Bioethanol Production from Characterized Pre-treated Sugar Cane Trash and Jatropha Agrowastes

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Abstract

Relying on second-generation bioethanol made from lignocellulosic substances is now an imperative goal for the entire world. However, the physical and chemical preparation of this kind of lignocellulosic feedstock is one of the main disadvantages of high ethanol yield. In order to increase the yield of fermentable sugars, pretreatment is an essential process step that alters the lignocellulosic structure and improves its accessibility for the expensive hydrolytic enzymes. In this context, the chemical composition of sugar cane trash (dry leaves, green leaves, and tops) and jatropha (shell and seed cake) was determined to be mainly cellulose, hemicellulose, and lignin. Hydrogen peroxide and sodium hydroxide were applied in an attempt to facilitate the solubilization of lignin and hemicelluloses in five agrowastes. The extraction of hydrogen peroxide was much better than that of sodium hydroxide. A comparative study was done using SEM, EDXA, and FTIR to evaluate the difference between the two methods. The pretreated wastes were subjected to saccharification by commercial cellulases (30 IU/g substrate). The obtained glucose was fortified with nutrients and fermented statically by *Saccharomyces cerevisiae* F-307 for bioethanol production. The results revealed the bioethanol yields were 325.4, 310.8, 282.9, 302.4, and 264.0 mg ethanol/g treated agrowastes from green leaves of sugar cane, jatropha deoiled seed cake, tops sugar cane, dry leaves of sugar cane, and jatropha shell, respectively.

1. Introduction

Biofuels have environmental friendliness, attracting interest from all over the world. As a carbon-neutral energy source that is renewable, unlike fossil fuels, which would cause global warming, biofuels do not upset the balance of air molecules in the atmosphere. Reducing reliance on traditional fossil fuels by employing biofuels is among the most feasible approaches. The world's most abundant biomass, lignocellulose, may be found in practically all extant plants as leaves, peels, bodies, branches, etc. As fossil fuels are used up, there is a growing need for renewable energy, particularly biofuels [1]. Therefore, the manufacturing of lignocellulosic bioethanol is unquestionably a method of supplying energy, particularly for nations.

Agriculture and agro-industrial wastes, as well as inexpensive lignocellulosic biomass supplies. These materials can range from sawdust to poplar trees, sugarcane bagasse to brewer's leftovers, grasses and straws to grain stems, leaves, husks, shells, and peels from corn, sorghum, and barley. Despite using these materials to create valuable products, lignocellulose wastes continue to build annually in enormous amounts, posing environmental issues [2]. The polysaccharides in lignocellulose wastes are inherently shielded by their structure from enzyme and chemical hydrolysis, making chemical and biological conversion of lignocellulose to other products, including ethanol, more challenging. Lignins are involved in the cross-linking of cellulose and hemicellululose in the matrix. These characteristics of lignins increase the strength and hardness of the lignocellulose structure. Pretreatment, which removes lignins from lignocellulose and improves the penetration of hydrolysis agents, is therefore an essential stage in the process of turning biomass into bioethanol [2]. Before lignocellulose is hydrolyzed and fermented, pretreatment procedures are done. Before-hand care To make cellulose more easily hydrolyzed, more amorphous areas should be present. To pretreat lignocelluloses, many physical, chemical, and biological techniques are applied. use of chemicals It is well known that lignin can be dissolved in aqueous acidic and alkaline solutions. For the most efficient and
practical lignocellulosic bioethanol production method, acidic and alkaline pretreatments of lignocellulose are used [3, 4]. Acidic pretreatments: Sulfuric acid and hydraulic acid are frequently used, although they are not particularly advised due to the production of furfural compounds during the pretreatment process, which prevents the growth of microorganisms during the fermentation process [5]. The loss of carbohydrates from hydrolysis is reduced when lignocellulose is first treated with an alkaline solution. Additionally, it facilitates a later hydrolysis, inhibits the production of furfural, and helps to eliminate acetyl groups. Because of its affordability and excellent efficacy, NaOH is the most often used alkali for lignin removal in lignocelluloses [6, 7]. Single pretreatment technique doesn't seem to be able to produce the desired outcome. It has long been practiced to combine several pretreatment techniques. The polysaccharide-enriched material is hydrolyzed to hexoses by enzymes after lignocellulose has undergone pretreatment [8, 9]. Cellulases, a general term for a variety of enzymes that are isolated from microorganisms, are what are used in commercially available products to hydrolyze cellulose. These enzymes cleave glycosidic bonds in carbohydrates, often via inverting or retaining processes, the latter of which advances. Microorganism development during fermentation is facilitated by enzymatic hydrolysis. The single sugars that were released as a result of enzymatic hydrolysis are metabolized by fermentation microorganisms to produce bioethanol.

The aim of the work is to produce bioethanol as renewable energy by using different agricultural wastes (dry leaves, green leaves, tops of sugar cane, shell, and deoiled seed cake of jatropha). The main biopolymers (cellulose, hemicellulose, and lignin) were extracted by using different alkaline solutions (sodium hydroxide and hydrogen peroxide). SEM, EDXA and FTIR were used to compare the effects of the two methods. The results showed that the pretreatment with H₂O₂ was more efficient than NaOH. The application of extracted methods was a new trend that improved the biomass of bioethanol production by decreasing the inhibitors of fermentation processes.

2. Materials and Methods

2.1. Materials

2.1.1. Collection of agrowastes

Sugar-cane trash (dry leaves, green leaves, and tops) and jatropha seed cake will be collected from El Menia area of Upper Egypt. The collected agricultural wastes will be dried in open air (in direct sunlight) and then grinding to 0.5 mm mesh. After the sugarcane harvest, the trash remaining consists of three main components, namely, dry leaves, green leaves, and tops [10] which dried in open air almost for seven days to remove water. Jatropha seeds collected after separating from shell, these seeds squeezing in EL Hussein Presses of kernel cold to remove oil to obtain jatropha seed cake.

2.1.2. Cellulase enzyme

Enzyme, cellulase, was gained from commercially available sources and were of industrial grade from Stem Enzym GmbH and Co. KG.Germany.

2.2. Methods
2.2.1. Chemical composition of the untreated and treated agrowastes

According to AOAC [11], we will determine the chemical composition (e.g., moisture, ash, wax, crude lipids, and low-molecular-weight carbohydrates (LMWC) of agricultural wastes. According to Jayme and Knoll [12], low-molecular-weight carbohydrates can be determined by dissolving in 85% ethanol for 24 hours and examined by paper chromatography, which is done after soxhlet extraction. Spots were detected by spraying the papers with aniline-phthalate reagent [13] and aniline-xylene. Quantitative screening of glucose, arabinose, and xylose was determined using the phenol-sulfuric acid method [14]. Thereafter, the color density was measured at wavelengths of 480 nm and 490 nm for pentoses and hexoses, respectively.

2.2.2. Total carbohydrate determination

Using strong acid hydrolysis, which extracted cellulose and hemicellulose as glucose [14], measured the total carbohydrates in agricultural wastes using the following procedure: Carefully, 0.5 mg of sample was stirred with 0.5 ml ice cold 80% H$_2$SO$_4$ at ambient temperature for 15 hours, it was diluted with a mixture of cold distilled water (up to 13 mL). The solution was hydrolyzed then heated in a sealed tube for 6 hours in a water bath that had been previously been boiled. To neutralize the hydrolyzate solution, the determined amount of BaCO$_3$ was added. Washing with water was used to filter the neutralized solution. A cation exchange resin called Amberlit IR-120 (H$^+$) was used to treat the filtrate. Using the phenol sulfuric acid technique, the extract's total carbohydrate content was calculated. This method's specifics were as follows: 1 ml of the resulting diluted solution was added to 1 ml of 5% phenol solution after the appropriate dilution. After blending, 5 ml of concentrated H$_2$SO$_4$ was quickly added to the mixture, shook, and left to sit for 10 minutes at room temperature before being heated to 20 to 30°C (in a water bath) for 20 minutes. Then, using a 490 nm spectrophotometer, the color density was determined. UNICO 7200.

