

**PRODUCTION OF BIOETHANOL FROM AGRICULTURAL WASTE**

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**ABSTRACT**

This study investigates the potential of ethanol production from agro wastes. Agro waste from sugarcane *Saccharum officinarum* (sugarcane baggasse, sugarcane bark) and maize plant *Zea mays* (corn cob, corn stalk, corn husk) was subjected to a pretreatment process using acid hydrolysis was applied to remove lignin which acts as physical barrier to cellulolytic enzymes. Ethanolic fermentation was done using *Saccharomyces cerevisiae* for 5 days and the ethanol yield, specific gravity, pH and total reducing sugar were also determined. From the results, the specific gravity, sugar content and pH decreased over time while the Sugarcane baggasse, Sugarcane bark, Cornstalk, Corncob and Cornhusk gave maximum percentage ethanol yield of 6.72, 6.23, 6.17, 4.17 and 3.45 respectively at 72hrs Fermentation. Maximum yields of ethanol were obtained at pH 3.60, 3.82, 4.00, 3.64 and 3.65. These findings show/prove that ethanol can be made from the named agricultural waste and the process is recommended as a means of generating wealth from waste.

**Keywords:** bioethanol; fermentation; agro waste; *Zea mays*; sugar cane.

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**1. INTRODUCTION**

Ethanol as most important alcohol can be produced by converting the sugar content of any starchy material into alcohol with the evolution of carbon dioxide (CO<sub>2</sub>) under controlled



environmental conditions [1]. The process is an anaerobic fermentation in accordance with embden-meyerhoff pathway (EMP) catalysed by enzymes produced by bacteria and fungi. The fermentation process is essentially the same process used to make alcoholic beverages. In this process yeast and heat are used to break down complex sugars into more simple sugars, producing ethanol. Starchy materials are first hydrolysed to fermentable sugars, and subsequently fermented with the required yeast species to produce ethanol [2]. During the fermentation process, part of the sugar is assimilated by the yeast cells and part is transformed into glycerol, acetaldehydes and lactic acid [3]. Production of ethanol from ligno-cellulosic materials such as corncob, cornstalk, cornhusk, sugarcane bagasse and sugarcane bark though faces challenges, but can substitute bio-ethanol production from edible food substances. The energy produced is both renewable and available in large quantities throughout the world [3]. It would also allow agricultural land to be used more efficiently and at the same time prevent competition with food supplies. Until recently the problem was that the complex mixture of sugars that make up these left over materials could not be efficiently converted into ethanol by *Saccharomyces cerevisiae* because they have a very strong crystalline structure surrounded by lignin which makes it difficult for enzyme accessibility. However, these problems have been overcome through pre-treatments such as acid hydrolysis [4]. Ethanol produced from agricultural waste using separate hydrolysis and fermentation also had problems as the higher concentration of reducing sugars inhibited the growth of yeast [3]. This study reports on the production of ethanol from agricultural waste obtained from sugar cane and maize plant. The pH and the total reducing sugar of the final ethanol products were also determined.

