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Research Article

Production and characterization of biodiesel from Allamanda (*Allamanda cathartica*) oil using lipase as catalyst

Evans C. Egwim[*1], Solomon I. Isege[1], Stephen Ochigbo[2], George Akpan[3]

[1]Department of Biochemistry, Federal University of Technology. P.M.B 65, Minna, Niger State, Nigeria.

[2]Department of Chemistry, Federal University of Technology, Minna, Niger State, Nigeria.

[3]Chemical Engineering Department, Federal University of Technology, Minna, Niger State, Nigeria.

Correspondence: Evans C. Egwim; evanschidi@gmail.com +2347065809474.

ABSTRACT: Biodiesel was produced from allamanda oil by transesterification of the oil using lipase as catalyst. The amount of enzyme, time and temperature were found to have an immerse effect on biodiesel synthesis. The conversion increased with temperatue up to 40 ^{0}C. About 97% yield of biodiesel was obtained using 0.5mg/ml of lipase with 1: 4 oil to ethanol molar ratio at 40 ^{0}C for 2 hours. The fuel properties of allamanda oil ethyl ester showed that the specific density of biodiesel produced was 0.88g/ml while that of the standard value was 0.85-0.89 mg/ml. The specific gravity of the diesel was 0.87 mg/ml which was within the range 0.86-0.88 mg/ml for standard values. The viscosity of the biodiesel produced was 4.6, the refractive index of the biodiesel was 1.46, the flash point of the diesel produced was 117, and this value was well above 100 for a typical biodiesel as provided by the national soy diesel development board for biodiesel. All the fuel properties compared favorably with international biodiesel specification as provided by American Society for Testing of Materials (ASTM) and National Soy Diesel Development Board for Biodiesel.

KEYWORDS: **Biodiesel, Transesterification, Allamanda oil, Lipase,**

INTRODUCTION

Biodiesel has been defined as the monoalkyl esters of long-chain fatty acids, preferentially methyl and ethyl esters, derived from renewable feed such as vegetable oils or animal fats. Its properties are close to diesel fuels, and therefore biodiesel becomes a strong candidate to replace the diesel fuels (Srivastava and Prasad, 2000). Due to increases in crude oil prices, limited resources of fossil oil, environmental concerns, population increase, and hence, higher energy demand, biodiesel represents a promising alternative fuel for use in compression ignition (diesel) engines. The biodiesel advantages over conventional fuels are its lower toxicity, high biodegradability, substantial reduction in emissions, considerable reduction in carbon monoxide (CO), polyaromatic hydrocarbons, smoke and particulate matter. It is obtained from renewable resources (vegetable oils) consuming more carbon dioxide from the atmosphere during the production than is added to it by their later combustion. Therefore, it reduces the carbon dioxide content of the atmosphere and hence, reduces the greenhouse effect. Furthermore, the Sulphuric acid is reduced (Soumanou and Bornscheuer, 2003).

The industrial process of biodiesel production is usually carried out by heating an excess of alcohol usually methanol or ethanol with vegetable oils under different conditions in the presence of an inorganic catalyst (Mittebach, 1990). This chemical process is known as transesterification or alcoholysis. The most commonly used catalyst are alkali hydroxides (NaOH, KOH), carbonates and corresponding sodium and potassium alkoxides. A disadvantage of alkali-catalysed procedures is that the homogenous catalysts are removed with the glycerol layer after the reaction and cannot be reused. Furthermore, neutralization to prevent toxic wastes is necessary and the purification of glycerol is more difficult when large amounts of catalyst have to be removed. Another disadvantage is the partial saponification reaction, which produces soap. The soap lowers the yield of esters and makes the separation of ester and glycerol difficult (Fuduka *et al.*, 2001). Besides, the use of more expensive refined oils is necessary because oil fats is relatively high constituting about 80% of the total cost of the biodiesel production (Bender, 1991).