2.2.2.1. Qualitative examination

Using a solvent system of n-butanol-acetone-water (4:5:1), the ethanol extract was decolored by boiling it with charcoal, concentrating it under low pressure at 45°C, and examining the results using paper chromatography Whatman No. 1. Reference [12]. Chromatography was used to analyze real samples of xylose, arabinose, glucuronic acid, and glucose. By spraying the sheets with the aniline-phthalate reagent, which is made up of 1.66 g of o-phthalic acid and 0.91 ml of aniline diluted in a solution of 48 ml n-butanol, 48 ml diethyl ether, and 4 ml water, [13] asserts that spots were observed. The chromatogram was air dried following chromatographic separation, dipped in 50 ml of aniline-phthalate reagent, to be heated in an oven for 10 minutes at 105°C to produce the colored parts.

2.2.2.2. Quantitative Determination

According to the modified approach of Wilson [15], the hydrolysis sugars were quantitatively determined. The individual chromatography spots were separated into short strips, dropped into a test tube containing 4 ml of eluting agents (0.7 N HCl in 80% ethanol), and shaken for thorough elution. Using the spectrophotometer UNICO 7200, the absorbance of the resulting colored solutions was measured at 390 nm for pentoses and
490 nm for hexoses sugars. By comparing the sugar amounts to relevant standard curves created under the same circumstances, the sugar amounts were identified.

2.2.3. Alkali pretreatment

2.2.3.1. Sodium hydroxide

Five grams of sugar cane (dry leaves, green leaves, and tops) and jatropha (shell and deoiled seed cake) were treated individually at 90°C for one hour (15 ml/g of liquid to solid ratio) three times in a 75 ml solution of sodium hydroxide with a pH of 12. The reaction mixture was filtered, the residue (crude cellulose) was washed with water several more times until it reached neutrality, and the filtrate was neutralized pH 7–6 by hydrochloric acid while being cooled. After filtering, hemicellulose is left behind and lignin is present in the filtrate; to extract lignin, hydrochloric acid was added till pH 1.5. There was lignin left over after filtering. At 105°C, all of the separated residues (cellulose, hemicelluloses, and lignin) were dried.

2.2.3.2. Hydrogen peroxide

Alkaline hydrogen peroxide (H₂O₂) solution was prepared by adding 5% H₂O₂ in distilled water and adjusting the pH to 11.5 with NaOH (liquid to agrowaste, 20 ml/g), then the reaction mixture was placed on a shaker at 40°C and 150 rpm for 6 h. The reaction mixture was filtrated. The crude cellulose was washed with distilled water and dried in a hot-air oven at 60°C [16].

2.2.3.3. Bleached cellulose

Sodium hypochlorite (2 – 1 w/w) in an appropriate quantity of distilled water at 80°C for two hours, followed by filtering, was used to create bleached cellulose from crude cellulose. In accordance with Ragab et al. [17], the cellulose residue was completely dried at 70°C after being extensively rinsed with distilled water till neutralization.

2.2.4. Chemical analysis

2.2.4.1. FTIR spectroscopy

The isolated products were characterized and identified using FT-IR. Using a Bruker Vectra 22 FT-IR Spectrometer with a Dura Sample IR IITM detector, IR spectra were immediately acquired from the powdered cellulose, hemicelluloses, and lignin onto a detector prism. In the wave number range of 4000 – 400 cm⁻¹, all spectra were recorded at a spectral resolution of 4 cm⁻¹.

2.2.4.2. SEM analysis

Three-dimensional (3D) structural information is of central importance in biological research on many long-scales. There are excellent methods to obtain atomic, molecular structures, electron microscopic organelles and light-microscopic resolution tissue. The resolution is enough to trace even the thinnest axons and to distinguish synapses. Stacks of several hundred parts were collected, 50–70 nm thick [18]. The surface morphology of samples was examined using scanning electron microscopy (JEOL 5410) microscope with an
accelerating voltage conducted at 10 kV. Samples were gold coated using a Hitachi coating unit IB-2 coater under a high vacuum, 0.1 Torr, high voltage, 1.2 kV and 50 mA.

2.2.4.3. Energy Dispersive X-Ray analysis (EDXA)

EDXA is an x-ray spectroscopic method for determining elemental compositions (qualitative and quantitative analysis).

2.2.5. Assay of cellulolytic enzymes

2.2.5.1. Exo-1, 4- glucannase (FP-ase) assay

Determination of FP-ase activity was measured according to Mandels et al. [19] by mixing 1 ml of supernatant, 1ml of 1% Whatman filter paper no.1 (strips of 1x6 cm) suspended in 0.05M sodium citrate buffer pH 4.8 and incubated at 50°C for 30 min. The color of reaction was developed by adding DNS reagent and the produced fermentable sugars was measured at 540 nm against a reagent blank [20]. One unit of enzyme was defined as the amount of the enzyme which released 1 µg of glucose.

2.2.5.2. Endo-1, 4- glucannase (CMC-ase) assay

Determination of carboxymethylcellulase (CMC-ase) was measured according to Mandels et al. [19] by mixing 1ml of supernatant, 1% CMC dissolved in 0.05M sodium citrate buffer pH 4.8) and incubated at 50°C for 30 min. The color of reaction was developed by adding DNS reagent and the produced fermentable sugars the absorbance was measured at 540 nm against a reagent blank [20]. One unit of the enzyme was defined as the amount of enzyme which released 1 µg of glucose per ml per min.

2.2.6. Enzymatic saccharification of treated lignocellulosic

The materials were shaken at 130 rpm and 50°C to initiate saccharification processes. The first thing that was done was. Using 10–50 IU/g of cellulosic material, the enzymatic reactions were carried out in 100 ml of 0.5 M sodium citrate buffer solutions at pH 4.8. Following the hydrolysis reaction's incubation period, the substance was filtered through filter paper, and the hydrolyzed sample was separated for further analysis to identify the reducing sugars. Three duplicates of the experiment were carried out.

2.2.7. Quantification of reducing sugars

Miller's technique [20] was used to calculate the total content of reducing sugars produced after hydrolysis. Based on the absorbance at 490 nm, this technique operates. Using glucose as the standard, a calibration curve was compared to quantify the quantity of reducing sugars contained in each sample in triplicate.

