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Biodiesel production and characterization from micro algae (*Chlorodesmis fastigiata*)

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ABSTRACT

The steady rise in energy demand around the global coupled with the environmental pollution associated with fossil fuel have given rise to an increase in search for alternative sources of energy that is sustainable and environmentally friendly. Biodiesel is a renewable, clean energy that can be produced from vegetable oils and animal fats. This research was aimed at synthesizing and analyzing biodiesel from micro green algae (Chlorodesmis fastigiata), to determine its potential as an alternative to petrol diesel. The algae samples were collected, identified, cultured, flocculated, harvested, sun-dried and ground prior to the oil extraction. Soxhlet extraction method was used to extract the oil from the algae biomass with n-hexane as the solvent. The oil was characterized of acid value, % FFA, % moisture content, iodine value, density and was converted to biodiesel via transesterification reaction. The biodiesel produced was also characterized of Acid value, Saponification value, Iodine value, Peroxide value, SG etc. The oil yield of the algal oil was 18.77%, Acid value was 3.40mg/g, Specific Gravity was 1.230. The biodiesel yield was 92%, other properties of the biodiesel were acid value 2.20mg/g, SG was 0.984, flash point was 105°C, Cloud point – 6°C, Pour point was -10°c, Cetane number was 49, pH was 6.7 e.t.c. The values of the properties gotten from the analysis were within the American standard (ASTMD6751) and this showed that biodiesel from Chlorodermis fastigiata (green algae) can serve as a good alternative to petro-diesel.

Keywords: *Biodiesel, Chlorodesmis fastigiate, acid value, saponification value, flash point, cetane number.*

INTRODUCTION

Most of the energy that the world is using is derived from non-renewable fossil fuels. The utilization of fossil energy has been on the increase following the quick development of the society, urbanization and industrialization [1]. Due to increased demand for energy, coupled with the global warming association with the emission of greenhouse gases from fossil fuels, global energy security challenges, increasing price of crude oil and rapid diminishing supply of fossils fuels are some major key factors leading to the search for alternative sources of energy to fossil fuels [2]. Some of the notable alternative sources of energy capable of serving as an alternative to fossil fuel include: water, solar, wind energy and biofuels. Biodiesel is one of the examples of biofuel. This research was aimed at synthesis and characterization of biodiesel from green algae to determine if it can serve as an alternative to Fossil fuel.

Biodiesel is a renewable, clean bioenergy as it can be produced from vegetable oils, animal fats and micro-algal oil [3]. Biodiesel has many advantages like it is biodegradable, renewable, less-toxic compared to fossil fuel and has a low emission profile [3]. Biodiesel commonly consists of esters formed by methanol or ethanol and long-chain saturated and unsaturated fatty acids such as Palmitic acid, Lauric acid, Linoleic acid, Oleic acid etc [4].

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The advancement of biodiesel can be divided into three, viz: First – generation biodiesel and was synthesized using edible crops such as soybean, corn sugar cane etc. as feedstocks. These feedstocks exhibited low yield and strained the resources required for societal sustenance [5]. Second-generation of biodiesel were mainly synthesized using Lignocellulose such as straw, hay and other non-foods, as feedstocks. The application of these feedstocks of second generation addressed the limitations of production associated with first-generation biodiesel, however, it was costly and provided low calorific value owning to the features and collection cost of Lignocellulose. Third-generation biodiesel is produced from microalgae, which produce lipid through carbon fixation during photosynthesis and have a yield of lipid to 70% [6]. In addition, microalgae also exhibit a short growth cycle and high yield, and their use as feedstock for biodiesel production does not threaten human food supply. These advantages of microalgae make it an optimal alternative feedstock for biodiesel production. As a bio energy source, microalgae exhibit high photosynthetic efficiency and high yields of biomass and lipid [6]. Microalgae can live on non-arable land such as beaches, saline and alkali soils, and it could also grow in waste water and sea water [7].

