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Research article

A comparative evaluation of fermentable sugars production from oxidative, alkaline, alkaline peroxide oxidation, dilute acid, and molten hydrate salt pretreatments of corn cob biomass

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Abstract: Production of high value-added products from lignocelluloses is an economically sustainable alternative to decreasing dependence on fossil fuels and making the chemical processes environmentally friendly. In this study, different methodologies of alkaline ($Ca(OH)_2$ and NaOH), dilute acid (10%w/w H_2SO_4), hydrogen peroxide (H_2O_2), alkaline peroxide oxidation (H_2O_2/$Ca(OH)_2$ and H_2O_2/NaOH), and molten hydrated salt (MHS) mediated ($ZnCl_2.4H_2O$) pretreatments were employed in the hydrolysis of corncob amenable to enzymatic hydrolysis. Optimal enzyme hydrolysis temperature (considering 45 and 50 ℃) and time (2, 24, 72, and 96 h) were investigated for each pretreatment procedure to ascertain the concentrations of glucose, xylose, and total sugar present in the corncob. At 45 ℃ and 96 h, NaOH alkaline pretreatment achieved the best optimum total sugar production of 75.54 mg/mL (about 54% and 88% increments compared to dilute acid pretreatment (35.06 mg/mL total sugars) and MHS (9.32 mg/mL total sugar) pretreatment respectively). In this study, total sugars production increased appreciably at 45 ℃ and longer hydrolysis period (96 h) compared to hydrolysis at 50 ℃ (with maximum total sugars production

of 18.00 mg/mL at 96 h). Scanning electron microscopic imaging of the untreated and treated samples displayed cell wall distortion and surface disruptions.

Keywords: pretreatment; lignocellulose; polysaccharide; enzymatic hydrolysis; reducing sugars; scanning electron microscope

1. Introduction

With the worldwide increasing energy demand worldwide and the accompanying environmental degradation brought about by conventional energy sources, it is essential that we look inwards for alternative sources of energy and chemicals [1]. Therefore, the conversion of plant biomass into useful products such as biofuels and biochemicals has been increasing due to improved economic feasibility of conversion processes [2]. As an important resource, lignocellulose biomass provides sustainability, and its economically friendly [3]. Currently, we can categorize biofuels into first and second generations. The first generation biofuels are obtained from sugars, starch, and plant oils. These are derived from feedstocks such as cereals, sugarcane, sugar beet, grapes, soya, palm, and sunflower [4]. The second generation biofuels are from lignocelluloses such as softwoods, hardwoods, municipal wastes, and agricultural wastes [5]. The major components in lignocellulosic biomass are hemicellulose, cellulose, and lignin while ash and the extractives are minor constituents. Cellulose and hemicelluloses are polymeric. Therefore, accessing useable products, fuels and chemicals, from them needs pretreatments. The polymeric (cellulose and hemicelluloses) parts of lignocelluloses can be hydrolyzed to form fermentable sugars. Lignin is a binding sheath in the lignocellulosic complex. It hinders hydrolysis, preventing the release of the polymers for decomposition or degradation. As a result, the pretreatment step is required for the rupturing of the lignocellulsic complex, thereby releasing the polymers and lignin before conversion of cellulose and hemicellulose into usable products [2,6]. Biomass pretreatment technologies can be divided into different categories such as mechanical, chemical, physicochemical and biological methods or various combinations of these [7]. These pretreatments processes have been developed in overcoming the recalcitrance nature of biomasses. Examples of the biomass pretreatment processes include steam explosion, hot water, alkaline (e.g., NaOH, $Ca(OH)_2$, Na_2CO_3), dilute or strong acid (e.g., H_2SO_4, HNO_3, HCl), ammonia (e.g., ammonia fiber explosion, soaking in aqueous ammonia, ammonia recycled percolation), ionic liquids, oxidative delignification (with addition of oxidizing agents like ozone, oxygen or air, hydrogen peroxide), organosolv, and molten hydrate salts [8–11]. Lignin in biomass complex hinders enzymatic hydrolysis of the polysaccharides. Therefore, raw biomass must be pretreated in order to rupture the lignocellulosic complex held together by lignin. The pretreatment methods alter or damage the lignocellulose biomass complex, removing or reducing lignin contents, and expose the polysaccharides to enzymatic attack. A reliable pretreatment process must be able to depolymerize hemicelluloses, decrystallize and preserves the celluloses, remove lignin, have low energy input, reduce the formation of inhibitors, and must be cost effective [12]. However, there are bottlenecks (such as the use of expensive pretreatment agents, handling of raw materials, energy utilization) hindering the efficient use of these pretreatment methodologies. Corn cobs lignocellulose has high caloric value and low nitrogen and sulphur contents. Corn cob is a by-product of corn grain production. This means no additional input is