2.2.8. Bioethanol Fermentation

After scarification using commercial cellulase, the glucose syrup was evaporated to concentrate the sugar content to 10% (w/v). There were 100 mL of YPM medium (containing (g/L) malt extract, yeast extract, peptone, and sucrose) in each of four conical flasks with a 250 mL capacity was inoculated with a loop of the yeast strain S. cerevisiae F-307, sterilized with steam at 121°C for 15 min, cooled to room temperature, and then let to stand at 34°C for 24 h. Inoculating the ready fermentation vessels at 1% v/v with the preceding
yeast culture. After sterilization, the aforementioned sugar syrup was fortified with (g/L) yeast extract, malt extract, magnesium sulfate, and diammonium phosphate. It was then inoculated with the aforementioned \textit{Saccharomyces cerevisiae} inoculum at 1% v/v and allowed to stand at 34°C for 72 hours. By applying the following formula to determine how much sugar is consumed during fermentation and conversion to ethanol:

\[ 1 \text{ g of glucose produces 0.51 g of ethanol, which equals } 0.51 \times 100 \text{ mL ethanol, as described in Fadel et al. [21].} \]

2.2.9. Fermentation Efficiently

The calculation of fermentation efficiency involved dividing the amount of generated ethanol by the theoretical amount of ethanol and multiplying the result by 100 [22].

\[ \% \text{ Hydrolysis} = \text{glucose amount (w)} \div \text{initial substrate weight} \times 0.9 \times 1000 \]

3. Results and Discussion

3.1. Physiochemical composition

3.1.1. Chemical composition of sugar cane

The results were obtained of dry leaves which shown in (Table 1) such as moisture content (5.52%), wax (2.46%), low molecular weight (7.66%), ash (3.50%), lipid content (1.14%) and total carbohydrate (14.88%). The percentage of ash in this study which is lower than published by Gómez et al. [23]. The moisture on dried leaves measured in this investigation was in close agreement with the findings reported in Franco et al. [24] and lower than published in Gómez et al. [23] and the percentage of wax determined, was higher than the published results to [25]. The obtained results of low molecular weight were higher than published in Gómez et al. [23]. While result of green leaves in (Table 1) were in an average 11.19% of moisture content, 1.13% wax, 5.24% low molecular weight, 0.74% total lipid, ash (2.20%) and 11.15% total carbohydrate. The result recorded in ash determination was approximately that was determined in Gómez et al. [23]. The percentages of wax, low molecular weight, total lipid, ash, and total carbohydrate of the tops in this work were (15.00%, 0.83%, 10.84%, 0.12%, 2.70%, and 16.08%). The ash result was lower than the result mentioned by Franco et al. [24] where, ash was on average 5.4%, 4.1% and 4.6% for green tops, dry leaves, and bulk straw respectively. The moisture content of tops varies between 15.00–5.52% was lower than published Franco et al. [24]. Tops have seven times more moisture than dry leaves. Similary, Menandro et al. [26] reproted that green tops contained six times more moisture than dry leaves (68% and 11%, respectively). The levels of ashes (4.7% on average) were similar in both the tops and the dry leaves. However, the extractives content is higher in tops (25.7%) than in dry leaves (13.7%) [24]. The section of ash was, on average 5.4% and 4.1% for green tops and dry leaves, respectively and 4.6% of bulk straw. There were also significant differences for extractives: 67% for green tops and 33% for dry leaves, allowing to Menandro et al. [26]. Green tops also stored up to four times as many nutrients as dry leaves. Green tops also had a six times higher humidity and higher chlorine content, which reduced the efficiency of the milling operation. In addition, the quality of dry leaves was higher in lignin, cellulose and hemicellulose and tended to be a better feedstock for ethanol.
production in the second generation. Overall, the results show that dry leaves are preferable for the production of bioenergy while green tops are left in the field for nutrient recycling [26].

### 3.1.2. Chemical composition of jatropha

Results obtained of jatropha shell, the moisture content, wax, low molecular weight, total lipid, ash and total carbohydrate were (5.46%, 5.27%, 5.61%, 1.65%, 5.00% and 12.74%), respectively. Moisture content of jatropha shell was lower than Inekwe et al. [27] and the part was roughly to published results of Murata et al. [28]. The measurement of low molecular weight (Table 1) was lower than issued in Singh et al. [29]. Direct combustion of jatropha shell was also characterized by flame front instabilities and short combustion periods due to its higher ash content [30]. The firm results seed cake in an average 4.63% of moisture content, 4.51% wax, 4.27% low molecular weight, 1.98% total lipid, 2.00% of ash and 11.57% total carbohydrate. Moisture of jatropha seed cake in was higher than the result of dos Santos et al. [31] but almost to the result of dos Santos et al. [27]. Consistent with the results of dos Santos et al. [31] the extraction of lipids for the seed cake preparation was highly efficient, leaving only 1.15% residual lipids were something like to fraction of this study. The percentage determined of total carbohydrate which was similar to the result of dos Santos et al. [27]. The published dos Santos et al. [31] of ash was higher than determined in this study.

### 3.2. Alkali treatment

#### 3.2.1. Sodium hydroxide

The results were obtained of dry leaves which shown in (Table 1), Plus the percentages determined of cellulose, hemicellulose and lignin were (44.00%, 9.00% and 14.00%), respectively. In this study, the result of cellulose was approximately to the authors published [32] and highr than Franco et al. [24]. The obtained results of hemicellulose was lower than result mentioned by Gómez et al. [23]. In this study, the percentage of lignin, was higher than Gómez et al. [23] but was lower than the result obtained by Menandro et al. [26]. In (Table 1) results of green leaves were determined by the calculation of cellulose, hemicelluloses and lignin (41.09%, 11.05% and 18.04%) respectively. In addition to the percentage of the low molecular weight carbohydrates (Table 1) was higher than published in [23]. According to Gómez et al. [23] the results were lower than the obtained results of cellulose and lignin. Because the straw has two or three times the quantity of silica as the green leaves do, the plant's hardening and mechanical resistance are increased in these areas. The amount of lignin in dry leaves was lower than the green leaves in this study. Like, the amount of lignin in dry leaves was approximately 60% smaller than those found in the green leaves according to Gómez et al. [23].

At this study, the percentages of cellulose, hemicellulose and lignin of tops was shown in (Table 1). By comparison the levels of ashes (4.7% on average) were similar in both the tops and the dry leaves. However, the extractives content is higher in tops (25.7%) than in dry leaves (13.7%) [24]. The proportion of cellulose in this study was lower than Franco et al. [24] and approximately to the result of Menandro et al. [26] but the published results of hemicellulose were higher than result showed in (Table 1) and the percentage of lignin was lower than Menandro et al. [26].
Table 1
Chemical composition of sugar cane trash (dry leaves, green leaves & tops) and jatropha (shell and seed cake) after pretreated alkaline sodium hydroxide and hydrogen peroxide

<table>
<thead>
<tr>
<th></th>
<th>Treatment with NaOH</th>
<th>Treatment with H₂O₂</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cellulose</td>
<td>Hemicellulose</td>
</tr>
<tr>
<td>Dry leaves</td>
<td>44.00</td>
<td>9.00</td>
</tr>
<tr>
<td></td>
<td>± 0.56</td>
<td>± 0.50</td>
</tr>
<tr>
<td>Green leaves</td>
<td>41.09</td>
<td>11.05</td>
</tr>
<tr>
<td></td>
<td>± 0.74</td>
<td>± 0.49</td>
</tr>
<tr>
<td>Tops</td>
<td>33.53</td>
<td>12.48</td>
</tr>
<tr>
<td></td>
<td>± 0.50</td>
<td>± 0.27</td>
</tr>
<tr>
<td>Jatropha Shell</td>
<td>41.53</td>
<td>09.84</td>
</tr>
<tr>
<td></td>
<td>± 0.77</td>
<td>± 0.62</td>
</tr>
<tr>
<td>Jatropha Seed cake</td>
<td>36.97</td>
<td>7.23</td>
</tr>
<tr>
<td></td>
<td>± 0.88</td>
<td>± 0.25</td>
</tr>
</tbody>
</table>

In (Table 1) results of shell, in keeping with Odetoye et al. [33] the percentage of cellulose was approximately to obtain in this study and higher than determined by Singh et al. [29]. And the amount of hemicellulose in this work 9.84% was average to publish in and lower than published in Ewunie et al. [30]. The section of lignin in table (13.00%) was higher than circulated in [29] and lower than result mentioned by Ewunie et al. [30]. Seed cake in this work, the percentage determined of cellulose, hemicellulose and lignin was (36.97%, 7.23% and 26.95%) respectively in (Table 1). The percentage of cellulose determined in this work was higher than obtained in [31, 34]. While the result of hemicellulose of this work was approximately to Shuhairi et al. [34] and the obtained result of lignin in (Table 1) was approximately according to the result of Shuhairi et al. [34] and lower than result of dos Santos et al. [31].