Microalgae can be utilized to produce gaseous fuels like hydrogen and biogas, or liquid fuels such as ethanol and liquid hydrocarbons [8]. Micro algae can also be used to produce biodiesel by transesterification [9], Methane production via anaerobic digestion [10]and production of hydrocarbon substances via gasification and pyrolysis [11]. Therefore, all these makes microalgae the most promising alternatives to replace fossil fuels.[6].

MATERIALS AND METHODS

Sample Collection and Identification:

The algae samples were collected from a pond in Awka, Nigeria with the aid of clean plastic containers having sufficient habitat water in order to maintain the natural environment for the algae. Identification of the samples was done at Botany Department of Nnamdi Azikiwe University, Awka Nigeria. The algae samples were cultured in an open pond in a medium of some chemicals that acted as nutrients. According to the method described by [12].

Oil Extraction Procedure:

The algae biomass was harvested after culturing, sun dried for one week, ground to powder before the oil extraction. Soxhlet extraction method was used to extract the oil from the algae biomass. 20 g of the ground powdered algae biomass was weighed into a thimble with electronic weighing balance (W_o) and inserted into a Soxhlet extractor apparatus. 300 mL of n hexane was added to 500ml extraction flask (W_1) and was fitted into the Soxhlet apparatus carrying the condenser. It was heated gently and allowed to reflux after soaking the sample for 3 hrs. After the extraction, the flask with the extracted sample was removed. The process was and repeated until all the ground algae sample were completely exhausted. The extracted oil was separated from the solvent in the flask using rotatory evaporator (W_2). The % oil content was calculated using the equation below.

$$\%oil\ content = \frac{W_2 - W_1}{W_0} \quad 1$$

Where W_1 is the initial weight of flask W_2 is the final weight of the flask W_o is the weight of the sample.

Production of Biodiesel (Transesterification):

About 2 g of NaOH was dissolved in 300 mL of methanol under constant stirring. The resultant solution (methoxide) was poured into heated 50 mL of algal oil and kept under constant stirring using a magnetic stirrer for 6 hrs. This was to ensure the completion of the transesterification reaction. Thereafter, the mixture was transferred into a separatory funnel and allowed to settle overnight in order to attain proper separation. The biodiesel (methyl-esters) was separated from glycerol and the produced biodiesel was first washed with a solution of 0.1% aqueous citric acid to remove the catalyst and subsequently washed 6 times with water to improve its purity. The residual water was removed by drying the oil in the oven at 110 °C for 3 hrs.

Characterization of Algae Biodiesel:

The Physical properties of algal biodiesel produced were determined using (AOCs) standard test methods and the method described by (150 3195). The physical properties analyzed were Density (g/ml), Viscosity (Cst), Flash point, Fire point, cloud point, pour point, and Moisture content.

Saponification Value:

Exactly 2g of the sample was weighed into a 250 mL of conical flask, 25 mL of alcoholic KOH was added and dissolved in the sample completely. The solution was boiled for about 30 min on a boiling water bath. It was cooled to room temperature after which 2 drops of Phenolphthalein indicator was added. The solution was titrated against standard 0.5 N HCl until the pink colour disappears.

The process was repeated for blank in the absence of oil.

$$\text{Saponification Value} = \frac{(Blank + Titre)x\ 100}{Weight\ of\ Sample} \quad 2$$

Peroxide value:

About 10 g of the sample was weighed into a conical flask and 30 mL of acetic acid chloroform was added. The conical flask was shaken very well in order to dissolve the sample after which 0.5 mL of KI solution was added. The conical flask was allowed to stand for 1 minute and swirled at intervals. After a minute 30 mL of distilled water was added and was titrated with standard thiosulphate solution using starch as indicator. Also, a blank was carried out in the same way without the sample. The PV was calculated as:

$$PV = \frac{(T - B) \times N \times 1000}{g} \quad 3$$

Where: T = titration volume for sample;
B = Titration volume for the blank;
N = Normality of thiosulphate used; g = weight of the sample.