## 2. RESULTS AND DISCUSSION

### 2.1. Results

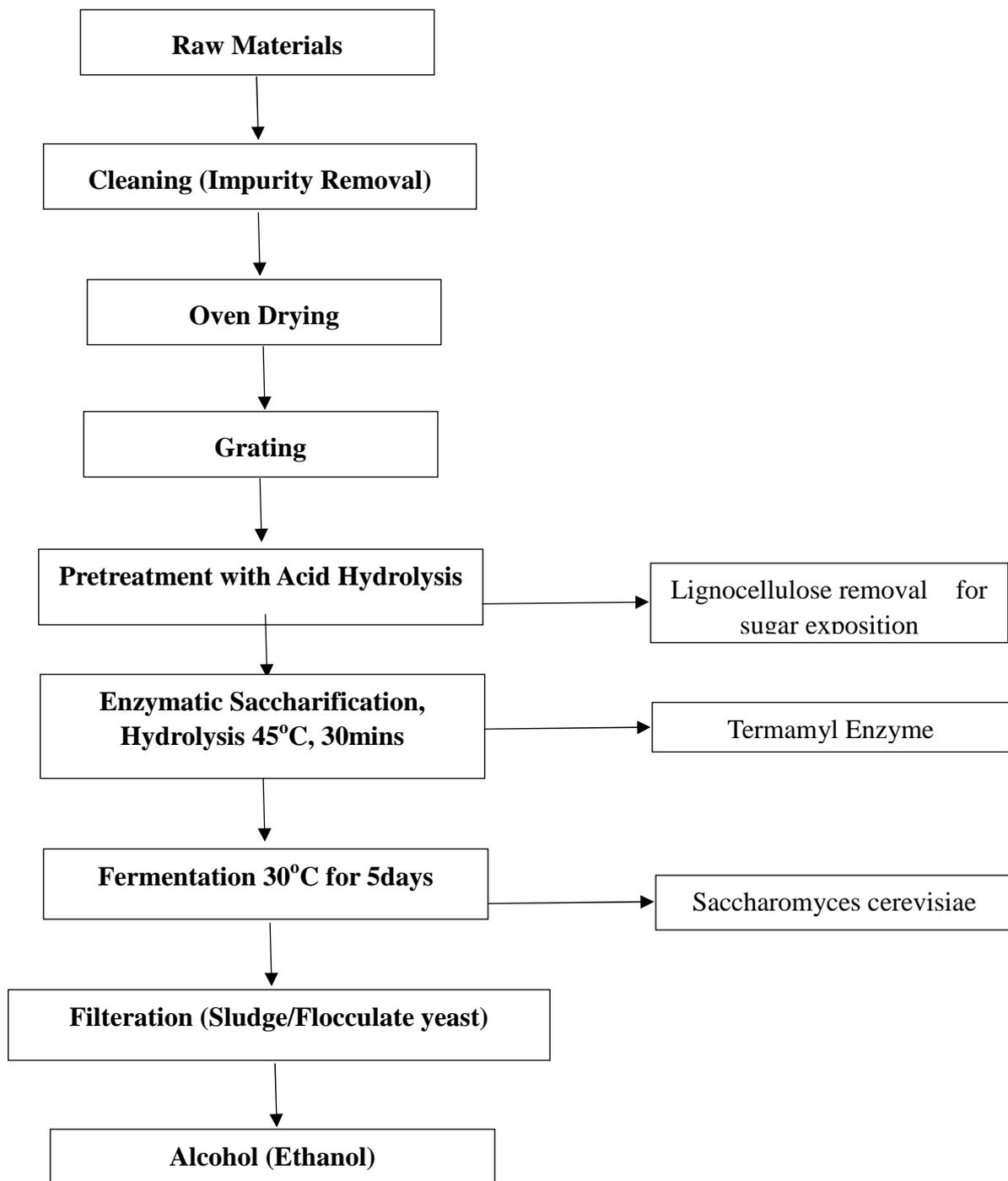
Figure 1 shows the flowchart of ethanol production that was adopted in the processing of the 5 different raw materials. Each point in the production line are critical control points that must be carefully monitored for quality control of products and reproducibility of the process. The physical parameters of the raw materials prior to processing is also shown in table 1 with the raw materials labelled A, B, C, D and E. The pH, Specific gravity, sugar content and ethanol yield was monitored. From the results, the pH, Specific gravity and total reducing sugar

decreased over the production time for each of the agro-waste products as shown in figure 2, figure 3 and Table 2 respectively. Figure 4 shows the ethanol yield of samples expressed in percentage (%) which increases for each of the raw materials reaching its peak at 72hrs and then declined. Table 3 also shows a comparison of the raw materials efficiency for production based on ethanol yield and total reducing sugars obtained from the mean for each of the agro-waste products.