3.2.2. Hydrogen peroxide

In the Table 1 below, we saw that the result of cellulose, hemicellulose and lignin after treated by alkaline hydrogen peroxide which, given highest percentage of holocellulose and removal high amount of lignin compared of the other traditional method which remove the small amount of lignin that will become as inhibitor to microorganisms in fermentation. The percentage of cellulose, hemicellulose and lignin in the dry leaves of sugar cane was 80.62%, 3.24% and 16.14% respectively, while green leaves was 75.41% of cellulose, 1.48% of hemicellulose and 23.11% of lignin. In connected with the result of sugar cane tops 71.42% of cellulose, 3.24% of hemicellulose and 25.34% of lignin. According to the results published in [16], the percentage after alkaline hydrogen peroxide was 50.55% which the result of this work was higher than published. After pretreated of jatropha shell and deoiled seed cake by H₂O₂, the result obtained that jatropha shell 67.42% of cellulose, 18.61% of lignin while deoiled seed cake 72.42%, 7.72% and 19.86% were the
percentage of cellulose, hemicellulose and lignin respectively. \( \text{H}_2\text{O}_2 \) has been used to disrupt and destroy the lignocellulosic structure of biomass for decades. However, because it creates potent hydroxyl radicals, its impact on biomass digestibility and delignification was pH dependant. This classic chemical pretreatment approach has been used to produce fermentable sugar and bioethanol from lignocellulosic feedstocks such as sugarcane bagasse, rice husks or hulls, wheat straw, and other lignocellulosic feedstocks [16]. The authors suggested that high alkalinity of sodium hydroxide deformed and soluble the amorphous cellulose in the agrowastes so that cellulose yield was low. The yield of extracting cellulose by hydrogen peroxide (\( \text{H}_2\text{O}_2 \)) was higher than by the sodium hydroxide methodology. In contrast, lignin percentage was higher than in case of hydrogen peroxide (\( \text{H}_2\text{O}_2 \)) method so the process of hydrogen peroxide (\( \text{H}_2\text{O}_2 \)) is better than sodium hydroxide process because we decrease of lignin percentage which play as inhibitor in fermentation process.

### 3.3. Monosaccharide constituents

#### 3.3.1. Sugar cane trash (dry leaves, green leaves and tops)

Dry leaves monosaccharide constituents in this study were 15% D-xylose, 10% L-arabinose, 20% D-fructose, 25% D-glucose, 20% D-galactose, 5% D-glucoronic acid and 5% D-glactouronic acid which presented in (Table 2). While monosaccharide constituents of green leaves were (30%, 20%, 40%, 10%) D-xylose, D-fructose, D-glucose and D-glactouronic acid, respectively. Chloroplast degradation and eventual depletion of complete chlorophyll are among the earliest occurrences during the senescence of the leaves [35]. In the dry and green leaves, the neutral monosaccharides fucose, rhamnose, arabinose, galactose, glucose, and xylose were examined. Arabinose and xylose are monosaccharides released from arabinoxylan, which is one of the most abundant hemicelluloses in cell walls of sugarcane. Arabinoxylan (dry leaves) was not damaged in the senescence process. Thus, it was very likely that the detected glucose in sugarcane leaves was derivative from the mixed linkage \( \beta \)-glucan (\( \beta \)-glucan), another hemicellulose found in sugarcane cell walls. However, measures have not been taken to remove starch from the cell-wall preparation; thus, starch can also contribute to glucose. Furthermore, it is probable that a small portion of the glucose and xylose could not be discarded from xylloglucan, which also occurred in sugarcane leaf cell walls. A small amount of galactose (~2.5 per cent) and only traces of fucose and rhamnose have been found. In these cases, it was presumed that these monosaccharides are derived from pectin polymers (apart from some of the arabinose described above). Our findings show that no substantial changes in large hemicellulose or pectin cell-wall polymers occur during the sugarcane leaf senescence (yellowing of leaves) [36]. Co-variance study, according to the authors shown this in the ‘in-leaf’ gradient of senescence, the monosaccharides rhamnose, arabinose, galactose and glucose are positively correlated while xylose was negatively correlated to all the above monosaccharides. The percentage of tops Monosaccharide constituents was 5% D-xylose, 5% L-arabians, 30% D-fructose, 30% D-glucose, 15% D-galactose, 10% D-glucoronic acid and 5% D-glactouronic acid which exposed in the (Table 2).

#### 3.3.2. Jatropha (shell and seed cake)

The ratio shown in the (Table 2) monosaccharide constituents of jatropha shell was 5% D-xylose, 5% D-fructose, 80% D-galactose and 10% D-glactouronic acid. The predominant hemicellulosic sugar was xylose, followed by mannose, galactose and arabinose. That's an alignment with most of the previous studies.
significantly higher content of arabinose was recorded only for Brazilian shell than that of mannose and galactose [37]. Galactose represented 31.6% of the sugars contained in the extract, followed by glucose (21.7%), arabinose (16.3%) and rhamnose (11.9%) [37]. In (Table 2) represented jataropha seed cake monosaccharide constituents were 85% D-glucose, 10% D-galactose and 5% D-galactouronic acid.

Table 2
<table>
<thead>
<tr>
<th>Alternate constituents of raw biomass after acid hydrolysis (% w/w)</th>
</tr>
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<tbody>
<tr>
<td>Sugar-cane trash</td>
</tr>
<tr>
<td>Dry leaves</td>
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<tr>
<td>---</td>
</tr>
<tr>
<td>15.00</td>
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<tr>
<td>10.00</td>
</tr>
<tr>
<td>20.00</td>
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<tr>
<td>25.00</td>
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<td>20.00</td>
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<td>5.00</td>
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<td>5.00</td>
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</tbody>
</table>

3.4. Scanning electron microscopy (SEM)

3.4.1. Sugar cane trash

Scanning Electron Microscopy (SEM) was utilized to analyze the morphology of untreated and treated sugarcane leaf which the surface of treated sample showed the broken fibril. SEM on dry leaves of sugar cane, observed that the smooth like fibers (Fig. 1A). Ultrastructure observation in green leaves found that the chloroplasts were near the cell wall and well organized in most instances, and the chloroplast thylakoids were organized in the chloroplasts in order. As a result of the continuous stress of the drought, the leaves became dry, plasmosis developed, the chloroplasts moved toward the center of the cell and eventually changed from long and oval to nearly round, and the amount of starch increased as the water content of the leaves decreased and the tips of the leaves began to curl. The native samples are rigidly packed and compact [38].

SEM dry leaves of sugar cane which are represented in (Fig. 1B), appeared to have cellulose fibers and very small fibers of hemicellulose and lignin that indicating of remove them after NaOH pretreatment process. The surface of dry leaves is ruptured after pretreatment by the aggregate impact test apparatus setup on the leaves, and rough and oval shaped form fibers such as material have been elongated [39]. In green leaves of
sugar cane after pretreatment and obtained cellulose fibers aggregate with removal of hemicellulose, lignin and chloroplast (Fig. 1E). While sugar cane tops (Fig. 1H) observed that the structure slanted with fibers of cellulose and small fibers of hemicellulose and lignin after pretreatment. The unpretreated samples have a compact rigid structure while the pretreated samples showed a distorted structure. The distorted structure and increase in surface area of the pretreated sugar cane tops improve the hydrolysis efficiency [38].

