Production and Biodegradability of Biodiesel From *Lagenaria Siceraria* Seed Oil

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Abstract: - **The production, physicochemical properties and biodegradability of biodiesel from the seeds oil of *Lagenariasiceraria* (calabash) was carried out. Oil was extracted from the seeds using soxhlet extractor with n-hexane; then trans-esterified using single step alkali hydrolysis to biodiesel. The biodiesel produced was analyzed for its physicochemical and fuel properties using ASTM methods and other methods. The specific gravity (SG), density, flash point, cloud point, pour point and percentage yield of the methyl esters were found to be 4.68, 0.86 g/ cm^3, 138°C, 4°C, 0°C and 59.44%, respectively. The biodegradability of the biodiesel assessed by the standard CO2 evolution method, revealed that Lagenariasiceraria biodiesel is ultimately biodegraded (59.39%).This suggest that Lageneriasiceraria seed oil is a potential source of environmental friendly biodiesel.**

***Keywords:- Lagenariasiceraria,* Biodiesel, Biodegradation, Environment, Inoculums**

I. INTRODUCTION

Biodiesel is produced from vegetable oils or animal fats and an alcohol, through Trans-esterification reaction. Biodiesel is a renewable fuel made from any biologically based oil, and can be used to power any diesel engine. Now accepted as an environmentally friendly alternative to petroleum diesel that has received worldwide acceptance as a substitute and also a blending agent to fossil diesel. It is a mono-alkyl ester which is produced mainly by transesterification of vegetable oils or animal fats. This reaction is carried out by heating feedstock (oil or fat) and alcohol with suitable catalyst. Over time, edible oils were used but this has resulted to food crisis causing the research on biodiesel production to shift to non-edible seed oils and even the waste oils. (Ibrahim *et al,* 2016).

Energy is an essential driving factor to socioeconomic development in our present society. Its impact touches all aspect of human endeavors such as agriculture, health, education, transportation among others. Petroleum based fuels are the major fuel source used in transportation sector in most of the developing nations. Its combustion generates emissions which are nuisance to environment and adversely affect human health. It has been established that these emissions are carcinogenic (BBC,2005). However, climate change and increase in pump price has also redirected research interest to renewable energy resources. The renewed interest to the quest for greener fuels sources is a topical issues that gain wide societal and political interest especially for its reduced green house emissions, biodegradability, sustainability as well its competitive nature to fossil fuels and food supply (Amish *et al.,* 2009; Mushtaq *et al*.,2009). Therefore, it is imperative to examine the biodegradability of biodiesel fuel and their biodegradation rate in natural environment so as to have an idea of how persistent they would be when discharged into the environment.

Biodiesel is derived from vegetable oil or fat via transesterification reaction. However, increasing interest and the used of petro diesel necessitate the search for other viable feedstock from the abundant and versatile renewable resource especially plant seeds. Cucubitacea family is among the abundant crop domesticated and grown at wild in most tropics especially in Nigeria.

Lagenariasiceraria a member of such family, a monocotyledonous flowering plant commonly known in Hausa language as 'kwarya or duma' and calabash in English. It possesses simple leaves which are 400mm long and 400mm broad, oval shape and whitish seeds embedded in a spongy pulp, 7 – 20 mm long (Welman, 2005). They were widely grown in Northern part of Nigeria for excavation of domestic utensil and food containers and 12 to 16 inches high with 20inch diameter, when dried have approximately ½-inch thickness.(Ibrahim *et al,* 2016).The fruits of these species contain vast number of seeds that have no commercial application in the locality they were produced. *Lageneriasiceraria* species (including Calabash) are among the cultivated crops in the north-western region of Nigeria that have no established large scale application. It has relatively short maturity time of about 3-4 months and produces large amount seeds with up to 50% inedible oil content and thus with stable price that may not subject to distortion by food prices. Ibrahim *et al.,* 2016).

Figure 1 (a) Bottle Shape**(b)** rounded Shape of popular Calabash in Northern Nigeria

Rounder varieties are called calabash gourds. They grow in a variety of shapes: they can be huge and rounded, small and bottle shaped, or slim and serpentine, more than a metre long. There are also the hard, hollow fruits of the calabash tree, the fruit pulp though bitter is edible, Crescentiacujete, whose fruits are also used to make utensils, containers, and musical instruments. In some countries, the dried shell of the fruit is used to make bowls and fruit containers, decorated with paintings or carvings.(Ibrahim *et al.,* 2016). This paper is aimed at production of biodiesel from *LagenerariaSiceraria* seed oil, evaluate its fuel quality and to determine its biodegradability.

