Quality Assessment of Corn Cob Monomeric Sugars for Biofuel Production

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Abstract: **- The optimal condition for quantitative release of monomeric sugars such as xylose, glucose and arabinose from corn cob biomass were investigated. In this study, acid hydrolysis using dilute sulfuric acid was adopted for liquefaction of the biomass under different conditions. Experimental parameters such as acid concentration (0.4 %w/v, 0.6 %w/v and 4 %w/v), particle size (1.11μm, 1.14 μm and 1.17 μm), reaction temperature (80 ^{0}C, 100 ^{0}C and 120 ^{0}C) and contact time (45 min, 60 min and 120 min) were varied in order to establish the best hydrolysis conditions for the release of sugars as well as conditions under which emergence of sugar degradation products (furfural and 5-hydroxymethylfurfural (HMF) were minimal. The concentration of the monomeric sugars at each run of the hydrolysis was evaluated using a dual wavelength Uv-visible spectrophotometer. The effects of the hydrolysis**

on surface morphology and structural composition of the pre-hydrolysed and post-hydrolysed biomass were monitored using Scanning Electron Microscope (SEM) and Fourier Transformed Infra Red (FTIR) Spectrophotometer. From the hydrolysis experiments, the optimal reaction conditions for quantitative recovery of xylose, glucose and arabinose were obtained with 4 %w/v sulfuric acid, 120 ^{0}C, reaction temperature, 2 h reaction time and 1.11 μm particle size of the biomass. The hydrolysate was fermented for ethanol production using in-house yeast (*Saccharomyces cerevisea)* generated from waste fruits. The FTIR spectrum of the fermented liquor showed the product obtained was an alcohol. The result of this study showed corn cob as a promising feed stock for biofuel production.

Keywords: **Hydrolysis, Monomeric sugars, Degradation products, Fermentation and Ethanol**

I. INTRODUCTION

Conversion of Lignocellulosic biomass into highly value-added products is an emerging trend in green energy research. However, as essential as this development, in view of its environmental management and value- addition advantages, the conversion process is still challenging and tasking. Nevertheless, this field of research, continually attracts global research attention due to its many other intrinsic benefits such as guaranteed sustainable source of alternative energy , eco-friendly bioproducts, job opportunities, amelioration of climate change effects e.t.c. Since, increase in population proportionally increases energy consumption and other resources, there is need for alternative sources of energy to meet up with the ever- increasing demands. Energy generation and global food production need to be increased by 60% by 2050 in order to meet the needs of the world [9]. At present, most of the countries in the world that specialize in chemical manufacturing, fuels production and power plants depend majorly on fossil-based feedstocks such as crude oil, natural gas, coal, chemicals, etc. [5]. Apart from the dwindling reserves of these feedstocks, environmental degradation associated with their processing and consumption are a lot of concerns globally at present. Hence, the need for alternatives and sustainable sources.

Maize cobs are the by-product of maize crop, consisting of the central fibrous rachis of the female inflorescence (the maize "ear"). While the whole maize ear (with the grains, with or without the husks) is also sometimes called a maize cob. The technological advancement in maize processing in the 20th century resulted in astronomical increase in the volumes of waste emanating from this crop, which trend is still on-going. For instance, about 180 kg of cobs are obtained from each ton of maize shelled [12]. In the United States of America, about 50 million tons of cobs were produced annually in 2000s, besides the fraction left rotten on farm lands [13]. African countries, like Nigeria, generated a total of 3.5Million metric tons of maize cob biomass residue in 2004 [26].

Maize cobs are highly fibrous product with many agricultural and industrial applications. The wastes find vast applications in biofuels production, poultry liters and other animals and soil conditioner. The crop residue has a promising potential as feedstock for bioenergy and biochemical production [13]. Annually, approximately 204 million dry metric tons of maize cobs are returned to the soil as waste in maize plantation [19]. There are concerns associated with environmental pollution resulting from disposal of maize cob wastes [26]. The chemical properties and physical characteristics of corn cobs make the feedstock suitable for biofuel and biochemical production.

A group of studies found that maize cobs containing 32.3-45.6% cellulose, 39.8% hemicelluloses mostly composed of pentosan, and 6.7-13.9% lignin. Cellulose is a polysaccharide of glucose units that serve as the main structural component of the cob's cell walls. Hemicellulose is a less complex polysaccharide that can easily be broken down to simpler monosaccharaides, such as simple sugars which can be converted into value added products [2].The use of dilute acids to catalyze the hydrolysis of hemicelluloses into

fermentable sugars is a well-known and effective method. The most commonly used acids include H_2SO_4, H_3PO_4, HCl or HNO_3. When this method is used, the composition and concentration of the hydrolysis product depends on the type of raw material used and the operational conditions employed. The amount of sugar recovered from the raw material is dependent on the reaction time, temperature and concentration of acid [20].

