PRODUCTION AND CHARACTERIZATION OF BIOETHANOL FROM SUGARCANE MOLASSES

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ABSTRACT

The dependence on oil (fossil fuel) across most industrial sectors, especially transportation, is unsustainable in both the short and long term. The fossil fuel depletion and its environmental impacts drive industries to explore alternative fuel and promote the adoption of renewable energy sources like bioethanol. This study aims to produce and evaluate the performance characteristics of bioethanol from sugarcane molasses as an alternative to fossil fuel. This study, bioethanol was produced from sugarcane molasses obtained from Savannah Sugar Company Limited Numan, Nigeria. The properties of the bioethanol were characterised and compared with ASTM standard properties for bioethanol. The result revealed key properties such as moisture content (25.95%), Brix (35.69°Bx), viscosity (436.5 cm^2/s), and density (1.183 g/cm^3). Fermentation of the molasses using Saccharomyces cerevisiae, Aspergillus Niger, and Zymomonas mobilis was carried out over 120 hours. After fermentation, bioethanol yield was 18.4% by weight, and subsequent fractional distillation produced bioethanol with a density of 795 kg/m^3, kinematic viscosity of 3.17 mm^2/s at 40°C, octane number of 109, and calorific value of 31.5 MJ/kg. Based on the result of the study, sugarcane molasses is a good substrate to produce bioethanol that can be used for internal combustion engines instead of fossil fuels that pollutes the environment with its emissions.

Keywords: Fossil fuel, Industrial, Transportation, Environmental, Savannah Sugar Company

1 INTRODUCTION

The provision of affordable and clean energy is one of the seventeen Sustainable Development Goals (SDGs), which are paramount in most countries in Africa such as Nigeria [1]. Energy is a fundamental input in the development of any human society and is one of the most powerful needs of the 21st century [2]. However, the amount of energy required per capital to foster or sustain development depends largely on the state of development, the local resources, the social and economic model chosen by the country, and other factors, it also drives all economies around the world and has done a great deal in improving the standard of human life [3]. In recent times, most people have depended on local or imported natural resources, such as fossil fuels. However, the environmental impact of fossil fuels - including global warming, ozone layer depletion, El Niño weather patterns, and widespread migration - has significantly driven the development of alternative energy sources. Among these, renewable options like biofuels have gained particular emphasis. Additionally, the growing demand for gasoline, fueled by population growth, has contributed to ozone layer depletion [2]. The increasing global concern over air pollution and the limited availability of gasoline are fueling interest in environmentally friendly alternative fuels [4]. Worldwide, there is a rising awareness that something must be done to alleviate the environmental damage caused by gasoline fuel [5]. As a result, alcohols are seen as appealing alternative fuels due to their abundance in natural and man-made sources, typically, the types of alcohols that dominate the others are ethanol and methanol. Fuel price fluctuations, rising oil usage, and the shortage of petroleum fuels have sparked interest in alternative, renewable fuels such as alcohol-based fuels. In recent years, ethanol has emerged as a widely used renewable [6]. Over the years, the energy demand has increased in African countries due to rapid population growth, an increase in small-scale (cottage) industries, and local technological inventions [4]. This demand is high in Nigeria the most populous black nation in the world, with a population of over 200 million people. Unfortunately, this demand is rarely met, as access to affordable and clean energy in Nigeria faces several challenges [1]. Consequently, the inaccessibility to renewable energy is because of problems which include poverty, decline in economic growth, poor research and development, and socioeconomic imbalance. Nigeria is rich in conventional energy resources, which come mainly from crude oil, coal, and natural gases. However, apart from the challenges the energy industry faces, conventional resources have other issues such as being nonrenewable and may facilitate environmental degradation. This has drawn attention to finding alternatives to the conventional energy resources in Nigeria [1]. The current dependence on oil across most industrial sectors, especially transportation, is unsustainable in both the short and long term. Its ongoing (fossil fuel) depletion and environmental impacts drive industries to explore alternatives and promote the adoption of renewable energy sources like bioethanol.

1.1 Biofuel

Yami [7] defined biofuels as liquid or gaseous fuels produced from agricultural and forest products and the biodegradable portion of industrial and municipal waste for either transport or burning purposes. According to Yami [7], biofuels may be solid, liquid or gaseous and include all kinds of biomass and derived products used for energetic purposes. Biofuels usually originate from plant oils, sugar beets, cereals, organic waste and the processing of biomass [8]. The term "biofuel" was used by the United Nations Conference on Trade and Development to describe any liquid fuel made from plant resources that can be used to replace petroleum-derived fuel [9]. The term "biofuel" was used by the United Nations Conference on Trade and Development to describe any liquid fuel made from plant resources that can be used to replace petroleum-derived fuel [10]. Also, it was reported by Bunmi [11] on Natural Resource and Extraction Program (NAREP); Biofuels was defined by the Standard Organization of Nigeria (SON) and the Department of Petroleum Resources (DPR) as bioethanol, biobutanol, and biodiesel, as well as other biomass-based fuels used in automotive, thermal, and power generation. Biofuel production from biomass resources is one of the preferred routes through which the transportation sector can be made “green” [12]. As a result of this, the demand for biofuel has been on the increase and thus there is need for a corresponding increase in its production to meet this ever-increasing demand [13]. According to Nabel *et al.*, [14], the most common types of biofuels are: straight vegetable oils, biodiesel and bioethanol.