needed except for its collection and transportation. Corn cob represents about 16% of the total stover biomass, and has about 14% grain yield. In this study, different pretreatment techniques namely; alkaline, dilute acid, hydrogen peroxide, alkaline peroxide oxidation (APO), and inorganic salt pretreatments were employed in the hydrolysis of corncob amenable to enzymatic hydrolysis. The pretreatments using acids, alkaline mixtures, and oxidative delignification processes are widely exploited for lignocelluloses hydrolysis to produce value-added commodities like sugars. Optimal enzyme hydrolysis temperature and time was investigated for each pretreatment procedure to ascertain the concentrations of glucose, xylose, and total sugar present in the corncob. Scanning electron microscopic imaging was used to monitor the morphological changes of raw and treated biomass.

2. Materials and methods

2.1. Materials

Dry Corncob was sourced from a local farm in Keffi, Abuja, Nigeria. The harvested material was stored at room temperature (varying between 23 ℃ and 26 ℃) until further processing. Screened sample fraction size of 1.18 mm was used for this study. Cellulase enzyme (*Trichoderma reesei*, with the specific enzyme activity of 700 units/g was purchased from Sigma-Aldrich (Chemie GmbH), while all other standard chemicals including dextrose (D-glucose), sulphuric acid, sodium hydroxide, calcium hydroxide, and hydrogen peroxide were analytical grades· The compositional analysis of the raw material was determined as reported earlier [13–15]. In summary, extractives were estimated by extracting 2.5 g of dried biomass with 150 mL acetone in a Soxhlet extractor. The operating temperature was 70 ℃ for a run period of 4 h. The %(w/w) of extractives was calculated based on the loss of weight (at 105 ℃) between the dried raw biomass and extracted sample. The hemicellulose content was determined by boiling for 3.5 h about 1 g of the extracted biomass sample in 150 mL of 0.5 mol/L NaOH solution. The %(w/w) of hemicellulose was the difference between the sample weight before and after the biomass treatment. Lignin content was estimated as reported by Sluiter et al. [16]. Mineral components were determined by ashing at 575 ℃ in a muffle furnace. The cellulose content was calculated by difference, assuming that extractives, hemicellulose, lignin, ash, and cellulose are the only components of the entire biomass. The corn cob lignocellulose used in this study contained 35.45%w/w cellulose, 37.42%w/w hemicellulose, 22.41%w/w lignin, 2.50%w/w ash, and 2.16%w/w extractives. All Chemicals used in this study were purchased from Sigma-Aldrich (Chemie GmbH).

2.2. Biomass quality parameters determination

The thermal behavior of lignocelluloses is crucial during the utilization of lignocelluloses as fuels, chemicals, and as energy resources. The higher heating value (HHV) was measured with an oxygen bomb calorimeter (equipped with DryCal 2010 software). The ASTM D2015 procedure was used for the measurement [17]. The proximate analysis, which indicates the quality of lignocelluloses as energy resource was estimated with the determination of the moisture content, volatile matter (VM), fixed carbon (FC), and ash contents. Using a Perkin-Elmer STA 6000 Simultaneous Thermal Analyzer equipped with Pyris series-STA 6000 instrument viewer software, the weight fraction variation with temperature was carried out through three sequential steps of drying,

devolatilization in nitrogen, and oxidation in oxygen using the ASTM D-5142-02a procedure [18]. Both nitrogen purge gas and oxygen for oxidation were operated at 400 kPascal. The elemental compositions of carbon and sulphur were determined using a LECO SC632 (equipped with LECO SC632 sulphur/carbon determinator software), operated at the furnace temperature of 1350 ℃. Nitrogen and hydrogen were estimated with Flash 2000 CHNS/O analyser (Thermo Scientific, USA). The weight percent of oxygen was obtained by the difference of C, H, N, S, from 100% [19].

2.3. Corn cob biomass pretreatments

The pretreatment methods were labelled as (i) hydrogen peroxide oxidation (**A):** 1%v/v H_2O_2, (ii) sodium hydroxide treatment (**B):** 0.1 g NaOH/g dry biomass, (iii) calcium hydroxide treatment (**C):** 0.1 g $Ca(OH)_2$/g dry biomass, (iv) sodium hydroxide- hydrogen peroxide (alkaline hydrogen peroxide (APO)) (**D):** 1%v/v H_2O_2 and 0.2 g NaOH/g dry biomass addition, (v) calcium hydroxide-hydrogen peroxide (alkaline hydrogen peroxide) (**E):** 1%v/v H_2O_2 and 0.6 g $Ca(OH)_2$/g dry biomass addition, (vi) dilute acid (**F):** 0.1 g (10%w/w) H_2SO_4/g dry biomass, (vii) inorganic salt (molten hydrate salt) (**G):** 0.1 g $ZnCl_2.4H_2O$/g dry biomass.