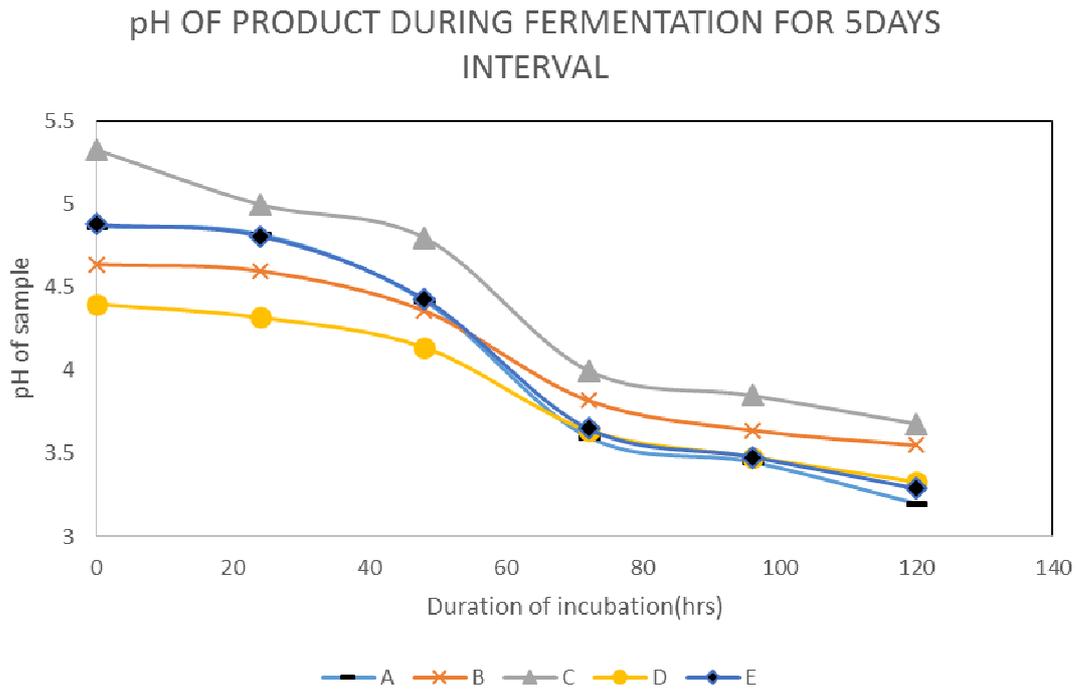
**Table 1.** Physical parameters of assessing the raw materials before/after fermentation

<b>PHYSICAL PARAMETERS</b>					
<b>RRaw materials</b>	<b>OG</b>	<b>Colour</b>	<b>Bitterness</b>	<b>Moisture</b>	<b>pH</b>
A	12.05	5.50	0.04	4.75	4.87
B	11.24	4.10	0.14	4.00	4.64
C	11.20	3.85	0.14	3.65	5.33
D	9.52	2.50	1.25	5.80	4.40
E	9.38	1.95	1.85	6.95	4.88

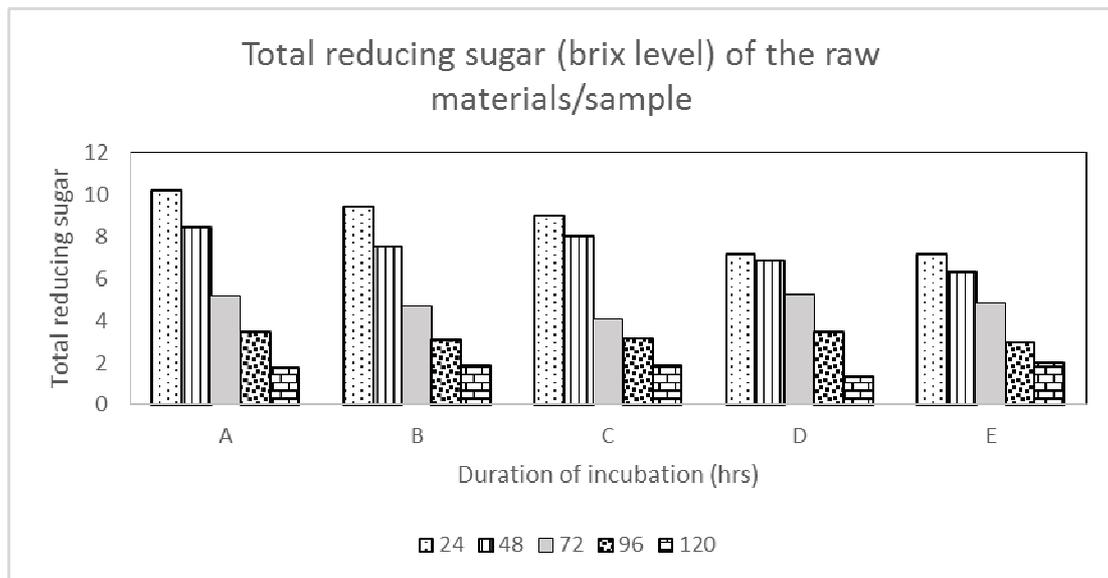
**Key:** OG-Original gravity; A -Sugarcane baggasse; B – Sugarcane bark; C – Corn stalk; D - Corn cob; E - Corn husk



**Fig.1.** Flowchart of production of ethanol from 5 different raw materials (Corncob, cornhusk, cornstalk, sugarcane bark, sugarcane bagasse)



