Comparative Study of Bioethanol Produced from Different Agro-Industrial Biomass Residues

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Abstract

Bioethanol was produced from the three different agro-industrial biomass residues, i.e., sugarcane bagasse (SB), rice husk (RH) and corn cob (CC)) at 35°C, 120hr with 90g of each substrate. 2% H_2SO_4 was used for hydrolysis of the samples while 3g of yeast (*saccharomyces cerevisiae*) was used for fermentation. Simple distillation was used for the distillation of the fermented broth. The concentration of reducing sugar and ethanol, quantity of produced bioethanol as well as the physical properties (pH, density, viscosity and flash point) was investigated. SB has the highest concentration of reducing sugar and ethanol as well as the quantity of produced bioethanol. The pH of bioethanol generated from all the three substrates are within the bioethanol standard value while the density, viscosity and flash point were higher than bioethanol standards. It was concluded that both SB, RH and CC has the potential of bioethanol production in commercial quantity under well-chosen production conditions.

1. Introduction

The ever-increasing demands for energy due to rapid increase in global population, industrialization, and geopolitical factors have called for the search for alternative and carbon neutral sources of energy [5, 16]. For many years now, the primary sources of energy have been non-renewable fossil fuels, oil, natural gas, and coal. However, these energy sources are inadequate to fulfil today's most significant requirements of the societies from the environmental and public health perspectives. The widespread application of conventional energy resources has contributed to serious challenges, including global warming and climate change by releasing greenhouse gases (GHGs) like carbon dioxide (CO_2), methane (CH_4), nitrous oxide (N_2O), and chlorofluorocarbons [10]. These adverse impacts have overshadowed the previous justifications used, including increasing petroleum prices, finite nature of fossil fuels, and have encourage the government and non-government agencies to find environmentally friendly, renewable, and sustainable energy resources for transportation, heating, and electricity generation [14].

According to Naik *et al.* [13], renewable energy is now capturing a significant headline worldwide due to concerns about declining supplies of fossil fuels, escalating population and industrialization triggering ever-increasing demand of fuels. The higher price of oil has attracted the greater attention to biofuels, especially bioethanol, biodiesel, biohydrogen, to list a few. More so, the importance of alternative energy source has become even more necessary not only due to the continuous depletion of limited fossil fuel stock but also for a safe and better environment [7].

Bioethanol is the most used biofuel, which is an alternative to fossil fuel and is mainly produced by the hydrolysis of cellulose from lignocellulosic biomass and by the fermentation of sugars of different lignocellulosic sources [3]. The biodegradability and reduced toxicity of bioethanol, for which biomass is used as a primary substrate as well, are its main advantages over fossil fuels [15].

Currently, biomass covers about 10% of the world's primary energy demand. Against a backdrop of rising crude oil prices, depletion of resources, political instability in producing countries and environmental challenges, biomass has high potential to replace the supply of energy worldwide [1]. The available sources are plant biomass which is an abundant and renewable source of energy-rich carbohydrates which can be efficiently converted by microbes into biofuels of which, bioethanol is widely produced on an industrial scale today [1].

Advantages of bioethanol include high-octane rating resulting to increased engine efficiency and performance, low boiling point, broad flammability, higher compression ratio and heat of vaporization, comparable energy content, reduced burning time and lean burn engine [4]. However, the disadvantages include high production cost resulting from high cost of feedstock, enzymes, detoxification and ethanol recovery.

When bioethanol is produced from edible feedstocks such as corn and sugar cane, it is called first generation (1G) bioethanol and second-generation (2G) bioethanol if the feedstock is a lignocellulose. Examples of these lignocelluloses biomass is switch grass, cornstalks, wood, herbaceous crops, wastepaper and paper products, agricultural and forestry residues, pulp and paper mill waste, municipal solid waste and food industry waste [18]. The 2G-bioethanol has a greater potential to reduce the greenhouse gases emission compared to 1G -bioethanol. The third generation (3G) bioethanol is obtained when algae are used as the feedstock. Algae bioethanol is gaining traction possibly due to high carbohydrate content and absence of lignin in most available algae. With this kind of feedstock, the cost of pre-treatment is expected to reduce as the complex lignin removal process is eliminated [2]. The fourth generation (4G) bioethanol is obtained from the modification of *E. coli* gene altercations through the application of metabolic engineering or systems biology strategies [9].