1.1.1 Bioethanol

Bioethanol is a distilled liquid derived from sugar fermentation in sugar cane and cereal crops (sugarcane, corn, beet, cassava, wheat sorghum) [12]. Any biological feedstock having a high amount of sugar or sugar-convertible components like starch or cellulose can be used to produce bioethanol [14]. Bioethanol may be manufactured from a wide range of biomass (plant) resources and can be used to substitute premium, super, and lead-free gasoline in a highly challenging chemical process [13]. Most of the time, it is blended with petrol/gasoline in varying proportions, while certain nations are currently producing vehicles that run mainly on ethanol [15]. Bioethanol is the biofuel with the largest global production capacity and is also the most advanced in terms of government policies on biofuel blend with gasoline [14]. This has made bioethanol the most demanded biofuel now [16]. Ethanol is a compound of carbon and hydrogen atoms, with a hydroxyl group having chemical formula C_2H_5OH anad the chemical structure is shown in equation 1, also known as ethyl alcohol or hydroxyl ethane. Its molecule is small and light, as compared to most gasoline components. The electrochemistry of the ethanol molecule is slightly exceptional being polar at one end and non-polar at the other.

$$H - \underset{H}{\overset{H}{C}} - \underset{H}{\overset{H}{C}} - OH \quad (1)$$

It participates in hydrogen linkage with other ethanol molecules or other polar substances due to presence of hydroxyl group. The polar end helps ethanol to be miscible in water or other polar compounds, and the non-polar end is advantageous in mixing with non-polar substances, such as gasoline. Generally, ethanol is produced in two forms: anhydrous, keeping water content less than 1%, or hydrous, having water content up to 10%, therefore, it is in the intention of this research work to produce hydrous bioethanol from sugarcane molasses. Purity of ethanol above 96% cannot be achieved through conventional distillation. To convert hydrous ethanol into anhydrous, separate technique as azeotropic distillation using 20–25% extra energy is used [17]. In the first generation, bioethanol is generally produced using starch- or sugar-based crops such as barley, corn, wheat, sugarcane, sugar beet, and sweet sorghum by means of a simple fermentation of sugar extracted from such crops [18]. During the process, CO_2 is extracted from carbohydrates, which have a C/H/O ratio of 1:2:1 [19]. Ethanol, with its high (C + H) to O ratio, retains most of the original energy content. Table 1 shows the physical properties of ethanol [19]. Bioethanol produced from sugar-based feedstocks has the greatest potential of replacing fossil fuel as transportation fuel due to the low cost of production, low carbon emission and higher yield in litres of bioethanol per hectare compared to starch-based crops used for production of bioethanol [18].

Table 1. Physical Properties of Bioethanol [19]

Properties	
Molecular formular	C_2H_5OH
Molar mass	46.01 g mol^{-1}
Exact mass	46.041864814 g mol^{-1}
Appearance	Colorless liquid
Density	0.789 g/cm^3
Melting point	-11 °C
Boiling point	78 °C

Nigeria National Petroleum Corporation (NNPC) was given the authority to establish an environment for the take-off of a domestic ethanol business in August 2005 as part of a government directive on an automobile biomass program for the country. The goal is to gradually reduce the country's reliance on imported gasoline, reduce pollution, and develop a financially viable business that can generate long-term local employment [20]. Furthermore, the Nigerian biofuel policy projects a 90% gasoline with 10% ethanol blend for gasoline engines, and an 80% diesel with 20% ethanol blend for diesel engines, with the Nigerian National Petroleum Corporation (NNPC) enforcing the regulations. The Department of Petroleum Resources (DPR) estimates that Nigeria's daily gasoline consumption is 38.2 million liters per day. To fulfill the ethanol-gasoline blends objective, Nigeria will need 3.82 million liters of fuel ethanol per day. Given the country's current status, this is a major responsibility for bioethanol production. To fulfill these goals, a lot of research has been done on bioethanol production, physical characteristics, engine performance, engine emission, and engine durability all over the world [21]. According to reports by [1, 22, 23], bioethanol is technically practical, economically viable, and environmentally favourable. Currently, substantial research is being undertaken in several countries in the areas of bioethanol production optimization, ethanol characterization, ease of transportation, storage, and improvement of engine performance and emission characteristics using a blend of bioethanol and gasoline as fuel [4].

1.2 Feedstock for bioethanol production

Biological feedstock that contains appreciable amounts of sugar or materials that can be converted into sugar, such as starch or cellulose, can be fermented to produce bioethanol to be used in gasoline engines

[2]. Bioethanol fermentation is considerably the largest-scale microbial process. Regardless of the simple or complex substrates utilized as microbial carbon sources acquiescent for conversion to ethanol, all types of substrates firstly result in the formation of hexoses, pentoses or glycerol (after the enzymatic, physical, chemical or mechanical pretreatment) that will be fermented by the relevant microorganisms in order to be converted into bioethanol [19].

1.2.1 Bioethanol from sugarcane molasses

Molasses is a dark reddish coloured jelly-like like viscous material which is generally used because it is rich in all salts except nitrogen, which is normally employed in the actual growth of yeast cells. Molasses is defined as waste product of sugar industry of which further extraction of sugar is uneconomical, contains about 45–50% fermentable sugars [24]. Because of the ease with which this can be fermented into ethanol and its low price have made this raw material ideal for ethanol production. Molasses are the final effluent (final molasses) and by-products of sugar manufacture. Due to their origin, sugarcane and molasses are different. Black strap molasses is molasses produced from raw sugar factories from cane or beet. Often, only term (molasses) is used for molasses from a beet factory whereas black strap is preferably used for cane molasses [19]. Refinery (final) molasses (refinery black strap, barrel syrup) is derived mainly from cane refineries, where white sugar is produced from raw sugar. Molasses is classified into following three grades namely, first grade Molasses which contains more than 50% total reducing sugar (TRS), Second grade Molasses which contains 40% to 50% total reducing sugar and lastly Third grade Molasses which contains less than 40% total reducing sugar. Molasses is an agricultural product, and its composition varies with the variety of maturity of cane with the climate and soil condition [19]. In addition, processing condition in the sugar factory may also bring about changes in the composition of molasses. So, only average values of main components of sugarcane black strap molasses are shown in Table 2.

