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Development of Heterogeneous Catalyst from Waste Cow Bone Using *Parinarium Macrophylum* Seed Oil for Biodiesel Production

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Abstract: *The production of biodiesel from oil seeds as feedstock is gaining more attention for the purpose of having alternative fuels without interfering with the food chain. In this study, biodiesel was produced from oils extracted from the P. macrophylum feed, which served as a feedstock. The physicochemical properties of the extracted seed oil were characterized for % yield (45.0%), specific gravity (0.82), refractive index (0.92), pH (4.52), colour (brown), acid value (4.3), saponification (108), iodine value (110), peroxide value (5.3) and viscosity (6.7). The cow bones sample were crushed into fine powdered of size, 105 µm and calcined at 500* ^{0}*C. The calcined cow bone was characterized for TGA, XRF, XRD, BET, SEM and IR. The optimization of the biodiesel production process parameters was done experimentally for the characterize seed oil extracted using the calcined calcium oxide catalyst obtained from the cow bone. The optimized parameters gave methanol to oil ratio (6:1), temperature (55* ^{0}*C), catalyst concentration (1:8 wt%), reaction time (60 min) and agitation speed (350 rpm). The biodiesel yield for the optimized parameters ranged from 75 to 85%. The properties of the produced biodiesel were within the recommended biodiesel standards (ASTM 6751). The cloud point (-10.5* ^{0}*C) of the produced biodiesel indicate that the product can be used in cold temperature region without blending. This study has revealed that P. macrophylum seed is a good feedstock for biodiesel production. Based on these findings, it is recommended that this plant should be domesticated.*

Keywords*: P. mcrophylum seed oil, waste cow bone, transesterification, ASTM standard*

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1.0 Introduction

The use of conventional fossil fuel is continuously gaining relevance as a driver for various human activities such as the production of goods, transportation and other needs (Galadima and Muraza, 2016). However, the global energy crisis and the possible future depletion of conventional fossil fuel, associated with environmental problems are some of the impactful challenges against the utilization of fossil fuels. In view of these shortcomings and the need to seek for alternative and green sources of energy, the global concern is strongly directed towards renewable sources of energy (Chouhan & Sarma, 2015; Muhammad *et al.,* 2015).

The current global economy significantly depends on the production of goods and services and their transportation to where they are needed. Consequently, all these activities rely on fossil fuels ranging from power generation, operations of industrial machines and heavy-duty vehicles. Apart from coal, nuclear power, natural gas and hydroelectricity that serve at different capacities in the provision of energy, the transportation sector alone has been reported to constitute over 90% dependence on fossil fuels (petroleum) with more than 60% annual global fuel consumption (Borges and Diaz, 2016) Renewable sources of energy such as biofuels are generally considered as a better alternative to the to traditional fossil fuel due to their eco-friendliness, ease of accessibility of raw materials and relatively low cost of production (Rabiah *et al.*, 2016).

At an industrial scale, biodiesel is a good alternative to fossil fuel. Biodiesel can be ~~is~~ produced by the reaction of methanol and vegetable oils using an alkaline catalyst (KOH and NaOH). However, application of homogenous catalysts has been seen as a major problem because the separation of the catalyst is difficult and excess water is required for the washing of the product. Also, the catalyst may not be suitable for re-use and can accelerate the corrosion of metallic components (Eddy and Odoemelam, 2008), . Despite challenges associated with the choice of catalyst, a brief survey of the production cost of biodiesel reveals that it is still higher than the cost of production of diesel oil from fossil fuel (Avhad and Marchetti 2015). The high cost is attributed to raw materials and processing techniques that are required for purity. The application of more effective catalysts and the use of local raw materials can reduce production cost. Therefore, the present study is aimed at optimizing biodiesel production by using heterogeneous catalysis from waste cow bone using neou plant (*P. macrophylum*) seed oil.

2.0 Materials and Methods

2.1 Sample collection and preparation

Neou plant seeds, which were the raw materials for the study, collected from Sabon gida area in Niger State. The collected samples were washed to remove impurities and dried to constant weight at room temperature. The dried samples were pulverized into uniform powder using a manual blender. Soxhlet extraction was employed to obtain oil from the seeds. The powdered material (100 g) of the sample of *P. microphylum* was weighed and transferred into a Soxhlet extraction column. The extraction column was plugged with cotton wool at the base with the tap was closed. *n*-hexane was poured into the extraction column (containing the sample) and covered with a plastic wrapper which was held together firmly with a rubber to prevent evaporation of the *n*-hexane. The system was allowed to stand for 48 h at room temperature.

The tap at the base of the extraction column was opened after 48 h and the extracted oil in each case was collected in a 1000 ml beaker. The solvent in the oils was removed using a rotary evaporator. The extracted oil and *n*-hexane were heated in the oven at 55 ^{0}C for 2 h.

The percentage oil yield was calculated using equation1

$$Oil\ Content = \frac{Weight\ of\ oil\ extracted}{Weight\ of\ seed} \times \frac{100}{1} \qquad (1)$$

2.3 Refining and characterization of the extracted oil

The extracted oil is pretreated to remove the dirt and inert materials by the application of degumming and neutralization processes.