Iodine value:

Exactly 0.2g of the sample was weighed into 500 mL conical flask. About 20 mL of chloroform was added and the sample was dissolved completely. It was kept in dark for 1 hour. 20ml of KI solution was added to the sample and was mixed properly. It was titrated against 0.1 N $Na_2S_2O_3$ solution using starch as an indicator with vigorous shaking. A blank was carried out similarly in the absence of the sample.

$$\text{Iodine number} = \frac{A \times N \times 0.1269 \times 100}{\text{weight of oil}} \quad 4$$

Where, A = mL of $Na_2S_2O_3$;
N = Normality of $Na_2S_2O_3$.

Free fatty acid analysis:

About 2 g of the sample were dissolved in 20 mL of ethanol inside a conical flask. 3 drops of phenolphthalein indicator were added to the solution and was titrated against 0.1 N NaOH. It is calculated as follows:

$$FFA = \frac{V\ NaOH \times N\ NaOH \times MW \times 100}{Sample(g) \times 1000} \quad 5$$

Where: V NaOH = Volume of sodium hydroxide used;
N = Normality of sodium hydroxide;
MW = Molecular weight of predominant fatty acid.
Moisture content and pH were determined according to the method used by [13].

RESULT AND DISCUSSION

Table 1: Physicochemical properties of algae oil (Chlorodesmis fastigiata)

Properties	Values
Colour	Green
Solubility	Immiscible
Oil Yield (%)	13.77
SG	1.230
Acid Value (mg/g)	2.40
FFA (%)	1.20
Moisture Content (%)	0.98

Table 2: Result of Physicochemical properties of Chlorodesmis fastigiata biodiesel with American (ASTM) and European (EN) standards.

Properties	Algal Biodiesel	EN 14214	ASTMD 6751
(%) Biodiesel yield	92	-	-
Specific Gravity	0.884	0.860 - 0.900	0.870 – 0.900
Viscosity cSt/s(40°C)	8.00	3.50 – 5.00	1.90 – 6.00
(°C) Flash Point	105	>130	>100 – 170
Fire Point (°C)	115	>140	>140
Pour point (°C)	3	-	-
Cloud point (°C)	-1	-	-
Acid value (mg/g)	1.80	0.500	0.500
% FFA (%)	0.98	0.500	0.600
Peroxide value (meq/kg)	5	-	10max
pH	6.7	-	-
Cetane value	49	>47	>50
Iodine value	82	120MAX	120MAX
Moisture content (%)	0.89	0.05	<1
Saponification value (mgKOH/g)	85.20	<100	<100

The result of the physicochemical properties of the algae oil were shown in Table 1. The colour of the algal oil is green, this maybe as a result of the pigment that was present in the algae biomass. The algal biomass recorded a relatively high oil yield 13.77 % and this makes it a great potential for biodiesel production. The acid value and % FFA were 2.40 mg/g and 1.20 % respectively. This is relatively low and therefore did not require any other pretreatment to reduce the acid value before transesterification reaction [14]. High amount of FFA (%) may result to emulsification and may present great difficulty during separation of the biodiesel from glycerol and during washing of the biodiesel after transesterifications reaction [15]. However, the Acid value and % FFA of the algae oil were higher than value recommended by American (ASTM) and European standard (EN). The acidity of the algal oil is likely from the fatty acid composition of the oil unlike that of fossil fuel that is as the result of its sulfur content [16]. In a study by [17] reported 0.46 mg KOH/g for ASO biodiesel.

The physicochemical properties of the algal biodiesel were shown in table 2 and it was compared with European biodiesel standard (EN 14218) and American biodiesel standard (ASTM D6751). The biodiesel yield that was gotten after the transesterification of the algal oil was 92 %. This implies that almost all the starting algal oil was relatively trans esterified. This was higher than 78 % as reported by [18] for ASO biodiesel but lower than 98 % as reported by [19] in biodiesel production from Vitex doniana (Black plum).