II. MATERIALS

The materials used for this study include; conical flask, burette, magnetic stirrer, separating funnel, density bottle and the host of others was obtained pyrex. The reagents used were analytical grade methanol, potassium hydroxide, sulphuric acid, was obtainrd from (Scharlauchemie, Spain, Burgoyne, India, BHD-Analar, England), Glacia acetic acid, Hydrochloric acid, n-hexane, Carbon tetrachloride Phenolphthalein,calcium oxide catalyst (Cartivalues, Reidel-de Haen,Germany, BHD-Analar, England) and ethanol (Merck.Lab. Germany). All chemicals were of analytical grade and were used without further purifications.

III. METHODS

Dried seeds of *Lageneriasiceraria* were obtained from Garta, Michika Local Area in Adamawa State between the months of February and April, 2018. These were identified by a Botanist at Biological Sciences Department (A.T.B.U) Bauchi, Bauchi State.

Seeds of *LageneriaSiceraria* were ground to a coarse texture using a commercial grinder and 298.3g were fed to a Soxhlet extractor fitted with a 500ml round bottomed flask. The oil extraction was done at a temperature of 60°C for 12hrs using *n*-hexane as a solvent. The solvent systems (n-hexane) containing the oil and the extracting solvents were differently dried in vacuum using a soxhlet extractor at 60 °C which was heated in a water bath. The concentrated oils were placed on a table over night to exclude every traces of solvent remaining. Oil quality parameters of the extracted oil such as iodine value (IV), peroxide valve (PV), Kinematic Viscosity, acid value, free fatty acid (FFA) and Saponification Value. The amount of oil extracted was determined using the equation as follows

$$\text{oil yield} = \frac{\text{wt of extracted oil}}{wt\ of\ sample\ seed} \times 100$$

Preparation of Fatty Acid Methyl Ester (Biodiesel)

Methanol (200ml) was added to 1.00g of KOH in a conical flask with a slight heating and slow stirring until completely dissolved.The mixture was added to the oil (90ml) with slow stirring and this continued for about 2 hours 15 minutes after which the mixture was slowly transferred into a separation funnel and allowed to stand overnight. The mixture separated into two distinct layers, the glycerol layer (the lower layer) was drained off. Water was slowly added to the FAMES, and swirled slowly so as not to form an emulsion and the water drained off with remaining glycerol. This process was repeated severally until the product was clear. The FAMES was then dried to obtained a pure biodiesel. The yield was calculated as follows:

$$(\%)\ \text{biodiesel} = \frac{wt\ of\ biodiesel}{\text{wt of LLSO}} \text{x}\ 100$$

Fuel Properties Test

The parameters tested includes density, viscosity, and flash point.

Flash point

Determination of flash point was done by measuring 1 ml of biodiesel in to a 100 ml conical flask. the flask was heated slowly on a hot plate at a constant rate. The flash point was taken as the lowest temperature an applied test flame caused the vapor above the flask to make a pop sound. The same procedure was repeated using that of oil extract parameter.

Density

The density of the *n*-hexane extract was determined using measuring cylinder. The measuring cylinder was first weighed empty, and then weighed filled with the oil. The weight of oil was determined by taking the difference between the weight of the empty measuring cylinder and weight of the measuring cylinder filled with oil. These were used to calculate the density of the *LegenariaSiceraria* seed oil extracts from the extracting solvent using the relation:

Weight of the extracted oil

$$\text{Density} = \frac{\text{wt of extracted oil}}{equall\ vol.of\ the\ oil} X\ 100$$

Kinematic Viscosity

A graduated burette was filled to mark with *Legenaria Siceraria* seed oil, the tap of the burette was opened and the oil was allowed to flow until it reaches the 10ml mark of the burette. The time taken for the flow (flow time) was recorded. The viscosity of the LSSO was determined after triplicate run of the sample and the mean value recorded. The kinematic viscosity was calculated as follows:

Biodegradability of biodiesel

The approach in this work followed the U.S. Environmental Protection Agency (EPA) standard method for determining the biodegradability of chemical substances (EPA, 1992). Shaker Flask System. A 2-L Erlenmeyer flask (fig.2.1)containing 900 mL deionized distilled water (DIW), 100 mL of inoculum (acclimation medium), 1 mL of each stock solution (Table 4), and 10 mg L–1carbon from the test compound. A reservoir holding 10 mL of calcium hydroxide solution was suspended in the flask to trap the CO_2. After inoculation, the test flasks were sparked with CO_2 free air to ensure that the trapped CO_2came only from the micro-organism's metabolizing the test substances. The flasks were sealed and incubated with shaking in a dark room.

