



Prospect of Defatted Cake of *Balanite Aegyptiaca* and *Lagenaria Vulgaris* Seed as Feedstock for Bioethanol Production

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Abstract

Efficacy of the defatted seed cakes of Balanite aegyptiaca and Lagenaria vulgaris as feedstocks for bioethanol production was studied. The cakes were found to contained carbohydrate and crude protein contents of 43.81%, 16.19% and 48.41%, 17.59% for Balanite aegyptiaca and Lagenaria siceraria respectively. Hydrolysis of the cakes with both dilute and concentrated teraoxosulphate (VI) acid yielded syrups which upon fermentation with Saccharomyces cerevisiae gives bioethanol. The percentage bioethanol yield of 53.33 and 64.25% (v/v) were obtained from syrup of Lagenaria vulgaris seeds cakes hydrolyzed with dilute and conc. H₂SO₄ respectively. The hydrolysate of Balanite aegyptiaca seed cake obtained using dilute and conc. H₂SO₄, yielded 47.71 and 62.30 % (v/v) ethanol respectively. The distillates gave positive test for alcohols implying that defatted seed cake of Balanite aegyptiaca and Lagenaria vulgaris could be use as raw materials for bioethanol production.

Keywords: *Hydrolysis; Hydrolysate; Lagenaria vulgaris; Balanite aegyptiaca; Fuel prospects.*

1.0 INTRODUCTION

The increasing demand and unending scarcity for fuel commodity in West African sub-region especially in transportation sector prompted many researchers to the quest for alternative fuels. Several efforts to address issues of policy, challenges and opportunities regarding alternative fuels had being a topic of discuss over a period of time, but yet significant impact of overcoming the persistent energy shortage need is far from being actualized. Thus, assessment and exploitation of the endowed natural resources could assist in solving this long lasting problem. Across the global, high demand for energy, uncertainty of petroleum resources

and concern about global climatic changes were among the main derivers to the resurgence in the development of an alternative liquid fuels (Saha *et al.*, 2005). Huge deposits of biodegradable output from industries, agricultural waste, forestry and household are dumped in most of the urban areas of developing nation especially in Nigeria. Transformation of these waste products through biochemical process to liquid biofuels like bioethanol can safeguard the environment from pollution as well as generating some incentive to teeming unemployed youths. Bioethanol is a form of liquid fuel generated from carbonaceous material. It was found to have an efficient

calorific content capable of been used in automobiles (Wyman and Hinman, 1990).

Balanite aegyptiaca belongs to the family *Balanitaceae*, it is widely distributed in semi-arid and arid regions. The plant mostly grown on sandy and clay soils (www.saabbiopower.co.uk). It is a small tree reaching a height of around 10m with a stem

diameter of around 30cm. Its canopy may be deciduous for part of the dry seasons. The tree is a good source fire wood, charcoal, and edible fruit (www.saabbiopower.co.uk).

The aim of present study is to determine the bioethanol potential of defatted cake of *Balanite aegyptiaca* and *Lagenaria vulgaris* seeds via both dilute and concentrated acid hydrolysis.

2.0 MATERIALS AND METHODS

2.1 Samples Collections and Treatment

Balanite aegyptiaca seeds were collected in the premises of Usman Danfodiyo University Sokoto, while *Lagenaria vulgaris* seeds were procured in *Sayyinawal* village, Shagari Local Government Area of Sokoto State. The seed kernels were removed, crushed separately into suitable sizes, and then soaked into n-hexane over-night to extract their oil constituents. The residues were sundried separately, ground, sieved through a 20mm mesh and stored at room temperature in a polythene bag prior to commencement of the analysis.

2.2 Determination of proximate composition

Proximate parameters were determined using AOAC (1998) methods from which the available carbohydrate was calculated using a relationship reported by James (1995).

2.3 Dilute Acid Hydrolysis

Two steps dilute acid hydrolysis of the samples was carried out based on the method reported by Sun and Cheng (2002) and Sanchez, et al., (2004). To thirty grams (30g) of defatted *Balanite aegyptiaca* in 250cm³, 100cm³ of diluted H₂SO₄ (0.75%) was added. The resulted slurry was heated at 50°C for 10min, cooled to room temperature and further heated at 190°C for 10 minutes. The content was then kept for 2 hours; then distilled using Vacuum distillation to obtain the sugar syrup.