Table 2. Main components of cane black strap molasses [19]

Composition	Percentage
Sugar	36.55
Sucrose	22.75
Invert Sugar	11.05
Other	2.75
Organic	7.75
Glutamic acid and pyrrolidine carboxylic acid	1.2
Other N	1.95
Organic acids	3.5
Pectin etc.	1.35
Inorganic	5.85
K_2O	2.65
Na_2O	0.05
CaO	0.1
MgO	0.5
$SO_2 + SO_3$	1.15
P_2O_5	0.4
Others	0.45
Total	100

2 MATERIALS AND METHOD

The materials, equipment and the chemicals that were used in this study are: Sugarcane Molasses, *Saccharomyces Cerevisiae, Aspergillus Niger and Zymomonas mobilis,* Amonium Chloride (NH_4Cl), Yeast extract, Incubator and Shaker, Shaking Flask, Spectrometer, Gas Chromatograph, Flame Ionization

Detector (FID), Oven, Thermometer, Measuring cylinder, Weighing balance, Conical flask, Electric weighing machine, Stop watch, density bottle, stopper, pH meter, Peltier Cloud and Pour point tester, Optical detectors, Bunsen burner, Single Cylinder Four Stroke Spark Ignition Engine, Internal Combustion Engine Instrumentation, Gas Automatic exhaust emission analyzer.

2.1 Materials Collection and Preparation

Sugarcane Molasses is the base material and was collected from Savannah Sugar Company in Numan Local Government Area of Adamawa State. The sugarcane molasses was transported to the process center in Chemistry Department, Abubakar Tafawa Balewa University Bauchi, Nigeria. *Saccharomyces Cerevisiae*, *Aspergillus Niger and Zymomonas mobilis* were cultured in Microbiology Department of Abubakar Tafawa Balewa University Bauchi.

2.2 Production of Bioethanol

36.25kg of sugarcane molasses (25 liters) was diluted with 10kg of lime water (calcium hydroxide) which was added to it which served as a flocculant and coagulated the impurities present in the molasses. The content in the container was then allowed to settle for 5 hours, the sugarcane molasses was decanted to remove impurities. 6kg of *Saccharomyces Cerevisiae, Aspergillus Niger and Zymomonas mobilis* was diluted with the sugarcane molasses, the solution was stirred properly and was allowed to settle for an incubation period of 120 hours under anaerobic condition (absence of oxygen) [25]. Measurement of temperature and pH was carried out at 12-hour intervals. During the process of fermentation in the closed container, microbial growth was observed every 24 hours. The solution was analyzed for bioethanol after commencement and addition of Saccharomyces Cerevisiae, Aspergillus Niger and Zymomonas mobilis at 12-hour time intervals of 12, 24, 36, 48, 60, 72, 84, 96, 108 and 120 hours using a gas chromatography method, the solution was occasionally stirred to ensure even fermentation. The fermentation process was monitored using a hydrometer. From 96 – 120 hours of fermentation, a constant specific gravity was observed which indicates fermentation is complete [26, 27]. The produced bioethanol was taken for distillation to remove water content and impurities. Fractional distillation was carried out and more purified bioethanol with low moisture content was achieved [28].

2.3 Characterization of Molasses

The properties of the sugarcane molasses that were measured include moisture content, brix (sugar content), viscosity, refractive index, and density.

2.3.1 Determination of moisture content

The moisture content was determined according to ASTM D1364-94 standard test method [29]. An empty dish with lid open was heated for 30 minutes in an oven at 105°C, then it was removed from the oven, the lid was replaced and placed in a desiccator at room temperature then weighted. 20g of molasses was placed in the dish and weighted with the lid on it. It was returned to the oven, with the open state for exactly 3 hours. The lid was replaced and remove to the desiccator and weight at room temperature and moisture content of the sugarcane molasses was determined using equation 2 [30].

$$Moisture\ percentage = \frac{100\ (M_2 - M_3)}{M_2 - M_1} \qquad (2\}$$

Where M_1 = mass of dish (g); M_2 = mass of dish + sample before drying (g); M_3 = mass of dish + sample after drying (g) [31].

2.3.2 Determination of brix (sugar content)

The brix was determined according to ASTM D1218-12 standard test method for refractive index and refractive dispersion of hydrocarbon liquids [29]. A sample of molasses was filtered to remove any particulate matter, and it was then transferred to a refractometer using a pipette. The refractometer was calibrated using distilled water, the refractometer was place in a temperature-controlled environment, and it was allowed to equilibrate for a few minutes. The reading of the refractive index of the sample will be taken using the refractometer. The brix of the molasses was determine using equation 2 [32].

$$Brix = \frac{(nD - 1.333)}{0.013} \tag{3}$$

Where nD is the refractive index of the sample [33].

2.3.3 Determination of viscosity

The viscosity of the molasses was determined using the ASTM D789-00 [34] standard test method for determination of viscosity using a stormer viscometer or using a U-shaped viscometer tube. The viscosity is generally given in centipoises (mpa) or centistock (cm^2/s), or as a relative viscosity [29]. The sample and distilled water were measured at 30°C using a water-bath. The viscosity of the molasses was determined using equations 4 and 5.

$$Kinematic\ viscosity = \frac{Flowtime\ of\ sample\ at\ 30°C \times 1.0038}{Flowtime\ of\ water\ at\ 30°C} \tag{4}$$

Where: 1.0083 = viscosity of water at 20°C.

$$Relative\ viscosity = \frac{T - T_o}{T_o} \tag{5}$$

Where T = flow time of the sample, T_o = flow time of distilled water at the same temperature [33]. Viscosity is often expressed in centipoise (cP) or Pascal-seconds (Pa·s).

