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COMPARATIVE STUDY OF BIOETHANOL POTENTIALS PRODUCED FROM YAM PEELS AND BAMBARA NUT SHELLS

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ABSTRACT

In this study, Yam peels and Bambara nut shells were hydrolyzed with 0.25m, 0.50m, 1.00m, and 2.00m concentration of dilute sulphuric acid (H_2SO_4) respectively and the reducing sugar concentration were determined using the dinitrosalicylic acid (DNS) colorimetric method. The results revealed that there is no significant difference ($p<0.05$) in the yields of the reducing sugar obtained from the two substrates at different treatment conditions. Confirmatory tests and other fuel properties such as; specific gravity, boiling point, flash point, pour point, cloud point, octane number FTIR and GCMS analysis were also determined in order to ascertained that the distillate produced were actually ethanol with respect to the standard from WHO/DPR and ASTM.

Keywords: Acid hydrolysis; Bambara nut shell; fermentation; DNSFTIR; GC/MS; ASTM; WHO; DPR.

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INTRODUCTION

Whether cost-effectively advanced or at different stages of advancement, most nations are facing with the difficulty of discarding and treatment of wastes. There are several ways of treating the wastes (e.g. by reducing its vastness or by recuperating and reprocessing it into useful substance) to meet sanitary standards. Ethanol fermented from renewable sources for fuel or fuel additives are known as bio-ethanol. One of the greatest tasks for humanity in the twenty-first century is that fossil fuels are depleting day by day and considered as limited and non-renewable energy. In addition, the availability of these non-renewable energy resources will certainly drop as a result of the escalation in energy demands and the limitation of energy resources. According to Croockes *et al*. (2006), the production of oil is expected to deteriorate in the next 10-100 years.

The long-time period blessings of the usage of discarded excesses as lignocellulosic feed stocks could be to bring collectively a sustainable strong waste control approach for a number of lignocellulosic waste materials, which contribute to the moderation in greenhouse gases through unrelenting carbon and nutrient recycling, lessen the capacity for water, air, and soil infection related to the land utility of organic waste materials, and to boost the feedstock supply of raw substances for the bioethanol industrialized enterprise (Rabah *et al.,* 2011).

The purpose of this research work is to optimize and compare the potential of bioethanol produced from yam peels and Bambara nut shell.

MATERIALS AND METHODS

Sample collection

Samples of the Yam peels and Bambara nut shell were acquired from Kaboji, Mashegu Local Government Area, Niger State, Nigeria.

Sample Preparation

The samples were sundried clumsily for two weeks and grinded to powder using a mortar and pestle to upturn the surface area for hydrolysis process.

Preparation of Reagents

Exactly 1.40ml of concentrated H_2SO_4 was precisely measured and reassigned into 100ml volumetric flask and up to the mark using distilled water for preparation of 0.25M. For 0.50M, 1.00M and 2.00M about 2.80ml, 5.60ml and 11.10ml were to be precisely measured and reassigned into 100ml volumetric flask and made up to the mark using distilled water.

2M Sodium hydroxide, exactly 80g of NaOH was accurately measure and conveyed into 1000cm^3volumetric flask and up to the mark using distilled water (Rabbah *et al.,* 2011).

Dinitrosalicylic Acid (DNSA), 60ml of the 2M NaOH was accurately measured and dispensed into a conical flask followed by addition of 3g of DNSA into the conical flask. About 50g of potassium sodium tartrate (Rochelle salt) were later weighed and dissolved into 100ml of

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distilled water. The potassium sodium tartrate salt solution will then be added to the DNSA solution and the mixture was made up to 200ml with distilled water (Sheikh *et al.,* 2016).

Experimental Design

The experimental design were conducted using mat lab software using (RSM)

Run Order	Concentration (M)	Time (min)	Temperature (^{o}C)
1	0.25	80	100
2	0.25	60	70
3	0.25	105	70
4	0.50	90	40
5	0.50	60	100
6	0.50	105	70
7	0.50	75	60
8	1.00	90	60
9	1.00	105	40
10	1.00	105	100
11	1.00	60	80
12	2.00	80	40
13	2.00	105	70
14	2.00	75	80
15	2.00	90	100

Preparation of standard curve

The standard curve was calibrated by adding 2ml of DNSA reagent into 0.10, 0.20, 0.30, 0.40 and 0.50ml of glucose standard solution followed by heating over water bath for 5min. The solution would be allowed to cool in cold water before measuring the absorbance using UV-visible spectrophotometer at 540nm (Nair *et al.,* 2017).

