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Biodiesel Potentials of *Chlorella vulgaris* Oil

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ABSTRACT

The sustainability of energy can be achieved through the use of renewable energy sources such as biodiesel from *Chlorella vulgaris*. Energy security is one of the driving forces behind research into biofuels especially, algae to biodiesel research. In this work, photosynthetic microalgae, *Chlorella vulgaris,* were cultured using the Walne's enrichment medium in a photo-bioreactor for biodiesel production. The solvent extraction, using n-hexane, gave 37% oil content. Fossil diesel was used as reference standard while considering the various fuel parameters. The results of fuel properties revealed that *Chlorella vulgaris* biodiesels have kinematic viscosities at 40 ^{0}C, 70 ^{0}C and 100 ^{0}C of 4.967±0.003, 4.087 ± 0.033 and 2.933 ± 0.033 mm^2/s respectively, calorific value: 43333.333 ± 0.0333 kJ/kg, cetane number: 51.333 ± 0.033, relative density: 0.821 ± 0.000 g/ml, sulphated ash: 0.012 ± 0.000 %wt, carbon residue of 0.024 ± 0.000 wt%, cloud points of 4.333 ± 0.333 ^{o}C, flash point was 98.667 ± 0.333 ^{o}C, and pour point was -4.333 ± 0.333 ^{o}C. The results also showed that the biodiesel from *Chlorella vulgaris* and its blends have potentials as substitutes for conventional diesel and complied to the requirements of American Standards for Testing and Materials for biodiesel (ASTM D6751-07b).

Keywords: Biodiesel, *Chlorella vulgaris,* Methyl esters and Photo-bioreactor.

INTRODUCTION

There is increase in awareness and importance attached to environmental issues. Global warming and resulting climate change are generating a growing concern; thereby arousing interest in biomass utilization so as to ameliorate these environmental problems [1]. The utilization of oil extracted from algae (algae oil) for biofuels production will reduce drastically the emission of green house gases which are responsible for the world's biggest challenge of global warming and the resulting effects. The level of carbon dioxide in the atmosphere today are higher than they have been about 100 years ago and these levels are increasing yearly [2]. To reduce the increasing concentration of these gases, there is need for an alternative and environmentally friendly fuels such as biodiesel produced from algae oils and other renewable sources. The dwindling reserve of conventional energy resources and their associated environmental problems have increased the awareness to seek other alternative renewable and sustainable resources for fuel production [3]. According to Food and Agriculture Organization's reports, esculent plants containing oils are used for biodiesel among which about 84% is from rapeseed oil and the remaining is from sunflower 13%, palm oil 1%, soybean and others 2% [4]. Davoodbasha *et al*. [5] discussed the effective, economic and eco-friendly conversion of algal oil into biodiesel using calcium oxide (CaO) nanocatalyst. CaO nanoparticles were used as alkaline catalyst for the transesterification of the indigenous green algae, *Chlorella vulgaris* into biodiesel. The catalyst was subjected to different calcinations temperature and their effect on complete transesterification of algal oil at different concentrations was also studied. Conversion of bio-oil extracted from *Chlorella vulgaris* micro algae to biodiesel via modified superparamagnetic nano-biocatalyst was also studied by Nematian *et al*. [6-7].

Microalgae have been suggested as a good option for biofuels production [8]. *Chlorella vulgaris* is a genus of single-cell green algae, belonging to the *phylum chlorophyta*. It is spherical in shape, about 2 to 10 micro meter in diameter, and is without flagella. *Chlorella vulgaris* contains the green photosynthetic pigments chlorophyll-a and chlorophyll-b in its chloroplast [9]. Through photosynthesis, it multiplies rapidly, requiring only carbon dioxide, water, sunlight and small amount of minerals to reproduce [10]. The name *chlorella* is taken from the Greek *Chloros*, meaning green, and the Latin diminutive suffix *ella*, meaning small [11]. *Chlorella vulgaris* is a type of algae that grows in fresh water. Hundreds of microalgae strain capable of producing high content of lipid have been screened and their lipid production metabolism have been characterized [12]. Under optimum growing conditions micro algae will produce up to 15000 gallons of oil/acre/year and micro-algae are the fastest growing photosynthesizing organisms. They can complete an entire growing cycle in few days having up to 54% oil content [13]. The generalized set of conditions for culturing microalgae is shown in Table 1 [14].