2.3.4 Determination of refractive index

The refractive index of molasses can be determined using the ASTM D1218-12 standard test method for refractive index and refractive dispersion of hydrocarbon liquids [32]. A sample of molasses was filtered to remove any particulate matter, and it was transferred to a refractometer using a pipette. The refractometer was calibrated using distilled water, the refractometer was placed in a temperature-controlled environment, and it was allowed to equilibrate for a few minutes. The reading of the refractive index of the sample was taken using the refractometer [29].

2.3.5 Determination of density

The Density test was carried out according to ASTM D891 standard test method. This method involves using a pycnometer to measure the mass and volume of the molasses. The empty pycnometer was weighed using an analytical balance and was recorded as W_1. The pycnometer was filled with distilled water and is weighed as W_2. The temperature of the water in the pycnometer was measured using a thermometer as T_1. The pycnometer was emptied, dried and weighed as W_3. The pycnometer was filled with molasses and weigh it accurately using an analytical balance and was recorded as W_4, the temperature was measured and recorded as T_2 [30]. The density of the sugarcane molasses was determined using equations 5, 6 and 7.

$$V = \frac{W_2 - W_1}{\rho W} \tag{6}$$

Where V= volume of the pycnometer, W_1 = weight of empty pycnometer, W_2 = weight of pycnometer filled with distilled water, W_3 = weight of empty pycnometer after drying, W_4 = weight of pycnometer filled with molasses, T_1 = Temperature of water inside pycnometer, T_2 = Temperature of molasses inside pycnometer. The mass of the molasses is calculated as:

$$M = W_4 - W_3 \tag{7}$$

Where M = mass of the molasses sample. The density of the molasses is calculated as:

$$\rho m = \frac{M}{V} \tag{8}$$

Where ρm = density of molasses, M = mass of the molasses sample, V = volume of the pycnometer [33].

2.4 Characterization of bioethanol

The produced ethanol was characterized according to the ASTM standards to determine its properties; flash point, pour point, cloud point, ethanol concentration, ash content, water content and viscosity.

2.4.1 Determination of flash point

Pensky Martens (ASTM D93) method was used to determine the flash point of the bioethanol [7]. The bioethanol sample was placed in a brass cup in such a quantity as to just touch the prescribed mark on the inside of the cup. The cover is then fitted into position on the cup. A hotplate was used to provide heat to the lower side of the apparatus. A flame was then inserted into the brass cup at every 2°C temperature rise of the bioethanol and the sample was continuously stirred. As the sample approaches the temperature of the flash, the injector burner is light on and then injected into the sample at about 12-second intervals until a distinct flash is observed within the container and the injector burner is put off. At this point, the close flash point is noted with the aid of a thermometer. The flash point was then recorded [28].

2.4.2 Determination of pour point

The pour point of each sample was determined in accordance with the ASTM D97 standard. A portion of the bioethanol was poured into a test tube and the mercury point of the thermometer with calibration below 1°C, was inserted in the test tube. The setup was inserted in a beaker containing ice and left to solidify. When the solidification was confirmed, the test tube was removed and tilted and closely observed till it started to flow. The lowest temperature at which the bioethanol was observed to flow was recorded as the pour point [35].

2.4.3 Determination of Cloud Point

Cloud points were determined according to the ASTM D2500 standard. A portion of bioethanol was poured into a test tube and the mercury point of the thermometer with calibration below 1ºC was inserted in the test tube. The setup was inserted in a beaker containing ice and the bioethanol was observed closely. After some time, the bioethanol was observed to form a cloud of gel. The temperature was recorded as the cloud point [28].

2.4.4 Determination of ethanol concentration

This was determined using a refractometer with the aid of the refractive index method. The refractive index value was cross-referenced to the standard table of ethanol concentration and the value was then confirmed.

2.4.5 Determination of ash content

The test was carried out according to ASTM D482-87 standard. Some samples of bioethanol is put on a metal plate and was place over an ignited burner until the entire organic matter is charred using destructive distillation method. It was transferred into a muffle furnace and maintained at 550°C for a few hours until grey ash is obtained, after which it was allowed to cool down in a desiccator. The ash residue is weighed, and its value was recorded as the ash content of the test fuel [28]. The percentage ash content of the bioethanol is calculated as the ratio of mass of the ash to the mass of the sample.

2.4.6 Determination of density

The test was carried out according to ASTM 1298 standard. The density bottle has been washed, dried, and weighted. Certain amount of bioethanol sample was measure into the density bottle and the circulating bath was burn at a certain temperature in a Gallenkemp Ballistic Bomb Calorimeter, after which the weight of the bioethanol was measured using weighing balance [7]. Equation 8 was used in measuring the density of the produced bioethanol.

$$\rho = \frac{M}{V} \tag{9}$$

Where ρ is the density in kg/m^3, M is the mass in kg and V is the volume m^3.

2.4.7 Determination of octane number

The test was carried out in accordance with ASTM D2700-86 standard which is an engine-based test. The octane number was measured using a portable octane meter adapter.

3 RESULTS AND DISCUSSION

3.1 Characterization of molasses

The result of the characterization of major properties of sugarcane molasses was repeated thrice, and the average of each parameter investigated is presented.

3.1.1 Moisture content of molasses

Moisture content is one of the basic properties to be tested in any material to be utilized as a feedstock for bioethanol production [28]. It has been reported that for sugarcane molasses to be suitable for the production of bioethanol, the moisture content must be within the range of 22-33% value higher than the set standard limit will alter the efficiency of the fermentation process [28, 30, 32]. The moisture content of sugarcane molasses was determined by the oven drying method (ASTM D1364-94 standard), the experimental results are shown in Table 3 below. Equation 1 was used to compute the moisture content of the molasses; it was found to be 25.95% which is within the set limit and is in accordance with ASTM standards.