The refractive index of the wild seed oils was measured using the Abbe refractometer. The experiment was conducted at 20 ^{0}C (Alamu *et* al., 2008). The specific gravity of wild seeds oils was evaluated at 25 °C using a specific gravity bottle (Odeinde *et al.*, 2009).

$$Specific\ Gravity\ of\ Test\ Sample = \frac{W_3 - W_1}{W_2 - W_1} \qquad (2)$$

where W_1 is the weight of the empty specific gravity bottle, W_2 is the weight of water + Specific gravity bottle and W_3 is the weight of the test sample + Specific gravity bottle

The state and the colour of the oil was noted using visual inspection at room temperature according to the method described by Oderinde *et al.* (2009).

The washed and cleared silica dishes were dried in an oven and cooled in desiccators. 5 cm^3 of the sample was put into the appropriate silica dish and weighed accurately. Each dish and its contents were placed in an oven at a temperature of 105 ^0C to dry for 1 h. Each dish was weighed and repeated until a constant weight was obtained.

The moisture content was calculated using the relationship described by AOAC (2006)

$$\%\,moisture = \frac{m}{m_i} \times \frac{100}{1} \qquad (3)$$

where m is the mass of the sample after drying to constant weight and m_i is the initial mass of the sample,

2.4 Determination of acid value

Diethyl ether and ethanol of about 25 cm^3 respectively was mixed in a 250 ml beaker. The resulting mixture was added to a conical flask containing 10 g of the sample and 1 cm^3 of phenolphthalein (1%) was added to the mixture. The mixture was titrated with aqueous 0.1 M NaOH shaking constantly until a pink colour that persists for 15seconds was observed.

The acid value was determined by the formula:

$$Acid\ value = \frac{Titre\ volume \times 5.61}{Weigh\ \ of\ sample\ used} \qquad (4)$$

The FFA figure is usually calculated as oleic acid (0.1 cm^3 sodium hydroxide ≡ 0.0282 g oleic acid) in which case the acid value = 2 × FFA (Oderinde *et al.,* 2009).

2.5 Determination of peroxide value (PV)

The sample (1 g) was weighed into a conical flask. 1g of powered potassium iodide (KI) and 20 cm^3 of solvent mixture (2 volume of glacial acetic acid + 1 volume chloroform) was subsequently added to the conical flask. The mixture was shaken vigorously and allowed to stand in the boiling water for 30 sec; and immediately poured into another flask containing 30 cm^3 freshly prepared 5% potassium iodide solution. The previous conical flask was rinsed twice with the 25 cm^3 of distilled water and poured into the flask. The mixture was titration stand were it was titrated with 0.002 M sodium thiosulphate solution using a starch indicator. The above procedure was repeated for the blank in absence of the sample (AOAC, 2006).

The peroxide value (PV) is given by:

$$PV = \frac{2(a-b)}{Weight\ of\ oil} \qquad (5)$$

where a is the titre value of sample and b is the titre value of blank.

2.5 Determination of saponification value

For saponification value of the sample oils was weighed into a Quick Fit Conical Flask and 0.1 M of ethanolic potassium hydroxide was added. The mixture was then heated under reflux with constant shaking to allow uniform temperature. Then the hot soap solution was titrated with 0.1 M of HCl using Phenolphthalein as an indicator. A blank was determined under the same condition and the saponification value of the oil was calculated as recommended by the AOAC (2006).

2.6 Preparation and characterization of heterogeneous catalyst from waste cow bones

The heterogeneous catalysts were prepared from the waste cow bone collected from Abattoir in Bida town. The produced catalyst was subjected to characterization for its morphology, surface area, elemental composition and spectral properties using TGA, SEM, XRF, BET and IR techniques.

2.6.1 Preparation of catalyst from waste cow bone for transesterification process of biodiesel

The cheapest source of heterogeneous catalysts is waste cow b one. This material is quite cheap and readily available. The best way to exploit this waste is by using it to produce a catalyst for biodiesel production. The method for preparing this catalyst includes; pretreatment, calcinations, activation and characterization.

2.6.2 Pretreatment

The waste cow bones were collected from Abattoir in Bida and its environs. The collected waste cow bone was washed and dried in a hot air oven at 105 ^0C for 24 h.

The dried waste *cow bone was pulverized in a grinder until they become a powder form.*

2.6.3 Calcinations

The powdered sample from waste cow bone was calcined in an electric furnace at 100, 200, 300, 400 and 500 ^{0}C for 5 h respectively. The weight loss after calcinations process was characterized by electric balance. The % weight loss was calculated according to the equation;

$$\%\, weight\ loss = \frac{w_i - w_f}{w_i} \times \frac{100}{1} \qquad (6)$$

where w_1 is the initial weight and w_f is the final weight . The % yield = 100 - % weight loss

For activation, the calcined waste cow bone was soaked in deionized water for 1 h before being calcined again under 250 ^{0}C for 2 h in electric furnace. The resulted catalyst was then sieved with a meshed size of 106 – 125 nm and kept in desiccators. The produced catalyst was characterized using the following techniques: XRF, BET, XRD and TGA.

2.7 Characterization of the catalyst

The solid catalyst was subjected to several physical and chemical characterizations using various techniques, such as elemental analysis by X-ray fluorescence (XRF). The surfaces are porosity; pure volume and crystallinity of the catalyst were determined by BET. The weight loss of the catalyst was determined by TGA and finally the morphology of the catalyst was determined using SEM analysis.