SEM analysis of dry leaves after pretreatment by H$_2$O$_2$, appear as keeping of crystanalnty of cellulose and hemicellulose removed high percentage of lignin and the same result obtained in green leaves and tops (Fig. 1C, F, I). The internal structures of the pretreated sample showed distortions that indicate the breakdown of the lignin, hemicellulose, and cellulose complex. The deformed shape can enhance the effectiveness of acid hydrolysis by increasing the accessible surface area [38].

### 3.4.2. Jatropha (shell and seed cake)

When applied of SEM analysis of jatropha shell presented that rod like fiber with groove slightly appearance in (Fig. 2A) which agree with published of the untreated shell (control) micrographs showed an intact rod-like fibril structure and a smooth exterior surface. From the internal view of the shell, a surface layer covering the structure of the fibril cell can be seen. In the untreated shell, no singular or loose fibres were found. SEM pictures of shell that were handled showed big difference between pretreated and untreated. According to (Fig. 2D) which appeared of SEM analysis of jatropha deoiled seed cake, fibers of cellulose, hemicellulose and lignin as smooth fibers and accepted with the papper of Shuhairi et al. [34].

After NaOH pretreatment of jatropha shell which resulted in removal of lignin and hemicellulose to obtain cellulose fibers pure which appeared as rod with groves fibers (Fig. 2B). Pretreated shells had a loose, twisted fibre structure and pores that were possibly due to biomass delignification. The study of the SEM supports the enzyme hydrolysis information which showed high yields of fermentable sugar. There is better interaction between the enzyme and biomass because hydrolysis of the jatropha shell, destructed the outer surface layers of the jatropha shell, distortion and loosening of the fibre structure, and formation of pore structure helps. Though the removal of not need fibers of hemicellulose and lignin to take of cellulose fibers jatropha deoiled seed cake that considered in this study after pretreatment. The extracted sample displayed an irregular and disturbed surface structure substantially different from the raw seed sample, primarily due to the breaking up of the lignin carbohydrate matrix during the extraction phase. This results in the aggregation of such lignin complexes that are concentrated on the surface of the sample collected.In order to delignify the biomass and increase the usable surface area of cellulose and hemicellulose to increase enzymatic hydrolysis Shuhairi et al. [34]. SEM analysis of shell and deoiled seed cake of jatropha after pretreatment by H$_2$O$_2$, appear as keeping of crstanalnty of cellulose and hemicellulose and removal high amount of lignin removed.

### 3.5. EDXA analysis

Qualitative and quantitative elements of sugar cane trash (dry leaves, green leaves and tops) which determined by EDXA and give these percentages. Firstly, dry leaves of sugar cane (47.19% C, 0.76% N, 45.03% O, 0.35% Na, 0.20% Mg, 0.10% Al, 1.13% Si, 0.69% P, 1.15% Cl and 3.40% K) and element of carbon and oxygen had high percentages observed. In addition to the dry leaves, in this study, determined of sugar cane
green leaves and small ratio of Mg, Na, Al, P, Cl, K which presented in (Table 3) and high percentages of C, N, O and Si. EDXA analysis showed tops of sugar cane contained the percentages of quantitative elements such as C was approximately equal to the ratio of O and minor traces of Mg, Na, Al, P, Cl, K which presented in (Fig. supplmentary data). The tops had the maximum N, K, P and Ca, while the dry leaves contained more of Mg. This work showed that the highest N content in tops, whereas dry leaves had the lowest N content which equivalent to results of Franco et al. [24]. The content of N, P and K in green leaves were higher than their in other parts of sugarcane greatly, but the content of Ca, Mg and S in bone-dry leaves were higher than their in other parts. The highest content of Zn and Cu appeared in green leaves, and Fe appeared in underground parts and the accumulation of N, P and K in stem accounted for about 60%, and in green leaves and tip accounted for 20%-25%. Ca, Mg and S mainly accumulated in stem, bone-dry leaves and green leaves and tip [40]. In sugar cane dry leaves the nutrients Ca, S, Mg, B, Mn and Al were seen to have accrued. The leaves had a comparatively higher abundance of Ca, S, Mn and Al. In the various areas of the leaf, Ca, and Al had varying accumulation amounts between 50 and 75 percent from the tip to the base of the leaf. Mg and B showed modest accumulation (25% in average). Interestingly, Mg showed also high accumulation levels in the base of the leaves about duplicate compared to the other portions of the leaf.

Qualitative and quantitative elements of sugar cane trash crude cellulose dry leaves after NaOH pretretment (Fig. supplmentary data) (42.11% C, 2.13% N, 52.19% O, 0.72% Na, 0.69% Mg, 0.16% Al, 1.02% Si, 0.19% P, 0.12% Cl and 0.11% K). Crude cellulose green leaves contain elements differentiate which carbon 56.54%, it was the highest percentage and the small were N, Na, Mg, Si, P, Cl. EDXA analysis crude cellulose tops showed that contained mainly C, N, O, Na, Mg, Al, Si, P, Cl, K which presented in (Table 3).

In (Table 3) presented the quantitative elements after H2O2 pretreatment of dry leaves (45.30% C, 1.04% N, 48.41% O, 0.16% Na, 0.13% Mg, 0.12% Al, 1.43% Si, 0.22% P, 0.10% Cl− and 0.12% K). while the green leaves, there are variation was determined in (Table 3) (53.06% C, 1.74% N, 32.80% O, 0.06% Na, 0.04% Mg, 0.14% Al, 3.28% Si, 0.25% P, 0.05% Cl and 0.29% K).
Table 3
Qualitative and quantitative elements of sugar cane trash (Dry leaves, Green leaves and Tops) (weight %)

<table>
<thead>
<tr>
<th>Element</th>
<th>Dry leaves</th>
<th>Green leaves</th>
<th>Tops</th>
<th>Dry leaves</th>
<th>Green leaves</th>
<th>Tops</th>
<th>Dry leaves</th>
<th>Green leaves</th>
<th>Tops</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>47.19</td>
<td>49.58</td>
<td>42.70</td>
<td>42.11</td>
<td>56.54</td>
<td>42.38</td>
<td>45.30</td>
<td>53.06</td>
<td>50.43</td>
</tr>
<tr>
<td>N</td>
<td>0.076</td>
<td>0.159</td>
<td>2.20</td>
<td>2.13</td>
<td>0.99</td>
<td>1.94</td>
<td>1.04</td>
<td>1.74</td>
<td>1.43</td>
</tr>
<tr>
<td>O</td>
<td>45.03</td>
<td>38.50</td>
<td>41.07</td>
<td>52.19</td>
<td>32.56</td>
<td>53.62</td>
<td>48.41</td>
<td>32.80</td>
<td>42.10</td>
</tr>
<tr>
<td>Na</td>
<td>0.35</td>
<td>0.05</td>
<td>0.09</td>
<td>0.72</td>
<td>0.87</td>
<td>0.55</td>
<td>0.16</td>
<td>0.06</td>
<td>0.54</td>
</tr>
<tr>
<td>Mg</td>
<td>0.20</td>
<td>0.01</td>
<td>0.27</td>
<td>0.69</td>
<td>0.04</td>
<td>0.23</td>
<td>0.13</td>
<td>0.04</td>
<td>0.16</td>
</tr>
<tr>
<td>Al</td>
<td>0.10</td>
<td>0.12</td>
<td>0.29</td>
<td>0.16</td>
<td>2.97</td>
<td>0.06</td>
<td>0.12</td>
<td>0.14</td>
<td>0.16</td>
</tr>
<tr>
<td>Si</td>
<td>0.13</td>
<td>8.96</td>
<td>10.96</td>
<td>0.26</td>
<td>0.12</td>
<td>1.43</td>
<td>3.28</td>
<td>0.29</td>
<td></td>
</tr>
<tr>
<td>P</td>
<td>0.69</td>
<td>0.72</td>
<td>0.59</td>
<td>0.19</td>
<td>0.22</td>
<td>0.21</td>
<td>0.22</td>
<td>0.25</td>
<td>0.21</td>
</tr>
<tr>
<td>Cl</td>
<td>1.15</td>
<td>0.09</td>
<td>0.47</td>
<td>0.12</td>
<td>0.25</td>
<td>0.12</td>
<td>0.10</td>
<td>0.05</td>
<td>0.15</td>
</tr>
<tr>
<td>K</td>
<td>3.40</td>
<td>0.37</td>
<td>0.56</td>
<td>0.11</td>
<td>1.39</td>
<td>0.18</td>
<td>0.12</td>
<td>0.29</td>
<td>0.21</td>
</tr>
</tbody>
</table>