Dilute acid pretreatment involved making a slurry of 12.5 g dry corn cob biomass and 200 mL solution of 10%w/w H_2SO_4 in a 250 mL Erlenmeyer flasks. Thermal air bath in a convection oven provided the heating. The reaction temperature was kept at 120 ℃ for 60 min reaction time for all the pretreatments. The reaction was stopped at the end of pretreatment and slurry was separated into liquid and solid fractions through vacuum filtration. The solid fraction was washed with distilled water until the pH of the washed liquid approached 7. Treated wet corn cob biomass was stored adequately for the enzymatic hydrolysis evaluation. During the alkaline pretreatments, sodium hydroxide (0.1 g NaOH/g dry biomass) and calcium hydroxide (0.1 g $Ca(OH)_2$/g dry biomass) were used as the pretreatment agents. For the hydrogen peroxide pretreatment, 1%v/v H_2O_2 was added to make up the 200 mL reaction mixture without addition of any other pretreatment agent. The alkaline peroxide oxidation (APO) involved the combination of the alkaline solutions (0.2 g NaOH/g dry substrate and 0.6 g of $Ca(OH)_2$/g dry substrate) and 1%v/v hydrogen peroxide solution in the reaction mixture. The addition of the alkalis was to maintain the pH of the reaction mixture at 11.5 [14,20]. The inorganic salt mediated pretreatment involved the use of 0.1 g zinc chloride ($ZnCl_2$)/g dry substrate in the reaction mixture on the milled corn cob [11,21]. Pretreatment parameters and conditions remain the same for all the other pretreatment techniques as described for the dilute acid pretreatment.

2.4. Enzymatic hydrolysis

The efficiency of substrate conversion was determined through enzymatic hydrolysis of the pretreated solid fractions, except the dilute acid treated biomass. The hydrolysis slurry contained wet pretreated 2% biomass loading (20 g/L dry biomass content), 5 mL of 0.1 M sodium citrate buffer (pH 4.8) in 20 mL culture tubes. The *Trichoderma reesei* cellulase enzyme system was added at a loading of 25 FPU/g dry biomass. The total volume of the mixture was kept at 10 mL for all the experimental runs. Samples were drawn periodically at 2, 24, 72, and 96 h for quantitative determination of glucose and xylose. Hydrolysis was made to occur at 45 ℃ and 50 ℃ temperatures in a shaking incubator operated at 130 revolutions per minute. Comparing enzymatic hydrolysis at

two temperatures (45 ℃ and 50 ℃) was to understand the effect of varying temperature on sugar yields. This is because cellulase enzymes activity is said to be optimum at 50 ± 5 ℃ [22]. At reduced hydrolysis temperatures, hydrolysis rate may be slower, as against denaturation of enzymes at increased temperature [23].

2.5. Fermentable sugars in hydrolysate

The concentrations of the glucose, xylose, and total sugars from the hydrolysates of the cellulase hydrolyzed treated corncob were determined. For each hydrolysis period, 0.5 mL aliquot was sampled and analysed for reducing sugars. The 3,5, dinitrosalicylic acid (DNS assay) was used to quantify the glucose content using glucose as standard [24]. For each aliquot, 1.5 mL of DNS reagent was added into the test tubes. The contents of the tubes were heated in a boiling bath for 5 min. When the contents of the tubes were still warm, 1 mL of 40% Rochelle salt solution was added, cooled, and the intensity of the colour was measured with a UV-Vis spectrophotometer at a wavelength of 540 nm. When considering xylose concentration in mixture, the phloroglucinol assay method was used. 5 mL of phloroglucinol solution was added to 0.2 mL of aliquot in a test tube. Heating was made to occur at 100 ℃ for 4 min. The change in colour intensity of mixture was measured at 554 nm in a UV-Vis spectrophotometer. Considering dilute acid pretreated sample, the fermentable sugars estimation was carried out on the filtrate from the pretreatment process, there was no enzymatic hydrolysis on acid treated biomass. Acid pretreatments directly rupture the lignocellulosic complex, and liberate sugars in the process.

2.6. Scanning electron microscope imaging

Microscopic imaging is an efficient method to understand the quality of exposing raw lignocelluloses to pretreatment and hydrolysis. In order to understand cellulose accessibility by enzymes, scanning electron microscopic (SEM) imaging was used to evaluate the transformation brought to the surface and wall structures by pretreatments. The raw and pretreated corn cob materials were washed with distilled water and air-dried for 96 h and subsequently stored in plastics for SEM analysis. The morphological changes were monitored on a FEI Quanta 200 scanning electron microscope. Air-dried samples were placed on aluminium stubs using conductive carbon tape and latter sputter coated with carbon and gold-palladium at 5 nm scale. The SEM was operated under vaccum between 3.9×10^{-4} to 2.2×10^{-3} Pascals with a voltage of 30 kV.

