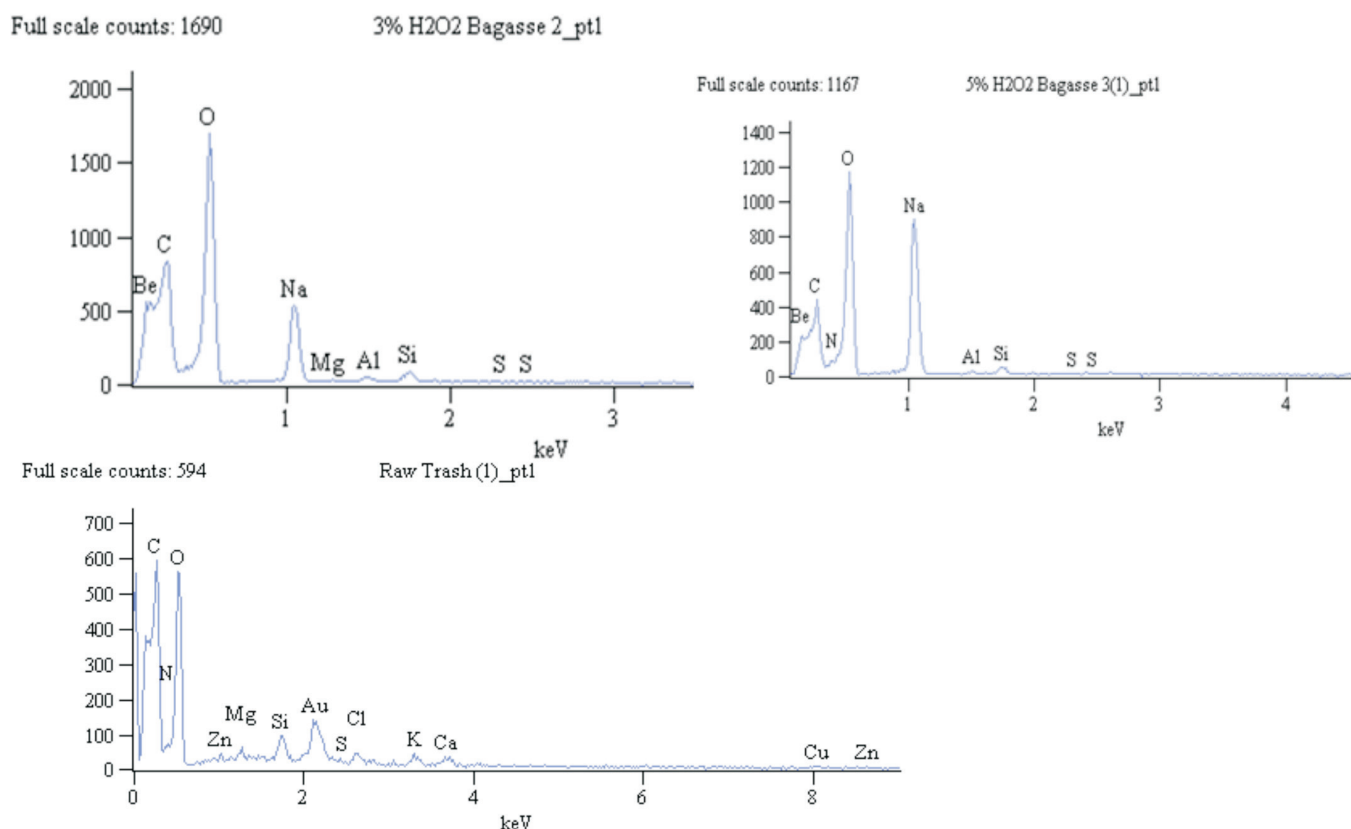


Figure 6 EDS results for 3 %, 5 % alkali peroxide pretreated trash and untreated material.

structure. There was quite a significant reduction in the quantities of lignin in particular with also some reduction in hemicellulose. Pretreatment of bagasse yielded significantly higher cellulose levels after pretreatment with low concentrations of alkali peroxide. This represents an increase in available exposed cellulose for use in further processes of enzyme hydrolysis and fermentation. The drawback is that this increase in cellulose percentage fraction can also mean that the crystalline structure of the cellulose was not disturbed, which could slow downstream processes of enzyme hydrolysis and subsequent fermentation. However, these slight changes in cellulose quantities are also an indication that alkali peroxide pretreatment conserves the quantities of cellulose and does not result in a loss in cellulose as compared to other pretreatment processes such as acid hydrolysis. Thus follow-up processes of enzyme hydrolysis and subsequent fermentation will yield higher levels of reducing sugars and bioethanol, respectively.