X-ray fluorescence (XRF)

Calcine waste cow bone ash was analyzed for elemental composition using X-ray fluorescence (XRF), and a Philips 1404 Wavelength Dispersive Spectrometer fitted with a Rh tube was used.

Thermo gravimetric analysis (TGA)

Thermo gravimetric analysis (TGA) was conducted using thermogravimetric analyzer

2.8 *Production of biodiesel*

The transesterification procedure for *P. microphylum* shows that the oil was found to have low free fatty acid content. The process for converting the oils to their methyl esters involved only single step base-catalyzed transesterification process (Chinmoy *et al.,* 2019).

The transesterification reactions under the batch tests were carried out with 100 g of the oil sample in a 500 ml three necked flask, place in a fume cupboard. The appropriate catalyst (CaO) was dissolved in methanol and the resulting solution was added to the oil in the reactor. The oil sample was heated in a hot plate equipped with a mechanical stirrer and the reaction was monitored immediately after the addition of the reactants timed. The reaction was carried out under reflux equipped with a magnetic stirrer for mixing the oil methanol and catalyst at a stirring speed of 600 rpm. After that the reaction had to be stopped by cooling the reactor to room temperature and the product mixture by using a suction flask. The mixture was placed in the separating funnel and allowed to stand overnight to ensure that the separation of the methyl esters and the glycerol phase occurred completely. The glycerol phase (bottom) was removed and left in a separate container. After the glycerol layer has been eliminated, the methyl ester layer was washed with two volumes of water to remove methanol, catalyst and the glycerol residuals until clear. Finally the methyl esters (biodiesel) were dried with sodium sulphate (25 wt% Na_2SO_4) based on the weight of oil. The methyl ester samples are then placed in an ice bath until required for analysis. Experiments were undertaken in duplicate to ensure that erroneous results were quickly identified within a series of samples (Sharman and Gupta, 2008).

2.9 Characterization of the fuel

The quality of the final biodiesel produced from heterogeneous catalysts was characterized for its properties to ascertain if they meet ASTM Standard.

2.9.1 Flash point determination

The flash point of the biodiesel was determined by pouring the methyl ester into a metal container and heated. At the interval, the flame was passed over the surface. Immediately, a flash appeared on the surface of the sample, a reading of the thermometer was then taken according to the method described by Oderinde *et al.* (2009).

2.9.2 Cetane number determination

The fatty acid methyl ester composition of the produced biodiesel was used to estimate the cetane number using correlations developed by Bamgboye and Hansen (2008). The obtained cetane number was then compared to cetane numbers obtained from ASTM standard D613.

2.10 Acid value determination

In a typical procedure, a known amount of sample dissolved in an organic solvent is titrated with a solution of potassium hydroxide with known concentration and with phenolphthalein as a colour indicator. Each titration was repeated four times and the standard deviation was calculated. The acid value was estimated using equation 7

$$AV = \frac{C \times V \times 56.1}{Y\,m} \qquad (7)$$

where Y is the acid value, V is the consumed volume of KOH (ml), C is the concentration of KOH (mol/l), and the *m* is the mass of the tested sample (g) and 56.1 is the molecular weight of KOH. It can also be calculated as 1.99 x FFA % (Alamu *et al.,* 2008).

2.11 Determination of free fatty acid (FFA)

The free fatty acid is the acid value expressed in percentage was estimated using a titrimetric method (Alamu *et al*., 2008).

2.12 Iodine value determination

The ASTM D5768-02 (2006) method reported by Oderinde *et al.* (2009) was adopted for the determination of iodine value

2.13 Density

Density is the mass per unit volume of any liquid at a given temperature. Specific gravity is the ratio of the density of a liquid to the density of water (Srivastava and Prasad 2017). The density of the oil sample at 15 °C was determined using the ASTM standard D1298.

2.14 Saponification value

In order to estimate the saponification value (SV) of the oil, a sample of fatty acid was first saponified by adding 0.5 mol/L potassium hydroxide ethanol, and followed by the addition of excessive potassium hydroxide for titration ~~is titrated~~ with 0.5 mol/L hydrochloric acid until the endpoint is reached. The SV was calculated using equation 8

$$SV = \frac{(V_1 - V_2) \times 56.1 \times c}{m} \qquad (8)$$

where, **V**, is the volume, in milliliters, of specification hydrochloric acid solution required for the blank; **V**, is the volume, in milliliters, of specification hydrochloric acid solution required for the fat; **c** is the concentration of the specification hydrochloric acid solution, in moles per liter; **m** is the mass, in grams of the sample taken.

3.0 Results and Discussion

3.1 *Physicochemical properties of P. macrophylum (Neon plant) seed oil*

The physicochemical properties of oil obtained from *P. macrophylum* seed oil are presented in Table 1.

Table 1: Result of Physicochemical Properties of *P. macrophylum*

Properties	Values
Yield (%)	45
Specific gravity	0.82
Viscosity	6.7
Moisture content (%)	4.5
Refractive index	0.82
pH	4.52
Colour	Brown
Acid value	4.3
Saponification value	108
Iodine value	110
Peroxide value	5.3

After the extraction, it was found that the seed contains 45% of oil, which was higher than 28.5% reported by (Kyari, 2015). The variation in the oil yield maybe due to the solvent used, difference in variety of plant and cultivation climate. It is also evident from the results presented in Table 1 that *P. macrophylum* seed oil contains an appreciable quantity of the oil that is enough for the commercial production of biodiesel. The acid value (AV) of the oil was 4.3 mgKOH/g, which represents the total acidity of the oil and probable include contributions from all the constituent fatty acids content of the glyceride molecule. It is a measure of the extent to which the triglycerides in the oil can be decomposed by lipase action to free fatty acids; Acid value depends on the degree of rancidity and therefore serves as an index of freshness (Encinar *et al.,* 2017). The acid value of oil may be used as a measure of quality.

