Biotechnology Journal International

22(1): 1-11, 2018; Article no.BJI.44261
ISSN: 2456-7051
(Past name: British Biotechnology Journal, Past ISSN: 2231–2927, NLM ID: 101616695)

Effects of Sun and Oven Drying Techniques on Quality of Oil Produced from *Chlorella vulgaris* (Microalgae) Biomass

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Authors' contributions

This work was carried out in collaboration between all authors. Authors IAY, JA and MBY designed the study, wrote the protocol and wrote the first draft of the manuscript. Authors IAY and JA performed the statistical analysis. Authors IAY, HD and FLC managed the analyses of the study. Authors IAY and MBY managed the literature searches. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/BJI/2018/44261
Editor(s):
(1) Dr. Kuo-Kau Lee, Professor, Department of Aquaculture, National Taiwan Ocean University, Taiwan.
Reviewers:
(1) G. Jakasania Ronak, Junagadh Agricultural University, India.
(2) Lim Jun Wei, Universiti Teknologi Petronas, Malaysia.
(3) Ige Ayodeji Rapheal, Kebbi State University of Science and Technology Aliero, Nigeria.
Complete Peer review History: http://www.sciencedomain.org/review-history/26988

Original Research Article

Received 30 July 2018
Accepted 04 October 2018
Published 01 November 2018

ABSTRACT

This study involved the molecular identification of *Chlorella vulgaris* microalgae obtained from Kaduna State University Fish Pond for biodiesel production potential. The DNA of *Chlorella* microalgae was extracted and subjected to PCR. The molecular weight of the PCR product obtained was 1.8kb using 18S rDNA primer sets and BLAST analyses revealed 95% identity with *Chlorella vulgaris*. The *Chlorella vulgaris* was cultured in open aquaria tanks at the Department of Biological Sciences, Nigerian Defence Academy. The biomass harvested was subjected to varying timings of

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sun and oven drying techniques (25-35ºC for 72hours and 60ºC for 12hours respectively) before extraction of oil from the biomass using solvent extraction method. The values for the density (0.854 and 0.867 cm^3), specific gravity (0.875 and 0.876), acid value (0.414 and 0.384 mgKOH/g), saponification value (173.3 and 170.1 mgKOH/g), kinematic viscosity (5.200 and 3.870 mm^2/g at 40ºC), flash point (114 and 115ºC) and cetane number (54.00 and 47.70) for the sun and oven dried biomass oil respectively were found to be in accordance with the ASTM standard values for biodiesel and fossil diesel. GC-MS analyses of the oil extracted using the two drying methods showed that the fatty acid profiling of the oil obtained from sun dried processed biomass had C14:0, C15:0, C16:0, C18:0, C18:1 cis9 and C22:1ω9 while the oven dried biomass oil had C14:0, C16:0, C19:0, C11:1, C18:1 cis9 and C22:1ω9. Drying methods, therefore, had influenced on the composition of saturated and unsaturated fatty acids. The oven dried biomass oil possesses high monounsaturated fatty acids when compared to sun dried biomass oil though the most important fatty acids (C14:0, C16:0 and C18:1) found in standard biodiesel were present in both. The results suggested that *Chlorella vulgaris* microalgae can be sustainably harvested for the production of biodiesel, both drying techniques can be employed for effective extraction but oven dried biomass oil was found to be of high quality because of the balanced in saturated and unsaturated fatty acid compositions and have an easy mode of operation but it required instrumentation.

Keywords: Chlorella vulgaris; biodiesel; biomass; sun drying; oven drying.

1. INTRODUCTION

1.1 Background of the Study

Research into the development of sustainable energy resources and the reduction of carbon dioxide emissions is thriving due to soaring oil price and global climate change. Emphasis on the development of renewable, biodegradable, and environmentally friendly industrial fluids, such as diesel and other fuels have raised the need to search for alternative renewable fuels [1]. Among the options for renewable energy, biofuels produced from biomass feedstock are of most interest to the global energy structure [2,3].