**Fig.2.** pH of product during fermentation for 5days interval

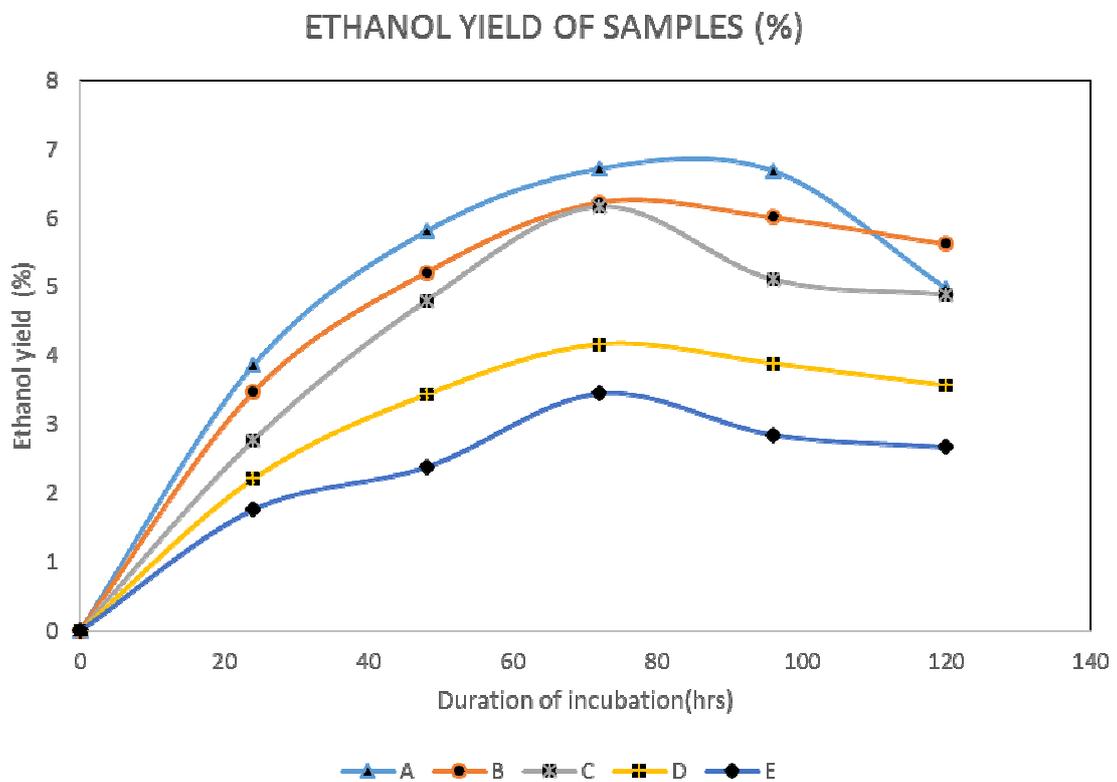


**Fig.3.** Total reducing sugar (brix level) of samples during fermentation for 5days interval

**Table 2.** Specific gravity of sample during fermentation for 5days interval

Duration of incubation(hrs)	SG OF SAMPLE OP (IN DEGREE PLATO)				
	A	B	C	D	E
24	1.3220	1.3001	1.1502	1.0020	1.0000
48	1.1340	1.0000	0.9993	0.9986	0.9958
72	0.9991	0.9965	0.9975	0.9845	0.9901
96	0.9983	0.9957	0.9820	0.9210	0.9843
120	0.9842	0.8643	0.8443	0.8389	0.9001

**Key:** SG-Specific gravity of sample; A-Sugarcane bagasse; B-Sugarcane bark; C-Cornstalk; D-Corn cob; E-Corn husk



**Fig.4.** Ethanol yield of sample

**Table 3.** Evaluation of the raw materials

Duration of incubation (hrs)	ETHANOL YIELD					TOTAL REDUCING SUGAR				
	A	B	C	D	E	A	B	C	D	E
24	3.86	3.47	2.77	2.21	1.76	10.21	9.43	9.00	7.20	7.15
48	5.81	5.21	4.80	3.44	2.38	8.45	7.55	8.02	6.85	6.35
72	6.72	6.23	6.17	4.17	3.45	5.22	4.69	4.10	5.28	4.86
96	6.69	6.01	5.11	3.89	2.85	3.47	3.11	3.15	3.48	3.00
120	4.98	5.62	4.89	3.57	2.67	1.76	1.87	1.89	1.34	1.98
TOTAL	28.06	26.54	23.74	17.28	13.11	29.11	26.65	26.16	24.15	23.34
Mean	5.612	5.308	4.748	3.456	2.622	5.822	5.330	5.232	4.83	4.668