Bioethanol is considered as the most promising biofuel to replace gasoline, especially due to its properties. It possesses a low volumetric energy density, meaning that more volume of bioethanol/km (up 50%) will be consume compared to the conventional gasoline [6].

Lignocellulosic biomass is being considered as feedstocks for bioethanol production due to relatively low cost of acquisition, availability and sustainability of supply. This biomass has the capacity to increase the current production rate of bioethanol and is being speculated to produce approximately 442 billion litres per year of bioethanol globally. It is worthy to understand that the use of bioethanol as a source of energy would be more than just complementing for solar, wind and other intermittent renewable energy sources in the long run [11]. Therefore, bioethanol is considered a potential substitute for the conventional gasoline and can be used directly in vehicles or blended with the gasoline, thereby reducing greenhouse gas emissions and consumption of gasoline. Thus, this study aim to conduct comparative study of bio-ethanol produced from different agro-industrial biomass residues, with the major objective of finding the feedstock potential for commercial bioethanol production.

2. Materials and Methods

2.1. Research materials

Sugarcane bagasse (SB), rice husk (RH) and corn cob (CC) were used for the study. Their choice for the study was based on their lignocellulosic composition, furthermore they are agro-industrial biomass residues (by-products of agriculture or its related industry), thus consider as waste with little or zero used.

The substrate (SB, RH and CC) were collected in polythene bags from Sokoto central market, Sokoto State, Nigeria. *Saccharomyces cerevisiae* was used as yeast for the fermentation process. The yeast was obtained at meat and vegetable market Sokoto state, Nigeria.

2.2. Bioethanol production

Pre-treatment

Samples were dried (for five days at room temperature) to eliminate moisture in them. This was followed by size reduction of the samples (grinding to powder) using pestle and mortar and then sieved through 36 μm mesh.

Hydrolysis

90g of powder substrate (for each sample) was mixed in 2% H_2SO_4. The substrate vessels were capped with cotton wool wrapped in aluminium foil and sterilized at 121°C for 15 mins. They were then allowed to cool and hydrolyse for five days. The content of each flask was filtered, and pH value was adjusted to 5 by adding NaOH (to prevent microorganisms from dying in hyper acidic condition) before fermentation. Glucose assay was carried out using Benedict's test to confirm the presence of reducing sugar prior to the fermentation processes.

Fermentation

3g of yeast (*saccharomyces cerevisiae*) was added to peptone water to activate the yeast and then added to the hydrolysed substrate. The substrates were properly covered with cotton wool wrapped in aluminium foil strapped with a masking tape. The samples were shaken and taken to the incubator for a period of five days at a temperature of 35°C.

Distillation of the fermented broth

The distillation of fermented broth was carried out-out using simple distillation. The

fermented substrate was dispensed into a round bottom flask that was fixed in a distillation column and enclosed in a running tap. The flask was heated on a heating mantle at 78°C for 30 minutes. The distillates were collected at the end of the distillation column.

2.3. Analysis and Characterization

Determination of reducing sugar concentration

Reducing sugar was determined using Bertrand method and spectrophotometer. This was carried out by adding $2cm^3$ of dinitro salicylic acid (DNS) reagents in $1cm^3$ of each sample in a lightly capped test tube. The mixture was heated at 90°C in water-bath for 15 minutes till red-brown coloration is developed. After the solution was cooled to room temperature, the absorbance of the solution was recorded with the help of UV spectrometer at 540nm. The above procedure was repeated for each of the filtered substrate.

$$C.R.S. = \frac{\text{Absorbance of sample}}{\text{Absorbance of glucose standard}} \times \text{Conc. of standard}$$

Determination of ethanol

Two drops of acidified 0.1M potassium dichromate ($K_2Cr_2O_7$) were added to the $2cm^3$ of the distillate produced, it was then heated for 30 minutes in a water bath. The content of the test tube changed to green colour indicating the presence of ethanol.