Table 1: A generalized set of conditions for culturing microalgae

Parameters	Range	Optima
Temperature (^{0}C)	16 -27	18 – 24
Salinity (g.L^{-1})	12 -40	20 -24
light intensity (Lux)	1,000 – 10,000	2,500 – 5000
Photo period (light: dark, hours)	-	16:8 (min) 24:0 (max)
pH	7 - 9	8.2 – 8.7

Source: [14].

Information on the flash point, relative density, carbon residue and viscosity of *Cucurbita pepo* seed oil and its methyl ester in regard to its potential to serve as biodiesel feedstock was provided by Ajiwe [8]. However, more information on the fuel properties of the feedstock is still required for commercial biodiesel production [15].

To obtain biodiesel, the algae oil, vegetable oil or animal fat is subjected to a chemical reaction termed transesterification [16]. In transesterification reaction, the oil is reacted in the presence of catalyst with an alcohol which is often methanol to give the corresponding alkyl esters of the fatty acid mixture that is found in the parent algae oil, vegetable oil or animal fat. Scheme 1 depicts the transesterification reaction for producing biodiesel [17].

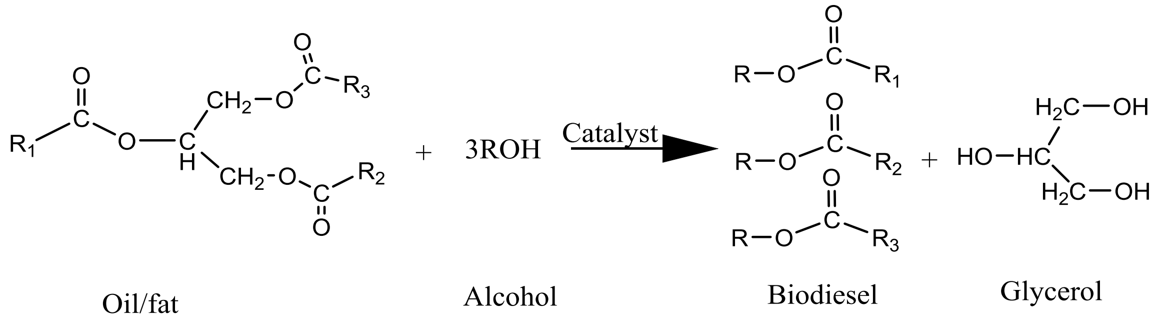

Fig 1: The Transesterification reaction. R_1,R_2 and R_3 are mixture of various fatty acid chain, The alcohol used for biodiesel production is usually methanol (R=CH_3)

Biodiesel can be produced from a great variety of feedstock. These feedstock include most oils from strains of algae, vegetable oils such as soybean, cotton seed, palm, peanut, rapeseed, sunflower, safflower, coconut oils and animal fats usually tallow as well as waste oils or used frying oils. The choice of feedstock depends largely on availability and oil contents of the feedstock [18].

Biodiesel is miscible with petroleum diesel in all ratios. In many countries, this has led to the use of blends of biodiesel with petro-diesel instead of 100% biodiesel. Often blends with petro-diesel are denoted by acronyms such as B20, which indicates a blend of 20% biodiesel with 80% petro-diesel [19]. The non transesterified algae oils, vegetable oils and animal fats should also not be called biodiesel [20].

Some of the American Standards for Testing and Materials for the reference Diesel and Biodiesel are shown in Table 2. This specification covers biodiesel grades for use as a blend component with diesel fuels. This specification prescribes the required properties of biodiesel or diesel fuels. All diesel and biodiesel fuel samples must have values within the specified range [21].