Worldwide biodiesel production is mainly from edible oils such as soybeans, sunflower and Carniola oils. Since Nigeria is not sufficient in the production of edible oils, non edible oil seeds available in the country are required to be tapped into for the production of biodiesel. With the abundance of forest and plant based non-edible oils in the country-*Jatropha curcas* (Jatropha) and *Allamanda cathartica* (Allamanda), no much attempt has been made to use esters of these non-edible oils as substitute for diesel except jatropha. Base catalysed transesterification reaction of Allamanda and jatropha oils is much slower, requiring a higher temperature and due to the formation of soap in the processing steps, the separation of by-products is often difficult as result of high level of free fatty acids (Egwim *et al.*, 2012; Berchmans and Hirata, 2008).

However, we have shown earlier that lipase catalysed transesterification of Jatropha oil, increased biodiesel yield at a lower temperature and reaction time (Egwim *et al.*, 2012). Thus, the objective of this work is to produce biodiesel from allamanda oil using lipase as catalyst, characterize the biodiesel produced in order to determine its fuel properties, and to optimize the yield of the diesel by varying reaction parameters such as temperature, enzyme concentration and time influencing the enzymatic synthesis of the biodiesel.

MATERIALS AND METHODS

Extraction of oil

The allamanda nuts were cracked to remove the seeds; the seeds were then dried and homogenized using mechanical pressure (mortar and pestle) (Egwim and Ibrahim, 2012). The homogenized seeds were then subjected to pressure using bare hands and with the aid of hot water o extract the oil.

Lipase Assay

The enzyme assayed was determined titrimatically using the method of Vorderwubecke *et al.* (1992). Into each of six 25-ml Erlenmeyer flasks, 10 ml of 95% (v/v) ethanol and 2 to 3 drops of 1% (w/v) thymolphthalein indicator was added. Into a 50ml Erlenmeyer flask with stopper, 50 ml of 5% (w/v) olive oil/gum Arabic emulsion substrate was added and pre-incubated 15 minutes in a 37 ^{0}C water bath with magnetic stirring. Enzyme, 0.1 mg/ml, was added to initiate lipolysis on the emulsion substrate; the timer was started, and stirred. At five suitable reaction intervals 5, 10, 15, 20, and 25 min, 5 ml reaction mixture was removed and transferred to a separate flask containing titration cocktail prepared in the first step. The content of each flask was titrated with 0.05 N NaOH using a burette until a light blue color appeared. In to the 25 ml Erlenmeyer flask containing titration cocktail, 5 ml phosphate buffered olive oil/gum Arabic emulsion substrate was added and mixed properly. The contents were then titrated with NaOH.

Determination of the amount of fatty acids released per minute µmol fatty acid/ml sample is calculated as follows:

$$[\text{Fatty Acids}] = \frac{(V(NaOH)_S - V(NaOH)_B) \times N \times 1000}{5\ ml}$$

where $V(NaOH)_S$ is volume of NaOH for sample, $V(NaOH)_B$ is volume of NaOH for blank, N is normality of the NaOH titrant used (0.05 in this case).

Transesterification

Transesterification of the oil was carried out according to the method suggested by Abigor *et al.* (2000). Oil sample (1 ml) to 4 mol of alcohol with different concentration of enzyme were incubated and stirred using a magnetic heating stirrer.

Table 1: Quantity of free fatty acid released per unit time of lipase reaction

Sample	Time (Mins)	Titre Value (ml)	Fatty Acids (μmol/ml)
Blank	0.0	1.6	0.0
1	5.0	2.0	4.0
2	10.0	2.2	6.0
3	15.0	2.4	8.0
4	20.0	2.7	11.0
5	25.0	2.9	13.0