Biofuels are solid, liquid or gaseous fuels derived from organic matter. They are generally divided into primary and secondary biofuels. Primary biofuels such as fuel wood are used in an unprocessed form primarily for heating in cooking or electricity production, secondary biofuels such as bioethanol and biodiesel are produced by processing biomass and are used in vehicles and various industrial processes. The secondary biofuels can be categorised into three generations: first, second and third generation biofuels on the basis of different parameters, such as the type of processing technology, type of feedstock or their level of development [4].

First generation biofuels correspond to those issued from food-based crops. They mainly correspond to ethanol-based fuels obtained from the fermentation of sugars (corn, beet, sugar cane, etc.), vegetable oil-based fuels (raw oil, biodiesel and renewable diesel produced from catalytic hydro deoxygenation) from oleaginous plants (colza, palm, canola, etc.) and biogas emitted from raw material or landfills [5-7]. However, the fact that food resources could be used to produce biofuels shows economic and environmental limitations. The most common concern related to the current first generation biofuels is that as production capacities increase, so does their competition with agriculture for arable land used for food production. The increased pressure on arable land currently used for food production can lead to severe food shortages. In addition, the intensive use of land with high fertiliser and pesticide applications and water can cause significant environmental pollution [8].

Second generation biofuels are the cellulosic-based biofuels obtained from non-food crops materials (wood, leaves, straw, etc.) i.e. the woody part of plants that do not compete with food production. Sources include agricultural residues, forest harvesting residues or wood processing waste such as leaves, straw or wood chips as well as the non-edible components of corn or sugarcane.

However, converting the woody biomass into fermentable sugars requires costly technologies involving pre-treatment with special enzymes, meaning that second generation biofuels cannot be produced yet economically on a large scale [9].

Third generation biofuels are microorganisms (yeast, fungi) biofuels and algae-based fuels like

vegetable oils, bio-oil, jet-fuels, bio-hydrogen, biodiesel, renewable diesel and many others [10,11]. Third generation biofuels derived from microalgae are considered to be a viable alternative energy resource that is devoid of the major drawbacks associated with first and second generation biofuels [4,12,13]. Commercialisation of microbial technology for biofuel production remains intricate and questionable due to many factors concerning the life cycle assessment and techno-economic feasibility of microorganisms-based biofuels [14].

Microalgae are diverse group of aquatic organisms, are thallophytes (plants lacking roots, stems, and leaves) that have chlorophyll *a* as their primary photosynthetic pigment and lack a sterile covering of cells around the reproductive cells [9]. The mechanism of photosynthesis in these microorganisms is similar to that of higher plants. Microalgae are generally more efficient converters of solar energy because of their simple cellular structure. In addition, because the cells grow in aqueous suspension, they have more efficient access to water, CO_2, and other nutrients [12].

Chlorella vulgaris is a green microalga from the family Chlorellaceae, it thought to have potential for biodiesel production [15]. *Chlorella vulgaris* compared with conventional crop plants that are usually harvested once or twice a year, have a very short harvesting cycle (1– 20 days depending on the process), allowing multiple or continuous harvests with significantly increased yields [8].

Recently, microalgae has received great attention as a feedstock for biofuels due to its rapid growth compared to regular terrestrial plants and the efficiency with which it captures carbon dioxide from the atmosphere [16]. The growth of microalgae has few territorial restraints and the resulting microalga biomass contains valuable components, such as proteins, sugars and lipids [17]. Moreover, lipid-deprived residues can also be used as substrate in the production of other biofuels, such as hydrogen, bioethanol and methane [18]. Biomass is a source of energy, which can be used to produce 1st, 2nd and 3rd generation biofuels.

Biodiesel from *Chlorella vulgaris* is gradually gaining acceptance in the market as an environmentally friendly alternative diesel fuel [19]. However, for *Chlorella vulgaris* biodiesel to become established and continue to mature in the market, various aspects must be examined and overcome. One of the key issues is improving the efficiency of the production process such as improving lipid yield by investigating effective suitable harvesting and drying techniques for a particular algae as well as reducing time and energy consumption.

This study investigates and presents the effect of sun and oven drying techniques on quantity and quality of oil produced from *Chlorella vulgaris* in order to address the challenge of scaling up biodiesel production process from pilot scale to industrial scale.