The % moisture content of the algal biodiesel was 0.89%, which was slightly higher than the standard level of ASTM D6751 (0.05%). High moisture content in biodiesel may cause various problems like water accumulation and microbial growth in fuel handling, storage and transportation equipment [20]. The specific gravity of biodiesel relies mainly on the composition of fatty acid compounds and varies with their fatty acid composition and their purity [21]. It is a very important properties of biodiesel. High specific gravity in biodiesel may lead to incomplete combustion and particulate matter emission [21]. The specific gravity of the algal biodiesel was 0.884 which was within the limit of both the American and European standards of biodiesel.

The Iodine value is the measure of degree of saturation or unsaturation in oil. It is a very useful property in analyzing the oxidative rancidity and chemical stability properties of different oil and biodiesel fuels. Oil with Iodine vale above 125 are classified as drying oils; those with Iodine value of 110 are classified as semi-drying oils and those with iodine value below 110 are classified as non-drying oils. Drying oils are susceptible to becoming rancid and should not be preserved for too long [21]. The biodiesel Iodine value was 82 and it was within the standard of both European and American standard. Also, the algal biodiesel is a non-drying oil.

Kinematic viscosity is one of the parameters that mostly affects the performance of the automobile engine because it deals with the movement or mobility of the fuel. It has been revealed that low viscosity fuel produces very subtle spray and cannot get into the combustion cylinder thus forming the fuel rich zone, which leads to the formation of soot [22]. However, higher viscosity turns atomized fuel into larger droplets with high momentum and has the ability to collide wall relatively and this may lead to an increase in deposits and fuel emissions [23]. The algal biodiesel had a viscosity at 40 °C of 8.00 cst which was higher than the limit recommended by both American and European standard (1.90 – 6.00). High viscosity at low temperature can cause unsuitable fuel injection and pumping [24].

Saponification value signifies the amount of saponifiable units per unit weight of oil. A high saponification value signifies a higher proportion of low molecular weight fatty acids in the oil and vice versa [21]. The algal biodiesel had a saponification value of 85.30 mgKOH/g which is within the standard limit of ASTM. A research by Eriola *et al.,* [25] reported a higher value of saponification value (190 mg KOH/g) from Beniseed oil.

Biodiesel cold-weather properties are measured by the cloud point (CP), pour point (PP) and the cold filter plugging point (CFPP). The Cloud point is the temperature at which tiny solid particles can be observed as the fuel cools. The Pour point of a diesel is the temperature at which the fuel loses its flow properties. The result of the Cloud point and Pour point of the algal biodiesel were 3 °C and -1 °C respectively, and were within the ASTM and EN limits.

Peroxide value is the majorly effectively used parameter to determine the level of oxidation in oils and fuel [26]. Biodiesel with high peroxide values (10meqO_2/kg) are regarded unstable and easily become rancid whereas oils and lower peroxide value (10meqO_2/kg) shows that the oil/fuel are stable against oxidation [27]. The algal biodiesel had a peroxide value of and it's within the recommended limit of ASTM and EN.

Cetane number is a measure of fuel's ignition delay. Higher cetane fuels will likely have shorter ignition delay. Fuels with low CN tend to cause diesel knocking and shows rapid rise in gaseous and particulate exhaust emissions due to occurrence of incomplete combustion [28]. The algal biodiesel had cetane number of 49 and it is within the limit of American and European standard of biodiesel. A research by Rachimoellah (2009) [29] reported the cetane index of ASO biodiesel to be 47.