The sugar syrup obtained was over limed by adding dried lime (Ca(OH)₂) until when the pH reached 10.0 under constant stirring at room temperature for 30 minutes. After over liming, 1cm³ of concentrated H₂SO₄ was added to neutralize the content and then centrifuged. The

precipitate formed during neutralization was also removed.

The over limed hydrolysate was further detoxified using activated charcoal (1.5% w/v) with constant stirring at room temperature for 30mins and the sugar syrup was recovered using vacuum filtration. The residue left in first stage was transferred into a conical flask for second acid hydrolysis. The residue was treated with 100cm³ of diluted sulphuric acid as done in the first stage.

2.4 Concentrated Acid Hydrolysis

Thirty grams (30g) of the samples were weighed and treated with 100cm³ of 70% concentrated H₂SO₄ and heated at 50°C for 4 hours. 100cm³ of distilled water was added to dissolve and hydrolysate the substrate into sugar constituents. The solid residue from the first- stage was de-watered and soaked in 100cm³ (40%) H₂SO₄ for 50 minutes at 100°C for further cellulosic hydrolysis. The mixture formed was pressed to obtain the second acid sugar. The two sugar streams from the two hydrolysis stage were then fermented separately for subsequent ethanol production. The same treatment was applied to the sample of *Lagenaria vulgaris* cake.

2.5 Fermentation Process

The sugar syrups obtained were fermented separately using 7.5g of commercial yeast (*S. cerevisiae*) for a period of one week Brook (2008) and Oyeleke et al., (2009). The fermented liquors were distilled up.

2.6 Quantification of Bioethanol Yield

The volume of the ethanol yield was expressed in g/l, by multiplying the volume of the distillate (bio-ethanol) by the density of ethanol (0.8033g/ml).

2.7 Qualitative tests on the distillates (bio-ethanol)

The two samples distillates were separately tested with litmus papers, a piece of sodium metal, ethanoic acid, acidified potassium heptaoxodichromate (VI) ($K_2Cr_2O_7$) and potassium permanganate ($KMnO_4$) solutions in order to ascertain their reactivity as described by *Gusau (1994)*.

2.8 Infrared Spectroscopy Analyses of the Distillates

The analysis was conducted using FTIR-8400s (Shimadzu Japan). The machine was switched on for 30 minute to warm. Background of the spectrum was also measured before the drops of the samples was introduced into the sample part

and scanned between the range of 400 and $4000cm^{-1}$. The resulted spectrum was manipulated to remove background noise.

3.0 RESULTS AND DISCUSSION

The samples proximate composition (Table 1) revealed that defatted cake of *Lagenaria vulgaris* contain moisture ($5.50\pm 1.00\%$), ash ($6.50\pm 0.75\%$), crude fibre ($2.5\pm 1.03\%$), crude protein ($17.59\pm 0.18\%$) while defatted cake of *Balanite aegytiaca* had moisture ($3.5\pm 0.120\%$), ash (5.5 ± 1.50), crude fibre (1.00%), crude proteins ($16.19\pm 0.13\%$), and crude lipid ($33.00\pm 0.050\%$)

Table 1: Proximate Composition of the Defatted Cakes Samples.

Parameter (%)	<i>Balanite aegytiaca</i>	<i>Lagenaria vulgaris</i>
Moisture Contents	3.5 ± 0.120	5.5 ± 1.00
Ash contents	5.50 ± 1.50	6.5 ± 0.75
Crude Fiber	1.00 ± 0.20	2.5 ± 1.03
Nitrogen	2.59 ± 0.12	2.81 ± 1.26
Crude Proteins	16.19 ± 0.13	17.59 ± 0.18
*Carbohydrate	43.81 ± 1.05	48.41 ± 0.97

*carbohydrate obtained by difference

The results show high percentage carbohydrates (48%) in *L. vulgaris* than the values obtained from *B. aegytiaca* (43%). This shows that the

cakes are good source of carbohydrate that could be utilized for bio-ethanol production (*Giorgos et al., 2013*).

Table 2: Effect of Acid concentration on Bioethanol Yield (% v/v).

Cake Sample	Dilute acid	Conc. acid
<i>B. aegytiaca</i>	47.71(52 cm ³)	62.30 (170 cm ³)
<i>L. siceraria</i>	53.33 (40cm ³)	64.25 (160 cm ³)

The results presented in Table 2 revealed the bioethanol yield of the substrates using both dilute and concentrated acid hydrolysis methods. Dilute acid hydrolysis yielded 52cm³ of the distillate for *B. aegytiaca* and 40cm³ for *L.siceraria*. While concentrated acid hydrolysis

resulted to 170cm³ of the distillate for *B. aegytiaca* and 160cm³ for *L. vulgaris*.