3. Results and discussion

3.1. Characterization of the raw corn cob

The compositional analysis of the raw biomass is as provided in Table 1. The 72%(w/w) polysaccharide (cellulose and hemicellulose) content makes corn cob biomass a viable precursor for fuel and chemicals production. Total lignin content was 22.41%(w/w). This high amount of lignin in the biomass provides a strong point for developing an efficient pretreatment methodology that will be able to rupture the lignocellulosic complex thereby exposing the polysaccharides for enzymatic attack in order for the useful compounds to be released from the complex [14].

Table 1. Compositional analysis of raw biomass (%w/w).

Components		
Extractives		2.161
Cellulose		35.45
Hemicellulose		37.42
Lignin	AIL[a]	20.35
	ASL[b]	2.06
Ash content		2.56

Note: [a]Acid insoluble lignin. [b]Acid soluble lignin.

Lignin functions as the cellular glue, which provides compressive strength to the plant tissue and the individual fibres. The strong bond between hemicellulose and lignin generally prevents easy accessibility of the cellulose fraction during enzymatic hydrolysis [25,26].

3.2. Quality parameters values for raw biomass

Biomass quality parameters are essential standard measures for biomass quality for processing. The parameters assess the fuel characteristics and biocommodity potentials. High values of fixed carbon and volatile matter indicate that biomass will require long combustion thereby increasing high caloric value of biomasses. The overall energy balance and the economy of the conversion process are negatively affected by high moisture content [27]. High moisture values reduces corresponding higher heating values. In Table 2, the proximate values indicates that the corn cob biomass is a potential fuel and energy resource because of the high values of volatile matter (78.77%w/w), fixed carbon (16.81%w/w), and higher heating value (17.29 MJ/kg). The low content of ash in the biomass contributed to the high values of volatile matter and fixed carbon. The volatile matter and fixed carbon values are affected by the presence of ash in lignocelluloses, which reduces the final biomass caloric value.

Table 2. Biomass quality parameters values.

Proximate analysis	Moisture content	Volatile matter	Fixed carbon	Ash	
	3.802	78.77	16.81	0.62	
Ultimate analysis	Sulphur	carbon	Hydrogen	Nitrogen	Oxygen
	0.22	42.06	5.83	0.17	51.73
Higher heating value	17.29 MJ/kg				

The carbon, hydrogen, and oxygen contents are the most important components of lignocelluloses [28]. Since carbon and hydrogen are oxidized to carbon dioxide and water, they increase the higher heating values while at the same time oxygen is reduced which negatively affects HHV [28]. The low levels of sulphur and nitrogen (Table 2) indicate that their oxidation to SO_2 and NOx emissions are going to be negligible. This makes the corn cob biomass suitable for combustion and biocommodity processing.

3.3. Enzymatic hydrolysis at 45 ℃

During enzymatic hydrolysis, polymeric fragments undergo conversion to fermentable sugars for enzymatic and microbial consumption. Features such as ambient reaction conditions, lack of inhibitory product formation and higher sugar yield has made enzymatic hydrolysis the preferred hydrolysis pretreatment over chemical hydrolysis [29]. At temperature of 45 ℃, the optimal glucose recovery (68.23 mg/mL) (Figure 1) was achieved in sodium hydroxide pretreated sample, **(B)**, after 96 h followed by the H_2O_2/$Ca(OH)_2$ pretreated sample, **E**, (45.54 mg/mL) after 24 h. Pretreatment D produced the highest xylose concentration (5.61 mg/mL) at 24 h hydrolysis time. The trend follows that more of the sugars were produced at low temperature and prolong hydrolysis period. Generally, different factors may affect the production of sugars during the enzymatic hydrolysis process. The type of pretreatment method and structural changes of lignocelluloses have great influence on how fermentable sugars can be liberated from the complex. During the enzymatic hydrolysis process of lignocelluloses, predicting a single optimum for a particular pretreatment methodology is impossible [30]. This is because some other factors hinder the efficiency of the cellulase complex in the reaction medium. End products of the hydrolysis like glucose, cellobiose, xylose, and furan compound may inhibit the enzyme activity, and negatively affects the hydrolysis process [13,30]. Xylose concentration reduced to a minimum value of 0.13 mg/mL for pretreatment F for dilute sulphuric acid pretreatment (data not shown). At 45 °C, cellulose conversion is most achieved in sodium hydroxide pretreated samples followed by calcium hydroxide pretreated samples. The concentration values of each sample are summarized in Figures 1 and 2.