Hydrolysis

Each run involve 1g of the Bambara nut shells in 250ml beaker, follow by addition of 150ml H_2SO_4 concentration as specified in the table 2.4. The mixture will then be heated and stirred concurrently over a magnetic stirrer for a period of time at particular temperature as specified in the matrix design table 2.4. After hydrolysis of the Bambara nut shells, a solution of sodium hydroxide was used to neutralize the pH within 4.5 to 6.0 (Mohd *et al.,* 2017).

Uv-spectrophotometer

The quantitative determination of glucose for bio-ethanol production was done using the UV-spectrophotometer at constant wavelength of 550 nm, using distilled water as blank. UV-spectrophotometer uses discrete wavelengths of light to determine the concentration of certain compounds in a sample.

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Determination of Glucose content

Spectroscopic method was employed in the determination of the glucose content present in the hydrolyzed samples using DNSA by the method reported by Ranken (1984), with a little modification which is briefly described as follows; 2ml of the DNSA reagent was added to 1ml of a sample. The mixture was then heated for about 5min over a water bath, and was allowed to cool in cold water. The absorbance of the sample was obtained using UV-visible spectrophotometer at a wavelength of 540nm. The glucose concentration was obtained with the help of the glucose standard curve.

Fermentation of the Hydrolyzed Samples

Fermentation of the hydrolyzed yam peels and Bambara nut shells were carried out using a method described by Adeeyo *et al*., (2015) with some modifications.

After *p*H of the hydrolysed samples were regulated between 4.5-6.0 using sodium hydroxide solutions, about 1.0g of yeast and 2.0ml of the hydrolyzed samples were added to 20ml of warm water which is shaken for about 5minutes to activate the yeast. This were added to 150ml of the hydrolyzed sample in a bottle and then closed tightly. The mixtures were then left for 96hours to ferment. The procedure will be repeated for 3.0g, 5.0g and 7.0g of yeast respectively (Adeeyo *et al.*, 2015).

Instrumental Analysis

The instrumental analysis was conducted at National Research Institute for Chemical Technology Zaria, Kaduna State, Nigeria.

While only Gc/Ms analysis was conducted at Shimadzu Training Center for Analytical Instruments (STC) Lagos.

Percentage yield of the Samples

The Percentage Yield of the samples was determined using the following equation.

$$\text{Percentage Yield} = \frac{\text{Quantity of ethanol produced x Specific Gravity}}{\text{Amount of Sample used}} \text{ X } 100$$

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RESULTS AND DISCUSSIONS

Results

Table 1: Percentage Glucose Yield

Run Order	Concentration (M)	Time (min)	Temperature (^{o}C)	Glucose Yield (A)	Glucose Yield (B)
1	0.25	80	100	0.4512	0.4840
2	0.25	60	70	0.2668	0.3928
3	0.25	105	70	0.4864	0.4784
4	0.50	90	40	0.2496	0.4100
5	0.50	60	100	0.4872	0.4588
6	0.50	105	70	0.2508	0.4808
7	0.50	75	60	0.2976	0.3832
8	1.00	90	60	0.1224	0.3940
9	1.00	105	40	0.1688	0.3536
10	1.00	105	100	0.4812	0.4812
11	1.00	60	80	0.3160	0.4668
12	2.00	80	40	0.4856	0.4352
13	2.00	105	70	0.4868	0.4800
14	2.00	75	80	0.4876	0.4816
15	2.00	90	100	0.1996	0.4792

Table 2: Physicochemical Characteristics of the Bioethanol Produced

Parameter	Sample (A)	Sample (B)	ASTM Standard
Specific Gravity	0.88000	0.9080	0.789-0.801
Boiling point	69	65.0	78.2
*p*H	6.68	6.24	6.5-9.0
Appearance	Cloudy, without any particles	Clear, without any particles	Clear, without any particles

Table 3: Fuel Properties of Bioethanol Produced

Parameter	Sample (A)	Sample (B)	ASTM Standard
Flash Point	16.50	18.40	16.50 - 16.70
Octane Rating	101	108	96 above
Pour Point	-7.40	-7.80	-5.0
Cloud Point	-4.30	-5.10	-23