The percentage bio-ethanol yield were 47.71% and 62.30% for defatted cake of *Balanite aegytiaca* seeds while 53.33% and 64.25% were for defatted cake of *Lagenaria vulgaris* seeds.

This indicate higher percentage yield from carbohydrate content. *Lagenaria vulgaris* seeds due to its high

Table 3: Qualitative Tests of the Distillates.

Test	Observations	Inference
Distillates + Acidified KMnO_4	The purple colouration, decolourised immediately.	Presence of OH
Acidified+ $\text{K}_2\text{Cr}_2\text{O}_7$ to the distillates	The orange colour, changed to light green immediately.	Presence of OH
Distillates+ litmus paper followed by blue litmus paper	No effects on both litmus papers	Neutral to lit paper
Distillates + CH_3COOH + few drops of conc. H_2SO_4	A pleasant fruity scent character was observed	Presence of OH
Distillates + sodium metal	A vigorous effervescence with evolution of colourless gas, which does not have any effect on litmus paper	Presence of OH Group

Table 3 shows the result of the qualitative analysis on the sample distillates. The distillates obtained decolorized the purple coloration of the KMnO_4 which indicates the presence of OH group. The distillates also change the orange coloration of $\text{K}_2\text{Cr}_2\text{O}_7$ to greenish, indicating the

presence of OH group. The distillates were also neutral to litmus papers.

Evolution of hydrogen gas was noticed on the reaction of the distillate with sodium metal which also is an indication of OH-groups reaction.

Table 4: Infrared Spectra Absorption peaks of the distillate from the defatted seeds cake of *Balanite aegyptiaca*.

Dilute acid hydrolysis			
Absorption peak cm^{-1}	Bond type	Appearance	Possible compound
3348.54 cm^{-1}	O-H	Broad	Alcohols, phenols
2928.04 cm^{-1}	C-H	Medium-strong	Alkyl
2858.60 cm^{-1}	C-H	Medium-strong	Alkyl
Concentrated acid hydrolysis			

3346.61 cm^{-1}	O-H	Broad	Alcohols, Phenols
1197.83 cm^{-1}	C-O	Medium	Alcohols
551.66 cm^{-1}	C-X	Weak-medium	Chloroalkanes
489.94 cm^{-1}	C-X		Chloroalkanes
437.86 cm^{-1}	C-X		Chloroalkanes

The results in Table 4, showed the absorptions peaks obtained from the interpretation of the I.R. The absorption spectrum shows an absorption at (3348.54 cm^{-1}) which is broad signifying the presence of alcohols, with a specific type of

bond at higher concentration falls between 3200-3400 cm^{-1} region of OH. The absorption spectrum at 1197.83 cm^{-1} fall within the range of 1150-1200 cm^{-1} indicating C- O bond which could likely be an alcohol.

Table 5: Infrared Spectra Absorption peaks of the distillate from the defatted cake of *Lagenaria vulgaris* seeds.

Absorption Peak cm^{-1}	Bond	Appearance	Possible Compound
3356.25 cm^{-1}	O-H	Broad	Alcohols, phenols
2926.11 cm^{-1}	C-H	Medium-strong	Alkyl
2856.67 cm^{-1}	C-H	Stretch	Alkanes
2330.09 cm^{-1}	C-H		Alkanes
Using Dilute method of Sulphuric Acid			
3350.46	O-H	Broad	Alcohol
2353.23			
1637.63	N-H	Strong	Primary
1197.83	C-O	Medium	Alcohol, phenol
565.16	C-X	Weak-medium	Chloroalkanes
540.09	C-X	Medium-strong	Bromoalkanes
435.93			

The IR analyses of the distillate had shown the presence of carbon hydrogen bond as well as presence of hydroxyl group. This implied that

the distillate contains functional group presence in ethanol.

4.0 CONCLUSION

Results presented in this research work had established that bioethanol can be produced from defatted cake of *Balanite aegyptiaca* seed and defatted cake of *Lagenaria vulgaris* cakes using commercial yeast. However, utilization of

this feedstock can improve as well as reducing the persistent fuel shortage owing to the fact that ethanol can be mixed with petrol in various proportion and burnt smoothly in combustion engines with no modification.

5.0 REFERENCES

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