2. METHODOLOGY

2.1 Isolation of Microalgae

A strain of *Chlorella vulgaris* was isolated from Department of Biological Sciences fish pond, Kaduna State University (KASU), Kaduna, Nigeria as describe by Mutanda et al. [20].

2.2 Molecular Characterisation of *Chlorella vulgaris*

The DNA of *Chlorella vulgaris* microalga was extracted using Bioland mini prep extraction kits.

18S rDNA primer sets were designed and procured from Bioneer Company i.e. SS5 (forward, 5'-GGTGATCCTGCCAGTAGTCATATGCTTG-3') and SS3 (reverse, 5'-GATCCTTCCGCAGGTTCACCTAC GGAAACC-3') were used for PCR reaction [21].
Following PCR reactions, the quality of the PCR products were determined through 1% standard Agarose gel electrophoresis (Agarose Gel Electrophoresis, n.d).

The DNA obtained was sequenced in DNA laboratory kaduna, using BECKMAN COULTER CEQ 2000XL DNA analysis system and the sequences obtained were compared for homology against sequences on the GenBank at the NCBI website for Identification of the microalgae.

2.3 Cultivation of *Chlorella vulgaris* Microalgae for Production of Biomass

The *Chlorella vulgaris* isolate was grown in a 1 litre conical flask containing 500 ml of nutrient

medium. The conical flask was placed under 12hours of natural light at a temperature of 18°C to 28°C and harvested after 20 days by filtration. The resulting microalgae were then transferred and cultured under natural light in open rectangular transparent aquaria of about 35 litres capacity. Bristol medium was used as nutrient media for the culture of the microalgae at a temperature of 18°C to 28°C following manufacturer's protocol. This was based on the method described by Ehimen, et al. [22].

Sedimentation and filtration were done to obtain thick biomass slurry. Harvesting of biomass was initiated on day 42 by gravitational settling to remove the bulk amount of water followed by filtration. Thick biomass slurry was obtained.

2.4 Drying Techniques

The harvested wet *Chlorella vulgaris* biomass were dried using two different drying techniques namely: sun drying and oven drying. Thick slurry of wet biomass obtained from the 35L microalga culture after gravitational settling followed by filtration were subjected to different drying techniques. *Chlorella vulgaris* biomass was sun-dried on a drying bed lined with white plastic of 1500 µm thickness at a temperature of 25–35°C for 72 hours as described by Rwehumbiza, et al. [23]. For oven drying, wet biomass was placed in an oven overnight at a temperature of 60°C for 12hours as described by Lee, et al. [24]. The dried *Chlorella vulgaris* biomass was crushed using a mortar and pestle and the dried *Chlorella* powder was stored in desiccator to avoid absorption of moisture. The culturing and drying techniques were repeated to accumulate a sufficient amount of dried biomass for oil extraction experiment.

2.5 Oil Extraction

A solvent extraction method was used for the oil extraction. This was done using Soxhlet assembly. The dried *Chlorella vulgaris* (powdered form) weighing 200 g was fed to a soxhlet extractor containing n-hexane in the round bottom flask which serves as the solvent. The soxhlet is then connected to a reflux condenser. The oil extraction was carried out for 6 hours at a temperature of 60°C after which the solvent evaporated over a water bath until completely evaporated using a method described by Bilal et al. [25].

2.6 Determination of the Quality Parameter of the *Chlorella vulgaris* Crude Oil

The Density, Specific gravity, Acid value, Saponification value and Gas Chromatography – Mass Spectrometry were determined and compared with ASTM standard values. These were done at National Research Institute for Chemical Technology (NARICT) Zaria.