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FTIR results of the Bioethanol produced

FTIR Analysis result of the bioethanol produced from sample A

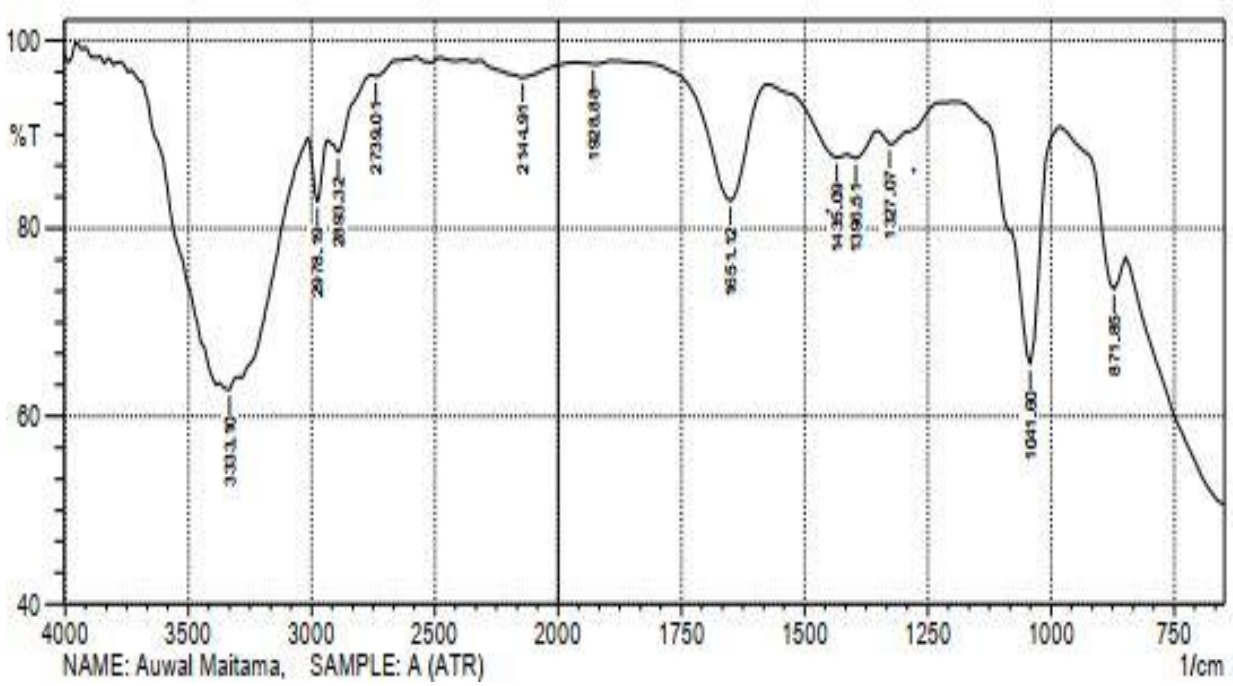

Fig (1).