Table 2: ASTM Specification for Diesel and Biodiesel

Property	Diesel	Biodiesel
Standard	**ASTM D975**	**ASTM D6751-07b**
Composition	HC(C_{10}-C_{21})	FAME (C_{12}-C_{22})
Kin. viscosity(mm^2/s) at 40^0C	1.9-4.1	1.9-6.0
Boiling point(^0C)	188-343	182-338
Flash point (^0C)	60-80	100-170
Cloud point (^0C)	-15 to 5	-3 to 12
Pour point (^0C)	-35 to -15	-15 to 16
Cetane number	40-55	48-60

HC= Hydrocarbon, FAME= Fatty Acid Methyl Esters; Source: [21]

The study is aimed at evaluating the biodiesel potentials of oil from *Chlorella vulgaris.* The use of biomass such as biodiesel from algae oil has potentials to greatly reduce greenhouse emissions resulting from the combustion of conventional diesel which biodiesel could be an alternative.

MATERIALS AND METHODS

Materials

The microalgae *Chlorella vulgaris* was collected from the National Research Institute for Chemical Technology, Zaria, Kaduna State. All the chemicals and reagents used were obtained from a chemical store in Zaria, Kaduna State, Nigeria, and they are of analytical grades

Oil extraction from the algae

The oil was extracted from dried *Chlorella vulgaris* with n-hexane using the standard method [22]. A clean, dried 500 cm^3 round bottom flask, containing few anti-bumping granules and 300 cm^3 of n-hexane was poured into the flask fitted with soxhlet extraction unit. The extraction thimble containing 200 g of the dried strain of *Chlorella vulgaris* biomass was placed into the soxhlet extraction unit. Extraction was carried out for six hours and the oil was recovered in a rotary evaporator and the residual solvent removed by drying in an oven at 70 ^oC for 1 hour. The round bottom flask with oil was cooled and the oil content calculated. The extraction procedure was carried out in three replicates to determine the percentage oil yield.

Biodiesel synthesis and dilution with fossil diesel

A three-necked glass reactor in a hot thermostat bath system was used as a batch reactor to produce biodiesel from the extracted oil samples. The transesterification was carried out using heterogeneous catalysis under reaction conditions of 60^oC reaction temperature, 3 hours of reaction time, 6:1 molar based alcohol to oil ratio, 5wt% Li-CaO catalyst and an agitation speed of 300 revolutions per minute method described in Atkins *et al* [23]. Centrifugation of samples was done at 5700 rpm for 30 minutes during which a clear phase separation was obtained. The samples due to differences in densities were separated into two distinct phases, biodiesel and glycerol with solid catalyst layers respectively. Samples for further analysis were prepared from the upper part of the clear biodiesel phase. Different dilutions of each biodiesel from *Chlorella vulgaris* and fossil diesel in the ratio of 10:90, 20:80, 30:70, 40:60, 50:50, 60:40, 70:30, 80:20 and 90:10 were prepared accordingly.

Analysis of fuel parameter of the *Chlorella vulgaris* oil, biodiesel and biodiesel blends with fossil fuel

The fuel parameter analysis such as relative density, sulphated ash, cloud point, carbon residue, flash point, pour point, viscosity at (40 ^0C, 70 ^0C and 100 ^0C), calorific value and cetane number of the *Chlorella vulgaris* oil, biodiesel and various blends were determined according to the American Standard for Testing and Materials [24]. The relative density of the samples was measured at 25 ^0C using the relative density bottle (ASTM D1298). Flash point was determined using Pensky Martin apparatus (ASTM D93). Kinematic viscosity was determined using

Redwood Viscometer (ASTM D445). The cetane number, copper strip, carbon residue and sulphated ash were determined according to ASTM methods: D613, D130, D189 and D874 respectively.

RESULTS AND DISCUSSION

The percentage oil contents of *Chlorella vulgaris* in this study was 37%. The oil content reveals good source of oil for biofuels production and compares well above *Jatropha curcas* with oil content of 32% as reported by Liu *et al* [25]. The results of the comparative fuel parameters of *Chlorella vulgaris* oil, its methyl esters, methyl ester/diesel blends and diesel are presented in Tables 3 and 4. The relative density of *Chlorella vulgaris* methyl ester was 0.821 ±0.000. This is within the ASTM specifications (0.86-0.9), the *Chlorella vulgaris* oil has 0.952 ± 0.000 relative density which is slightly higher than that of *Chlorella vulgaris* methyl ester as a result of the oil's high viscosity. The relative densities of all the blends are within the acceptable ASTM limits [25]. The sulphated ash content of the *Chlorella vulgaris* is 0.012 ± 0.000 which is lower than the maximum limit of the sulphated ash using the ASTM 874. The biodiesel test samples and all the blends had sulphated ash values within the prescribed limits.