The acid value of 4.3 mgKOH/g recorded for the *P. macrophylum* seed oil is lower than a value of 7.38 reported for breadfruit seed oil that is 7.38 mgKOH/g but higher than the AV of butter oil which is 1.79 mgKOH/g (Asuquo *et al.,* 2010). The value is also less than those of cashew nut. (10.7 mgKOH/g) and avocado seed oil (16.8 mgKOH/g.). (Akinhami *et al*., 2018). The moderate value for acid value of this oil suggests that the oil may be good for paint making (Akpan *et al.,* 2014). The lower the acid value of oil, the fewer is the fatty acid content and the better is the tendency towards rancidity it (Roger *et al.,* 2016).

Viscosity is a measure of the resistance of a fluid to deform under shear stress. It is an index that evaluates the thickness, or resistance to pouring. Viscosity describes a fluid's internal resistance to flow and is therefore a measure of fluid friction (Nzikou *et al*., 2010). The viscosity of the extracted oil was 6.7, which is within the recommended standard range of 6.3 to 8.8 St, with the exception of melon oil with a value of 15.14.

The refractive index which is the ratio of the velocity of light in a vacuum to the velocity of light in a medium is an indication of the level of saturation of the oil (Oderinde *et al*., 2009). The refractive index analysis shows 0.92 which is slightly less compared to the ASTM recommended range of 1.476 to 1.479.

The estimated moisture content of the oil was 4.5%; which is lower than those reported by Belewu *et al.* (2014) for castor oil (8%), rubber seed oil (8.6%) and shea butter oil (10%) and also comparable to 9.4% and 7.8% reported for oils from cowpea and green pea (Wang *et al.,* 2015). The higher the moisture content in the oil, the lower is the expected percentage of oil yield and the quality of the oil. Studies have shown that high moisture content can accelerate rancidity and could initiate some operational problems such as corrosion and pump plug-in causing deposited formation (Eddy and Odoemelam, 2008; Wang *et al.,* 2015).

The mean peroxide values of the oil were 5.3 meq/g, and is within the range of values reported for some plant seed oil such as *Q. undulate* (5.93±0.06 meq/g), and slightly lower than *A. afzelia* (6.82±0.02meq/g) and (8.57±0.15meq/g). The low concentration of peroxide observed for the oil is an indication of oxidative stabilities of the oil (Kyari, 2015). Therefore, the extracted seed oil may be a good substrate for biodiesel production. High peroxide values are indicative of high levels of oxidative rancidity of oils and also suggest the presence of low concentrations of antioxidants (Kyari, 2015). Peroxide value is the most common indicator of lipid oxidation.

Specific gravity is related to the density of the oil and can be expressed as specific gravity, at a given temperature (Muthu *et al.,* 2014). It is an important property in diesel engine performance because, fuel injection operates on a volume metering system. The specific gravity of the extracted oil was 0.82 and is in the range of values reported for some seed oil such as cottonseed oil (0.83g/cm^3) (Yahaya *et al.,* 2016) and sunflower oil. The specific gravity of most diesel fuel ranged from 0.85 to 0.88 g/cm, which is comparable to the value obtained for the studied seed oil. The slight difference in the density of the oil may be attributed to fatty acid composition of esther and purity of the feedstock (Azam *et al.,* 2015).

The pH for the extracted oil was 4.52, the value is within the range according to Codex Standard (2011) for biodiesel production. The pH for the oil extracted shows that the value is less than seven. This is due to the presence of weak fatty acid in the oils.

The colour for the extracted oil was brown, which is an acceptable appearance for oil meant for biodiesel production (Codex Standard, 2011)

Iodine value is a measure of the degree of unsaturation and it is an identity characteristic of oilseed that promotes it as an excellent raw material for biodiesel production. The iodine value for *P. macrophylum* seed oil is 110 gI$_2$/100 g. Iodine value can be used to quantify the amount of double bond present in the oil which reflects the susceptibility of the oil to oxidation. Oils with iodine value less than 100 gI$_2$/100 g of oil are said to be non-drying oils. Aremu *et al.* (2015) stated that the lower the iodine value the lesser the number of unsaturated bonds and the susceptibility of such oil to oxidative rancidity.

The saponification value is a measure of the tendency towards oxidation (during storage) and hence, the expected deterioration rate (Eddy and Ekop, 2006). The saponification value of the extracted oil was 108 mg KOH/g, which is moderate, compared to several seed oils.

3.2 Characterization of the catalyst

Some analytical parameters for the oxide (CaO) prepared from the waste cow bone are presented in Tables 2 to 4 and also in Figs. 1 to 3. The calcination was carried out at different temperatures. There was more than 30% weight loss for the cow bone at a given temperature and time. The yield was characterized by thermogravimetric analyzer (TGA), X-ray flourometer (XRF), Brunnar Emmet Teller analyser (BET, X-ray diffractometer), Fourier transformed infrared spectrophotometer (FTIR) and scanning electron microscopy (SEM).

