

## Sugar Cane Cellulosic Waste as a Biofuel Generating Agricultural Residue

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### Abstract:

This research has utilized cellulosic sugar cane for the production of bioethanol in order to diversify in economy by using supposedly waste as bioenergy resource. The sugar cane was obtained from Mofor sub-region of Udu Local Government Area, Delta state Nigeria. The cellulosic waste was obtained by mashing and extracting the juice from the cane sugar. The cane waste was initially pretreated with HCl to breakdown the lignin content after which hydrolysis using thermamyl( $\alpha$ -amylase) and amyloglucosidase was carried out. The reaction conditions were strictly adhered to in order to optimize the yield of bioethanol. The  $\alpha$ -amylase operating condition was maintained at a temperature range of 90-100°C, pH of 4.5 and 0.1% enzyme concentration while the activity of amyloglucosidase was maintained 50-60°C and pH of 4.5. The amount of reducing sugar obtained after saccharification was 18% which was diluted to 11% to enable the yeast cells act on it. After fermentation, the ethanol in solution was distilled and 87ml distillate obtained which gave 56% ethanol using the specific gravity table.

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### 1. Introduction

Overexploitation and dependency on oil and its products has slowed down the growth of the renewable energy sector. With great potentials from crops and crops residue which could be largely grown anywhere, there is need to make attractive to investors the current market, economy and government policy [1].

Known petroleum reserves are limit resources. Various studies put the date of the global peak in oil production between 1996 and 2035 [2]. Biomass energy technologies use waste or plant matter to produce energy with a lower level of greenhouse gas emissions than fossil fuel sources [3]. In developed countries there is a growing trend towards employing modern technologies and efficient bio-energy conversion using a range of biofuels, which are becoming cost-wise competitive with fossil fuels. There are several reasons for biofuels to be considered as relevant technologies by both developing and industrialized countries. They include energy security reasons, environmental concerns, foreign exchange savings, and socioeconomic issues related to the rural sector. Due to its environmental merits, the share of biofuel in the automotive fuel market is currently growing and will grow faster in the next decade. Advantages of biofuels are the following: biofuels are easily available from common biomass sources; they are clean and represent a carbon dioxide-cycle in combustion; biofuels have a considerable environmentally friendly potential; there are many benefits to the environment, economy and consumers in using biofuels; and they are biodegradable and contribute to sustainability [4].

Sugar cane biofuels are generating considerable interest around the world. They may represent a sustainable pathway for helping to meet the world biofuel production targets especially in the African continent that have largely depended on fossil fuels for energy and chemical production.

Biofuels made from sugarcane and its wastes hold the potential to solve many of the sustainability challenges facing other biofuels today.

In Costa Rica, a 240,000 litre per day distillery to produce alcohol from sugar cane has been installed. In Argentina, 3.5 million tons of sugarcane was diverted to alcohol production in 1978 [5]. Brazil in the past 30 years has become virtually energy independent and a leader in renewable energy.

With a largely diversified energy basis, Brazil (with a population of 192 million, according to the 2010 census) is a worldwide model on how a large country can efficiently establish a renewable and diversified energy matrix. According to the Brazilian Ministry of Mines and Energy, the energy produced in 2008 in the country was 46% renewable, with 15% derived from hydroelectric, 16% from sugarcane, 12% from coal and wood, and 3% from others sources. In sugarcane refineries, 20% of the energy used in boilers comes from *bagasse*, the remaining fibrous matter after juice extraction from sugarcane stalks [6,7].