Table 3. Moisture content of molasses experimental data

S/N	Weight	Amount (g)
1	Weight of empty watch glass (w_1)	33.813g
2	Weight of empty watch glass plus sample before drying (w_2)	35.813g
3	Weight of empty watch glass plus sample after drying (w_3)	35.294g

3.1.2 Brix (sugar content)

Brix is the amount of sugar content present in a sugarcane juice sample [36]. The brix was determined according to ASTM D1218-12 standard test method for refractive index and refractive dispersion of hydrocarbon liquids using equation 2 [37]. The brix content of the sugarcane molasses was determined to be 35.69 mmol/l which was found to be lower than the standard (75-85 mmol/l) range, this may be associated to the degree of refining and processing.

3.1.3 pH value

The pH value of the sugarcane molasses was found to be 7.9 which has a slight variation to the standard range 4.5 to 7.0. An appreciable pH can provide the enabling environment for the use of fungi in the fermentation process. Fungi aid fermentation effectively within the range of 4-6 [28]. The difference in pH values with other reported works such as Hawas *et al*., [38] can be associated with the type of feedstock, the pre-treatment method employed, and the geographical location where feedstock was obtained [28, 39].

3.1.4 Viscosity

The viscosity of molasses was determined in accordance with ASTM D789-00 [34] method using equations 2 and 3, and was found to be 436.5 cm^2/s.

3.1.5 Density

The Density test was carried out in accordance with the ASTM D891 standard test method and was computed using equation 7 and was found to be 1.183g/cm^3.

3.2 Production of Bioethanol

After fermentation of the sugarcane molasses using *Saccharomyces Cerevisiae, Aspergillus Niger and Zymomonas mobilis* as microorganisms, the experimental result shows 6kg (18.4%) bioethanol yield was obtained at a substrate concentration of 36.25kg, fungi concentration of 5kg, fermentation period of 120 hours and a temperature of 25°C as shown in Table 4. The experimental results indicate 18.4% yield of the produced bioethanol was lower than the expected 25-30% (ASTM standard) which is due to high sugar causing osmotic pressure that slows down the metabolic activities of the yeast thereby resulting in slower fermentation rate and lower bioethanol production. Also, yeasts are highly sensitive to pH, and a higher pH can inhibit their enzymatic activities thereby slowing down the fermentation [39].

Table 4. Bioethanol experimental data

S/N	Parameters	Value
1	Initial volume of molasses mixture (L)	25
2	Sugar content (^{0}Bx)	35.69
3	pH value	7.9
4	Fermentation duration (hr)	120
5	Bioethanol produced (L)	4.6
6	Bioethanol yield (%)	18.4

3.3 Characterization of Bioethanol

The produced bioethanol was characterized, and the following properties as shown in Table 5 were determined.

Table 5. Summary of the physicochemical properties of bioethanol and gasoline blends

Fuel Property	BE0	BE100
Density at (kg/m^3)	740	795
Specific gravity	0.740	0.795
Kinematic viscosity at 40^{0}C (mm^2/s)	4.87	3.17
Flash Point (^{o}C)	-26.9	11.4
Pour Point (^{o}C)	-23	-9
Cloud Point (^{o}C)	12	16.7
Octane Number	94.0	109
Calorific value (MJ/Kg)	48.50	31.50

3.3.1 Density and Specific Gravity

A vital consideration in ethanol fuel injection systems is density. It is necessary to keep the density value within acceptable limits (789 to 793 kg/cm^3) to provide the best air-fuel ratio for full combustion [22]. Particulate matter emissions and incomplete combustion can result from high-density bioethanol. The density of the bioethanol produced was found to be 795 kg/cm^{3,} which is slightly higher than the ASTM D891 range of 789 to 793 kg/cm^3 [22], 789 kg/cm^3 [28], 790 kg/cm^3 [40], and 777.2 kg/cm^3 [41]. The experimental result is slightly greater than the ASTM standard bioethanol test method (ASTM D891) range of 789 to 793 kg/cm^3 as reported by Deng *et al*., [22]. It is important to note that the slight disparity in density observed can be strongly attributed to differences in feedstock used, moisture content, fermentation process employed and presence of impurities [28]. The specific gravity of a fuel is the weight of the fuel compared to the weight of the same volume of distilled water at a given temperature [35]. It is a very important property of bioethanol which has relevance in blending with gasoline and gives an indication of the purity of a fuel. When fuel is mixed with another liquid, its specific gravity tends to either rise or fall based on the specific gravity of the unknown substance [41]. The specific gravity of the bioethanol produced is 0.795 which is within the ASTM bioethanol standard limit [22].

3.3.2 Flash point

This is a key property in determining the flammability of a fuel [42]. The flash point indicates the lowest temperature at which the fuel can vaporize to produce an ignitable mixture with air, it is therefore the tendency of a sample to form flammable mixture [28]. Sintali *et al*. [41] stated that liquids with flash points which are less than 37.8^{o}C are referred to as flammable and combustible liquids. The standard flash point of bioethanol is 12^{o}C, and the produced bioethanol has a flash point of 11.4^{o}C which can also be characterized as flammable and combustible fuel and showed a closed proximity to 12.5^{o}C as reported by Musa [43] and conformed to ASTM D93 standard test method. The result obtained shows that the bioethanol produced does not pose a hazard at low temperatures during storage [28].

3.3.3 Pour point

The pour point is an important characteristic of bioethanol that says the lowest operational temperature of the bioethanol [43]. The higher pour point of biofuel often limits its use as fuel for engines in cold climatic conditions [7]. The pour point was determined according to ASTM D97 standard test method. The experimental value obtained was -9°C which is by far lower than the 5.20°C stipulated by the ASTM standard. The result is a definite indication that the produced bioethanol can be used effectively and adequately even in a polar region when the ambient temperature is not below -9°C.