Table 2: data from thermogravemetric analysis

Calcination Time (hr)	Temperature (^{0}C)	Weight Loss %	Yield (%)
1	100	35.5	64.5
2	200	33.8	66.2
3	300	30.2	69.8
4	400	28.5	71.5
5	500	28.3	71.7

Results obtained for calcinations time, weight loss and percentage yield at various temperatures are presented in Table 2. The carbon and oxygen content in the cow bone might have been lost after calcinations under high temperatures. The weight loss was more than 28%for the waste cow bone at each temperature and time. The calcinations temperatures and time were progressively increased upto a maximum of 500 ^{0}C and 5h respectively and the percentage yield under this condition was 71.7%.

The crystalline structure of the cow bone changed after calcinations, which may be due to the decomposition of calcium carbonate (CaCO$_3$) to carbon (IV) oxide and and calcium oxide (CaO) (Variya *et al.,* 2010). As the temperature increase over the time, the yield was also observed to increases. High temperature increases the yield of heterogeneous catalysts.

After the calcinations, the calcium oxide produced was activated at 500 °C to improve the surface area of the catalyst. The catalyst was also characterized. The determination of the chemical composition of catalyst was performed using x-ray fluorescence (XRF) at a temperature range of 100 – 500 ˚C. The result shows that ash from waste cow bone mainly contained CaO (51.2%) and can be regarded as a favourable base catalyst in biodiesel production with high basic strength, minor toxicity and easy reactions with water (Joschek, 2016). The remaining 48.8% was made up of different metal oxides (MgO, SiO, P_2O_5, Na_2O, Al_2O_3, K_2O, FeO, Cr_2O_3, TiO_2, ZnO and SrO) in trace amounts. These oxides have been reported to be active materials for transesterification (Haberko *et al.,* 2016). The basic oxides (MgO, K_2O, Fe_2O_3) are useful in the enhancement of the basic strength of the catalyst, while the acidic components (SiO_2, SO_3, P_2O_5,) have the potential to mediate esterification of the feedstock's FFA content.

Table 3: Xray-Fluorescence XRF

Oxides compositions (%)	Cow bone
Fe_2O_3	0.254
CuO	0.023
ZnO	0.039
Al_2O_3	2.105
MgO	0.671
SiO_2	22.62
P_2O_5	2.351
SO_3	0.24
K_2O	0.254
CaO	51.2
TiO_2	0.101
Cr_2O_3	0.016
MnO	0.032
BaO	0.026
SrO	1.131

Table 4: Brunnauer Emmet and Teller (BET) Analysis

Properties	Calcined
Surface area (m^3/g)	92.8
Pore volume (cm^3/g)	26.5
Pure size (Å)	32.5

The catalyst prepared was characterized (BET) by using calcined form with a high pore size (32.5 Å), higher surface area (92.8 m^2/g) aided the reactants to diffuse easily into the interior of the catalyst (Niju *et al*., 2014). Sharma *et al.* (2012) reported that high pore size is desirable for better diffusion of reactant and product molecules. The pore volume o (26.5 cm^3/g) obtained is high compared to the results reported by Birla *et al*. (2012) who recorded a pore volume of (15.7 cm^3/g).

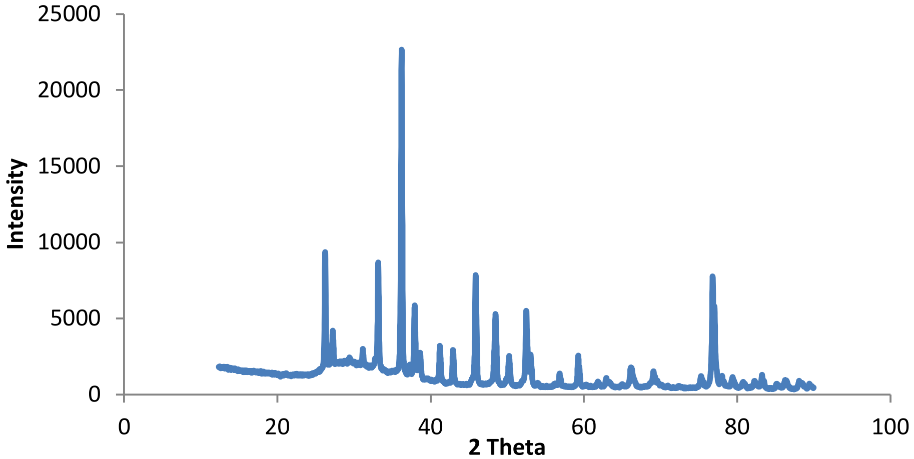

Fig. 1: XRD spectrum for the calcined cow bone

As mentioned earlier, the major components present in the calcined bone samples are CaO, hydroxyapatite, $Ca(OH)_2$, and β-$Ca_3(PO_4)_2$. The major characteristic peaks of CaO were observed in the samples, whose calcination temperature extended from 700 to 1000 ^{0}C. A similar observation was recorded for the peaks attributed to hydroxyapatite, CaO and $Ca(OH)_2$ calcined samples of ostrich bones derived catalyst (Khan *et al.,* 2020). The appearance of calcium hydroxide may be assigned to the hygroscopic nature of CaO. It has also been reported that hydroxyapatite present in calcined bones can potentially transform into beta tricalcium phosphate that is capable of as an active catalyst in transesterification reaction (Farooq, 2015).