Sugar cane is a crop that grows well in Nigeria and the crop has been abused and misused as it is eaten and scattered in the major streets of the town. The potentials of this crop have been underutilized and that's why this research wants to focus on the utilization as a method of diversification in economy and reduction in total dependency on petroleum. Some scientists have argued that if sugar crops are used for biofuel generation that it will affect the supply of food and lead to scarcity but this is not the case with sugar cane. The development of sugar cane farm for biofuel technology would create jobs, lead to sustainability and enhance productivity in the states and country. This source (*bagasse*) would not only achieve energy balance that is more than eight times more efficient than fossil fuels but would co-generate heat and electricity. The residue would equally be used as livestock feed, in paper manufacture and also used as insulated disposable food containers replacing Styrofoam. This work wants to reawaken the interest of the world especially the Nigerian Federal Government who initially mapped out some states for cassava production for biofuel technology but yet to set out with the mandate. Instead, this work is suggesting the use of sugar cane as a lead candidate instead of cassava as the processing is easier and the technology is indeed less complicated.

The sustainability of ethanol production as a component of the energetic base of a country is highly dependent on how the source crop is managed, and this also depends on the genetic background of cultivars available to farmers. When the sugar cane is genetically modified, it can give low lignin which yields more ethanol.

In an extensive production and utilization, the roadmap will draw upon the expertise of a carefully balanced group of invited scientists and other experts in the various required disciplines such as biology, systems and process engineering, modeling and analysis, sugar cane cultivation, molasses extraction and conversion, other sugar cane-based co-products, water and land use, policy and regulatory issues, etc. Input from these varied professions will help define activities needed to resolve uncertainties associated with commercial-scale sugar cane biofuel production.

## 2. Materials and Method

### 2.1. Sample Preparation

Sugar cane was obtained from a farm at Mofor, Udu Local Government Area of Delta state, peeled with knife and chopped into smaller sizes and crushed. The crushed or mashed sugar cane was squeezed and the juice extracted. The remains which is the cellulosic waste was rinsed severally with water and afterwards dried for four weeks. 1kg of the dried waste was ground with industrial blender and the resulting material served as sample for our research. The powdered material was stored at room temperature for further analysis.

### 2.2. Acid Pre-treatment

15 g of the sugar cane waste was dissolved in 500 ml of 0.5 M HCl concentration. The sample was soaked for 24 hrs and thereafter heated for 2 hrs. It was carried out in duplicate and labelled A and B. Sample A was filtered after pretreatment, dried and weighed while sample B was left that way.

### 2.3. Simultaneous Acid and Enzyme Hydrolysis

Before enzymatic hydrolysis, the pH of the solution was maintained at 4.5 using sodium acetate buffer to provide enabling environment for the activities of the enzyme. After pH adjustment, the enzymatic hydrolysis was carried out using 0.1%  $\alpha$ -amylase and the solution heated for an hour at a temperature of 90°C on both sample A and B. Sample B was now filtered, dried and weighed to know the amount of lignocellulose broken down by acid, heat pretreatment and  $\alpha$ -amylase hydrolysis. Saccharification was followed using 0.1% amyloglucosidase to both the filtrate from Sample A and Sample B in an incubator at temperature of 60°C for 24 hrs [8,9].

### 2.4. Test for Reducing Sugar Using Benedict's Solution

The quantitative sugar determination using Benedict's solution was done by adding 1.5 g  $\text{Na}_2\text{CO}_3$  to 10 ml of the Benedict's solution and heated using anti-bumping agents. 0.5% glucose standard was titrated against Benedict's solution and the volume that effected colour change was noted. The hydrolysed sample was also titrated against the Benedict's solution and the volume of the hydrosylate was recorded.

### 2.5. Fermentation of Hydrolysate

After saccharification, the hydrolysate was cooled and filtered. 1 g of yeast was added to the 818.18 ml filtrate of 11% reducing sugar concentration and rate of depletion of the sugar studied at time intervals [10].

### 2.6. Distillation

After five days, the saccharified and fermented sample was distilled and volume of distillate recorded.

## 3. Results and Discussion

### 3.1. Effect of HCl Pretreatment on Sample A

Pretreatment of sample A with 0.5 M HCl and heating for 2 hrs gave 7.14 g of unhydrolysed matter, showing that 7.86 g of sample A was hydrolysed by acid and heat. This shows that the percentage acid hydrolysed matter is 52.4% while the unhydrolysed matter is 47.6% and it is shown in Table 1.