The kinematic viscosities of both *Chlorella vulgaris*, its methyl esters and their blends at 40 ^{o}C, 70 ^{o}C and 100 ^{o}C were within the biodiesel specification and may be recommended for use in a diesel engine (D 7467). The results indicated that the kinematic viscosities increase as the temperature increases as seen in Table 3. The calorific value of fuel is the heat available from the fuel when it is completely burned, expressed as heat units per unit of fuel weight or volume. The calorific value of *Chlorella vulgaris* oil, its methyl esters and their blends showed an energy content similarity with conventional fossil diesel.

The *Chlorella vulgaris* methyl ester has a cetane number of 51.333 ± 0.333 whereas the raw *Chlorella vulgaris* oil has 37.000 ± 0.000 cetane number. The results reveal that the biodiesel have a higher cetane number than the petroleum diesel fuel. ASTM D-6751 specified the minimum value of cetane number 51. The cetane number values for all the blends are above the minimum limits which will ensure good cold start properties and minimize the formation of white smoke.

The sulphated ash is a measure of the residue from fuel/lubricant remaining after the sample has been carbonized, treated with acid and heated to a constant weight. The sulphated ash content of diesel is 0.067 ± 0.000% whereas that of *Chlorella vulgaris* methyl ester is 0.012 ± 0.000% which is below the maximum limit of sulphated ash using the ASTM D-6751.

The flash point of fuel is the minimum temperature at which it gives enough vapour to form a flammable mixture that ignites for a moment, when a tiny flame is brought near it. It can be seen that flash points of *Chlorella vulgaris* methyl esters were generally higher than that of fossil diesel. This could be attributed to the higher number of fatty acid methyl esters which are not volatile. High flash points signified the safety of the fuels during storage and transportation.

Table 3: Comparative results for fuel parameters of *Chlorella vulgaris* oil, its methyl esters, methyl ester/diesel and diesel

Variables	Relative density At 28 ^{O}C	Sulphated ash (%wt)	Cloud point (^{O}C)	Carbon residue (%wt)	Flash point (^{O}C)
Diesel	0.863 ± 0.000	0.067 ± 0.000	5.000 ± 0.000	0.042 ± 0.000	70.333 ± 0.333
CVO	0.952 ± 0.000	0.070 ± 0.000	5.000 ± 0.000	0.031 ± 0.000	84.000 ± 0.000
CVME	0.821 ± 0.000	0.012 ± 0.000	4.333 ± 0.333	0.023 ± 0.000	98.667 ± 0.333
10CVME:90D	0.860 ± 0.000	0.015 ± 0.001	3.000 ± 0.000	0.022 ± 0.000	82.333 ± 0.333
20CVME:80D	0.850 ± 0.000	0.015 ± 0.001	3.000 ± 0.000	0.001 ± 0.000	82.667 ± 0.333
30CVME:70D	0.871 ± 0.000	0.012 ± 0.000	3.667 ± 0.333	0.005 ± 0.000	83.667 ± 0.333
40CVME:60D	0.870 ± 0.000	0.021 ± 0.000	3.000 ± 0.000	0.004 ± 0.000	84.333 ± 0.333
50CVME:50D	0.881 ± 0.000	0.013 ± 0.000	4.000 ± 0.000	0.005 ± 0.000	83.667 ± 0.333
60CVME:40D	0.880 ± 0.000	0.012 ± 0.000	4.000 ± 0.000	0.004 ± 0.000	83.333 ± 0.333
70CVME:30D	0.891 ± 0.000	0.014 ± 0.000	3.667 ± 0.333	0.005 ± 0.000	83.000 ± 0.000
80CVME:20D	0.890 ± 0.000	0.015 ± 0.000	3.000 ± 0.000	0.005 ± 0.000	83.333 ± 0.333
90CVME:10D	0.891 ± 0.000	0.014 ± 0.000	4.000 ± 0.000	0.004 ± 0.000	83.667 ± 0.333