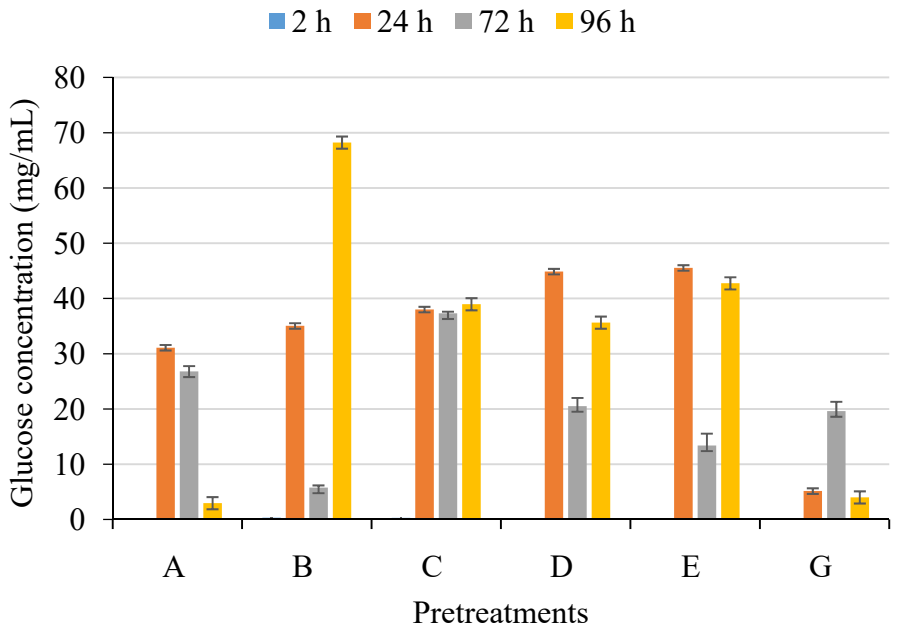


Figure 1. Glucose production at 45 °C hydrolysis period.

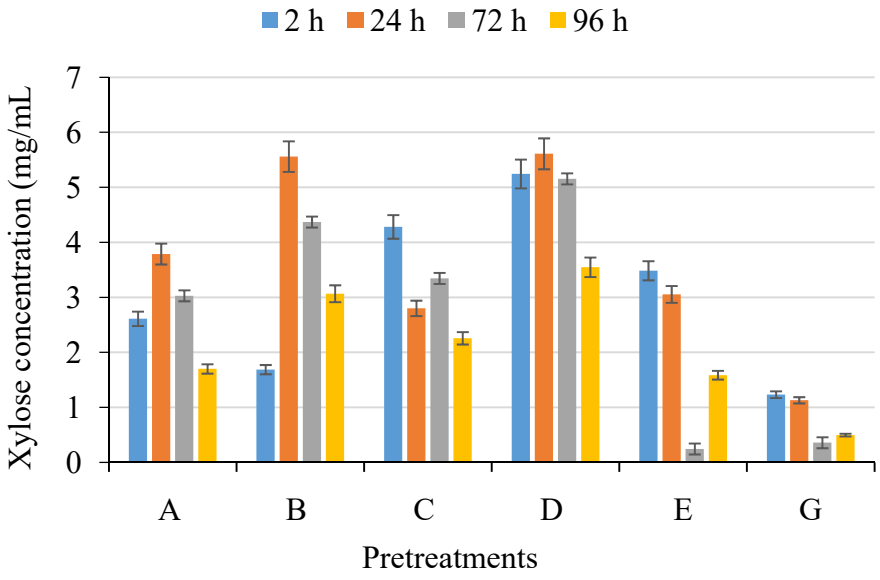


Figure 2. Xylose production at 45 °C hydrolysis period.

3.4. Enzymatic hydrolysis at 50 ℃

The optimum production of glucose (9.73 mg/mL) at 50 ℃ was obtained for the calcium hydroxide pretreatment sample **C** at 96 h followed by the H_2O_2/NaOH pretreatment biomass **D** (8.43 mg/mL) after 96 h. The minimum concentration of glucose was obtained during the H_2O_2 pretreatment sample **A** at 24 h (4.32 mg/mL). Looking closely, the release of xylose was more during the 50 ℃ hydrolysis temperature compare to glucose production. Pretreatment E (1%v/v) H_2O_2 and 0.6 g $Ca(OH)_2$/g dry biomass addition, the APO process), produced 11.63 mg/mL xylose concentration during the hydrolysis period of 24 h. This indicates that under the prevailing experimental conditions, more sugars were produced at shorter hydrolysis time. In addition, the molten hydrate salt pretreated sample, **G** (0.1 g $ZnCl_2.4H_2O$/g dry biomass), produced 10.56 mg/mL xylose after hydrolysis. The least concentration of xylose was recorded in the H_2O_2/$Ca(OH)_2$ pretreatment sample **D** at 2 h (3.94 mg/mL). The concentration values of each sample are summarized in Figures 3 and 4.