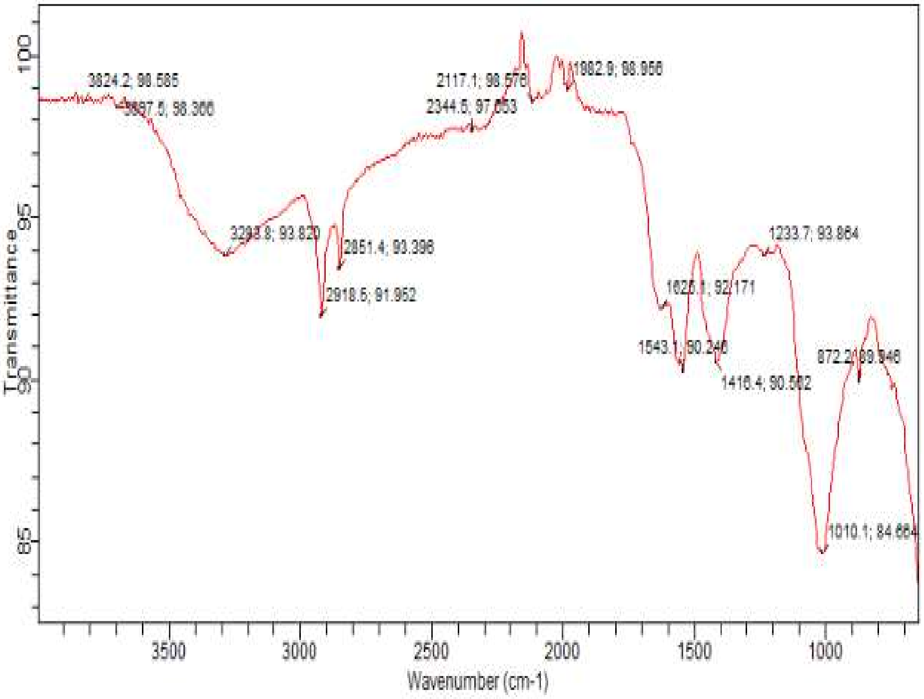

Fig. 2: FTIR spectrum for calcined cow bone

FTIR spectra were recorded for the cow bone that was calcines at 500 ^{0}C (Fig. 2). The calcined sample showed IR bands that corresponded to $CaCO_3$. The broad transmission band at 3824.2 cm^{-1} is attributed to OH stretching vibration from water. Sharp peaks at 2117.1 and 1982.9 cm^{-1} are typical characteristic peaks for C-O stretching and bending modes of $CaCO_3$. Similar peaks have been reported by Joshi *et al.* (2015) for catalysts derived from calcined crab shells.

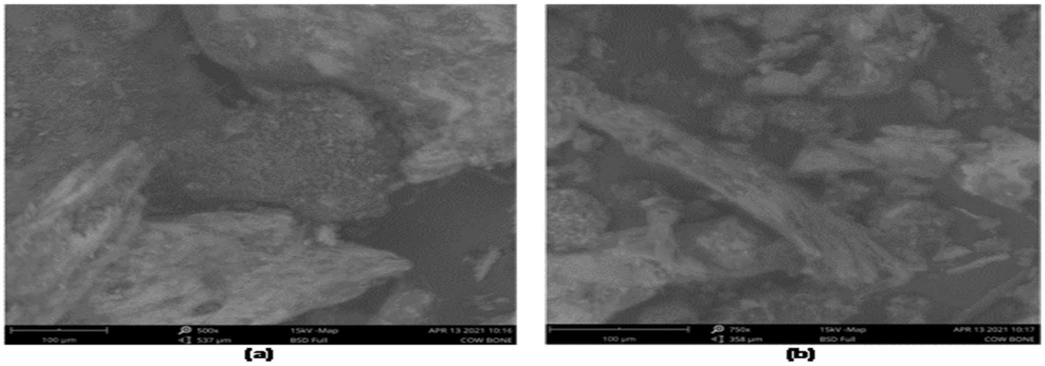

Fig. 3: Scanning electron micrographs of (a) calcined and (b) uncalcined of cow bone.

Scanning electron microscopy (SEM) was carried out to study the morphology of the waste cow bone calcined at 500 ^{0}C for 5 h. Natural waste cow bone exhibited irregular sizes of rod and spherical particles and layers of rod-like structures. After calcination of the waste cow bone at 500 ^{0}C, irregular sizes of semi-spherical particles and, porous surfaces were observed as shown in Fig.3 a and b. The porous surface obtained after calcination may be due to the release of water and gaseous CO_2 during decomposition of $CaCO_3$ to CaO (Madhuvilakku *et al.,* 2013). The stoichiometric ratio for transesterification requires 3 moles of methanol for each mole of oil to yield 3 moles of fatty acid methyl ester and one mole of glycerol. Since transesterification is a reversible reaction, excess methanol is required to drive the reaction towards the product. From Fig. 3, it is evidence that the more preferable ratio for the derived CaO is 6:1 because it gives the maximum biodiesel yield of 85.0%. After this maximum, decreasing yield with mole ratio was observed due to the dilution effect of alcohol. Also, a high concentration of methanol can slow down the separation of the methyl ester and glycerine phases (Tang *et al.,* 2013).