2.7 Transesterification of the Oil

The transesterification of *Chlorella vulgaris* oil was carried out using methanol. The process was carried out according to standard procedure using 6:1 molar ratio of methanol to microalgae oil for 1hr at 30°C along with 5 wt.% NaOH as a catalyst. Transesterification is the reaction of triglycerides to microalgae methyl esters (MME) and low molecular weight alcohols such as methanol or ethanol in the presence of catalyst [26]. At the end of the reaction, the mixture was allowed to cool at room temperature without agitation to achieve a two phase separation. The upper phase of the mixture is microalgae methyl ester (MME) i.e. the biodiesel. The lower phase consists of glycerol, excess methanol, catalyst, soap formed during the reaction, some entrained MME and traces of glycerides. The two phases were separated by decantation. This was based on a method described by Bilal et al. [25].

2.8 Determination of the Quality Parameter of the *Chlorella vulgaris* Biodiesel

The Kinematic Viscosity, Flash point and Cetane number were also determined and compared with ASTM standard values for biodiesel.

The Kinematic viscosity is the resistance of a liquid to deform by shear stress or tensile stress [27]. This was carried out at NARICT in Zaria using viscometer according to ASTM D-445.

The Cetane number is an indicator of combustion speed of diesel fuel i.e. measure of the ignition quality of diesel fuel [27]. The higher this number the easier it is to start a standard diesel engine. This experiment was carried out at NARICT in Zaria.

The Flash point is an indicator of combustion speed of diesel fuel i.e. measure of the ignition quality of diesel fuel [27]. The higher this number the easier it is to start a standard diesel engine.

This experiment was carried out at NARICT in Zaria.

2.9 Data Analysis

Data obtained were statistically analysed using graphpad prism 7 statistical software for significance at 95% level by analysis of variance (ANOVA) and Chi-square methods.

3. RESULTS

3.1 Molecular Characterisation Results

The DNA concentration ranged from 1- 10 µg. The molecular weight of the PCR product obtained was 1.8 kb with the 18S rDNA primers when matched with the DNA molecular weight ladder.

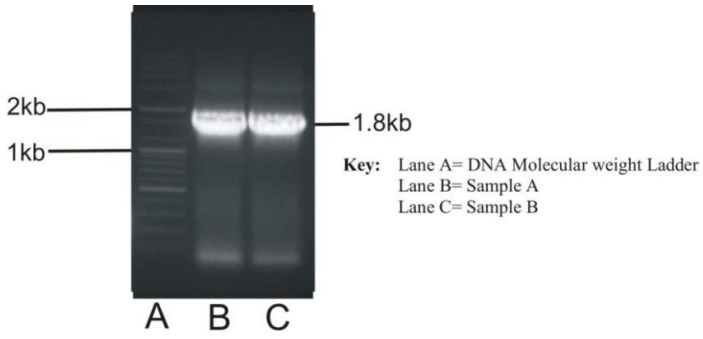

Plate 1. Gel Electrophoresis result of *Chlorella vulgaris*

After Sequencing reaction, viewing and editing; the following sequence was obtained for the sub-unit in fasta format;