Key: A-Sugarcane bagasse; B-Sugarcane bark; C-Cornstalk; D-Corncob E-Cornhusk

## 2.2. Discussion

The production of ethanol from agro-waste derived from two plants namely sugarcane (*Saccharum officinarum*) and maize (*Zea mays*) plant (sugarcane bagasse, sugarcane bark, corncob, cornhusk, and corn husk) involves the pretreatment of the agro-waste using acid hydrolysis to remove the lignocellulosic component to expose the simple sugars which the yeast can utilize [13]. Lignocellulosic biomass cannot be saccharified by enzymes to higher yields without a pretreatment, mainly because the lignin in plant cell walls form a barrier against enzymatic attack [14]. This pretreatment method was followed by a five days alcoholic fermentation brought about by *Saccharomyces cerevisiae* which utilizes the sugar content of the agro-waste as nutrients and ends up converting the sugar to ethanol under anaerobic condition (figure 3) [5]. The yeast undergoes several physiological changes during the fermentation process. There is a buildup of unsaturated fatty acids and sterols at the start of fermentation, which are vital nutrients for the yeast. The yeast consumes these nutrients and depletes the amount of sugar as the fermentation progresses. Fermentation was considered complete when the supply of sugar was almost completely converted to ethanol [15]. The raw materials were assessed before fermentation to determine if they are good substrates for fermentation, and were found to be suitable based on their colour, bitterness, moisture and pH conditions.

The pH of the broth for each of the raw materials generally decreased during the five days fermentation period with optimum pH for maximum ethanol production ranging from 3.6-4.0 at 72hrs incubation, this co-relates with the work of Nester *et al* [16] where saccharification increased from pH 3.5 to a maximum of 4.0. The reduced pH favours *Saccharomyces cerevisiae* which converts the sugar present in the medium to ethanol [17], and also provides acidic condition which prevents bacterial contamination during fermentation. As the pH decreases, the fermenting broth became more acidic, thus changing the metabolic activities of the yeast for increased ethanol production. The results in figure 3, shows the pattern of residual sugar during the fermentation period. The residual sugar in the fermentation media was observed to decrease with increase in fermentation time. This could be attributed to the utilization of the sugar as carbon source for the growth, energy and metabolic activities of the micro-organism (*Saccharomyces cerevisiae*) and subsequent ethanol production [18]. During these five days, the depletion of sugar was very rapid, This phase was believed to be the exponential phase which was the period of rapid cell multiplication indicated by active fermentation [19]. The sugar level decreased appreciably as the specific gravity decreases from the end of fermentation. The decrease in specific gravity could be attributed to the decrease in the total soluble solids as the sugar present in the broth was fermented to alcohol [20].

The results of the ethanol yield from the five different raw materials were shown in table 3. There is a maximum ethanol yield at 72hrs fermentation period in each of the raw materials. It was observed that at all concentration of the substrates; the ethanol yield increased steadily reaching the peak at 72hrs of fermentation and then declined [21]. The reason for this could be that the yeast was progressing to the stationary phase and could no longer utilize the limited sugar present in the sample [22]. When the composition of the wort is limited, an energy deprivation would occur and the fermentative capacity will drastically reduce, this co-relates with the work of Martin *et al* [2], where seven different strains of *saccharomyces cerevisiae* were tested for the ability to maintain their fermentative capacity during 24hrs of carbon and nitrogen starvation. Starvation was imposed by transferring cells, exponentially growing in anaerobic batch cultures to a defined growth medium lacking either a carbon or nitrogen source. After 24hrs of starvation, fermentation capacity was determined by addition