FTIR Analysis result of the bioethanol produced from sample B

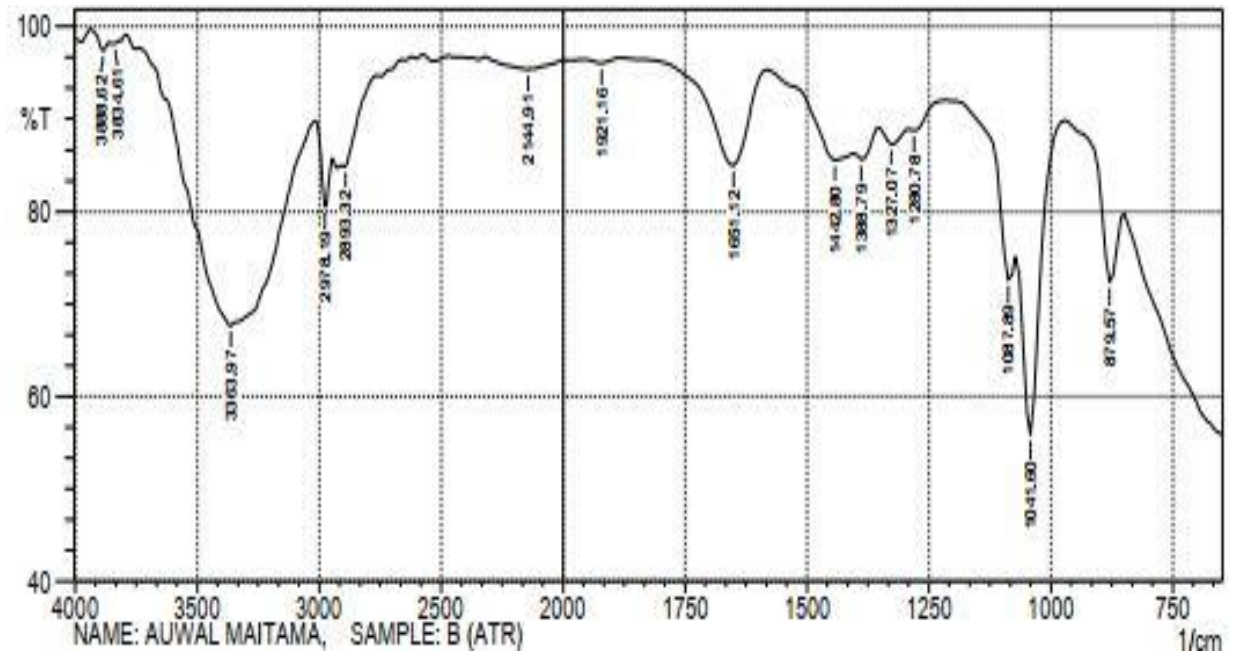

Fig (2).

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Results for Gc/Ms Analysis of Bioethanol produced.

Gas chromatography and mass spectroscopy was conducted to determine the end product of the bioethanol which was present in the entire sample.

GCMS Analysis result of the bioethanol produced from sample (A)