Flash point and Fire point are the parameter used to measure the flammability of a fuel. The Flash point of a flammable liquid is the lowest temperature at which it can form an ignitable mixture in air whereas the fire point is the temperature at which the vapor continues to burn after being ignited for at least 5 secs. It is recorded that the higher the Flash and Fire points in a fuel, the lower the risk the fuel will have during transportation and storage [29]. The algal biodiesel had Flash and Fire points of 105 °C and 115 °C respectively, and therefore posed less risk of fire outbreak during transportation and storage.

CONCLUSION

Microalgae used in this research (*Chlorodesmis fastigiata*) had a relatively high lipid content (13.77%). This made it a good feed stock for biodiesel production coupled with the fact that the algal biomass does not compete with food thereby eliminating food versus fuel debate. Moreover, the properties of the biodiesel such as cetane number, Iodine value, flash and fire point, cloud and pour point etc were all within the ASTMD6751 and EN14214 but kinematic viscosity at 40°C and % FFA were slightly above the recommended standard. This proves that biodiesel from *Chlorodesmis fastigiata* can serve as an alternative to Petro-diesel.

REFERENCES

1. Zhang X., Wu C., Hu C., Li Y., Sun X., Xu N. (2020). 'Lipid remodeling associated with chitooligo saccharides. Induced heat tolerance of marine macroalgae Gracilariopsis Lamaneiformis. Algal Res. 52: 102113.

2. Hou Q., Qi X., Zhen M., Qian H., Nie Y., Bai C. (2021). “Bio refinery roadmap based on catalytic production and upgrading %-hydroxymethyl furfural” Green chem. 23119-231.

3. Khan O., Khan M. E., Yadav A. K., Sharma D. (2017). “Ultrasonic assisted optimization of biodiesel production from eucalyptus oil energy source part A”. Recovery Utilization Environ Eff. 39 (13): 1323-1331.

4. Salic A., Jurinjak Tusek A., Gojun M., Zelic B. (2020). “Biodiesel purification in micro extractors: choline chloride based deepeutectic solvents vs water” sep. purify. Technol. 242.

5. Li J. and Guo Z. (2017). “Structure Evolution of Synthetic Amino Acids-Derived Basic Ionic Liquids for Catalytic Production of Biodiesel” ACS Sustain. Chem. Eng. 51237-1247.

6. Zhang S., Zhang L., Xu G., Li X., (2022) “A review on biodiesel production from microalgae: Influencing parameters and recent advanced technologies”. Front Microbial. 13:970028 Doi:10.3389/fmicb.2022.970028.

7. Zada S., Lu H., Khan S., Iqbql A., Ahmad A., (2021). “Biosorption of iron ions through microalgae from waste water and soil: Optimization and Comparative study” Chemoshpere 2020. 129172.

8. Sanghamitra S., Deshmukh S., Narayan K. P., (2020). “Effects of alternate nutrient medium on Microalgae biomass and lipid production as a bioenergy source for fuel production”. Materials Today: proceedings 28(2):659-664.

9. Vignesh P., Pradeep K. A., Ganesh N. s., Jayaseelan V., Sudhakar K. (2021). “Biodiesel and green diesel generation: an overview.” Oil gas sci. Technol. 76:6.

10. Qi W., Chen T., Wang L., Wu M., Zhao Q., Wei W. (2017). “High-strength fermentable waste water reclamation through a sequential process of anaerobic formation followed by microalgae cultivation” Bioresour. Technol. 227317-323.

11. Su G., Ong H. C., Gan Y. Y., Chem W., Chong C. T., Ok Y. S. (2022). “Co-pyrolysis of microalgae and other biomass wastes for the production of high-quality bio-oil: progress and prospective”. Bioresour. Technol. 344.

12. Faroog M., Ramli A., Subbarao D. (2013) biodiesel production from waste cooking oil using bio functional heterogenous solid catalyst”. Journal of cleaner production (59):131-140.

13. Ikeh, O.A., Okonkwo, N.A., Anarado, IL., Ejidike., L.C, Okpalaunegbu, C.A and Akubudike, E.H(2024). Physicochemical Assessment of Soils from Selected Metal Scrap Dumpsites in Anambra state, Nigeria. Journal of Materials Science Research and Reviews. 7(2): 197-203.