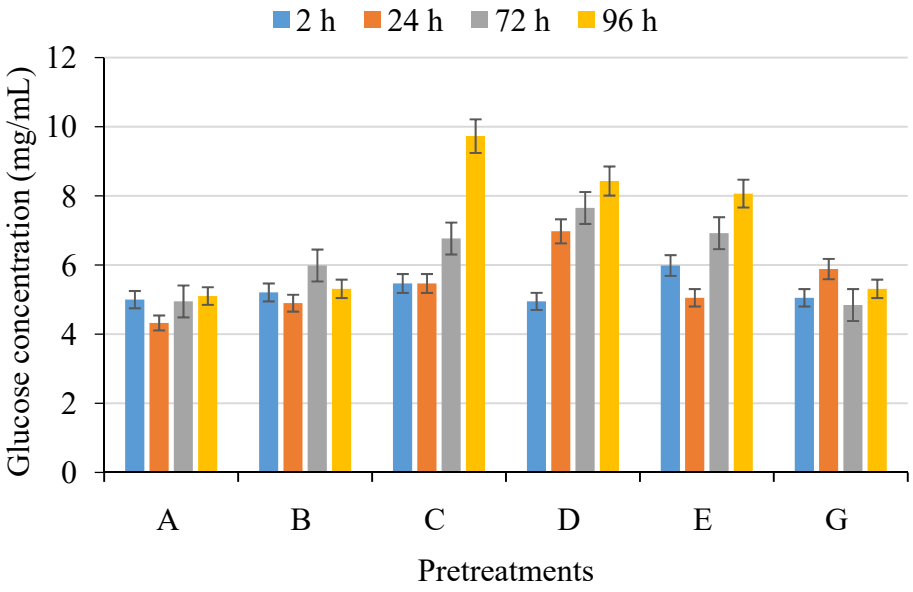


Figure 3. Glucose production at 50 °C hydrolysis period.

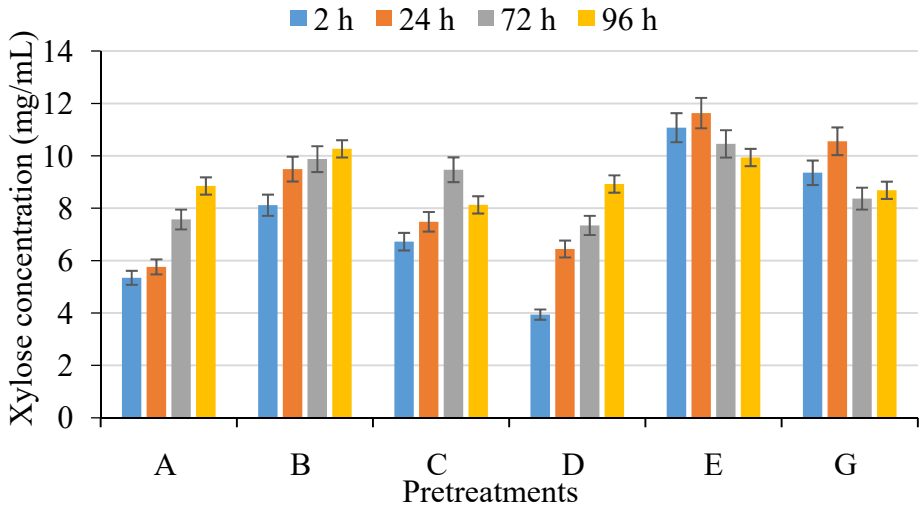
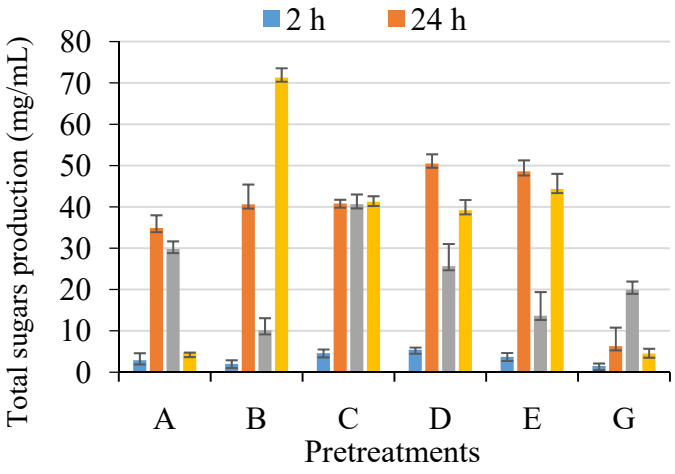


Figure 4. Xylose production at 50 °C hydrolysis period.