Measurement of CO_2 Evolution:

The quantity of CO_2evolved was measured by titration of the entire $Ca(OH)_2$sample 10 mL of $Ca(OH)_2$ plus 10 mL of rinse waterwith 0.1 N HCl to the phenolphthalein end point. After sampling, the reservoir was refilled with fresh $Ca(OH)_2$.All the samples were analyzed at least five times in a 28-day period to provide sufficient data to determine biodegradation trend with time. Three mL of 20% H_2SO_4were added on the day prior to terminating the test. The percent theoretical CO_2evolved from the test compound was calculated at any sampling time from the formula:

$$\% \ CO_2 \text{ evolution } = \frac{TF - CF}{C} \times 100$$

Where;

TF: represents milliliters (mL) of 0.1 N HCl required to neutralize the $Ca(OH)_2$from the flask with the test substance.

CF: represents milliliters (mL) of 0.1 N HCl required to neutralize the $Ca(OH)_2$from the control flask; and C is the theoretical volume of the HCl required to neutralize the CO_2 converted from the carbon.

For 10 mg carbon: C = 16.67 mL of 0.1 N HCl.

Preparation of inoculum

The method used for preparing the inoculum was that of (Zhang *et al.*, 1998).The soil samples were collected from biological garden, and the sewage from the boy's hostel both in Abubakar Tafawa Balewa University, Bauchi whereas the yeast was obtained from YelwanTudu, Bauchi, Nigeria.

Inoculum (Acclimation Medium).

To 1000 ml distilled water was added one gram of organic matter rich soil, 2 ml of activated (aerated) sewage mixed liquor, 50 ml of raw domestic sewage water, 25 mg/L of Difco vitamin-free casamino acids (Difco Laboratories, Mauston, Wis.), 25 mg/L of yeast extract, and 1 ml of each stock solutionI, II, III (Table1).

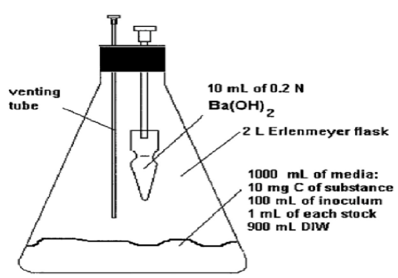


Figure 2.1–Shake flask system

Table.1:Medium employed for assay of CO_2 evolution from EPA (1992)

Stock solution	*compound*	*Concentration(g/dm³)*
I	NH_4Cl	*25*
	KNO_3	*15*
	$Na_2HPO_4.3H_20$	*75*
	$NaH_2PO_4.H_2O$	*25*
II	KCl	*10*
	$MgSO_2$	*20*
	$FeSO_4.7H_2O$	*1*
III	$CaCl_2$	*25*
	$ZnCl_2$	*0.05*
	MnCl	*0.5*
	$Cocl_2$	*0.001*
	H_3BO_3	*0.001*
	$Cucl_2$	*0.005*
	MoO_3	*0.4*

IV. RESULTS AND DISCUSSION

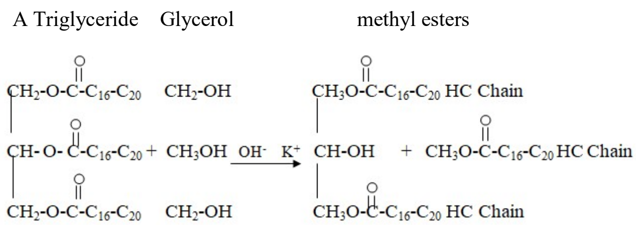
Oil Quality Parameters of Legenariasiceraria