The obtained results of jatropha shell NaOH pretreatment were 40.30% C, 1.23% N, 51.58% O, 2.81% Na, 0.31% Mg, 0.09% Al, 0.19% Si, 0.21% P, 0.20% Cl and 0.73% K while deoiled seed cake had the highest percentages of C and small traces of Na, 0.95% Mg, 0.26% Al, 0.17% Si, 1.31% P, 0.15% Cl and 0.37% K. Quantitative element of nitrogen was equal in both jatropha shell and deoiled seed cake which observed in (Table 4) and showed in (Fig. supplementary data). According to Kratzeisen and Müller [41] the ratio of C jatropha shell was 50.9% higher than determined in this work but O quantitative element was approximately to authors published.

Analysied crude cellulose samples of shell and deoiled seed cake H$_2$O$_2$ pretreatment by using EDXA, however they enclosed with different ratios as listed in (Table 4), these elements were C, N, O, Na, Mg, Al, Si, P, Cl, K. Qualitative and quantitative elements of jatropha shell showed in (Table 4) (48.97% C, 1.81% N, 44.39% O, 0.05% Na, 0.02% Mg, 0% Al, 0.03% Si, 0.25% P, 0.07% Cl, 0.58% K), the percentage C element is the highest compared to the other method of pretreatment. While the result of jatropha seed cake which presented in different elements obtained with different percentages and showed in (Fig. supplementary data).
Table 4
Qualitative and quantitative elements of jatropha (shell and deoiled seed cake) (weight %)

<table>
<thead>
<tr>
<th>Biomass</th>
<th>Raw materials</th>
<th>NaOH treatment</th>
<th>Hydrogen peroxide</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Shell</td>
<td>Deoiled seed cake</td>
<td>Shell</td>
</tr>
<tr>
<td>C</td>
<td>46.45</td>
<td>71.21</td>
<td>40.30</td>
</tr>
<tr>
<td>N</td>
<td>2.69</td>
<td>2.84</td>
<td>1.23</td>
</tr>
<tr>
<td>O</td>
<td>42.47</td>
<td>22.1</td>
<td>51.58</td>
</tr>
<tr>
<td>Na</td>
<td>1.94</td>
<td>0.90</td>
<td>2.81</td>
</tr>
<tr>
<td>Mg</td>
<td>0.53</td>
<td>0.21</td>
<td>0.31</td>
</tr>
<tr>
<td>Al</td>
<td>0.23</td>
<td>0.14</td>
<td>0.09</td>
</tr>
<tr>
<td>Si</td>
<td>0.37</td>
<td>0.27</td>
<td>0.19</td>
</tr>
<tr>
<td>P</td>
<td>0.75</td>
<td>1.07</td>
<td>0.21</td>
</tr>
<tr>
<td>Cl</td>
<td>1.00</td>
<td>0.46</td>
<td>0.20</td>
</tr>
<tr>
<td>K</td>
<td>3.58</td>
<td>0.79</td>
<td>0.73</td>
</tr>
</tbody>
</table>

3.6. FT-IR analysis

FT-IR spectroscopy yields “fingerprint” spectra usable as structural evidence. Common “nonpolymer” signals observed by means of FTIR spectroscopy are adsorbed water at about 1630-1640 cm\(^{-1}\) and CO\(_2\) at about 2340-2350 cm\(^{-1}\). The spectra of the agro-wastes before and after NaOH and H\(_2\)O\(_2\) pretreatment were affected by the FTIR spectra. In sugar cane and jatropha NaOH pretreatment (Fig. 3A and B), the most representative band is summarized as follows. The band widening at 1318 cm\(^{-1}\) can be ascribed to CH\(_2\) wagging vibrations in cellulose. A similar observation was reported earlier by Spiridon et al.\([42]\). An absorption band at 897 cm\(^{-1}\) is assigned to C-O-C stretching at the \(\beta\)-(1, 4) -glycosidic linkage in cellulose. A similar observation was reported earlier by Spiridon et al.\([42]\). An absorption band at 897 cm\(^{-1}\) is assigned to C-O-C stretching at the \(\beta\)-(1, 4) -glycosidic linkage in cellulose.

The asymmetric bending of CH\(_3\) was said to be responsible for the band at 1467 cm\(^{-1}\). The guaiacyl ring of lignin, which is present in the spectra of native biomass, has a characteristic band at 1516 cm\(^{-1}\) that was attributed to it and is associated to lignin removal. This band is absent in the pretreated sample, showing that the lignin is eliminated during pretreatment. The depolymerization of lignin during pretreatment may be the cause of the bands at 1516 cm\(^{-1}\) disappearing. The vibrational modes of -CH\(_2\)OH groups and the C-O stretching vibration and C-O bending of the C-OH groups of carbohydrates are caused by the band at 1045 cm\(^{-1}\) in the IR spectrum\([38]\). To evaluate the functional group alterations that happened after pretreatment, various absorption bands between 4000 and 600 cm\(^{-1}\) were seen. A modest absorbance band about 901 cm\(^{-1}\) in pretreated materials may be caused by C-O-C stretching of the \(-{(1,4)}\) -glycosidic linkage connecting the hemicellulose and cellulose\([43]\). The primary absorption band at 1038 cm\(^{-1}\) denotes the xylan region, and it can be attributed to the vibrational modes of the C-OH groups of carbohydrates, particularly
hemicellulose, that are -CH$_2$OH and C-O stretching paired with C-O bending [44]. The distinctive band seen at 1236 corresponds to the respective C = O vibration modes of the guaiacyl unit in lignin. The band at 1629 cm$^{-1}$ represents the bent stretch of the absorbed water molecules [45]. After H$_2$O$_2$ treatments, there was a decrease in band intensity (Fig. 4A and B), which demonstrated effective delignification. The -CH$_2$ vibrational mode is represented by the band at 1325 cm$^{-1}$, and the decrease in peak intensity during hydrolysis indicates that cellulose is effectively converted to monomeric sugars [46]. The conspicuous band at 1420 cm$^{-1}$ was attributed to cellulose's C-6 region's -CH$_2$ bending and scissoring mode [47]. The CH$_2$ stretching of cellulose is responsible for the band at 2886 cm$^{-1}$ in pretreated samples. The -CH stretching, symmetric, and asymmetric -CH$_2$ stretching vibrational modes are represented by a number of minor bands between 2980 and 2835 cm$^{-1}$. Three major biopolymers—cellulose, hemicellulose, and lignin—have C-H methyl and methylene groups, which are represented by the absorption peak at 2913 cm$^{-1}$. The presence of a carbonyl group is indicated by the absorption band at 2913 cm$^{-1}$. H bound OH stretching was connected to the broad absorption band that occurred at 3318 cm$^{-1}$ [48].