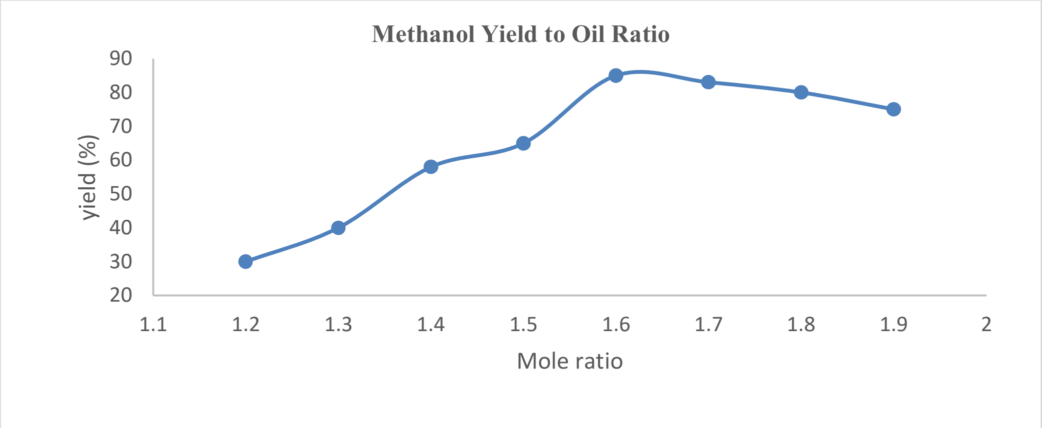

Fig. 3: Methanol to Oil Ratio of Produced Biodiesel

Optimization of Biodiesel Parameters

The reaction and yield of biodiesel were observed to be significantly dependent on the reaction temperature as shown in Fig. 4, which displays the effect of varying the temperature from 20 to 65 ^{0}C on the percentage yield of biodiesel. However, optimum yields were observed at temperatures that were below the boiling point of methanol but beyond this temperature, the decreasing yield was observed because of the saponification reaction of triglycerides (Tariq *et al.,* 2016). The maximum percentage yield of biodiesel obtained at 55 ^{0}C was 82.02%.

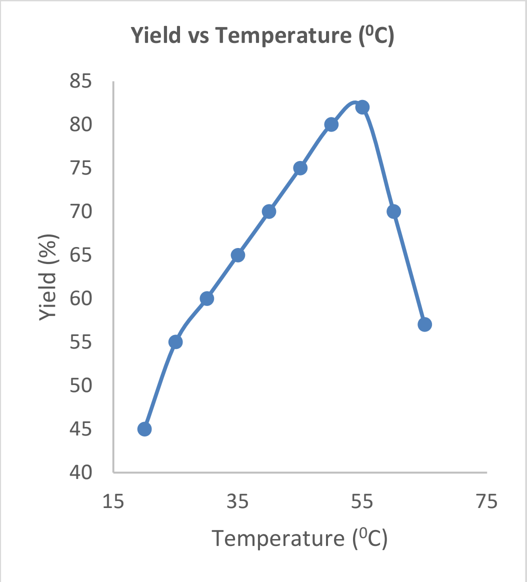

Fig. 4: variation of percentage yield with temperature

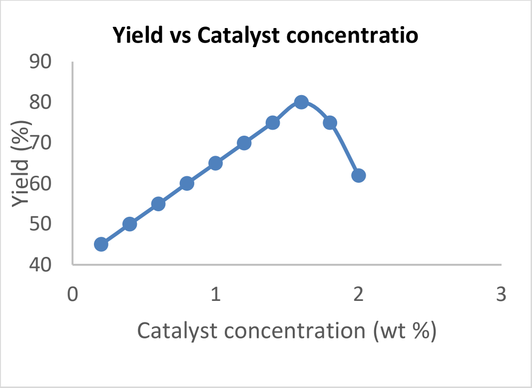

Fig. 5: variation of percentage yield with catalyst concentration

Fig. 5 shows the variation of percentage yield of methyl esters with the concentration of the catalyst. The effect of catalyst concentration on the conversion of oil was negligible when the mass ratio of CaO to oil was increased above 1.8 wt%. Increase in the concentration of the catalyst led to the generation of mixtures that highly viscous and was succeeded by mixing problem and higher demand for power consumption However, when the catalyst loading amount was minimal, maximum production yield was observed. The best catalyst concentration was 1.8 wt% of oil at 55 °C. This was in accordance with the result obtained by Lee *et al.* (Lee and Park, 2019). However, they used soybean oil as feedstock for biodiesel production.

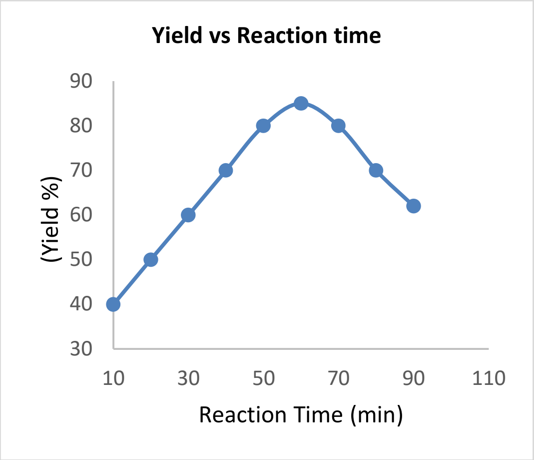

Fig. 6: variation of percentage yield with time

However, the effect of methanol to oil ratio and the effect of reaction temperature, the effect of reaction time also influenced the production of biodiesel as shown in Fig. 5, which reveals the observation made after the variation of the reaction time from 20 – 90 minutes and fixing the methanol to oil ratio and reaction temperature at 6:1 and 55 ^{0}C for the produced catalyst. It is evident from the plots that the maximum yields of 85.04% was obtained in 60 minutes. In the early stage of transesterification, the production of methyl esters gradually increased and the rate diminished and finally started to decrease after about 1 hour. This is because the transesterification reaction between oil and alcohol will form soap due to the reversible reaction when the reaction time increased (Achanai *et al.,* 2015).