Table 1: The results of the pretreatment effect on the substrates

	Sample A	Sample B
Total mass of sample(g)	15	15
Mass of hydrolysed matter(g)	7.86	11.36
Mass of unhydrolysed matter(g)	7.14	3.64
Percent hydrolysed(%)	52.4	75.73
Percent unhydrolysed(%)	47.6	24.27

### 3.2. Effect of HCl Pretreatment with $\alpha$ -Amylase on Sample B

When 15 g of the substrate was pretreated by acid and heat with further hydrolysis by enzymes, 3.64 g of unhydrolysed matter was obtained which indicates 75.73% hydrolysable matter and shown in Table 1.

### 3.3. Liquefaction and Saacharification

The two stage enzymatic hydrolysis treatment was carried out on the sample with the aim of first liquefying the starch at higher

temperature(90°C) using  $\alpha$ -amylase and then saccharification at lower temperature(60°C) by amyloglucosidase to convert the starch completely to monomeric sugars.

After saccharification of sample A, the reducing sugar concentration was determined to be 8.76% while that of sample B was determined to be 18%.

Sample B was further diluted to 11% with distilled water and 0.1% yeast cells was added to the solution. The rate of degradation of sugar with time during fermentation was monitored and the results shown in Table 2 and 3.

Table 2: Determination of reducing sugar present after saccharification of sample A

Time(hr)	Vol of sample used(ml)	% reducing sugar
0	41.5	8.67
1	46	7.83
2	51	7.06
4	85	6.32
8	92	5.00

Table 3: Determination of reducing sugar present after saccharification of sample B

Time(hr)	Vol of sample used(ml)	% reducing sugar
0	52	6.9
1	77	4.67
2	80	4.5
4	119	3.03
8	137	2.63

According to Figure 1, there was gradual depletion of the sugar in sample A which may be as a result of initial inhibitory compounds emanating from ineffective digestion and hydrolysis of the lignin and hemicelluloses. During the fermentation kinetics of sample B, it was found out that at the initial time of addition of the yeast cells, the sugar concentration drastically reduced to 6.9%. After four hours of reaction, there was no noticeable change from the graph in Figure 2 showing that there were less sugar for consumption by the yeast cells and presence of substantial amounts of different impurities like furfural, acetate and other phenolic compounds which may have acted as inhibitors and affect fermentation rate of the hydrolysate [11,12].

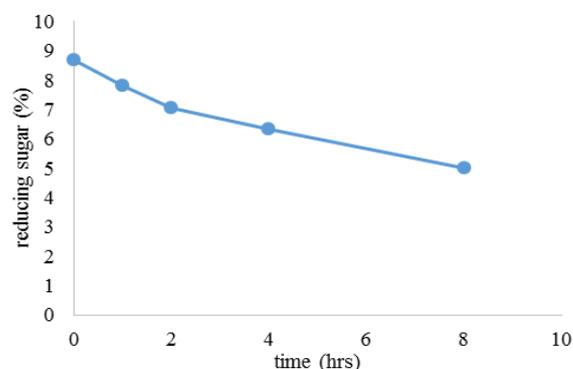


Figure 1: Plot of reducing sugar against time showing the rate of degradation of reducing sugar for sample A

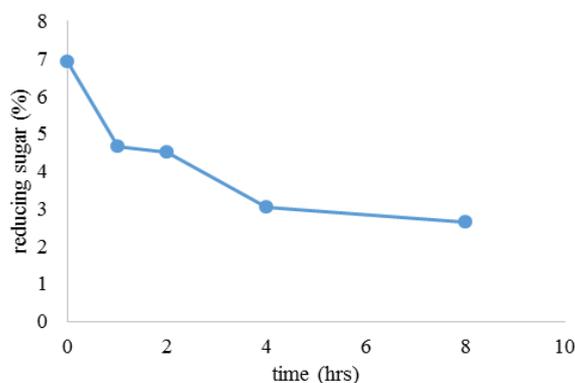


Figure 2: Plot of reducing sugar against time showing the rate of degradation of reducing sugar for sample B

After fermentation for 5 days, distillation was carried out and the volume of distillate obtained for sample B was 87 ml. specific gravity of the distillate was determined to be 0.927 using density bottle at room temperature. Using the ethanol reference table, the ethanol concentration was approximately 56%.