However, it has been reported that acid concentration is the most important parameter affecting sugar yield, while temperature is the most important factor affecting the formation of sugar degradation products [16], fermentation of highly concentrated acid hydrolysates can be inhibited by compounds present in the raw material or produced during the hydrolysis process, such as acetic acid (derived from acetyl groups), furfural and hydroxymethyl furfural (generated from pentose degradation) and lignin degradation products. In biomass conversion, it is important to seek efficient methods and conditions for achieving a controlled sugar extraction yield (mainly from hemicelluloses) with minimum cellulose degradation. Data from the preliminary analysis of pre-hydrolyzed biomass provides important information for better understanding the effects of the process [11].

Series of traditional methods are available for sugar analysis. Colorimetric methods with the dinitrosalicylic acid (DNS) assay, Orcinol-sulfuric acid method [6], Phenol-sulfuric acid method [8]. These methods had been reported effective for quantification of total reducing sugars, however, could not be used to quantify pentoses and hexoses separately. Hence, these methods will not give the needed guidance relative to the subsequent sugar fermentation. The Douglas method (which is the phloroglucinol-glacial acetic acid method), can be applied only for the determination of pentosans and/or pentoses. Among these colorimetric methods, the Douglas method has the advantage of higher measurement accuracy and relatively lower toxicity of its color reagent, phloroglucinol. Modern equipment such as gas chromatography (GC), high performance liquid chromatography (HPLC) and high-performance anion exchange chromatography (HPAEC) have also been used for determination and quantification of sugars. In GC method, sugars are converted into volatile derivatives; this procedure usually creates a large uncertainty in the sugar analysis [6]. Although HPLC and HPAEC are regarded as the best methods for sugars analysis, both qualitatively and quantitatively [15], they require high-cost analytical columns, eluent reagents, and instrument maintenance [6], developed a simple, rapid, and low-cost method for sugar analysis in biomass. The aim of this research work is to determine the optimum hydrolysis conditions for quantitative release of monomeric sugars from maize cob biomass using UV-Visible Spectrophotometric method.

II. MATERIALS AND METHOD

2.1 Sample Collection and preparation

Corn cobs were collected from First Lets Farm Maize Mills, Akure town in Ondo state Nigeria. The cobs were thoroughly cleaned, oven-dried for 5days at a temperature of 80 ^{0}Cand processed mechanically using mortar and pestle, after which it was milled using milling machine (Suzuki 369). Composition analysis (Proximate and Fibre fraction) of the cob were carried according to standard methods [1].

2.2 Optimization and Acid Hydrolysis of Corn cob

Two hundred grams (200 g) of maize cob were weighed in triplicate, and pretreated using screening and ultra-sonication. The milled samples were screened using analytical sieve with different particle sizes (1.11 ⍰m, 1.14 ⍰m and 1.17 ⍰m). 30 g of each particle size was added to 250 mL of water so as to obtain slurry after which the slurry was poured into a bigger trough. The slurry were homogenized thoroughly by stirring and sonicated for 30min using ultrasonic homogenizer

(Hielscher ultrasonic UP200S). After sonication, the samples were dewatered and stored for hydrolysis. Acid hydrolysis was carried out using a laboratory scale reactor built with a pressure pot and operated under reaction temperature of 80 ^{0}C, 100 ^{0}C and 120 ^{0}C, contact time of 45 min, 60 min and120 min and varying acid concentrations of 0.4 %w/v, 0.6 %w/v and 4 %w/v at different stages of the hydrolysis using automated stirrer under pressure. After the reaction time was over, the hydrolyzed samples were cooled and neutralized with NaOH until tested neutral to litmus. The hydrolysate was filtered and kept under refrigeration for further analysis.

2.3 Quantification of the Monomeric Sugars

Quantification of the monomeric sugars was carried out as described by Chi *et al*. (2013) [6]. Four grams (4 g) of phloroglucinol was dissolved in 220 mL of glacial acetic acid followed by addition of 60 mL absolute ethanol and 4mL of concentrated hydrochloric acid. 5 sets of each of the sugar standards (glucose, xylose and Arabinose) were prepared and also the standards of the Hydromethylfurfural and furfural which are the sugars degradation products were prepared. 1mL each of the sugar and degradation products standards were mixed with 20 mL of the prepared colour reagent in a 50 mL test tube and afterwards immersed in a hot water bath for 20 min after a colour change was observed it was allowed to cool under tap for 6 min. The spectra scanning was conducted for the standards and samples in the wavelength ranging between 200 nm and 800 nm. The reference sample (mixture of the coloured reagent and distilled water) was subjected to the same condition as stated above. The absorption wavelengths were noted and recorded for both the test and reference samples.

2.4 Analytical Procedures.

The surface morphology and structural composition of the feedstocks (pre and post hydrolysed) were investigated using

Scanning Electron Microscopy (SEM) (Tescam 600) and Fourier Transform Infra-red (Perkin Elmer).There after the photomicrograph and FTIR spectra were obtained. Identification and quantification of the monomeric sugars (glucose, xylose, and arabinose) was carried out using UV-Vis spectrophotometer (PerkinElmer Lambda XLS).