The oil quality parameters of *Legenariasiceraria* seed oil in comparison with that of Balanitesaegyptiaca (Jauro *et al*,2011) are shown on table 2. The average oil content obtained from *Legenariasiceraria* seed was 59.1% (Table 2) which is above that of *Balanitesaegyptiaca* (37.2%). This places *Legenariasiceraria* seed amongst the richest oil seeds. The iodine value of (17%) recorded for *Legenariasiceraria* indicates that the oil is a non drying type with a very low degree of unsaturation. Oils are classified in accordance with their iodine value as non- drying oils (I.V less than 100), drying (I.V. 130 and above) and semi drying oils (I.V. between 100 and 130). Based on research, the more unsaturated, the higher the iodine value and the more prone the oil to rancidity by oxidation. The low iodine value of the oil is highly advantageous because the oil would be stable to polymerization and oxidation. (Jauro *et al.*,2011)

The peroxide value of an oil or fat is used as a measurement of the extent to which rancidity reactions have occurred in an oil and fat during storage (Danjuma *et al*, 2009). The peroxide value of *Legenariasiceraria* seed oil was 38meq/kg which is above that of *Balanitesaegyptiaca (*Table 2). For fresh vegetable oil the peroxide value is known to be lower than 100meq/kg which may be due to the freshness of the seed. This indicates that the oils would not easily go rancid when properly stored and show a good potential for production of biodiesel. The acid value is used to quantify the amount of acid present in a chemical substance. The acid value of *Legenariasiceraria* was 0.28mgKOH/g, lower than that of *Balanitesaegyptiaca* 0.995mgKOH/g *(*Table 2). This indicates that it contains fatty acid composition of 0.14 mgKOH/g which is within the acceptable range (0-0.80 max) specified by the ASTM and that of European standard (0-0.5Max). This result reveals that the acid value is good enough for *Lageneriasiceraria* (Calabash) seed oil to serve as a good feedstock for the production of biodiesel. Oil with higher acid value has a greater tendency to corrode fuel tank, lining and pipeline (Farm energy., 2015).

The saponification value of *Lageneriasiceraria* seed oil was 126 mgKOH/g which is lower than that of *Balanites aegyptiaca*134.64meq/kg (Table 2). For a vast majority of oils used in biodiesel production, their saponification value are within the range of 130 to 193 meq/kg (Aliyu *et a.l,* 2011). This shows that the oil is suitable for use in biodiesel production.

Fig.2. A scheme of transesterification process of triglyceride with methanol using a base catalyst.



Production of Biodiesel (Transesterification)

Chemically, transesterification reaction is a typical substitution reaction. The process involves substituting the alkyl group of the esters with the alkyl group of the alcohol. In the case of fatty acid methyl esters (FAME) using *Lageneriasiceraria* seed oil, the alkyl group on the triglyceride (oil) is substituted with the methyl group of the alcohol (*fig*.2). The base (KOH) catalyst was dissolved in the alcohol to make it convenient for dispersing the solid catalyst into the oil. The methoxide produced ($KOCH_3$) was then mixed with the oil and the substitution reaction proceeds in a series of steps (Aliyu *et al.,* 2011). The percentage conversion of the *Lageneriasiceraria* seed vegetable oil (LSVO) to fatty acid methyl esters(FAMES) was 59.44%

Fuel Quality Parameters

Density: the density of the biodiesel was 0.86 g/cm^3, which is similar to that of *Lageneriasiceraria* seed vegetable oil (LSVO) 0.9, these are all within the ASTM standard 0.86 - 0.9 g/cm^3. The density of the *Lageneriasiceraria* seed oil was found to be 0.9g/cm^3, while that of the biodiesel was found to be 0.86 g/cm$^{3.}$. These shows that the biodiesel possess the required density for a good biodiesel

Table 2: oil parameters of *Lageneriasiceraria*in comparison with those of *Balanitesaegyptiaca*

PARAMETERS	*Lageneria seedoil*	*Balanites seed oil*	European standards	ASTM
%Yield	59.1%	37.2%		
Iodine value(mg/100g)	17± 0 . 4	42.28	40.2-153	
Peroxide value(meq/kg) Acid value(mgKOH/g) Free fatty acid% Saponification value(mg KOH/g) Relative density(g/cm^3) Cloud point(ºC) Pour point (ºC)	38± 0 . 2 0.28± 0 . 0 0 0.14± 0 . 0 0 126± 0 . 0 5 0.9± 0 . 0 1 -4± 0 . 0 0 -7± 0 . 0 0	8.00 0.995 0.5 134.64	 0-0.5max 0.4-4.5mg/g	 0.8max
Flash point	-7± 0 . 0 0			
Texture	viscous	viscous	viscous	
Smell/odour	pleasant	pleasant	pleasant	

Values are mean ±standard deviation(n=3)

Table 3. Fuel Quality Parameters of *Lageneriasiceraria* Vegetable oil (LSVO), Fatty Acid Methyl Esters (FAME) in comparison with the ASTM standard.