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Supplementary Material

Supplementary information is provided in the online supplement.

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Supplementary material to:

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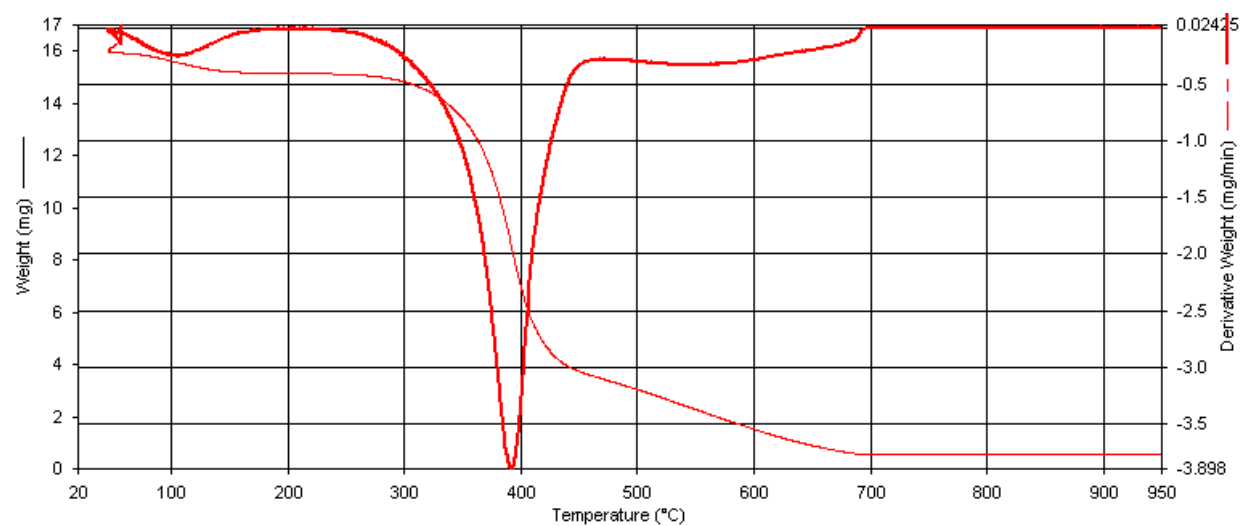
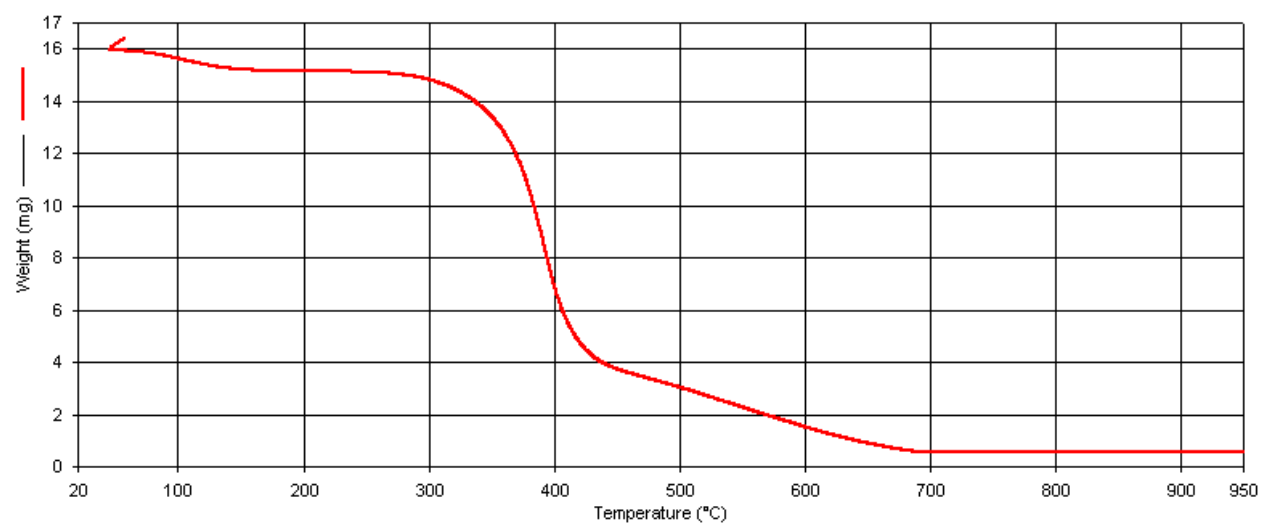
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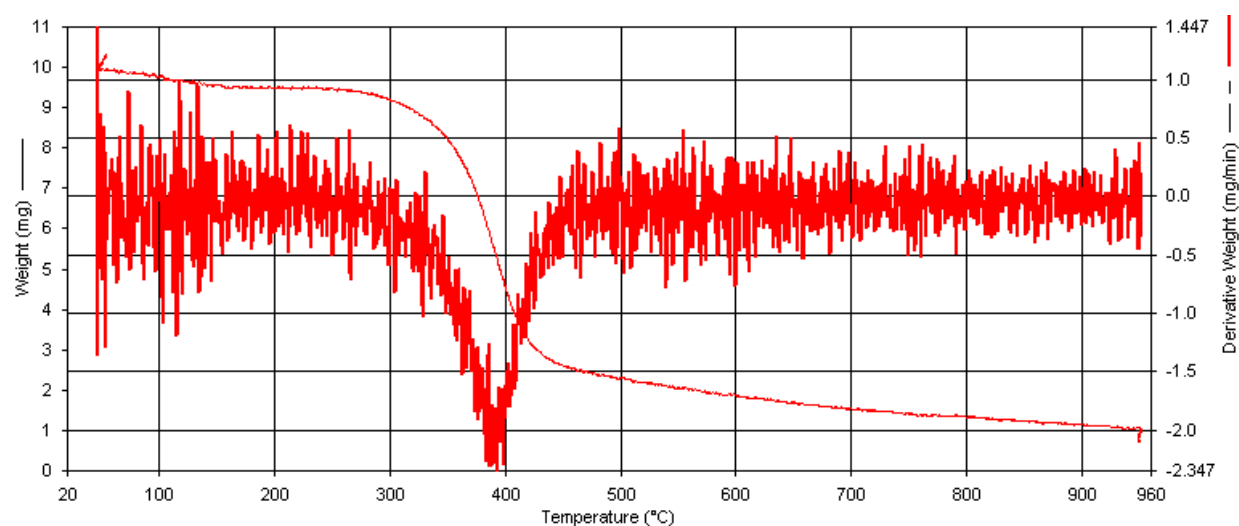
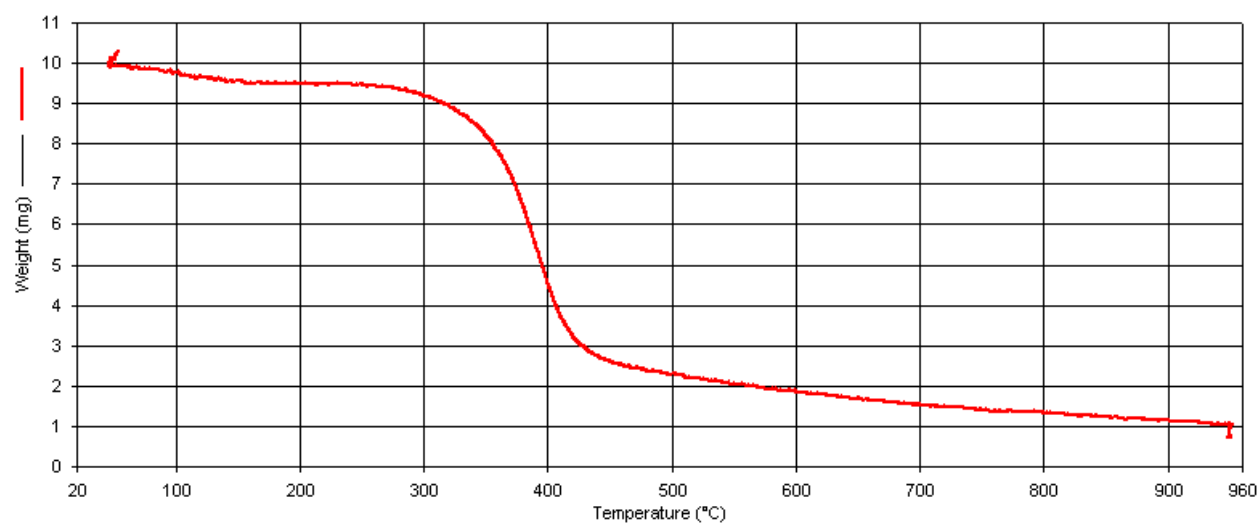
Annexure A