2.5 Isolation of Yeast Strain from Waste Fruits.

Isolation of saccharomyces was done as described by Armanul *et al*. (2016) [3]. Waste fruit samples (pineapple, banana and orange) were collected from fruit stand at the Federal University of Technology Akure. The juice was extracted by squeezing mechanically. Two milliliter (2 mL) of the juice was soaked in 250 mL yeast maintenance media (YMM) broth at 30 °C for 3 days. After 3 day of incubation, 100 mL suspension of the sample was spread on a plate containing YMM, consisting of 3 g yeast extract, 3 g malt extract, 5 g peptone, 10 g glucose, and 15 g agar, in 1: l water. The initial pH of the sample was 5.5, and was incubated aerobically at 27 °C for 3 days. Single colony formed was picked, and the cells were observed under a microscope.

2.6 Maintenance of the Culture Yeast

The culture of yeast was maintained by sub-culturing on slants using YMM, incubating for 48 h at 30 °C, and thereafter stored in a refrigerator at 4 °C for future use.

2.7 Fermentation of the Hydrolyzed Sample

The hydrolysate was fermented by inoculating 48 h old cells and incubated under shaking condition at 200 rpm. After 18-20 h, log phase biomass was inoculated into the fermentation medium at 10 % (v/v). Fermentation took place under static condition for 5 days. After which the fermented broth was distilled with simple distillation set-up at 78 ^{0}C. The bioethanol was further purified by refluxing the distilled liquor with CaO for 3 h and then re-distilled using another distillation set-up consisting of a Liebig condenser and fractionating column.

III. RESULTS AND DISCUSSIONS

Table 1 shows the composition analysis of corn cob. The values obtained were within the range of those reported for rice straw biomass [4]. Due to the high fibre content of the biomass, it can be considered as potential feedstock for generation of monomeric sugars and bioethanol production.

Table 1: Compositional Analysis of Corn Cob

Analysis	Results %
Moisture content	5.49±0.02
Ash	2.60± 0.04
Crude Fibre	4.30 ± 0.01
Crude Fat	33.53± 0.02
Crude Protein	4.22± 0.03
Total Carbohydrate	49.86± 0.03
Neutral Detergent Fibre	69.40± 0.02
Acid Detergent Fiber	30.60± 0.01
Hemicellose	38.80 ± 0.01
Cellulose	25.40±0.01

3.1 FT-IR Analysis

Figure 1 depicts the FTIR spectra of Pre and Post hydrolysed biomass of corn cob.The spectra show absorption bands at around 3400 cm^{-1}.This band indicated the presence of OH groups in lignin and carbohydrates. Lower band intensity was observed in the spectrum of the pre hydrolysed biomass while the band was broader and stronger for OH in the spectrum of the post hydrolysed biomass. The band found around 2900 cm^{-1} in the post hydrolysed spectrum signified the presence of C-H streching vibrations of CHO and could be attributed to the presence ofthe remnants of arabinose and xylose left in the biomass after hydrolysis. The band found at1700 cm^{-1}corresponded to carbonyl absorption band C=O and was found both in the pre andpost hydrolysedmaterial but have high intensity in the post hydrolysed biomass. The band around 1600 cm^{-1}confirmed the presence of C=C streching vibration of olefinicwhich was noticed in both samples. The bands around 1100cm^{-1}correspond to C-O stretch vibration, which was more intense and sharper in the spectrum of the Post hydrolyzed biomass than in the hydrolyzed samples.The result show that the acid hydrolysis technique adopted was effective as the cell walls of the fibre were broken down by the acid and released the hemicellulose portion of the biomass

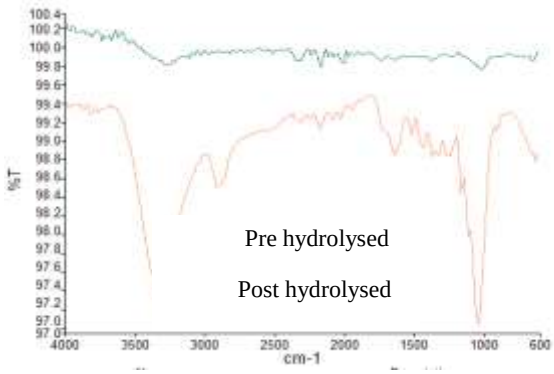


Fig. 1: FT-IR Spectrum of Pre and Post Hydrolyzed Cob

3.2 SEM Analysis

Figure 2 and 3 show the surface morphology of the Pre and Post hydrolysed sample.The result of the morphologies showed a well organised surface structure before the hydrolysis and more disorganized structure after the hydrolysis.The result implies that the hydrolysis was effective.