Fuel property	Fames	LSVO	ASTM standard	Limits
Colour	Dark yellow	Fale yellow		
%yield	59.44	59.1		
Rel.density	0.86± 0 . 0 1	0.9± 0 . 0 1	0.86	0.86-0.90
Flash point(ºC)	138	155	100min	130min
Cloud point(ºC)	4± 0 . 0 0	-4± 0 . 0 0		-1min
Pour point(ºC)	0± 0 . 0 0	-7± 0 . 0 0		-15-10
viscosity	6.5± 0 . 0 1	2 0 ± 0 . 3		3.5-5.0
Cetane number	87			51min

Values are mean ±standard deviation(n=3)

Table4. Carbon dioxide (CO_2) evolution from biodiesel and control using Inoculum

day	LSMES	CONTROL
0	0.00±0.00	0.00±0.00
7	19.79±6.42	1.00±0.00
14	26.21±23.23	3.6±6
21	59.39±23	3.2±1.2
28	49.19±4.58	3.17±1.00

Values are mean ± standards deviation (n=3)

Flash Point

The flash point is used in assessing the overall flammability of a material. Higher flash point indicate material that is less likely to ignites accidentally (Aliyu *et al*, 2011). ASTM standard requires a minimum 100°C. the flash point of *Lageneriasiceraria* methyl esters (LSMES) (138°C) is lower than that of *Lageneriasiceraria* vegetable oil (LSVO) (155°C) but both of them are greater than that of petrol diesel and are all within the ASTM standard (*Table* 3). This indicate that fatty acid methyl ester FAME will be safe for use since it will not ignite easily.

Cetane number

Cetane number (CN) is one of the primary indicators of diesel fuel and combustion quality. It is related to the ignition delay time and indicates how well a fuel will combust inside a compression engine. Biodiesel usually has a higher cetane number than the petro-diesel. The cetane number varies from a minimum value of 51min. The cetane number of pure biodiesel depends on its fatty acid profile; those from saturated fat will have a higher cetane number than those from unsaturated oils The cetane number of 87 for *LageneriaSiceraria* methyl esters (LSMES) (Table 4) is higher than that *of Luffa cylindrical* seed biodiesel (71.93) (Ozulu *et al.*, 2015). The cetane number of *LageneriaSiceraria* methyl esters (LSMES) has been found to be within the International standard for biodiesel for countries like Germany, Austria, Italy France and Sweden standard for biodiesel (Min.51) which reveals its feasibility to be used in these countries.

Cloud Point

Biodiesel tends to freeze at higher temperatures than petro-diesel. This is one of the major factors hindering the use of biodiesel. The cloud point (CP) is the temperature of the fuel at which small solid crystals can be observed as the fuel cools. The observed cloud point of *LageneriaSiceraria* methyl esters (LSMES) biodiesel was 4°C while that of *Lageneriasiceraria* vegetable oil (LSVO) -4°C. Both has been found to be within the International standard for biodiesel for countries like Germany, Austria, Italy France and Sweden and also conforms which reveals its feasibility to be used in these countries.

Biodegradability Test

The cumulative percentage of CO_2 evolved from *LageneriaSiceraria* methyl esters (biodiesel) and control within the 28 days period shown on (Table5), revealed that the maximum percentage CO_2 evolved within the 28 days period was 59.39%, while on the last day of the experiment it drops down to 49.19%. This indicates that there is a significant difference in the biodegradability of the biodiesel within the 28 days, 49.19% percentage of CO_2 evolved on the last day tells either the microbes in the media has died off or the available appreciable food in the biodiesel for the microbes has been considerably depleted. This shows that the biodiesel fuel is "ultimately biodegradable''.

V. CONCLUSIONS

Lageneria Siceraria is a rich sources of vegetable oil. The fuel quality parameters of the *LageneriaSiceraria* biodiesel such as flash point, density and cetane number are similar to those of the petrol-diesel and are within the general accepted standards. *LageneriaSiceraria* biodiesel is ultimately biodegradable compared to petrol-diesel (Jauro *et al*., 2011) which is partially degradable. This suggest that *LageneriaSiceraria* seed oil is a potential source of environmental friendly biodiesel.

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