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TGTGTGTGTG TGCTTGTCTC AAAGATTAAG CCATGCATGT CTAAGTATAA ACTGCTTTAT
ACTGTGAAAC CGCGCGCGCG TGCTGCCGTC AGTTATAGTT TATTTGATGG TACCTACTAC
TCGGATACCC GTAGTAAATC TAGAGCTAAT ACGTGCGTAA ATCCCGACTT CTGGAAGGGA
CGTATTTATT AGATAAAAGG CCGACCGGGC TCTGCCCGAC TCGCGGTGAA TCATGATAAC
TTCACGAATC GCATGGCCTT GTGCCGGCGA TGTTTCATTC AAATTTCTGC CCTATCAACT
TTCGATGGTA GGATAGAGGC CTACCATGGT GGTAACGGGT GACGGAGGAT TAGGGTTCGA
ATGCGCTAAG GGAGCCTGAG AAACGGCTAC CACATCCAAG GAAGGCAGCA GGCGCGCAAA
TTACCCAATC CTGACACAGG GAGGTAGTGA CAATAAATAA CAATACTGGG CCTTTTCAGG
TCTGGTAATT GGAATGAGTA AACCCATTGG CCCTTAACGA GGATCAATTG GAGGGCAAGT
CTGGTGCCAG CAGCCGCGGT AATTCCAGCT CCAATAGCGT ATATTTAAGT TTCCTTGGAT
AAAAAGCTCG TAGTTGGATT TCGGGTGGGG CCTGCCGGTC CGCCGTTTCG GTGTGCACTG
GCAGGGCCCA CCTTGTTGCC GGGGACGGGC TCCTGGGCTT CACTGTCCGG GACTCGGAGT
GCCGGTCAST ACTTTGAGTA AATTAAGGCG TTCAAAGCAG GCCTACGCTC TGAATACATT
AGCATGGAAT AACACGATAG GACTCTGGCC TATCCTGTTG GTCTGTAGGA CCGGAGTAAT
GATTAAGAGG GACAGTCGGG GGCATTCGTA TTTCATTGTC AGAGGTGAAA TTCTTGGATT
TATGAAAGAC GAACTACTGC GAAAGCATTT GCCAAGGATG TTTTCATTAA TCAAGAACGA
AAGTTGGGGG CTCGAAGACG ATTAGATACC GTCCTAGTCT CAACCATAAA CGATGCCGAC
TAGGGATCGG CGGATGTTTC TTCGATGACT CCGCCGGCAC CTTATGAGAA ATCAAAGTTT
TTGGGTTCCG GGGGGAGTAT GGTCGCAAGG ATTAATTGGC AAGGAATTGA CGGAAGGGCA
CCACCAGGCG TGGAGCCTGC GGCTTAATTT GACTCAACAC CCAGTTTAAT ACCAGGTCCA
GACATAGTGA GGATTGACAG ATTGAGAGCT CTTTCTTGAT TCTATGGGTG GTGGTGCATG
GCCGTTCTTA GTTGGTGGGT TGCCTTGTCA GGTTGATTCC GGTAACGAAC GAGACCTCAG
CCTGCTAAAT AGTCACGGTT GGCTCGCCAG CCGGCGGACT TCTTAGAGGG ACTATTGGCG
AAACCTCCCT GAAGCATGAG GCAATAACAG GTCTGTGATG CCCTTAGATG TTCTGGGCCG
CACGCGCGCT ACACTGATGC AAATTTAAAG CTTAGCCTTG GCCGAGAGGC CCGGGTAATC
TTTGAAACTG CATCGTGATG GGGATAGATT ATTGCAATTA TTAATCTTCA ACGAGGAATG
CCTAGTAAGC GGGGGGGGGG AGCTTGCGTT GATTACGTCC CTGCCCTTTG TACACACCGC
CCGTCGCTCC TACCGATTGG GTGTGCTGGT GAAGTGTTCG AGTGAGAGTT CGGGGGCGGT
CTCCGCTCTC GGCCGCCGAG CCAACCAATG AACCCTCCCA CCTAGAGGAA GGAGAAGTCG
TAACAAGGTT TCCGTAGGTG AACCTGCGGA GGCGGCTAAT
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Blast results of *Chlorella vulgaris*

BLAST revealed 95% identity with *Chlorella vulgaris* strain CCAP 211/11F 18S ribosomal RNA gene.

3.2 Drying of the *Chlorella vulgaris* Biomass

Sun drying technique took longer drying time of 72hrs when compared with the oven drying technique which took only 12hrs as presented in Fig. 1.

3.3 Oil Production Data of the Dried *Chlorella vulgaris* Biomass

Table 1 shows the results of the Physico-chemical properties for both sun-dried prepared biomass and oven dried prepared biomass oil in comparison with ASTM standards. The values for the densities (0.854 and 0.867), specific gravities (0.875 and 0.876), acid values (0.414 and 0.384) and saponification values (173.3 and 170.1) of the sun and oven dried biomass prepared oil respectively before esterification were found to be insignificant ($P>0.05$) and within the range for ASTM standard values for crude biodiesel and fossil diesel.

Table 2 present the fatty acids composition of the sun-dried *Chlorella vulgaris* crude oil. The GC-MS results indicate that the oil contained 66.7% saturated fatty acids ($C_{14}H_{28}O_2$, $C_{15}H_{30}O_2$, $C_{16}H_{32}O_2$ and $C_{18}H_{36}O_2$) than 33.3% unsaturated ($C_{18}H_{34}O_2$ and $C_{22}H_{42}O_2$).