3.3.4 Cloud point

Cloud point is described as the temperature at which a cloud crystal will first appear in a liquid that is cooled under prescribed condition. It provides information on the low temperature usability of a fuel under extremely cold conditions [44]. The experimental cloud point of the produced bioethanol is 16.7 ^{0}C which varies from 10 ^{0}C, 19.3 ^{0}C, and 22.5 ^{0}C as reported in literatures [28, 41, 43] respectively. The experimental cloud is lower than the ASTM standard which is 23 ^{0}C this implies that the fuel will perform satisfactory even in cold climatic conditions since the tendency for gel formation was low. Higher cloud point, however, can negatively impact emissions and engine performance in cold climates. It appears that using bioethanol fuel in an engine at a temperature lower than the fuel's cloud point can cause fuel filter clogging because of the formation of wax [45].

3.3.5 Kinematic viscosity

The efficient operability of car engines depends on the kinematic viscosity of the fuel used. The viscosity of a fuel must be given significant consideration for fuel injection combustion chambers system [28]. This property is a measure of the resistance of a substance (mostly liquids) to flow. The evaluation of fuel kinematic viscosity is required for the creation of actual fuel atomization and the engine power, and combustion efficiency are affected by injection viscosity [46]. The kinematic viscosity of the produced bioethanol was found to be 3.17 mm^2/s which is within the ASTM maximum standard value of 5 mm^2/s. Kinematic viscosity within the set of the limit must always be maintained to ensure efficient engine functionality. High injection pressure and pumping losses are experienced when highly viscous bioethanol is used, which will result in poor combustion [46] and emissions of unburned hydrocarbon [43].

3.3.6 Calorific value

The calorific value is the amount of energy produced when a fuel is completely combusted [45]. The calorific value of the produced bioethanol is 31.5 MJ/kg which is lower than gasoline (48.50 MJ/kg).

3.3.7 Octane number

The octane number of a fuel indicates its ability to resist preignition and burn evenly [45]. From Table 4.0 the experimental result showed the produced bioethanol has an octane number of 109 which conforms standard range of 108-110 ASTM D2699 standard test method [47].

4 CONCLUSIONS

Energy is a vital factor of consideration in terms of industrial growth and in the provision of required services that improve the quality of life of mankind. The search for alternative energy to either replace or complement the existing energy source which is fossil fuel led to the discovery and acceptance of biofuels as a renewable and environmentally friendly energy source. Bioethanol of the family of biofuel, has been proved in this study as a fuel with necessary potentials to replace fossil gasoline in the future. Based on the result of the study, sugarcane molasses is a good substrate for the production of bioethanol that can be used for internal combustion engines instead of fossil fuels that pollutes the environment with its emissions. The following conclusion can be drawn from the results of experimental analyses carried out in this study. Bioethanol was produced from sugarcane molasses using Saccharomyces Cerevisiae, Aspergillus Niger and Zymomonas mobilis as microorganisms. The obtained result of the characterization analyses showed clearly that the physio-chemical properties of the produced bioethanol and blends of bioethanol/gasoline fall within the ASTM specification which can be used safely in spark ignition engine and properties of the fuel were improved.

REFERENCES

1. Adewuyi, A. (2020). Challenges and prospects of renewable energy in Nigeria: A case of bioethanol and biodiesel production. *Energy Reports*, vol. 6, no. 4, pp. 77-88. https://doi.org/10.1016/j.egyr.2019.12.002.
2. Adeniyi, A.G. and Ighalo, J.O. (2019). A review of steam reforming of glycerol. *Chemical Papers*, vol. 73, no. 11, pp. 2619-2635. https://doi.org/10.1007/s11696-019-00840-8.
3. Wang, S., Liu, S., Zhang, J., Che, X., Wang, Z. and Kong, D. (2020). Feasibility study on prediction of gasoline octane number using NIR spectroscopy combined with manifold learning and neural network. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, vol. *228*, 117836. https://doi.org/10.1016/j.saa.2019.117836.
4. Adedayo, H.B., Adio, S.A. and Oboirien, B.O. (2021). Energy research in Nigeria: A bibliometric analysis. *Energy Strategy Reviews*, vol. 34, 100629. https://doi.org/10.1016/j.esr.2021.100629.
5. Elfasakhany, A. (2016). Engine performance evaluation and pollutant emissions analysis using ternary bio-ethanol–iso-butanol–gasoline blends in gasoline engines. *Journal of Cleaner Production*, vol. 139, pp. 1057-1067.
6. Saikrishnan, V., Karthikeyan, A. and Jayaprabakar, J. (2018). Analysis of ethanol blends on spark ignition engines. *International Journal of Ambient Energy*, vol. *39*, no. 2, pp. 103-107. https://doi.org/10.1080/01430750.2016.1269678.
7. Yami, A.M. (2021). Production and optimization of biodiesel from Neem and Yellow Oleander seed oils and performance evaluation of their blends in compression ignition engine. *Unpublished Doctor of Philosophy (PhD) thesis, Mechanical Engineering Department, Modibbo Adama University of Technology Yola, Nigeria*.
8. Gabisa, E.W., Bessou, C. and Gheewala, S.H. (2019). Life cycle environmental performance and energy balance of ethanol production based on sugarcane molasses in Ethiopia. *Journal of Cleaner Production*, *234*, 43–53.
9. Tian, Z., Zhen, X., Wang, Y., Liu, D. and Li, X. (2020). Comparative study on combustion and emission characteristics of methanol, ethanol and butanol fuel in TISI engine. *Fuel*, vol. 259, pp. 116-199. https://doi.org/10.1016/j.fuel.2019.116199.
10. Tan, Y.H., Abdullah, M.O., Kansedo, J., Mubarak, N.M., Chan, Y.S. and Nolasco-Hipolito, C. (2019). Biodiesel production from used cooking oil using green solid catalyst derived from calcined fusion waste chicken and fish bones. *Renewable Energy*, vol. 139, pp. 696-706.

https://doi.org/10.1016/j.renene.2019.02.110.