3.7. Activity of commercial enzymes

In spite of the activity of enzyme is labeled on the imported container, the assay must be detected firstly to avoid unfavorable can be happened during shaping and secondly to the proper dilution of enzyme from economically point data obtained refer to the possibility to dilute enzyme before application and this can make undifference in the cost of enzyme application reflect on the bioethanol production costs (Table 5).
Table 5
Determination of CMCase and Fpase activities of enzyme before application

<table>
<thead>
<tr>
<th>DIULATION (enzyme ul): dis water (ul)</th>
<th>6 CMC OD</th>
<th>AFTER equation U/ml/min</th>
<th>6 Fpase OD</th>
<th>AFTER equation U/ml/min</th>
<th>7 CMC OD</th>
<th>AFTER equation U/ml/min</th>
<th>7 Fpase OD</th>
<th>AFTER equation U/ml/min</th>
</tr>
</thead>
<tbody>
<tr>
<td>100:900</td>
<td>3.1043</td>
<td>64.28032</td>
<td>3.1370</td>
<td>64.95994</td>
<td>11.73211</td>
<td>0.7393</td>
<td>16.02755</td>
<td></td>
</tr>
<tr>
<td>90:910</td>
<td>3.1016</td>
<td>64.22421</td>
<td>3.0843</td>
<td>63.86466</td>
<td>10.94443</td>
<td>0.5627</td>
<td>12.13902</td>
<td></td>
</tr>
<tr>
<td>80:920</td>
<td>3.0976</td>
<td>64.14108</td>
<td>2.9505</td>
<td>61.08387</td>
<td>8.992886</td>
<td>0.5188</td>
<td>11.17239</td>
<td></td>
</tr>
<tr>
<td>70:930</td>
<td>2.9584</td>
<td>61.24806</td>
<td>2.8976</td>
<td>59.98444</td>
<td>8.358999</td>
<td>0.4415</td>
<td>9.470326</td>
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</tr>
<tr>
<td>60:940</td>
<td>2.9262</td>
<td>60.57884</td>
<td>2.7530</td>
<td>56.97919</td>
<td>7.432068</td>
<td>0.3935</td>
<td>8.413419</td>
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</tr>
<tr>
<td>50:950</td>
<td>2.9222</td>
<td>60.49571</td>
<td>2.6698</td>
<td>55.25003</td>
<td>6.96029</td>
<td>0.3177</td>
<td>6.744387</td>
<td></td>
</tr>
<tr>
<td>40:960</td>
<td>2.2833</td>
<td>47.21733</td>
<td>2.4794</td>
<td>51.29291</td>
<td>5.939835</td>
<td>0.3233</td>
<td>6.867693</td>
<td></td>
</tr>
<tr>
<td>30:970</td>
<td>2.1989</td>
<td>45.46323</td>
<td>1.5224</td>
<td>31.4034</td>
<td>4.805073</td>
<td>0.2210</td>
<td>4.61516</td>
<td></td>
</tr>
<tr>
<td>20:980</td>
<td>1.5587</td>
<td>32.15783</td>
<td>1.1945</td>
<td>24.58859</td>
<td>4.258475</td>
<td>0.1068</td>
<td>2.100602</td>
<td></td>
</tr>
<tr>
<td>10:990</td>
<td>1.0628</td>
<td>21.85144</td>
<td>0.8754</td>
<td>17.95668</td>
<td>3.982059</td>
<td>0.0747</td>
<td>1.393796</td>
<td></td>
</tr>
<tr>
<td>5:995</td>
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<td>14.99091</td>
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<td>8.500324</td>
<td>2.009734</td>
<td>0.0308</td>
<td>0.427167</td>
<td></td>
</tr>
</tbody>
</table>

Scarification of pretreated agrowastes

Degrading on and recycling the abundant cellulosic biomass in nature. From biotechnological standpoint, cellulases have a vital role to play in the generation of potentially sustainable energy sources such as glucose and bioethanol [49]. Figure 6 was shown that data, percent of hydrolyzing the five deferent agrowastes pretreated with H₂O₂ or bleached with sodium hypochlorite comparable to native cellulose using different levels from cellulase. However, pretreatment with H₂O₂ more suitable than that applied with sodium hypochlorite. The hydrolyzed % were differ between wastes and the application of 30 IU/g substrate was the best for all pretreated wastes. The more glucose was obtained from green leaves and deoiled seed cake followed by tops sugar cane and the lowest was from jatropha shell. pretreatment to remove lignins from lignocellulose and enhance the penetration of hydrolysis agents is a vital step in the process of converting biomass by enzymes to bioethanol [2]. Pretreatment of lignocellulosic biomass are applied to lignocellulose prior to hydrolysis and fermentation in order to increase the amorphous regions. There are three categories in the cellulase family: exo-1,4-glucanase (celllobiohydrolase or avicelase (EC 3.2.1.91), endo-1,4-glucanase (EC 3.2.1.4), and glucosidase (EC 3.2.1.21). The cellulose chain is broken down into individual sugars by their combined action [50]. Enzymatic hydrolysis combined with microbial fermentation is a more preferred technique with significantly improved performance [51]. Lignocellulose is saccharified by hydrolyzing the polysaccharide-rich material into single sugars (hexoses and pentoses) using enzymes after it has undergone
pretreatment. The commercially available cellulase, which is used to break down cellulose and hemicellulose, is actually a blend of many enzymes that were taken from microorganisms and given the generic name cellulase. These enzymes break down the glycosidic bonds found in carbohydrates, usually via inverting or retaining processes, the latter of which involves a two-step process that includes the production of a glycosyl-enzyme intermediate [52]. Microorganism development during fermentation is facilitated by enzymatic hydrolysis.

3.8. Effect of substrate concentration on the substrate hydrolysis by cellulose

Data presented in Fig. 7 was shown the effect of substrate percent in the reaction mixture on the yield of glucose released by the action of cellulase on substrate. The configuration of the substrate molecules affect the viscosity resulted in the reaction mixture [53], consequently affect movement of the enzyme to reach its active site. However, the data obtained reported that the yield of glucose can achieved at 3% (w/v) for all tested substrates. Santos et al. [54] used 1.5% of sugar cane bagasse for enzymatic saccharication to obtain reducing sugars for bioethanol production.