Table 3 present the fatty acids composition of the oven-dried *Chlorella vulgaris* crude oil. The GC-MS results indicate that the oil has a strike balance of 50% saturated fatty acids ($C_{14}H_{28}O_2$, $C_{16}H_{32}O_2$ and $C_{19}H_{38}O_2$) than 50% unsaturated ($C_{11}H_{20}O_2$, $C_{18}H_{34}O_2$ and $C_{22}H_{42}O_2$).

Table 4 shows the results of the Physico-chemical properties for both sun-dried and oven dried prepared biomass biodiesel in comparison with ASTM standards. A high kinematic viscosity (52.00) and cetane number (54.00) were observed in sun-dried biomass prepared biodiesel in comparison with the kinematic viscosity (3.870) and cetane number (49.70) of oven-dried biomass prepared biodiesel, though both values were within the ASTM standard values for biodiesel and fossil diesel.

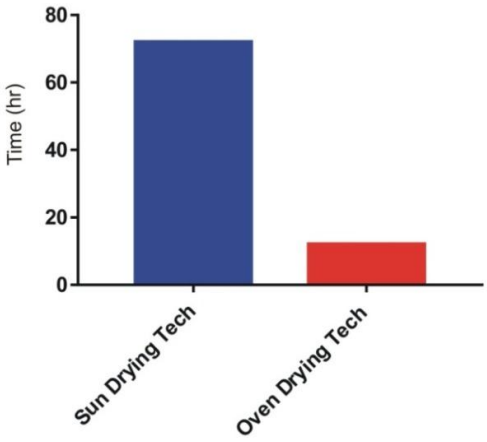

Fig. 1. Time taking for drying *Chlorella v.*

Table 1. Physico-chemical properties of crude *Chlorella vulgaris* oil in comparison with ASTM standards

Property	Density(cm^3)	Specific Gravity	Acid Value (mgKOH/g)	Saponification Value (mg KOH/g)
Sun dried Biomass oil	0.854[a]	0.875[a]	0.414[a]	173.3[a]
Oven dried Biomass oil	0.867[a]	0.876[a]	0.384[a]	170.1[a]
Biodiesel ASTM Standard	0.876-0.900	0.881[a]	0.500 max	-
Fossil Diesel ASTM Standard	0.876-0.900	0.850[a]	0.500 max	-

Data are represented as mean of triplicate values at P=0.05

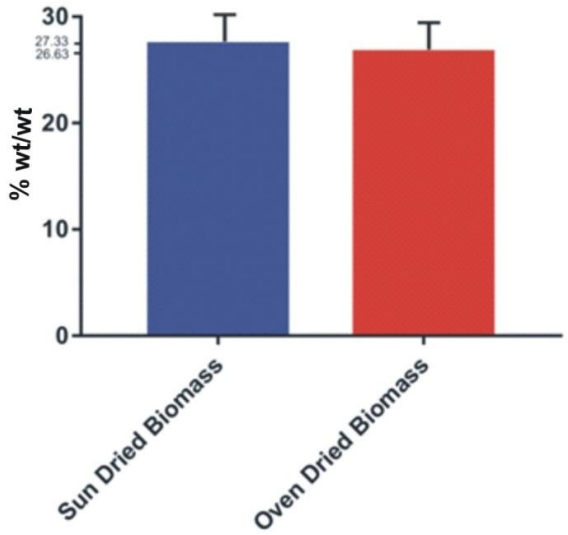

Fig. 2. Quantity of oil (wt/wt) recovered from *Chlorella v.*

Table 2. Fatty acids composition of sun dried prepared *Chlorella vulgaris* biomass oil