11. Bunmi, A. (2021). National Resource and Extraction Program media oil and gas, Fellowship of the premium Times Centre for Investigation Journalism.

12. Kumar, S., Shrestha, P. and Salam, P.A. (2013). A review of biofuel policies in the major biofuel producing countries of ASEAN: production, targets, policy drivers and impacts. *Renew Sust Energ* Rev., vol. 26, pp. 22-36.

13. Uría-Martínez, R., Leiby, P.N. and Brown, M.L. (2018). Energy security role of biofuels in evolving liquid fuel markets. *Biofuels, Bioproducts and Biorefining*, vol. 12, no. 5, pp. 802-814. https://doi.org/10.1002/bbb.1891.

14. Nabel, A.N., Maram, T.H., Abou, K., Youssif, M.A. and Mona Y.M. (2017). Biofuels from Vegetable Oils as Alternative Fuels Advantages and Disadvantages, *Egyptian National Scientific and Technical Information Network*, vol. 5, pp. 289-365. DOI:10.1201/9781315120829-18, https://www.researchgate.net/publication/319852971.

15. Susmozas, A., Martín-Sampedro, R., Ibarra, D., Eugenio, M.E., Iglesias, R., Manzanares, P. and Moreno, A.D. (2020). Process Strategies for the Transition of 1G to Advanced Bioethanol Production. *Processes*, vol. 8, no. 10, 1310. https://doi.org/10.3390/pr8101310.

16. Grand View Research (2024). Fuel ethanol market size, share & trends analysis report by product (starch-based, sugar-based, cellulosic), by technology (wet mill, dry mill), by application (conventional fuel vehicles), by region, and segment forecasts, *2024–2030*. https://www.grandviewresearch.com/industry-analysis/fuel-ethanol-market.

17. Satari, B., Karimi, K. and Kumar, R. (2019). Cellulose solvent-based pretreatment for enhanced second-generation biofuel production: a review. *Sustainable Energy & Fuels*, vol. *3*, no. 1, pp. 11-62. https://doi.org/10.1039/c8se00287h.

18. Ayodele, B.V., Alsaffar, M.A. and Mustapa, S.I. (2020). An overview of integration opportunities for sustainable bioethanol production from first- and second-generation sugar-based feedstocks. *Journal of Cleaner Production*, vol. 245, 118857. https://doi.org/10.1016/j.jclepro.2019.118857.

19. Arshad, M., Hussain, T., Iqbal, M. and Abbas, M. (2017). Enhanced ethanol production at commercial scale from molasses using high gravity technology by mutant S. cerevisiae. *Brazilian Journal of Microbiology*, vol. 48, no. 3, pp. 403-409.

20. Nigeria biofuel policy and incentive (2021). Nigerian bio-fuel policy and incentives. *Official Gazette. Nigerian National Petroleum Corporation (NNPC)*.

21. Ugwu, C., Uzo, P., Ozoegwu, C. and Agwu, N. (2022). Biomass Resources in Nigeria and the Conversion Pathways. *IEOM Society International*, vol. 7, pp. 746-756.

22. Deng, X., Chen, Z., Wang, X., Zhen, H. and Xie, R. (2018). Exhaust noise, performance and emission characteristics of spark ignition engine fuelled with pure gasoline and hydrous ethanol gasoline blends. *Case Studies in Thermal Engineering*, vol. 12, pp. 55-63. https://doi.org/10.1016/j.csite.2018.02.004.

23. Dhande, D.Y., Sinaga, N. and Dahe, K.B. (2021). Study on combustion, performance and exhaust emissions of bioethanol-gasoline blended spark ignition engine. *Heliyon*, vol. 7, no. 3, e06380. https://doi.org/10.1016/j.heliyon.2021.e06380.

24. Arora, R., Behera, S. and Sharma, N. (2019). Evaluating the Pathway for Co-fermentation of Glucose and Xylose for Enhanced Bioethanol Production Using Flux Balance Analysis. *Publishing Research Inn Applied Microbiology and Biotechnology*, vol. 24, pp. 924-933. https://link.springer.com/article/10.1007/s12257-019-0026-5.

25. Bilyaminu, A., Nasir, I.A. and Umar, B. (2016). Optimization of bioethanol production from Nigerian sugarcane juice using factorial design. *Advances in Energy Research*, vol. 4, no. 1, pp. 1-11.

26. Raharja, R., Murdiyatmo, U., Sutrisno, A. and Wardani, A.K. (2019). Bioethanol production from sugarcane molasses by instant dry yeast. *IOP Conference Series: Earth and Environmental Science*, vol. 230, 012076, DOI:10.1088/1755-1315/230/1/012076.

27. Khoshkho, S.M., Mahdavian, M., Karimi, F., Karimi-Maleh, H. and Razaghi, P. (2022). Production of bioethanol from carrot pulp in the presence of Saccharomyces cerevisiae and beet molasses inoculum; A biomass-based investigation. *Chemosphere*, vol. 286, 1316. https://doi.org/10.1016/j.chemosphere.2021.131688.

28. Suleiman, B., Abdulkareem, S.A., Afolabi, E.A., Musa, U., Mohammed, I.A. and Eyikanmi, T. A. (2016). Optimization of bioethanol production from nigerian sugarcane juice using factorial design. *Advances in Energy Research*, vol. 4, no. 1, pp. 69-86.

29. Gambo, B.A., Robinson, I.E., Dandakouta, H. and Tokan, A. (2020). Monitoring and Evaluation of Exhaust Gas Emission of Gasoline - Cadoba Farinosa Forskk Bio-Ethanol Blended Fuel Samples Run on a Spark Ignition Engine. *International Journal of Engineering Research And*, vol. 9, no. 1, pp. 222-226. DOI:10.17577/IJERTV9IS010133.