of glucose and measurement of the resulting ethanol production rate. The results showed that at 24hrs of nitrogen starvation, reduced the fermentative capacity by 70-75percent depending on the strain. Carbon starvation on the other hand, provoked an almost complete loss of fermentative capacity in all the strains tested. The absence of ethanol production following carbon starvation occurred even though the cells possessed a substantial glucose transport capacity. Observed in carbon starved cells was almost surely a result of energy deprivation. Carbon starvation drastically reduced the ATP content of the cells to values well below 0.1 $\mu$ mol/g. While nitrogen starved cells still contained approximately 6 $\mu$ mol/g after 24hrs of treatment. Addition of a small amount of glucose at the initiation of starvation or use of stationary phase instead of log phase cells enabled the cells to preserve their fermentative capacity also during carbon starvation. The prerequisite for successful adaptation to starvation conditions are probably gradual nutrient depletion and access to energy during the adaptation period. The raw materials were compared based on their reducing sugar content and total ethanol yield, sugarcane bagasse containing the highest sugar available for utilization by the yeast gave the highest ethanol when compared with others. The results obtained shows that all the agricultural waste being studied could be used to produce ethanol.

### **3. MATERIALS AND METHODS**

#### **3.1. Sample collection**

Corn cob, corn husk, corn stalk, sugarcane bagasse and sugarcane bark were collected and processed. The sugarcane bark was separated from the bagasse by scraping off the bark with a knife. The sugar juice was then mechanically squeezed out to obtain the bagasse which is the residual dry fiber of the cane after cane juice has been extracted. A pretreatment procedure was done at Anthony van Leuwenhoek Research laboratory in Owerri, Imo State, Nigeria, while the enzymatic saccharification, fermentation and recovery of the alcohol was done at 33 Consolidated Breweries, Awommama, Imo State. The brewer's yeast obtained from 33 consolidated breweries was screened before use.

#### **3.2. Pretreatment of the cellulosic waste products**

Each of the waste products were oven dried at 30 $^{\circ}$ C for 96hrs and grind into semi powdered form using a stainless steel grinder and then stored in well labeled transparent polyethylene

bags at room temperature. Acid pretreatment was done by dissolving 50g of each substrate into 500ml of 5% H<sub>2</sub>SO<sub>4</sub> using a 500ml conical flask. The mixtures were hydrolysed by autoclaving at 121oC for 15minutes.The pretreated samples were then filtered using a 24cm pleated filter paper into a 500ml conical flask. The filtrates were finally incubated in a water bath at 50oC for 30min.The residue was washed with 1% NaOH to neutralize the acid and then with distilled water and finally dried in an oven at 70oC for 24h [5]. Acid hydrolysis was done to achieve delignification. The removal of lignin was necessary for cellulose to become readily available for the enzymes produced by the yeast to convert the glucose to ethanol [5].The filtrate obtained from the acid hydrolysis and heat pretreatment was used to determine the reducing sugar contents of each of the cellulosic waste [6].

### **3.3. Enzymatic hydrolysis and saccharification process**

After the cellulosic substrate was autoclaved for 15 minutes, prehydrolysis with the commercial enzyme, Termamyl was performed at 50oC. Two hundred and fifty milliliter (250ml) of each of the pretreated substrate was poured into a previously weighed mashing cup and ten milliliter of calcium chloride (CaCl<sub>2</sub>) solution was added into the mashing cup. Aliquot (0.2ml) of Termamyl enzyme was also added to the mashing cups containing each of the samples. This enzyme help break down the cellulose into simple sugar (glucose) which the yeast acted upon [7]. The mixtures contained in the mashing cup were then placed into a programmed thermostatic mashing bath at 45OC and the reaction mixtures were stirred continuously to mix. The mixtures were then allowed to boil for 30 minutes [8].

### **3.4. Wort production**

The volume of the samples in each beaker was made up to two hundred and fifty milliliters (250ml) by the addition of distilled water. It was then brought to a boil at 98oC for one hour to halt enzymatic activity. The resultant sample called mash was then cooled to 45oC and the volume of each mash made up by addition of distilled water. The mash was then filtered into a measuring cylinder by the use of 24cm pleated filter paper placed in a funnel. Two hundred and fifty milliliters (250ml) of the resultant liquid called wort was then added into 500ml sterile conical flask [8].