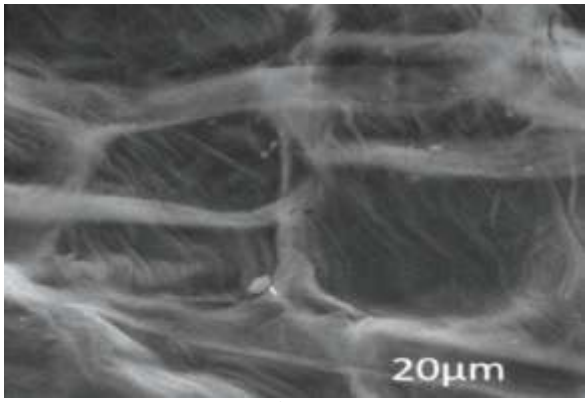


Fig. 2: SEM Images of Pre-Hydrolysed Cob.

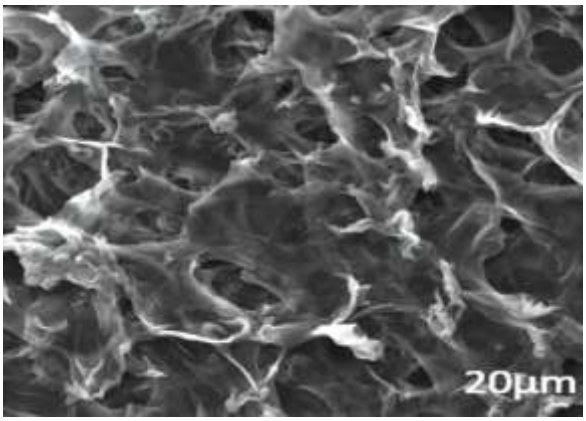


Fig. 3: SEM Images of Post-Hydrolysed Cob

3.3 Effect of Acid Concentrations on the Hydrolysis of Corn Cob

Figures 4 and 5 show the effects of acid concentration on the release of sugar during hydrolysis at 60 and 120 min respectively. Highest yields of monomeric sugars (glucose, xylose and arabinose) were obtained with 4 % sulphuric acid at 120 °C and 120 min. The glucose yield was 18.66%, Xylose 37.67% and Arabinose 26.60 %.The amount of sugars obtained at low acid concentration (0.4%w/v) was noticed to be lower than the amount realized at higher acid concentration (4%w/v) i.e. glucose increased from 0.4 to 4.11 g/L, xylose from 4.11 to11.3g/L and arabinose from 0.35 to 8.01g/L. Values obtained for glucose and arabinose in corn cob were comparatively higher than those of hydrolyzed sugar cane bagasse using 4 % phosphoric acid (arabinose 2.6 g/L and glucose 3.0 g/L) reported by Gamez *et al*. [10]. However, the concentration of xylose obtained was similar to 11.29 g/L obtained by Pattra*et al*. [18] but lower than 17.6 g/L reported by Gamez *et al*. [10]. Aguilar *et al*. [2] also reported 21.6 g/L xylose, 3 g/L glucose from the hydrolysis of sugar cane bagasse using 2% H_2SO_4 at 122^{0}C.

However, at acid concentration of 4 %, there was emergence of degradation products (furfural and HMF) of the sugars, which are inhibitory compounds. This implies that higher acid concentration did not favour further release of sugar rather; it encouraged formation of degradation products. The effective release of sugar from biomass is favoured at higher reaction temperature (160 ^{0}C), low acid concentration and low reaction time. However, under severe conditions such as high temperature, longer contact time and high acid concentration, the presence of degradation products will be evident as sugar concentrations will noticeably reduce [25]. The highest concentration of furfural (0.02 g/L) and HMF (0.03 g/L) were obtained under reaction conditions of 120 ^{0}C, 120 min and 4 % H_2SO_4. With the increase in acid concentration and reaction time, the yield of the monomeric sugars increased. The concentrations of the inhibitory products (furfural and HMF) were found to be lower than those reported to cause inhibition of fermentation [14]. Hence the hydrolysate obtained in this study could serve as substrates for fermentation process and can further be converted into value-added chemicals.