The effect of an increase in the speed of agitation was evaluated by plotting the variation in percentage yield with agitation speed as shown in Fig. 7. The figure reveals that the reactants contact during transesterification was relatively enhanced after agitation. The stirring speed was varied from 50 to 500 rpm while other parameters were kept at their optimal values. Phase changes were found to differ with ~~at~~ stirring speed.

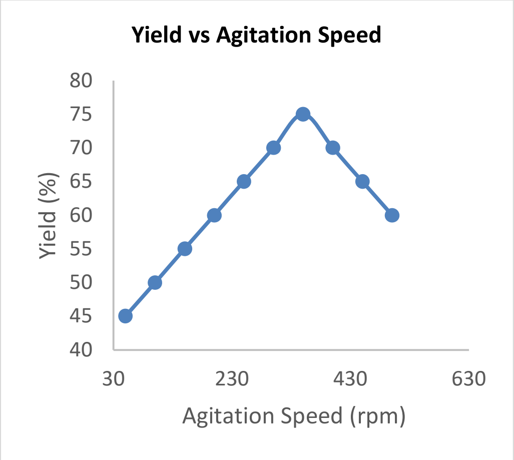

Fig. 7: The variation of percentage yield with agitation speed

At lower stirring speeds facilitated the approach to phase change to be slower than what was observed at higher speeds. From Fig. 7, it is seen that optimal conversion occurred when the stirring speed was 350 rpm (i.e 80% yield).

Table 5: Characterization of Fuel Properties of Biodiesel Produced

Biodiesel properties	Neon plant seed oil	ASTM 6751 standard
Flash point (^{0}C)		100 – 170
Cloud point	120.2	-13 – 12
Cetane number	43.2	48 – 65
Pour point (^{0}C)	-3.2	-1.5 – 10
Kinematic viscosity (mm^2/s)	2.8	2.3 – 6.0
Density (g/cm^3)	0.81	0.75 – 0.84

Cetane number is a measure of the ignition quality of diesel fuel and is a prime indicator of fuel quality in the realm of diesel engines. The cetane number of a fuel is related to the ignition delay time. It represents the time required for the injection of fuel into the cylinder. The result for the Cetane number of the biodiesel was 43.2. This value is in agreement with the report of Yahaya *et al.* (2016) for jathropha oil with 46.75 and hence the results are within the acceptable limit.

Pour point is the temperature at which the amount of wax from biodiesel is sufficient to gel the fuel. The evaluated pour point for the investigated biodiesel was 3.2 °C. This result suggests that the biodiesel produced is within the ASTM standard (-1.5 – 10.0 °C).

Density limits are presented in ASTM Standard (0.75 – 0.84 g/m^3 at 40°C) for biodiesel fuels (Badan, 2012). The density of the produced biodiesel was 0.81 g/m^3, as shown in Table 1. Therefore, the density of the produced biodiesel density is within the ASTM recommended range. The kinematic viscosity is the measure of the resistance to flow by a fuel and can also be used to select the profile of fatty acids in the raw material used for the production of the biofuel. Kinematic viscosity limits specified by ASTM standard for kinematic viscosity ranged from 2.3 to 6.0 mm^2/s at 40°C for biodiesel fuels (Badan, 2012). The kinematic viscosity of produced biodiesel (2.8 mm^2/s) was also found to fit this range, hence the produced biodiesel meets the requirement for kinematic viscosity.

Flash point property is an index for the required precautions in handling, transporting and storage of the fuel. The produced biodiesel met the ASTM standard for flash (100 – 170 ^{0}C) since the measured flash point was 120.2 ^{0}C.

Cloud point and pour point have implications on the use of biodiesel in cold weather. The cloud point is the commonest index for assessing the tendency of a fuel to crystallize. The produced biodiesel has cloud point value of -10.5 ^{0}C, which is within the ASTM standard of (-12 to -13^{0}C).

4.0 Conclusion

Biodiesel production from transesterification of *P. macrophylum* seed oil using cost-effective waste calcined cow bone is investigated was investigated in this study. The waste cow bone contains calcium carbonate ($CaCO_3$) that was calcined at 500 ^{0}C for 5 h and subsequently transformed to calcium oxide (CO_2) leaving the CaO to catalyzed the production of biodiesel from *P. macrophylum* seed oil. Overall biodiesel yield was 85%. Biodiesel properties were evaluated by standard method. All evaluated test parameters were in strong agreement with ASTM standards. Therefore-waste cow bone has a high potential to serve as a low cost, commercial and eco-friendly heterogeneous catalyst for the synthesis of high-quality biodiesel

5.0Conflict of Interest

The authors declared no conflict of interest.

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