Data presented in (Table 6) and illustrated in Fig. (9) clear the bioethanol yield from the obtained glucose from enzymatic saccharification of different pretreated lignocellulosic wastes fermented Saccharomyces cerevisiae F-307. The more bioethanol was obtained from green leaves and deoiled seed cake followed by tops sugar cane and the lowest was from jatropha shell. In spite that the jatropha deoiled seed cake had the highest glucose content (85%) compare to the green leaves (40%), it superior the jatropha deoiled seed cake in bioethanol yield. This means that in case of JDSC* most glucose was ex pense in the Saccharomyces cerevisiae growth, while in case of green leaves could use the D-xylose or fructose (Table 2). In general as it was seen in (Table 5), (Table 6), there is a noticeable difference of monosaccharides and metals between each of the different pretreated lignocellulosic wastes. This led to variation in the growth of the yeast and also in enzymatic production behavior; accordingly, the bioethanol production showed a clear variation depending on the type of pretreated lignocellulosic waste. For instance, high concentrations of Cl had an adverse impact on the growth and bioactivity of microorganisms, especially in the presence of Na, and could affect the production of some important enzymes [55]. The lowest Cl content, which was noticed in green leaves treated with H2O2 (0.05%) could interpret the high bioethanol production. It is a favorable choice of yeast to ferment sugar solution to bioethanol (Table 3). Also, EDXA table was shown that the oxygen reduction played a role in bioethanol yield, it was obvious that the GL*, JDSC* had low oxygen in compare to DL*, TSC* and jatropha shell. Also, as it was seen in (Table 6), the high content of silicon in green leaves might play a vital role in triggering important enzymes such as invertase, which had a positive effect on ethanol fermentation [56]. Saccharomyces cerevisiae is well known as both the most common and a classic yeast in the manufacture of bioethanol due to its tolerance to high ethanol concentrations and material’s inhibitors. its resistance to high ethanol concentrations and the inhibitors of the substance [57]. An extra nutrient needs to be supplied in order to offer an organic nitrogen source for the development of microorganisms throughout the fermentation process. Peptone, corn steep liquor (CSL), urea, and even the distillation waste from the bioethanol manufacturing process are among the materials that are used and studied [58]. The cost-effectiveness of lignocellulosic ethanol production has not changed. in an effort to
increase the yield of bioethanol fermentation. Different lignocellulosic wastes underwent varied physical pretreatments. Chemicals and biological processes were followed by enzymatic hydrolysis to produce glucose, after which Saccharomyces cerevisiae fermented the sugar syrup to produce bioethanol [58].

<table>
<thead>
<tr>
<th>Sample</th>
<th>Treatment</th>
<th>Ethanol mg/g substrate</th>
<th>Biomass mg/g substrate</th>
<th>Fermentation efficiency %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Green leaves</td>
<td>H₂O₂</td>
<td>325.4</td>
<td>58</td>
<td>86</td>
</tr>
<tr>
<td>Jatopha shell</td>
<td>H₂O₂</td>
<td>264.0</td>
<td>32</td>
<td>80</td>
</tr>
<tr>
<td>Tops sugar cane</td>
<td>H₂O₂</td>
<td>282.9</td>
<td>37</td>
<td>82</td>
</tr>
<tr>
<td>Dry leaves</td>
<td>H₂O₂</td>
<td>302.4</td>
<td>46</td>
<td>84</td>
</tr>
<tr>
<td>Jatropha deoiled seed cake</td>
<td>H₂O₂</td>
<td>310.8</td>
<td>48</td>
<td>84</td>
</tr>
<tr>
<td>Tops bleached</td>
<td>bleached</td>
<td>290.5</td>
<td>43</td>
<td>83</td>
</tr>
<tr>
<td>Dry leaves bleached</td>
<td>bleached</td>
<td>240.0</td>
<td>35</td>
<td>80</td>
</tr>
<tr>
<td>Green leaves bleached</td>
<td>Bleached</td>
<td>262.0</td>
<td>38</td>
<td>80</td>
</tr>
<tr>
<td>Jatropha shell bleached</td>
<td>bleached</td>
<td>310.8</td>
<td>51</td>
<td>81</td>
</tr>
<tr>
<td>Jatropha deoiled seed cake bleached</td>
<td>bleached</td>
<td>272.0</td>
<td>37</td>
<td>82</td>
</tr>
<tr>
<td>Cellulose</td>
<td>————</td>
<td>376.0</td>
<td>64</td>
<td>88</td>
</tr>
</tbody>
</table>

N.B. Green leaves (GL*)- Jatropha shell (JS*)- Tops sugar cane (TSC*)- Dry leaves (DL*)- Jatropha deoiled seed cake (JDSC*)- Tops bleached (TB)- Dry leaves bleached (DLB)- Green leaves bleached (GLB)- Jatropha Shell bleached (JSB)- Jatropha deoiled seed cake bleached (JDSCB)- Cellulose (CEL).

* means that pretreatment by H₂O₂-Without* means that bleached by sodium hypochlorite.

**Conclusion**

This work tried to discriminate the difference between the extraction of biopolymer of sugar cane trash and jatropha by the using each of sodium hydroxide and H₂O₂. This idea was implanted through comprehensive comparative studies to characterize the difference between the two methods. The results pointed to the priority of H₂O₂ in cellulose yield. In addition, the results showed that the bioethanol yield was much better in samples treated with H₂O₂.

**Declarations**

**Authors’ contributions**
Naglaa A. Elnagdy carried out the experiments; Tamer I.M. Ragab Conceptualization, Methodology, Validation, Investigation, Formal analysis, Writing original draft, Writing, Mohamed A. Fadel conceived and planned the experiments; Mohamed A. Abou-Zeid analysed the data; Mona A. Esawy Investigation, Writing—review & editing. All authors read and approved final version of manuscript.

Data Availability

The data used to support the study can be made available upon request at the corresponding author.

Ethics approval and consent to participate

The manuscript does not contain data collected from humans or animals.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Acknowledgments

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References


Figures

Figure 1

SEM analysis (Sugar cane – (A) raw materials of dry leaves, (B) dry leaves treated by sodium hydroxide, (C) dry leaves by $\text{H}_2\text{O}_2$, (D) raw materials of green leaves, (E) green leaves by sodium hydroxide, (F) green leaves by $\text{H}_2\text{O}_2$, (G) tops of raw materials, (H) tops treated by sodium hydroxide and (I) tops by $\text{H}_2\text{O}_2$)
Figure 2

jatropha shell- (A) raw materials, (B) treated by sodium hydroxide, (C) treated by \( \text{H}_2\text{O}_2 \) and jatropha deoiled seed cake- (D) raw materials, (E) treated by sodium hydroxide, (F) treated by by \( \text{H}_2\text{O}_2 \)
Figure 3

Sodium hydroxide pretreatment cellulose (A) sugar cane trash dry leaves, green leaves and tops, (B) Jatropha (shell and deoiled seed cake)
Figure 4

Hydrogen peroxide pretreatment cellulose (A) Dry leaves, Green leaves, Tops of sugar cane, (B) Jatropha shell and Deoiled seed cake
Figure 5

Bleached cellulose by sodium hypochlorite (A) Dry leaves, Green leaves, Tops of sugar cane, (B) Jatropha shell and Deoiled seed cake
Figure 6

Scarification of pretreated substrates using different levels of cellulase at 50°C for 24 hrs on a rotary shaker 100rpm. 1% substrate in citrate buffer 0.05M pH 4

N.B. Green leaves (GL*)- Jatropha shell (JS*)- Tops sugar cane (TSC*)- Dry leaves (DL*)- Jatropha deoiled seed cake (JDSC*)- Tops bleached (TB)- Dry leaves bleached (DLB)- Green leaves bleached (GLB)- Jatropha shell bleached (JSB)- Jatropha deoiled seed cake bleached (JDSCB)- Cellulose (CEL)

* means that pretreatment by H₂O₂- Without* means that bleached by sodium hypochlorite
Figure 7

Effect of substrate concentration on the substrate hydrolysis by cellulase (30 IU/g Substrate) at 50°C for 24 hr on a rotary shaker 100 rpm

*30 IU/g substrate in citrate buffer 0.05M pH 4.8

Figure 8

Effect of incubation time on the substrate hydrolysis by cellulase (30 IU/g Substrate) at 50°C for 24 hrs on a rotary shaker 100 rpm.
Supplementary Files

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