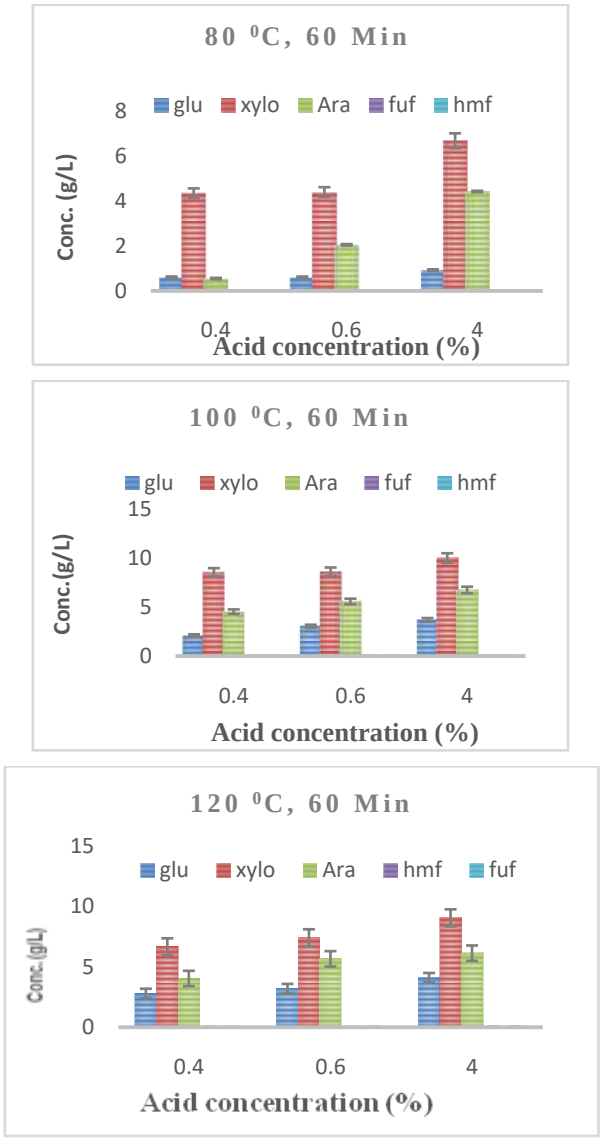
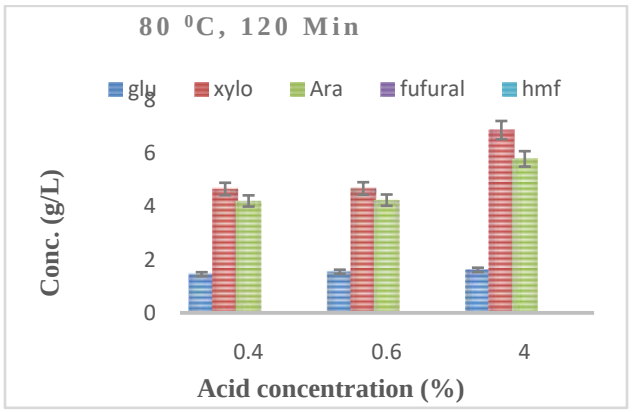
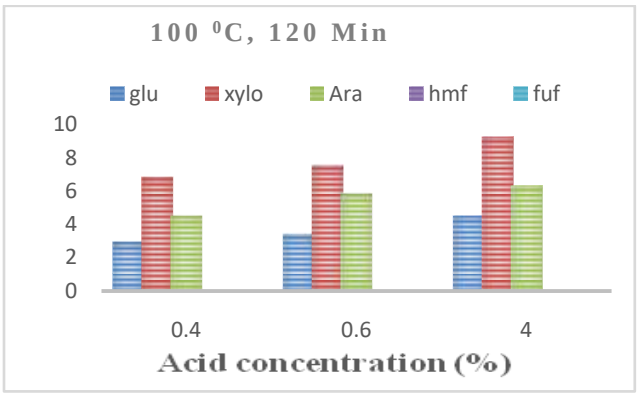


Fig. 4: Effect of acid concentration on sugar level at 80, 100 and 120 ^{0}C for 60 min.





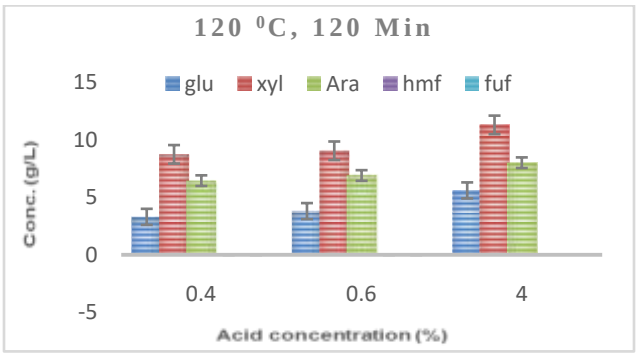
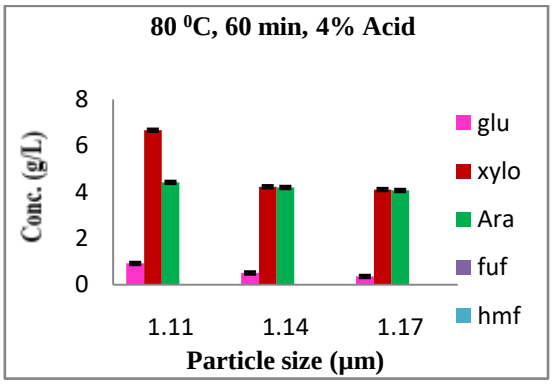
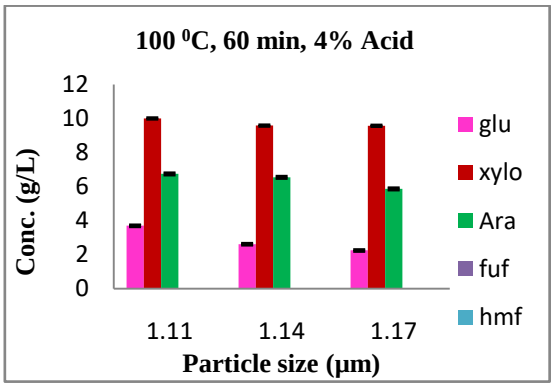


Fig. 5: Effect of acid concentration on sugar level at 80, 100 and 120^{0}C for 120 min.