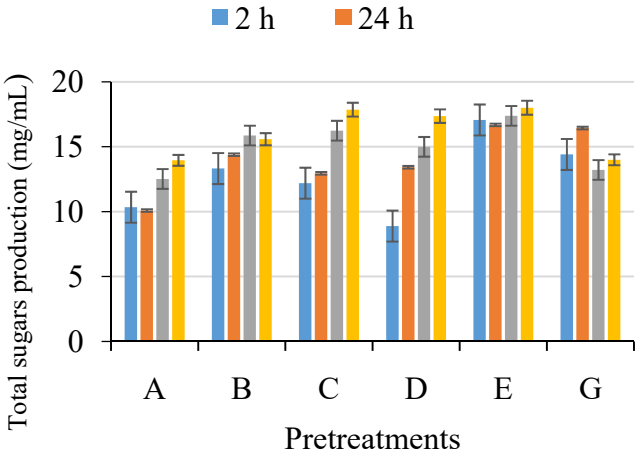
It should be noted that the production of reducing sugars did not follow a particular trend because the type of pretreatment process may greatly affect increasing or decreasing production of sugars. For example, pretreatment G (0.1 g $ZnCl_2.4H_2O$/g dry biomass), produced 4.01 mg/mL at 45 ℃ and 96 h (Figure 1) but 5.31 mg/mL at 50 ℃ and 96 h (Figure 3). Pretreatment B (0.1 g NaOH/g dry biomass) produced 68.23 mg/mL of sugar after 96 h and 45 ℃ (Figure 1). At 50 ℃, B produced 5.31 mg/mL after 96 h (Figure 3). This is around 94% efficiency of pretreatment B over molten hydrate salt, pretreatment G (considering 45 ℃ hydrolysis temperature). This information indicates that production of sugar is highly specific to the type of pretreatment method [13].

3.5. Total sugars production

In comparison, glucose concentration released after hydrolysis increased greatly at 45 ℃ compared to hydrolysis temperature of 50 ℃. For example, at 45 ℃ and 96 h hydrolysis time, glucose production was 68.23 mg/mL (pretreatment B) compared to highest glucose production (9.73 mg/mL) at 50 ℃ and 96 h hydrolysis time (pretreatment C). This is about 86% increment in hydrolysis at 45 ℃ compared to hydrolysis at 50 ℃. A better understanding of the hydrolysis process was evaluated by considering the total reducing sugar (TS) production. TS was estimated as the summation of glucose and xylose concentrations at the different enzymatic hydrolysis temperatures (45 and 50 ℃) and varying periods (2, 24, 72, and 96 h) (Figure 5).



(a)



(b)

Figure 5. Total sugar production (a) 45 ℃ (a) and (b) 50 ℃ enzymatic hydrolysis periods.

At 45 ℃, more total sugars were produced (73.54 mg/mL) at longer hydrolysis period (96 h) by pretreatment B and lowest for pretreatment D (5.16 mg/mL) for 2 h. Maximum total sugars production at hydrolysis temperature of 50 ℃ was 18.00 mg/mL (pretreatment E). The release of sugars at higher temperature and longer hydrolysis period may be hindered due to enzyme

denaturation at increasing hydrolysis temperature [13,31]. Dilute acid pretreated samples produced optimum total sugars (35.06 mg/mL)(data not shown), at 45 ℃ and 96 h. At 50 ℃ hydrolysis temperature, dilute acid pretreated biomass total sugar production was 14.27 mg/mL at 72 h. Li et al. [32], reported maximum sugar production of 53.7 g/L after 72 h (at 50 ℃ hydrolysis temperature) using a two-stage process (acid pretreatment and enzymatic hydrolysis on corn cob biomass). Compared to our study, the higher sugar production in their work may have been due to the two-stage process operation, and other variations of the conditions of the sugar production process. The one-stage acid pretreatment of lignocelluloses may reduce costs of the entire biofuel production process

3.6. Morphological changes of treated samples

Scanning electron microscopic images of the untreated and recovered treated solids are provided in Figure 6(a–d). Figure 6a, is the scanning electron microscopic (SEM) image of the untreated biomass, shows ordered structures. After pretreatments, Figure 6(b,c), the ordered structures have been extremely made irregular by pretreatments. Pores were open because of pretreatments giving a path for enzymatic attack. The SEM images display cell wall distortion with the alteration of micro-fibres. The images further reveal significant surface disruptions in the treated samples.