30. Zhang, L., Shen, Q., Pang, C.H., Chao, W., Tong, S., Kow, K.W., Lester, E., Wu, T., Shang, L., Song, X., Sun, N. and Wei, W. (2023). Life cycle assessment of bio-fermentation ethanol production and its influence in China's steeling industry. *Journal of Cleaner Production*, vol. 397, pp. 136-492. https://doi.org/10.1016/j.jclepro.2023.136492.

31. Uslu, S. and Celik, M.B. (2020). Performance and Exhaust Emission Prediction of a SI Engine Fueled with I-amyl Alcohol-Gasoline Blends: An ANN Coupled RSM Based Optimization. *Fuel*, vol. 265, 116922. https://doi.org/10.1016/j.fuel.2019.116922.

32. Baral, S. and Upadhyay, R. (2024). Environmental impact and energy balance assessment in ethanol production from sugarcane molasses. *Clean Technologies and Environmental Policy*, vol. 26, no. 1, pp. 45-58. https://doi.org/10.1007/s10098-023-02576-4.

33. Yahuza, I., Dandakouta, H., Ejilah, R.I., Dasin, D.Y. and Farinwata, S.S. (2018). Exhaust Emissions Characterization of a Single Cylinder Diesel Engine Fueled with Biodiesel-Ethanol-Diesel Blends. *International Journal of Energy Engineering*, vol. 8, no. 1, pp. 19-24. https://doi.org/10.5923/j.ijee.20180801.03.

34. ASTM D789-00 (2016). Standard test method for determination of relative viscosity of polyamide (PA) solutions. *ASTM International. West Conshohocken, PA* 2016.

35. Awad, O.I., Mamat, R., Ibrahim, T.K., Kettner, M., Kadirgama, K., Leman, A.M. and Saiful, A. I.M. (2018). Effects of fusel oil water content reduction on fuel properties, performance and emissions of SI engine fueled with gasoline-fusel oil blends. *Renewable Energy*, vol. 118, pp. 858-869.

36. Verma, N. and Kumar, V. (2021). Microbial conversion of waste biomass into bioethanol: Current challenges and future prospects. *Biomass Conversion and Biorefinery*, vol. 13, no. 2, pp. 6419-6456. https://doi.org/10.1007/s13399-021-01824-z.

37. Baral, S. and Upadhyay, R. (2024). Environmental impact and energy balance assessment in ethanol production from sugarcane molasses. *Clean Technologies and Environmental Policy*, vol. 26, no. 1, pp. 45-58. https://doi.org/10.1007/s10098-023-02576-4.

38. Hawaz, E., Tafesse, M., Tesfaye, A., Beyene, D., Kiros, S., Kebede, G., Boekhout, T., Theelen, B., Groenewald, M., Degefe, A., Degu, S., Admas, A. and Muleta, D. (2022). Isolation and

characterization of bioethanol producing wild yeasts from bio-wastes and co-products of sugar factories. *Ann Microbiol*, vol. 72, no. 1, pp. 1-39.

39. Mohammed, A.H., Mohammed, A.K., Kamar, F.H., Abbas, A.A. and Nechifor, G. (2018). Determination of ethanol in fermented broth bu headspace gas chromatography using capillary column. Rev Chim Burcharest-Orig Ed, vol. 69, no. 11, pp. 2969-2972.

40. Nwufo, O.C., Nwaiwu, C.F., Ononogbo, C., Igbokwe, J.O., Nwafor, O.M.I. and Anyanwu, E.E. (2017). Performance, emission and combustion characteristics of a single cylinder spark ignition engine using ethanol–petrol-blended fuels. *International Journal of Ambient Energy*, vol. *39*, no. 8, pp. 792-801.

41. Sintali, S.I., Innocent, C., Adekunle, A.M. and Juma, H.T. (2023). An exhaust emission characteristic in a spark ignition engine fueled by petrol-snot apple (Azanza garckeana) bioethanol blends. International Journal of Advance Research and Innovative Ideas in Education, vol. 9, no. 5, pp.993-999.

42. Hubert, K. (2019). Experimental investigation of the autoignition properties of ethanol-biodiesel fuel blends. *Fuels*, vol. 235, no. 1, pp. 1301-1308.

43. Musa, N.A. (2019). Production and fuel characterization of bioethanol from dika– nutshell. *Acta Technica Corviniensis: Bulletin of Engineering Tome XII [2019], Fascicule 2 [April – June]*, pp. 101-104.

44. Sakai, S. and Rothamer, D. (2019). Impact of ethanol blending on particulate emissions from a spark-ignition direct-injection engine. *Fuel*, *236*, 1548–1558.

45. Amir, H.N., Bahman N., Sina A., Gholamhossein J. and Amirhosein M. (2023): The Effect of Biodiesel, Ethanol, and Water on the Performance and Emissions of a Dual-Fuel Diesel Engine with Natural Gas: Sustainable Energy Production through a Life Cycle Assessment Approach: *International Journal of Energy Research*, pp. 1-24. https://doi.org/10.1155/2023/4630828.

46. Sirvio, K., Niemi, S., Help, R., Heikkila, S. and Hiltunen, E. (2018). Kinematic viscosity studies for medium–speed CI engine fuel blends. *Agronomy Research*, vol. 16, no. S1, pp. 1247-1256.

47. Catapano, F., Di Iorio, S., Luise, L., Sementa, P. and Vaglieco, B.M. (2019). Influence of ethanol blended and dual fueled with gasoline on soot formation and particulate matter emissions in a small displacement spark ignition engine. *Fuel*, vol. 245, pp. 253-262. https://doi.org/10.1016/j.fuel.2019.01.173.