3.4 Effect of particle size on the hydrolysis of corn cob

Figures 6 and 7 show the effect of different particle sizes (1.11 µm, 1.14 µm and 1.17 µm) on the release of sugar with respect to time and temperature. The highest concentration of monomeric sugars (xylose 13.16g/L, glucose 6.3g/L and arabinose 8.01 g/L) were obtained with smallest particle size 1.11µm. This implies that increase in surface area of the biomass enhanced the release of more sugar. As the particle size increased from 1.11 µm to from 1.17 µm, glucose concentration decreased from 6.3g/L to 5.6g/L, xylose from13.16g/L to 12.36g/Land arabinose from 8.3g/L to 8.2g/L. The concentration of the degradation product such as furfural increased from 0.03g/L to 0.08 g/L upon increase in the particle size of the biomass.





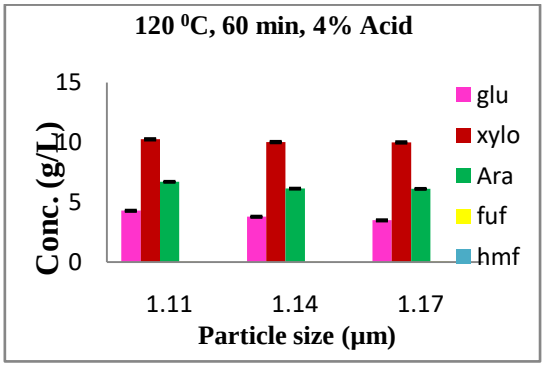
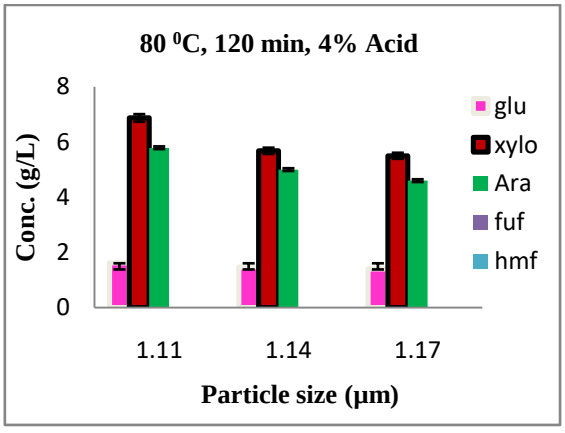
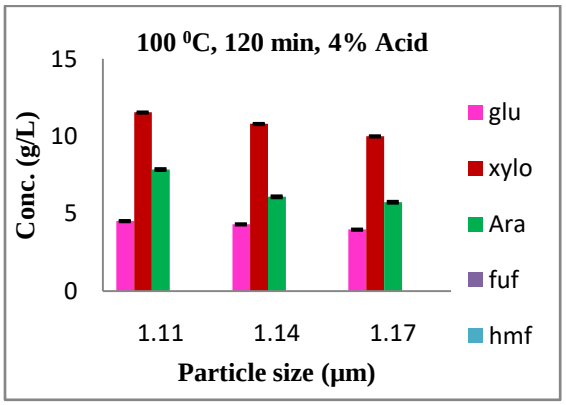


Fig. 6: Effect of particle size on sugar level at 80, 100 and 120^{0}C for 60 min.





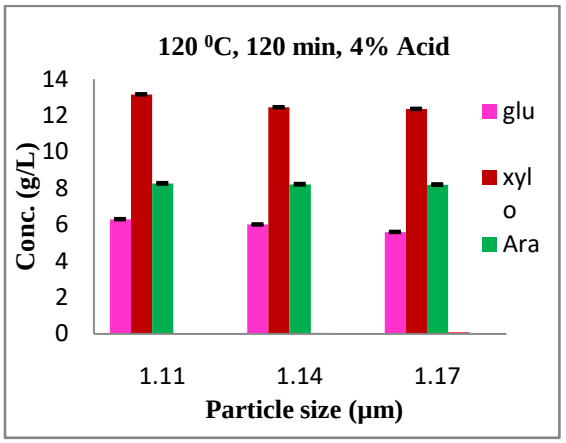
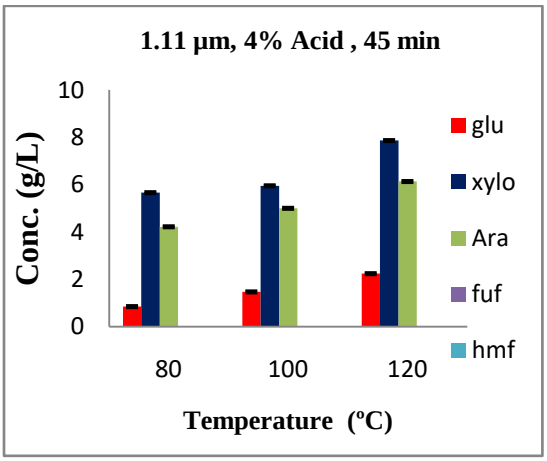
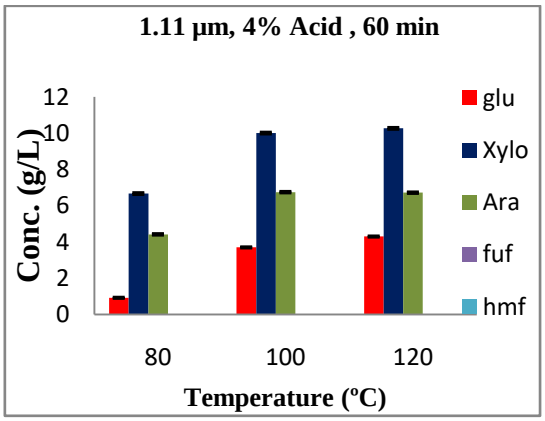