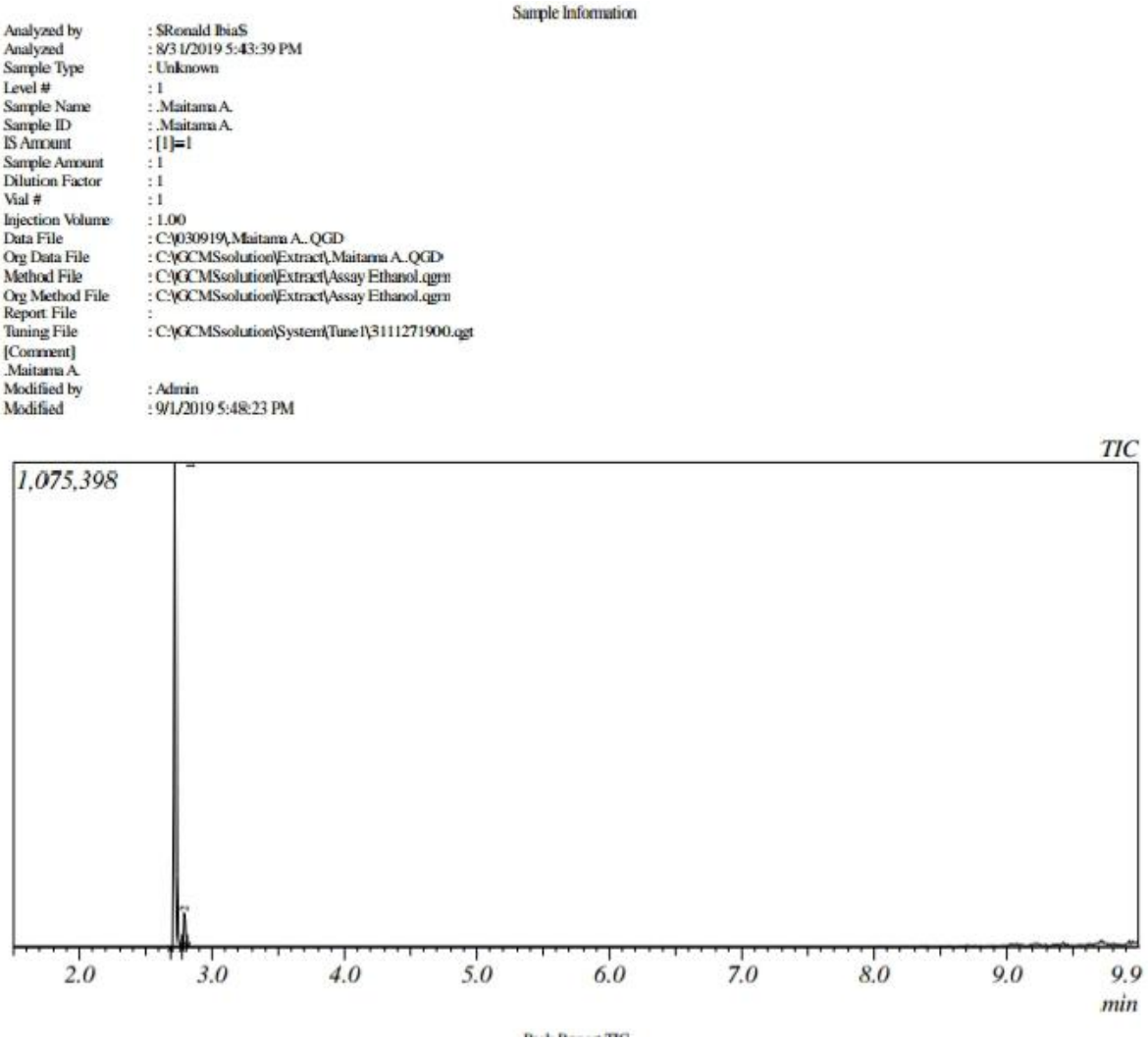

Fig (3).

Sample (A)

Line Number	Compound Name	Molecular Formula	Molecular Weight	Retention Time	Base Peak
1	Methoxymethane	C_2H_6O	46	296	45.05
2	2-Propanol	C_3H_8O	60	482	45.05

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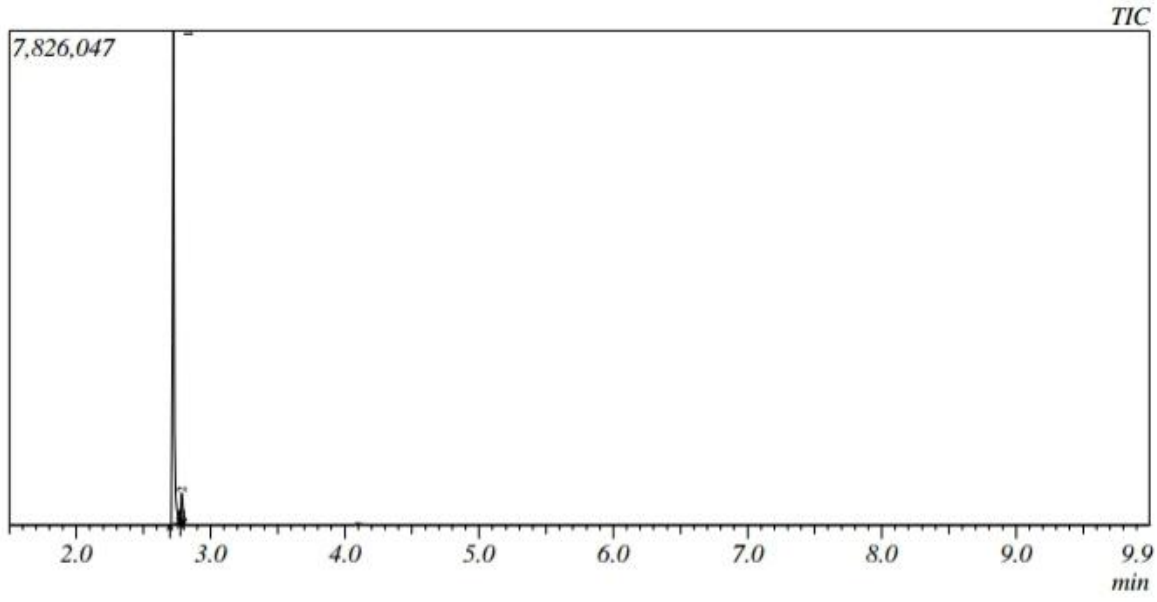
GCMS Analysis result of the bioethanol produced from sample B