CVME=*Chlorella vulgaris* Methyl Esters, 10CVME:90D= Ratios of 10CVME to 90Diesel,CVO= *Chlorella vulgaris* Oil, and D= Diesel,

Table 4: Comparative results for fuel parameters of *Chlorella vulgaris* oil, its methyl esters, methyl ester/diesel and diesel

Variable	Pour point (^{O}C)	Viscosity@40^{0}C (mm^2/s)	Viscosity @70^{0}C (mm^2/s)	Viscosity @100^{0}C (mm^2/s)	Calorific value (kJ/Kg)	Cetane no -
Diesel	-7.667 ± 0.333	3.387 ± 0.003	2.033 ± 0.003	1.433 ± 0.003	45125.333 ± 0.333	56.667 ± 0.333
CVO	5.667 ± 0.333	10.297 ± 0.003	6.687 ± 0.013	4.053 ± 0.023	44004.667 ± 0.667	37.000 ± 0.000
CVME	-4.333 ± 0.333	4.967 ± 0.033	4.087 ± 0.003	2.933 ± 0.033	43333.333 ± 0.333	51.333 ± 0.333
10CVME:90D	-3.667 ± 0.333	4.277 ± 0.007	3.943 ± 0.003	2.023 ± 0.003	44623.333 ± 0.333	54.000 ± 0.000
20CVME:80D	-3.667 ± 0.333	5.027 ± 0.003	3.670 ± 0.000	2.287 ± 0.003	44620.667 ± 0.667	50.333 ± 0.333
30CVME:70D	-3.000 ± 0.000	5.007 ± 0.003	3.780 ± 0.006	2.283 ± 0.003	43764.333 ± 0.333	52.000 ± 0.000
40CVME:60D	-3.667 ± 0.333	4.860 ± 0.040	3.937 ± 0.003	2.697 ± 0.003	44233.667 ± 0.333	51.000 ± 0.000
50CVME:50D	-4.000 ± 0.000	4.600 ± 0.000	2.967 ± 0.033	2.733 ± 0.003	44373.667 ± 0.333	50.667 ± 0.333
60CVME:40D	-3.333 ± 0.333	4.133 ± 0.033	3.983 ± 0.007	2.557 ± 0.007	44675.667 ± 0.667	52.000 ± 0.000
70CVME:30D	-4.000 ± 0.000	4.690 ± 0.010	2.933 ± 0.003	2.667 ± 0.067	44533.667 ± 0.333	51.333 ± 0.333
80CVME:20D	-3.667 ± 0.333	4.000 ± 0.000	3.327 ± 0.003	2.667 ± 0.003	44432.333 ± 0.333	52.000 ± 0.000
90CVME:10D	-3.000 ± 0.000	4.027 ± 0.003	2.993 ± 0.007	2.727 ± 0.027	44600.667 ± 0.333	50.333 ± 0.333

CVME= *Chlorella vulgaris* Methyl Esters, 10CVME:90D= Ratios of 10CVME to 90Diesel, CVO= *Chlorella vulgaris* Oil andD= Diesel,

CONCLUSIONS

The evaluations of the fuel parameters of biodiesel produced from *Chlorella vulgaris* oils revealed the similarities in fuel properties with that of the reference conventional diesel using the various American Standards for Testing and Materials (ASTM D6751-07b). The various blending of *Chlorella vulgaris* biodiesel and the petrodiesel have fuel parameters similar to that of the petrodiesel and thereby suggests a potential alternative to the petrodiesel. This work has shown that *Chlorella vulgaris* oils could be a suitable feedstock for biodiesel production since they are not edible and its use would not lead to food crisis. Further performance evaluation and emission studies of the fuel from *Chlorella vulgaris* oils are recommended.

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