TGA and DTG distributions

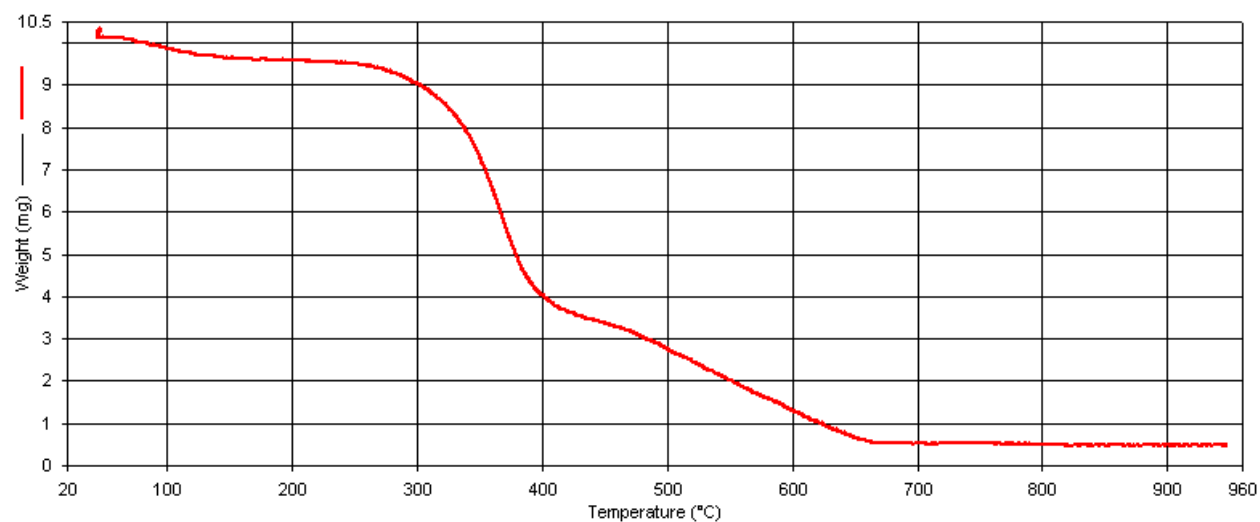
1g 48hrs

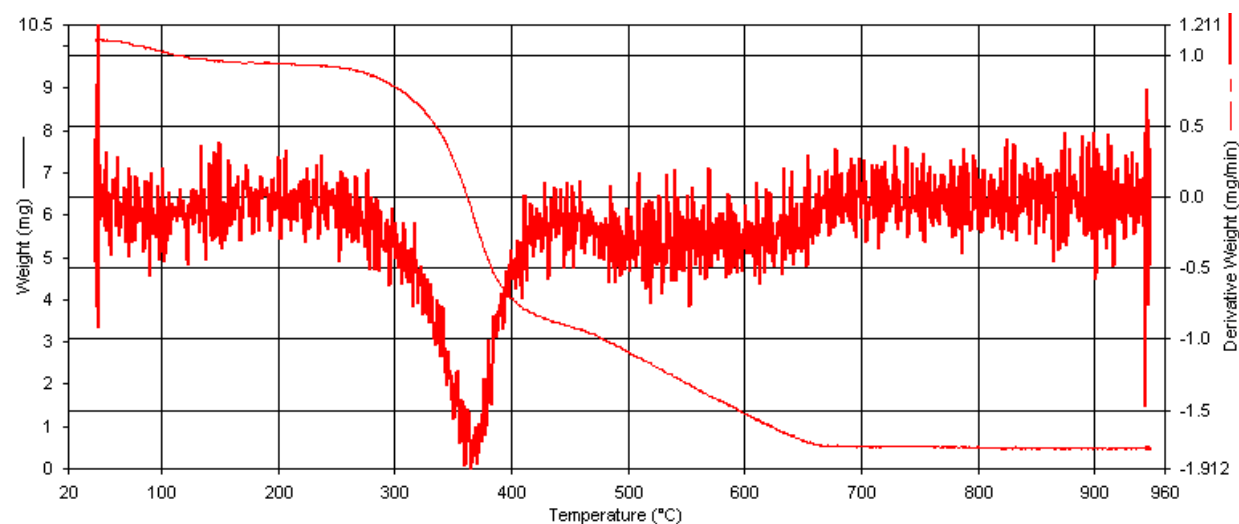


48hrs H₂O₂



24hrs





Og(original)