Determination of ethanol concentration

This was carried out by adding $3cm^3$ of potassium dichromate ($K_2Cr_2O_7$) followed by $3cm^3$ of distillate (bioethanol) and equalized with distilled water in a test tube. The mixture was heated using heating mantle for 15 minutes at 90°C to develop green colour. Then the absorbance of the sample was measured at 575nm using ultraviolet spectrophotometer.

$$\% \text{ of ethanol} = \frac{\text{Absorbance test} \times \text{concentration of standard}}{\text{Absorbance of standard}}$$

Determination of the quantity of bioethanol produced

The distillate collected over a slow heat at 78°C was measured using a measuring cylinder and expressed as the quantity of ethanol produced in g/cm^3 by multiplying the volume of the distillate by the density of ethanol.

Physical characterization of bioethanol

The pH, density, viscosity and flash point of the produced bioethanol was analysed using ASTM standard D1613, D1298-99, D445 and D93 respectively.

3. Results and Discussion

Table 1 and Figure 1 depict the reducing sugar concentration, ethanol concentration and quantity of produced bioethanol obtained from the three different substrates: sugarcane bagasse (SB), rice husk (RH) and corn cob (CC) at 35℃, 120hr with 90g of each substrate. The results obtained indicates that the highest concentration of reducing sugars is obtained from SB. The ethanol concentration and quantity of produced bioethanol is also higher in SB compared to RH and CC. This was expected due to the higher concentration of reducing sugar from SB. The fermentable sugars from SB have no doubt play a vital role in the high ethanol concentration and quantity of produced bioethanol from SB in contrast to RH and CC. In addition to the low concentration of reducing sugar, the accumulation of toxic compounds or secondary metabolites that are generated during fermentation process might be one of the reasons behind the low ethanol concentration and quantity of produced bioethanol from RH and CC.

Direct comparison of previous studies with present study is not possible due to differences in variables and source of substrates. However, previous studies by Irfan *et al*. [8] using three different substrates i.e. sugarcane bagasse, rice straw and wheat straw with *Sacchromyces cerevisiae* for bioethanol production found that sugarcane bagasse produced more ethanol compared to rice straw and wheat straw. This is in good agreement with the present studies. The work of Sasikumar and Viruthagiri [17] reported that maximum ethanol production can be obtained from pre-treated sugarcane bagasse under optimized process conditions in aerobic batch fermentation. This is also in good agreement with the present study to some extent.
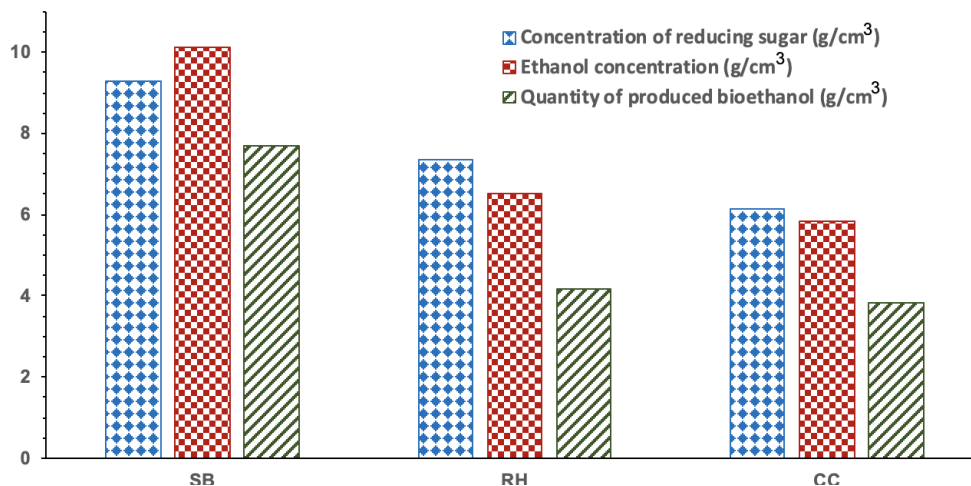
Figure 1. Concentration of reducing sugar, ethanol concentration and quantity of produced bioethanol in g/cm^3 (Note: SB = Sugarcane bagasse, RH = rice husk and CC = corn cob).