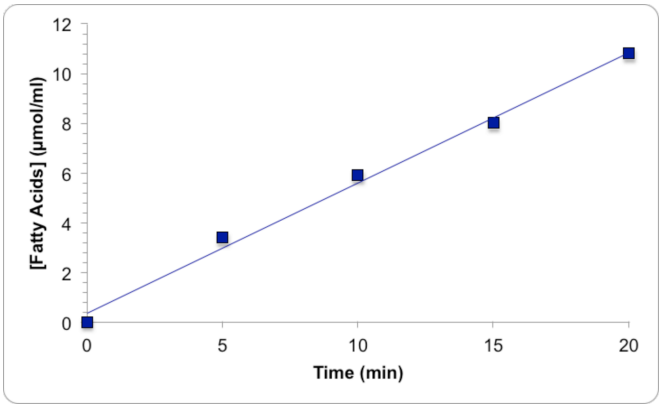

Figure 1: Linear regression of data in Table 1 to calculate the amount of fatty acid released per lipase reaction time.

At the end of the incubation, the alkyl esters were extracted with a mixture containing 4 volumes of hexane/ether (1:1, v/v) and 2 volumes of saturated NaCl. The biodiesel product was dried by passing the sample through glass wool to obtain a pure fraction.

Characterization of Biodiesel

The analysis of the resulting biodiesel was conducted in order to determine its properties and compare it with that of fossil diesel fuel.

Specific Gravity

The specific gravity of the biodiesel was determined by taking a known volume of the biodiesel and weighing it on a weighing balance. The same volume of water was taken and weighed. The ratio of the weight of the fuel of same volume of water was calculated to obtain the specific gravity of the diesel (Alhassan and Issa, 2000).

Density

The density of the oil was calculated using the pykinometer with the formula:

$$\mathcal{P}t = \frac{M_1 - M_0}{V_t}$$

Where M_0 is the mass in gram of the pykinometer or density bottle, M_1 is the gram of the pykinometer at a temperature t, and V_t = Volume in ml of the oil in the pyknometer (Egwim and Ibrahim, 2012).

Flash Point Flash point was determined according to the method suggested by ASTM (2003) using Pensk-Martens Flash point Analyser.

Viscosity Test

Same amount of oil was drawn into the stem of the canon fenske viscometer to the mark above the upper bulb of the viscometer. The time taken for the meniscus to fall between the upper and lower mark of the bulb through capillary tube of the viscometer was recorded and used to compute for viscosity.

Refractive Index

The refractive index was determined using an Abbe refractometer.

Regression Analysis of Percentage Yield versus Time, Temperature and Enzyme Concentration

The percentage yield of biodiesel was determined using the regression equation:

% yield of biodiesel=73.481+ 0.2 (T) + 1.5 (T) + 27.583 [E]

where Temperature is in ^{0}C, Time in Hours, and Enzyme concentration [E] in mg/ml.

RESULTS

The percentage yield of the oil after extraction was estimated to be 56.70%. The activity of the lipase was determined from the slope of the linear portion of the graph showing the amount of the fatty acid released against reaction time, where the units are μmol/(ml x min), equivalent to mM/min. The enzyme activity calculated was 9.3 x 10^{-3} mM/sec. (Table 1 and Figure 1). One unit of lipase was defined as the amount of enzyme that liberates one milimole of fatty acid per minute under the assay conditions.

Effect of Reaction Temperature on Biodiesel Yield

The result in Table 2 shows that as temperature increases biodiesel yield increased up to 40 ^{0}C and yield decreased as temperature was further increased.

Table 2: Effect of Temperature on Biodiesel Synthesis.

Volume oil (ml)	Temperature (^{0}C)	Percentage Yield (%)
10	30	78 ± 0.0
10	40	95 ± 1.4
10	50	82 ± 2.8

Reaction conditions: 0.1 mg/ml of lipase, 1:4 oil to ethanol molar ratio and at 2 hours. Data represents mean ± standard deviation of 3 replicates.