Fig. 7: Effect of particle size on sugar level at 80, 100 and 120 ^{0}C for 120 min.

3.5 Effect of temperature and reaction time on the hydrolysis of corn cob

Figures 8 and 9 show the effect of reaction temperature and time on the hydrolysis of corn cob. The total concentration of all the monomeric sugars increased when higher reaction temperatures and longer reaction times were used. Additionally, at a contact time beyond 120 min and reaction temperature above 120 ^{0}C, emergence of degradation products was noticed under these higher operating conditions.





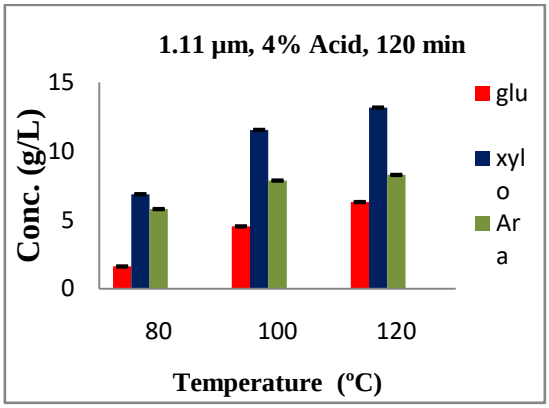


Fig 8: Effect of reaction temperature on sugar level

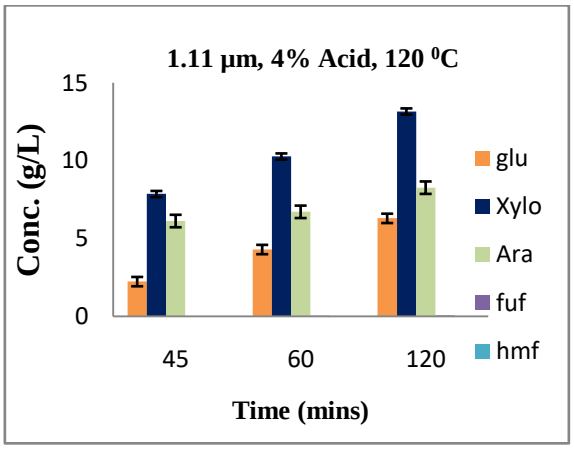


Fig 9: Effect of reaction time on sugar level

3.6 Selectivity of Monomeric Sugar

Figure 10 shows selectivity for glucose, xylose and arabinose. The highest concentrations of glucose, xylose and arabinose were obtained at 4 % w/v H_2SO_4, and at 1.11µm.The sugar with the highest concentration was Xylose (13.16 g/L) followed by arabinose (8.27 g/L) and then glucose (6.3 g/L). This reaction condition favours the release of xylose more than other monomeric sugar.