### 3.4. Discussion

It has been reported that when the juice from cane is expressed, we can get up to 70-90% alcohol per ton of sugar cane [5]. There are other by-products obtained but a major by-product of ethanol distillation is vinasse, a complex liquid residue derived from distillation of the fermentate that is rich in humic acids (soluble organic matter) and minerals (such as K and P). For every liter of ethanol distilled, 10-20 liters of vinasse is left over, which have been used as fertilizer in sugarcane fields, saving millions of liters of water and improving the physical conditions of the soil. Vinasse application to sugarcane fields allows the recycling of nutrients back to the soil. However, its application in the field requires careful procedures to avoid contamination of water tables [13]. Another potential destination of vinasse is biogas production [14].

Sugarcane second generation ethanol which is considered here (cellulosic sugar cane waste)

has not yet been used commercially, but many sugarcane breeding programmes are improving germplasm not only for sucrose yield but also for biomass yield in anticipation of upcoming technologies that may allow for efficient energy production from cellulosic residues which this laboratory scale production has demonstrated.

In Brazil, the sugarcane agribusiness accounts for more than US\$ 20 billion / year and is one of the main direct and indirect job generation sectors. The country produces 25% of the world's cane sugar and is the largest producer with about 31 million tons / year and exporter of about 19.5 million tons / year [6].

From the results of this present research, it can be inferred that heat and acid pretreatment solubilized the hemicelluloses thereby releasing pentose sugars which were converted to ethanol. Factors such as temperature, reaction time and acid concentration played important roles in obtaining high yields of ethanol.

In a previous work by Wong and Sanggari, 2014 [15], they reported that after subjecting sugarcane bagasse to different kinetic parameters, that higher concentration of ethanol (14.8% in water) was obtained at a temperature of 35°C and pH of 4.5. According to their report, the enzymes operates effectively at this temperature but denatures above 35°C and slows down the reaction below that temperature, while the ethanol concentration drops drastically at pH of 5.0. Elemike 2015 [8], also reported that 16ml of 32.5% ethanol was obtained from 15 g cassava cellulosic waste after pretreatment with 0.5 M HCl, and subsequent enzymatic hydrolysis and saccharification. When compared with the present study, the sugar cane wastes gave higher yield of ethanol though at an enzyme hydrolyzing temperature of about 60°C which may not be optimum and therefore stands out as a better substrate for bioethanol production. The fibrous network of the waste was distorted thereby releasing the hydrolysable components. The heat and acid pretreatment procedure enhanced the amount of maltose available for saacharification which correspondingly released more glucose for fermentation.

For a greater improvement of sugar cane for biofuel generation, the cultivars and genotype need to be critically evaluated at the biochemical and physiological level. Some genotype differ in their saccharification level making some more amenable to acid or enzyme hydrolysis and the others yield more cellulosic ethanol. It is well known that the sugar cane cell wall contains fibres rich in arabinoxytan, cellulose with lower amounts of beta-glucan and pectins but the sugar linkages and the overall architectures of sugar cane cell wall is still very vague [16].

### 4. Conclusion

In this research we have exploited the reported waste, in order to ascertain that actually it can be put to great use rather than discarding it as waste.

Acid pretreatment and enzyme hydrolysis with  $\alpha$ -amylase and amyloglucosidase yielded appreciable amounts of ethanol compared to acid hydrolysis alone as the enzymes broke down cellulose and hemicelluloses. 18% of glucose was obtained from 15 g of sugar cane bagasse and more fermentable sugars could be obtained when the quantity of the substrate is increased.

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