Table 3. Effect of Reaction Time on biodiesel Synthesis

Volume of oil (ml)	Time (Hrs)	Percentage Yield (%)
10	1.0	80.5 ± 2.1
10	2.0	95.0 ± 1.4
10	3.0	83.5 ± 2.1

Reaction conditions: 0.1 mg/ml of lipase, 1:4 oil to ethanol molar ratio and an optimum temperature of 40 ^{0}C. Data represents mean ± standard deviation of 3 replicates.

Table 4. Effect of Enzyme Concentration on biodiesel Synthesis

Volume of Oil (ml)	[Enzyme] (mg/ml)	Percentage Yield (%)
10	0.1	95.0 ± 1.4
10	0.3	96.2 ± 0.1
10	0.5	96.6 ± 0.3

Reaction conditions: 0.1 mg/ml of lipase, 1:4 oil to ethanol molar ratio, for 2 hours and at optimum temperature of 40 ^{0}C. Data represents mean ± standard deviation of 3 replicates.

Table 5. Model Summary showing R^2 Value

Model	R	R^2	Adjusted R^2	Std. Error
1	.128[a]	.016	-.124	0.028
2	.160[b]	.026	-.299	0.089
3	.524[c]	.275	-.160	0.084

a=Predictors: (Constant) ^{0}C; b=Predictors: (Constant), ^{0}C, Hr; c= Predictors: (Constant), ^{0}C, Hr, mg/ml, R^2 Value=30%

Effect of Reaction Time on Biodiesel Yield

The result in Table 3 shows that transesterification time was highest at 2 hours with decrease in yield observed with increased reaction time.

Effect of Enzyme Concentration on Biodiesel Yield

The effect of enzyme concentration was investigated. The result (Table 4) shows that the percentage biodiesel yield increased with increase in enzyme load with maximum yield of about 97%.

DISCUSSION

Biodiesel was produced from allamanda oil using lipase as catalyst in an enzyme catalysed transesterification process. Temperatures, reaction time and enzyme concentration of the reacting mixture influenced the yield of biodiesel produced from allamanda. The oil yield which was about 57% correlated with the yield obtained from allamanda seed as reported by Egwim and Ibrahim (2012). This makes allamanda a cheaper, non-edible source for biodiesel production as compared with other non edible sources such as Jatropha (40%) and Azadarichta (20%) (Mahanta *et al.*, 2008). Lipase catalyses also improved the percentage (97%) oil yield as compared with the use of NaOH (94%). This could be as a result of increased product separation and increase in release of fatty acids with increased time as shown in Table 1.

It is observed from Tables 2 and 3 that an optimum yield of 95% was obtained at a temperature of 40 ^{0}C and at 2 hours. An increase in temperature speeds up enzyme-mediated reactions just like any other catalyzed reactions.

However, enzymes are proteins, and their functions are dependent on their specific structure, and may become denatured when heated beyond an optimum temperature, hence; at 50 ^{0}C a decrease in biodiesel yield was observed, although transesterification process can occur at different temperature and time depending on the oil used.

From Table 4, it was observed that an increase in enzyme concentration from 0.1, 0.3, 0.5 mg/ml led to an increase in

Table 6. Coefficient Table showing the regression Equation Coefficients[a]

Model	Unstandardized Coefficients			Standardized Coefficients	
	B	Std. Error	Beta	t	Sig
(Constant)	81.078	23.599		3.436	.011
Temperature	.200	.589	.128	.341	.743
(Constant)	78.078	28.326		2.756	.033
Temperature	.200	.630	.128	.318	.762
Time	1.500	6.299	.096	.238	.820
(Constant)	73.481	26.999		2.722	0.42
Temperature	.200	.595	.128	.336	.751
Time	1.500	5.953	.096	.252	.811
Enz. Conc.	27.583	21.047	.499	1.311	.247

a= dependent variable: %, Enz.Conc= Enzyme concentration.