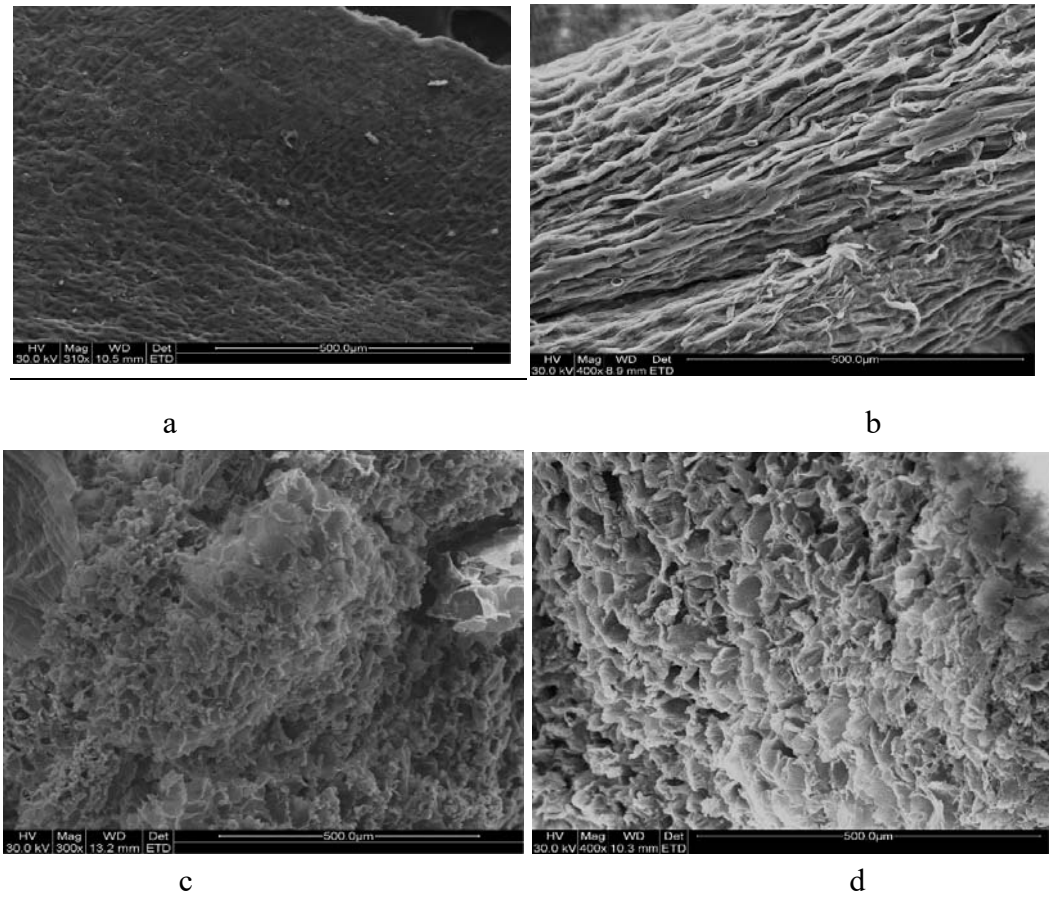


Figure 6. Scanning electron microscope images for the (a) untreated corn cob biomass, (b) 0.1 g $Ca(OH)_2$/g dry substrate, (c) 1%(v/v) H_2O_2 and 0. 2 g NaOH/g dry substrate, (d) 0.1 g H_2SO_4/g dry substrate.

4. Conclusions

Pretreatment of lignocellulosic biomass is a critical step in the production of biofuels and other chemicals from them. Basic needs to comprehend the essentials of different procedures are necessary because they help in settling reasonable decisions depending on the structure of the biomass substrate and the hydrolysis agent. Our findings in this study showed that more sugars were produced during the alkaline and oxidative aided alkaline solutions compared to acid and molten hydrate salt pretreatment conditions. At 45 ℃ enzymatic hydrolysis conditions, (96 h), highest total sugars produced was 71.30 mg/mL (pretreatment B) compared to dilute acid hydrolysis conditions (pretreatment F) that produced 35.06 mg/mL (at 96 h hydrolysis time) and molten hydrate salt (pretreatment G) that produced 19.97 mg/mL total reducing sugars at 72 h hydrolysis time. A higher enzyme hydrolysis temperature of 50 ℃ reduced total reducing sugars production (where maximum total sugars was 18.00 mg/mL achieved by pretreatment E) drastically compared to hydrolysis at 45 ℃. The least total sugars production (8.88 mg/mL) at 50 ℃ was noticed after pretreatment D and hydrolysis period of 2 h.

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Conflicts of interest

The authors declare no conflicts.

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