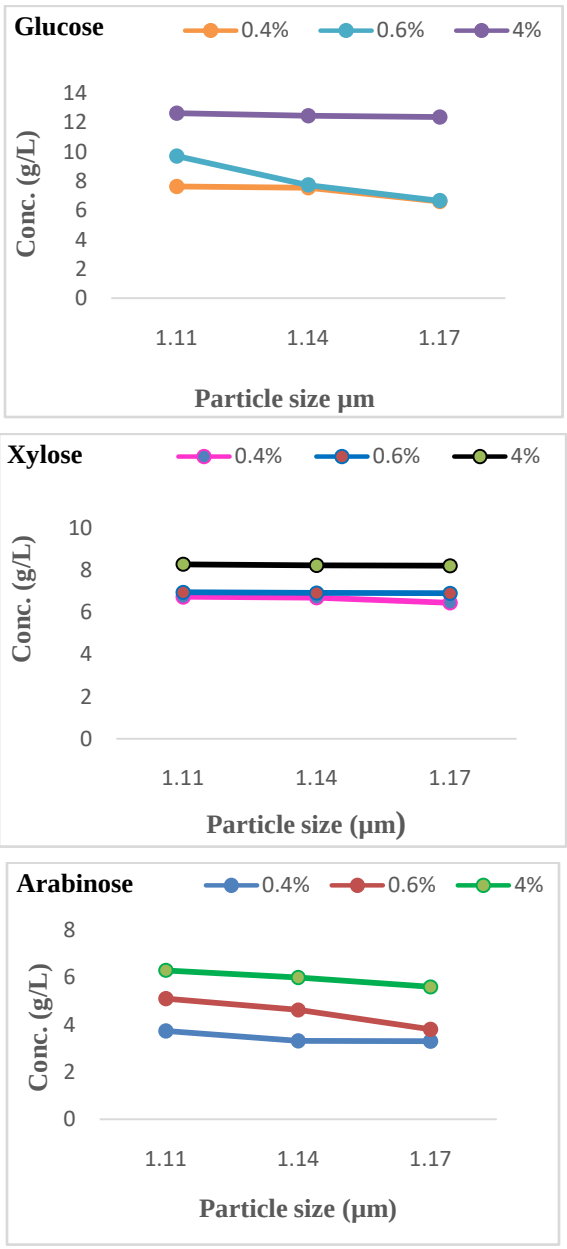


Fig. 10: Selectivity of glucose, xylose and arabinose at various acid concentration

3.7 Ethanol production

Table 2 shows the results of the physicochemical properties of the bioethanol produced from corn cob. The flash point obtained was 43 ºC, this value was comparable with the flash point of conventional ethanol (38 ºC) but comparatively lower than 27 ºC and 39 ºC reported for the same biomass [7]. The specific gravity was recorded as 0.8426 and also the refractive index as 1.3610. These values compared favorably with those of the conventional ethanol (S.G = 0.7974 and R.F = 1.3600) and with the value (1.3607) reported for cocoyam bioethanol [17]. Also, the calorific value (569.03Jg^{-1}) was in the range of the standard values. The results obtained showed that corn cob biomass is a promising lignocellulosic feedstock for bioethanol production. Other properties such as density, appearance, color and flavor conform favorably to the standard values obtained from conventional ethanol and bioethanol [23].

Table 2: Fuel properties of Corn Cob bioethanol

Chemical formular	Bioethanol C_2H_5OH	Conventional Ethanol C_2H_5OH
Molecular weight	46	46
Boiling point in ^0C	79	78
Specific gravity at 30 ^0C	$0.8426^b \pm 0.02$	$0.7974^a \pm 0.01$
Refractive Index	$1.3670^a \pm 0.01$	$1.3600^a \pm 0.01$
Alcohol by volume %	$80^a \pm 0.02$	$98^b \pm 0.00$
Flash point ^0C	$48^b \pm 0.02$	$38^a \pm 0.01$
Calorific Value in J/g^{-1}	$569.03^b \pm 0.01$	$567.03^b \pm 0.00$
Percentage Yield	85	----------

3.8 FTIR Analysis

Figure 11 depicts the FTIR spectrum obtained from the bioethanol produced with absorption band in the following regions: 3333.67 cm^{-1} (O-H stretch of alcohol), 2973.41 cm^{-1} (C-H stretch of alkane) and 1044.74 cm^{-1}, 1088.08 cm^{-1} (C-O stretch of alcohol).

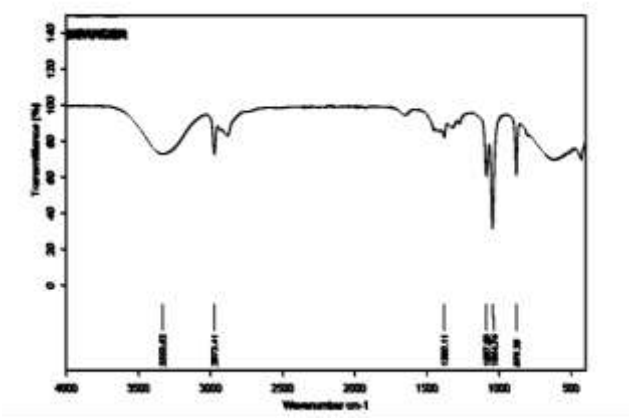


Fig. 11: FT-IR spectra of bioethanol produced in this study.

IV. CONCLUSION

The results from this study indicated that the optimal reaction conditions for the release of monomeric sugars (glucose, xylose and arabinose) from corn cob biomass were 4% sulfuric acid concentration, reaction temperature of 120 ^0C, reaction time of 120 min and particle size of 1.11µm.The availability of the biomass coupled with the high yield of the bioethanol product (85 %) derived from the biomass proved the resourcefulness of the biomass as potential feedstock for bio-based products such as biofuels. The effectiveness of the in-house yeast (*Saccharomyces cerevisiae*) in the fermentation

process showed that the process of bioethanol production from this biomass is self-sustaining.

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