Sample Information

Analyzed by	: $Ronald Ibia$
Analyzed	: 8/31/2019 5:58:25 PM
Sample Type	: Unknown
Level #	: 1
Sample Name	: .Maitama B.
Sample ID	: .Maitama B.
IS Amount	: [1]=1
Sample Amount	: 1
Dilution Factor	: 1
Vial #	: 2
Injection Volume	: 1.00
Data File	: C:\030919\.Maitama B..QGD
Org Data File	: C:\GCMSsolution\Extract\.Maitama B..QGD
Method File	: C:\GCMSsolution\Extract\Assay Ethanol.qgm
Org Method File	: C:\GCMSsolution\Extract\Assay Ethanol.qgm
Report File	:
Tuning File	: C:\GCMSsolution\System\Tune1\3111271900.qgt
[Comment]	
.Maitama B.	
Modified by	: Admin
Modified	: 9/1/2019 5:50:42 PM



Peak Report TIC

Peak#	R.Time	I.Time	F.Time	Area	Area%	Height	Height%	A/H	Mark	Name
1	2.724	2.700	2.767	6436670	94.01	7810134	94.28	0.82	MI	Methane, nitroso-
2	2.786	2.775	2.800	410243	5.99	473797	5.72	0.87	MI	Isopropyl Alcohol
				6846913	100.00	8283931	100.00			

Fig (4).

Sample (B)

Line Number	Compound Name	Molecular Formula	Molecular Weight	Retention Time	Base Peak
1	Ethanol	C_2H_6O	46	463	45.05
2	2-Propanol	C_3H_8O	60	482	45.05

Discussion

Acid hydrolysis of Bambara nut shells and yam peels at different acid concentrations and ambient temperature showed an increase in glucose concentration with time (Table 1). The concentration of glucose was higher when the concentration acid for sample A and B were 2M at

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70^{OC} for the period of 75 minutes and 0.25M H_2SO_4 at 100^{OC} for the period of 80 minutes were used respectively. There was a slight drop in glucose concentration for the biomass when hydrolyzed at 1M H_2SO_4 for both the samples. This could be ascribed to the actuality that at higher concentration of acid, glucose can be changed to levulinic acid and formic acid (Goh and Lee, 2010), which leads decline in glucose yield. These then suggest that uppermost glucose yield can be obtained at negligible acid concentration of 0.25M to 2.00M H_2SO_4 which as well serves as the most advantageous *p*H condition for yeast to metabolise its substrate (Mosie *et al.,* 2005). This corresponds with the findings of Abdullahi *et al.* (2014).

The fermentation was completed on the 5^{th} day as no observable change was noticed on the fermentation medium such as bubbles of CO_2. These observation suggested that maximum ethanol production corresponds with observation of Piotrowski *et al.* (2011), following the method described by Hendriks, and Zeeman (2009). 39.5% and 29.8% ethanol was produced in hydrolysates A and B respectively by the end of fermentation. This corresponds with the findings of Mathew (2013).

The general shortcomings of batch fermentation described by Reed (2014), may also affect yield, thereby suggesting continuous fermentation which increase the yeast population by recycling and the removal of ethanol during the fermentation as a better method. Separating the ethanol from the fermentation liquid by partial distillation recovered about 50% ethanol by volume which was reported by Rittmann *et al.,* (2008) on cassava waste. Additional distillation of the sample under controlled conditions could produce higher percentage of ethanol.

The specific gravity of the two samples being 0.88 and 0.91 were in total agreement with the standard obtained from ASTM range (0.78-0.85) it is noted that, the more the water content the higher the specific gravity of the liquid. And this indicates that it can be used as a solvent for chemicals and also in production of liquid detergents.It was observed that the boiling point which varies from 69^{o}c (yam peels) to 65^{o}c for (bambara nut shells) relate favorably with the standard boiling point of ethanol.

The *p*H of the two samples (yam peels and bambara nut shells) are 6.68 and 6.24 respectively, which implies that the experimental ethanol was neither acidic nor alkali. The *p*H below the specification can develop a risk for reflux.

The minimum temperature at which the fuel will ignite when exposed to an ignition sources. The results presented indicate that the flash point of the produced bioethanol was 21.50 and 19.60^{o}c for yam peels and bambara nut shells respectively. This implies that the bioethanol produced is less flammable than the standard ethanol fuel (Soibam *et al.,* 2017).

The cloud point is also an important property of bioethanol fuel, also it is a criterion for low temperature performance of a fuel. Cloud point is the lowest temperature at which a cloud of wax crystals first appear in the fuel when it is cooled (Lenihan *et al.,* 2010). The cloud point of bioethanol from yam peels is 19^{O}c while that of bambara nut shells is 20^{o}c which are in agreement with the ASTM standard (23^{O}c). This properties help to show the behavior of the bioethanol under a specified climate setting.