Table 1. Concentration of reducing sugar, ethanol concentration and quantity of produced bioethanol in g/cm^3 (Note: SB = Sugarcane bagasse, RH = rice husk and CC = corn cob)

Analysis	SB	RH	CC
Concentration of reducing sugar(g/cm^3)	9.27	7.35	6.13
Ethanol concentration (g/cm^3)	10.11	6.51	5.83
Quantity of produced bioethanol (g/cm^3)	7.68	4.17	3.82

Table 2 shows the physical properties test of bioethanol produced from the three substrates. The pH value depicts acid strength in bioethanol and can be used to determine the possibility of corrosion of bioethanol. The pH values of the bioethanol generated from the three substrates fall within the standard bioethanol pH as shown in Table 2. It is worth mentioning that the pH valuehas significant effect on fuel injector, engine cylinder and fuel pump. When a pH value is <6.5 they (fuel injector, engine cylinder and fuel pump)might stop working. Furthermore, when pH value is > 9.0 parts of the fuel pump plastic will stop working [12].

Density is a measure of the amount of matter contained by a given volume. The quality of ignition of fuel is significantly affected by density [12]. The density of the produced bioethanol (from all the three substrates)is higher than the standard value. The density of CC is the lowest, thus, the closest to the standard bioethanol value.

"Viscosity is the amount of the resistance of a fluid being deformed either by share stress or extensional stress". Viscosity is usually seen as thickness or resistance to flow. Table 2 shows that the lowest viscosity is from bioethanol generated from RH(1.785cSt). Furthermore, the viscosity of all the produced bioethanol is higher than the bioethanol standard value. Viscosity changes with change in temperature. As temperature decreases, viscosity increases and vice versa. Viscosity is a very vital property in the storage and use of fuel. High fuel viscosity (thick fuel) causes difficulty in pumping for the engine, difficulty in igniting the burner and difficulty in the flow of the fuel. High fuel viscosity also aggravates atomization. Thus, prompting the formation of carbon deposition on cylinder wall of the engine [12].

Flash point is a flammability of a fuel. It is the lowermost temperature in which a fuel ignites when exposed to an ignition source. Flash point is a very important property to ascertain the magnitude of hazards during travel or fuel storage. The results as presented in Table 2 shows that the flash point of all the produced bioethanol is very high compared to the bioethanol standard that is 12°C. This implies that the bioethanol produced is less flammable than the standard bioethanol fuel. There is a probability that the density had play a role in the high values seen in the flash points of the generated bioethanol.

Table 2. Physical properties test of produced bioethanol (Note: SB = Sugarcane bagasse, RH = rice husk and CC = corn cob)

Properties	SB	RH	CC	Bioethanol Standard
pH	7.2	6.8	8.3	6.5-9.0
Density(g/cm^3)	0.994	1.07	0.847	0.789
Viscosity(Cst)	1.900	1.785	2.197	1.525
Flash point (°C)	17	19	15	12

4. Conclusion

The present study showed that sugarcane bagasse (SB), rice husk (RH) and corn cob (CC) are both potential substrates for bioethanol production. However, SB has the highest concentration of reducing sugar and ethanol with highest quantity of bioethanol at the same production conditions. The pH values of bioethanol generated from all the three substrates fall within the bioethanol standard while the density, viscosity and flash point were not within the bioethanol standard. The later can be adjusted to the bioethanol standard by careful selection of production conditions.

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