biodiesel production from 95%, 96.2%, and 96.6% respectively. A previous study demonstrated that the yield of biodiesel increased when the amount of *Rhizopus oryzae* lipase was increased from 30 to 60 U (Ghamguia *et al.*, 2004). Similar results were reported for the methanolysis of rice bran oil catalysed by *Cryptococcus spp.* S-2 lipase (Abigor *et al.*, 2000; Kamini and Lefuji, 2001) transesterified palm kernel and coconut oils at a time of 4 hours under the same temperature using lipase as catalyst and ethanol, and obtained a maximum yield of 72%, and 35% of biodiesel respectively. The result of the yield of biodiesel from allamanda oil showed 96.6%. This yield is higher compared with other oil yield like soybean oil (80%) (Ma and Hanna, 1999), palm kernel oil (72%) and coconut oil (35%) (Abigor *et al.*, 2000) which are from edible sources, hence oil from allamanda (a non-edible plant), can be effectively used to replace these oils. This also correlates with previous study on the effect of lipase load on Jatropha oil by (Egwim *et al.*, 2012), it was noted that increase in enzyme load for both free and immobilized lipase increased the biodiesel yield. This could be due to the fact that the enzyme activity was saturated. If the saturated state was attained any further addition of the enzyme would not have a significant effect on the yield of the biodiesel.

The regression equation obtained with regards to temperature, enzyme concentration and reaction time in respect to the % yield of biodiesel from allamanda oil using lipase as catalyst was determined. This was carried out in order to predict results for subsequent productions of biodiesel from allamanda oil using lipase as catalyst either in large, medium or small scale by imputing the parameters to be determined into the equation as observed in the results. The R^2 was 30%, which implies that the optimization studies were low and requires further studies.

The result of characterization of oil presented in Table 7 shows that the density and specific gravity of the biodiesel produced from allamanda oil are 0.88g/ml and 0.87 g/ml which are within the range of 0.85-0.89 g/ml and 0.86-0.88 g/ml respectively specified for standard biodiesel. The implication of this finding is that the biodiesel produced from allamanda oil will have a better lubricating effect on the parts of a compressing ignition engine (Knothe, 2006). The viscosity limits present by both ASTM D6751 and EN 14214 are 1.9-6.0 mm^2/s and 3.5-5.0 mm^2/s at 40 ^0C respectively for biodiesel fuels. Viscosity is a key fuel property because it persuades the atomization of fuel upon injection into the diesel engine ignition chamber and ultimately influences the formation of engine deposits (Knothe and Steadley, 2005).

Table 7. Some parameters characterized in the Biodiesel produced.

Parameters determined	Standard Values	Experimental Values
	Biodiesel	Allamanda oil
Specific density	0.85–0.89 g/ml	0.88 g/ml
Refractive index	1.4600	
Viscosity	1.9–6.0 mm^2/s	4.6 mm^2/s
Flash point	100 ^0C	117 ^0C
Energy value	38 MJ/Kg	34 MJ/Kg

In the current study, the viscosity of biodiesel produced was 4.6mm^2/s and this is within the range for both the American and EU biodiesel specification ranges. Flashpoint of fuel is the temperature at which the fuel will ignite when exposed to a flame or spark. The flash point of the biodiesel produced from allamanda oil was 117 ^0C. This value is well above 100 for typical biodiesel as provided by the National Soy Board for biodiesel. This higher value of flash point is an advantage over fossil diesel, which has flash point of 52, in an event of crash. The energy values signifies if the biodiesel can fully power a diesel engine, the energy value of a typical biodiesel is about 38 MJ/Kg this is 9% lower than regular petro diesel (Knothe, 2006). The energy value of biodiesel produced from allamanda oil was 34 MJ/Kg, although it is lower than that of petro diesel; biodiesel undergoes a more complete combustion than petrodiesel thus the engine energy output partially compensating for the higher energy density of petro diesel. All the fuel properties compared favourably with international biodiesel specifications as provided by American Society for Testing of Materials (ASTM) and national soy diesel development board for biodiesel.

This current work has demonstrated that transesterification of allamanda oil with lipase may be used for the production of higher yield of biodiesel with acceptable quality.

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