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The pour point is the lowest temperature at which the fuel or bioethanol cannot be moved (freezing point). These properties are related to the use of bioethanol in the cold temperate regions especially in hail region or snow region (Lenihan *et al.,* 2010). The pour point was determined according to ASTM D97 and the value obtained as previewed in table 3 were -25^{O}c and -24^{O}c which is lower than the standard pour point of ethanol (5.30^{O}c).The result indicates that the bioethanol produced can be used even in Polar Regions where the atmospheric temperature is not less than 5^{O}c.

Octane number is the anti- knock index of a fuel, the higher the octane number, the lesser the anti-knock effect and vice-versa, Reported by Sheikh (2016). The value of the octane number obtained as previewed in table 3 were 101 and 104 respectively which are in agreement with the ASTM D97 standard which is 99.

It can be referred from the various analysis conducted on the bioethanol produced that the properties of the bioethanol produced compared favorably with some of the properties of ethanol. The variation in some of the properties can be attributed to the nature of the feedstock (yam peels and Bambara nut shells) used in this study.

The Fourier transform infrared spectrophotometer (FTIR) showed the presence of peaks range between 1750$cm^{-1}$and 1512 cm^{-1}, 2200 cm^{-1} and 2000 cm^{-1}, 3000 cm^{-1} and 2800 cm^{-1}, 3450 cm^{-1} and 3371 cm^{-1} which suggests the presences of NH bend, C=N, C-H stretch and O-H stretch for the first sample. While for the second sample transform infrared spectrophotometer (FTIR) showed the presence of peaks range between 1750 cm^{-1} and 1512 cm^{-1}, 3000 cm^{-1} and 2800 cm^{-1}, 3450 cm^{-1} and 3371 cm^{-1} which suggests the presences 0f N-H bend, C-H stretch and O-H stretch. Both the results suggest that all the functional groups present are majorly alcohol (O-H) at the absorption range.

The results of FTIR spectroscopic analysis are similar to the finding of Piotrowski, *et al.* (2011) who investigated structural changes in waste lignocellusic materials.

Gc/Ms Analysis Ethyl alcohol was obtained which is the main goal of the research, though other compounds were also present in the entire samples which are due to the presence of other organic compounds in the sample. The process of fermentation and enzymatic activities hinder the formation of the following constituents: propane, butanol and carboximide. Interestingly, most of these compounds present in the end of bioethanol production have fuel potentiality. Propane is a good source of liquid gasoline that belongs to alkane group. It is a three carbon gas which is formed as a result of decomposition of organic matter over a period of time. Propane is a clean and eco-friendly gasoline commonly used in America for cooking, grilling and automobile fuel. Propane emission reaches the standard of clean air set by Environment Protection Agency.

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CONCLUSION AND RECOMMENDATION

Conclusion

The aim of this research was to produce bioethanol using lignocellulosic yam peels and bambara nut shells. Pretreatment procedure conducted is dilute acid hydrolysis. Acid hydrolysis offers the advantage of taking a shorter period than that of enzyme hydrolysis. However, enzymes hydrolysis gave high amount of hydrolyzates biomass without requiring any neutralization. The absence of fermentation inhibitors resulted in more bioethanol being produced through enzyme hydrolysis than from acid hydrolysis after fermentation. Ethanol yield could have been higher if the yeast saccharomyces cerevisiae could ferment both pentose and hexose sugars. Dilute acid hydrolysis greatly improves the accessibility of cellulose. It would be beneficial to use 2M H_2SO_4 than 0.25M to achieve high yield of glucose in sample A (yam peels) and 0.25M H_2SO_4 than 2M in sample B to attain high yield of glucose since there was not much of a difference in the reduction of the degree of crystalline and the yield of glucose per gram of biomass. Lower temperature were employed in the dilute acid hydrolysis, and alkali neutralization with sodium hydroxide resulted in the highest amount of glucose being present in the hyrolyzates without requiring a further step of concentration which would have increased the cost. Bioethanol production remains one of the most promising possible substituent for fossil based fuel. Therefore, the need to make available, cost effective methods employed in its production if it is to be sustainable.

Recommendation

Future work in bioethanol production needs to be focused on producing enzymes that are more tolerant to harsh acid conditions or produce glucose faster. The same also applies to fermentation yeasts.

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