### **3.5. Microbial source**

The yeast *Saccharomyces cerevisiae* was obtained from 33 Consolidated Brewery,

Awommama Imo State, Nigeria. The strain was known to produce ethanol from starchy materials (European Brewery Convention manual). The yeast strain was characterized in order to ascertain the quality, viability, purity and fermentative capability. Cultural and microscopic characterization as well as few biochemical tests was done to confirm the identity of the yeast (*Saccharomyces cerevisiae*) with spherical and short oval budding cells [9,10]

### **3.6. Inoculum (yeast) development for fermentation process.**

The yeast inoculum was prepared as described by Scholar and Benedikte [9] and Suh et al. [11]. Two grams (2g) of dry brewer's yeast obtained from 33 breweries was grown on yeast peptone dextrose (YPD) agar plate at 30°C for 48hrs to activate the yeast and check for contaminants. A loopful of the yeast colony was transferred from the agar plate into 100ml of the 5% YPD broth and incubated at room temperature on a shaker at 130rpm for 48 hrs. Seven milliliters of the broth was centrifuged at 4500rpm for 5min. The supernatant was decanted, and the pellet was resuspended in 10ml of sterile distilled water twice, centrifuged and the supernatant decanted. The pellet was resuspended in 1/10th of 50ml citrate buffer of working solution for each flask and was used as its inoculums. This process was performed in a centrifuge tube to obtain pure yeast.

### **3.7. Alcoholic fermentation process**

Two (2) grams of the centrifuged yeast was dissolved into each of the wort sample contained in a well labeled 500 ml conical flask. It was aerated by shaking before closing with a cotton plug. Fermentation was allowed to take place in a shaker set at 250 rpm for 5 days at 30 °C [12]. At 24 hrs interval samples were aseptically taken from the fermentation media using a 5ml syringe, microcentrifuged at 10,000 rpm for 6 minutes to determine the reducing sugar content (brix level), pH, specific gravity and percentage alcohol by volume [12].

Sample analysis during alcoholic fermentation

### **3.8. Determination of ethanol production**

Ethanol production was analysed by a hydrometer (alcoholometer) which was calibrated to room temperature 20 °C. Each of the filtrate filled to the brim was injected into a cuvette. The cuvette was then placed in an alcolyser which act by absorbing the filtrate after which the result is displayed [12].

### **3.9. Determination of total reducing sugar, specific gravity and original gravity**

The concentrations of reducing sugar, specific gravity, original gravity of the samples were determined using a saccharometer (VLB Labo Tech D-13353 Berlin. Grad Celsius) [12]. The saccharometer work by determining the density of the fluid. The saccharometer was dropped into ten milliliter of the solution being measured into a cylindrical flask. Once the device stabilizes and stops bobbling, a reading was then taken from the device. The marks can correspond to brix, plato or bailing scales all of which are expressions of the percentage of sugar in a solution [12].

### **3.10. Colour Determination**

The colour and bitterness of the samples was determined before and fermentation by the use of spectrophotometer (Aurius 2000 series cecil instruments, UK pat no: 20210001). The spectrophotometer measures the transmission or absorption of light in liquids or solids as a function of wavelength. Absorbance is represented as optical density (O.D) of the solution. Ten milliliter of each of the sample was pipetted into a cuvette and was ran through a spectrophotometer at a wavelength of 430 nm. The result obtained through reading from the spectrophotometer was multiplied with constant 25 expressed in EBU (European brewery unit) (European brewery convention manual).

### **3.11. Determination of Bitterness**

Ten milliliter of each sample was pipette into 50ml flask. One milliliter of hydrochloric acid and twenty milliliter of iso-octane was added into the 50ml flask as well. The mixtures were then placed in a shaker for 5 minutes at 1250 rpm and the supernatant obtained by decanting. The resultant solution was placed in a dark cupboard for 25 minutes, removed and poured into a cuvette. The cuvette was then placed in the spectrophotometer at 275nm wavelength [12]. The result obtained was multiplied by the constant 50 and expressed in EBU (European brewery unit) (European brewery convention manual).

### **3.12. pH determination**

The pH of each of the sample was determined using a pH meter (Mettler-Toledo GmbH 2006). Ten milliliters of each of the solution was pipetted into a 50ml flask and electrodes are dipped into it. The function selector was then turned from standby to pH. And the pH of the solution was read and recorded for each of the samples [12].

#### 4. CONCLUSION

The result of this study shows that agricultural waste namely sugarcane baggasse, sugarcane bark, corncob, corn stalk, corn husk known to contain sugar are good substrates for ethanol production. Therefore the findings of this work suggest that ethanol can be produced from agricultural wastes rather than allowing it to contribute a nuisance to the environment. Therefore:

1. There should be the development of an environmentally friendly pretreatment procedure.
2. Highly effective enzyme systems for conversion of pretreated waste to fermentable sugars.
3. Effective microorganism to convert multiple sugars to ethanol.

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