14. Kumar S.P.J. and Banerjee .R.(2013): Optimization of lipid enriched biomass production from Oleaginous fungus using response surface methodology. Indian J.Exp. Biol. 51979-983.

15. Dermibas A. (2006) “Biodiesel production via non-catalytic SCF method and biodiesel characteristics”. Journal of Energy Conversion and management 47 (15): 2271-2282.

16. Ajiwe V. I. E. Ajibola V. O., Martins C. M. A. O. (2003). “Biodiesel fuels from palm oil, palm oil methylester and ester-diesel blends bull Chem. Soc. Ethiopia 17(1), 19-26.

17. Gidigbi, J.A.; Abubakar, A.B. (2023). "Optimization of Bio-Diesel made from Non-Edible Avocado Seed Oil (ASO) using Homogeneous H_2SO_4/KOH Catalyst." Int. Journal of New Chemistry, 10(4): 242-260.

18. Dagde, K.K. (2019). "Extraction of vegetable oil from avocado seeds for production of biodiesel." J. Appl. Sci. Environ. Manage. Vol 23 (2) 215-221. http://dx.doi.org/10.4314/jasem.v2312.3

19. Gloria I. N. and Anselem T. U. (2020). Biodiesel Production from Vitex Doniana (Black plum) seed oil, via a two-step catalyzed Transesterification. Bull chem. Soc. Ethiop. 34(1), 75-82. https//doi.org. /10. 4314/bese v34il.7.

20. Gerpen J.V. (2005) “biodiesel processing and production”. Fuel processing technology 86(10):1097-1107.

21. Onyezeka E. G., Nnaji J. C., Omotugha S. K., Garba M. H. (2020). “Physiochemical properties of biodiesel produced by transesterification of various esterified waste frying oils”. Niger J. Chem. Res. 25(1) 44-56.

22. Putri E. V. Din M. F. M., Ahmed Z., Jamalnddin H., Chelliapan S. (2011). “Investigation of microalgae for high lipid content using palm oil mill effluent (POME) as carbon source.” International Conference on environmental and Industrial Innovation Vol. 12.

23. Ezekwe C. C., Ajiwe V. (2014). The effects of temperature on the viscosities of biofuels from the vetia, gourd and bush melon. Recent Sci. Res. 5 (3): 611-617.

24. Shibi H.M.; Nehdi I.A.; Tan C.P (2014) "Production and characterization of biodiesel from Camelus dromedarius (Hachi) fat". Energy Convers. Manag. 78(5) 1-7.

25. Eriola B.; Tunde F.A.; Akinbiyi K.O.; and Seyi E.A (2012) "Statistical Approach to the optimization of Oil from Beniseed (Sesamum indicum) oilseeds. Journal of Food Science and Engineering 2: 351–357.

26. Canneddu G., Junior L. C. C., Tiexeira G. H. (2016). “Quality evaluation of shelled and unshelled macadamia nuts by means of near-infrared spectroscopy (NIR). Journal of food science, 81: 1613-1621.

27. Nana G. and Sun Y.(2019): Multiobjective optimization of sustainable waste cooking oil(WCO) for biodiesel supply chain network design. Journal of Advanced Transportation. Article ID 6640358. https://doi.org/10.1155/2019/6640358.

28. Nikul P., Padamnabhi N., Shailesh S., Anand P., (2013). “Identification of non-edible seeds as potential feedstock for the production and application of biodiesel” Journal of Energy and power 3:67-78.

29. Rachimoellah H. M., Dyah A. R., Ali Z., Susila W. (2009) 'Production and biodiesel through Transesterification of Avocado (*Persia gratissima*) seed oil using Base catalyst. Journal Teknik Mesin Vol. 11 No. 2 pp 85-90.