Fatty acid	Formula	Structure	Mol Wt(g)	Saturated/unsaturated fatty acids
Myristic acid	$C_{14}H_{28}O_2$	14:0	228	Saturated F.A
Pentadecyclic acid	$C_{15}H_{30}O_2$	15:0	242	Saturated F.A
Palmitic acid	$C_{16}H_{32}O_2$	16:0	254	Saturated F.A
Stearic acid	$C_{18}H_{36}O_2$	18:0	284	Saturated F.A
Oleic acid	$C_{18}H_{34}O_2$	18:1 cis9	282	Mono unsat. F.A
Erucic acid	$C_{22}H_{42}O_2$	22:1ω9	338	Mono unsat. F.A

Sundried Biomass oil Fatty acids profile

Table 3. Fatty acids composition of oven dried prepared *Chlorella vulgaris* biomass oil

Fatty acid	Formula	Structure	Mol Wt(g)	Saturated/unsaturated fatty acids
Myristic acid	$C_{14}H_{28}O_2$	14:0	228	Saturated F.A
Palmitic acid	$C_{16}H_{32}O_2$	16:0	254	Saturated F.A
Nonadecyclic acid	$C_{19}H_{38}O_2$	19:0	298	Saturated F.A
Undecyclenic acid	$C_{11}H_{20}O_2$	11:1	184	Mono unsat. F.A
Oleic acid	$C_{18}H_{34}O_2$	18:1 cis9	282	Mono unsat. F.A
Erucic acid	$C_{22}H_{42}O_2$	22:1ω9	338	Mono unsat. F.A

Oven dried Biomass oil Fatty acids profile

Table 4. Physico-chemical properties of synthesised *Chlorella vulgaris* biodiesel in comparison with ASTM standard

Property	Kinematic viscosity mm^2/g at 40ºC	Flash point °C	Cetane number
Sun dried Biomass Biodiesel	5.200[a]	114.0[a]	54.00[a]
Oven dried Biomass Biodiesel	3.870[b]	115.0[a]	49.70[b]
Biodiesel ASTM Standard	2.800-5.700	96.00-190.0	45.00-70.00
Fossil Diesel ASTM Standard	1.900-3.800	60.00-80.00	40.00-55.00

Data are represented as mean of triplicate values at P=0.05

4. DISCUSSION

One of our goals was to achieve positive DNA extraction, amplification and sequencing of Chlorophyceae (*Chlorella sp*) microalgae as a feedstock for biodiesel potential. The result of DNA extraction showed that DNA concentration ranges from 1-10 µg. The molecular weight of the PCR product was 1.8kb using a set of 18S rDNA primer sets. This is in consistency with result of [28]. Sequencing and BLAST revealed 95% identity with *Chlorella vulgaris* strain CCAP 211/11F 18S ribosomal RNA gene.

Water has to be removed from *Chlorella vulgaris* biomass slurry to increase its viability for effective lipid extraction. The time taken for removal of water using the two techniques varied. Sun drying took substantially longer to remove water from the *Chlorella vulgaris* biomass when compared to oven drying. The short term for the oven drying could be the presence of an air circulating fan in the oven that assisted in uniform distribution of heat and air. Among the two techniques, oven drying have an easy mode of operation but required instrumentation. Sun drying technique required a large drying surface, takes longer drying time, and risk the loss of bioreactive products but it is cheap [13].

Oil yields from *Chlorella vulgaris* biomass prepared by the two techniques showed no significant difference ($P>0.05$) in the lipid yield although sundried biomass oil volume was slightly higher than oven drying biomass oil. The insignificant differences could be because the species are the same and longer drying time does not affect the total % of oil yield. Similar findings were reported by Balasubramanian *et al*. [29] where biomass of *Nannochloropsis sp.* was dried using sun drying technique.

Oil characteristics such as density and specific gravity were studied. The variations in both the densities and specific gravities values are insignificant. This is in agreement with the previous study by Kelaiya et al. [30]. These properties compared favourably with the acceptable biodiesel and diesel standards.

A high acid value was observed in sun dried biomass oil compared to oven dried biomass oil. This indicates the presence of high amount of free fatty acids in the sundried biomass oil. This demonstrates that drying methods has an influence on the level of free fatty acids. The high level of acid value in sundried biomass oil extracted could be as a result of long term exposure to ultra violet radiation, enzyme degradation and sun light. This result is in agreement with study by Balasubramanian et al. [29] where they found similar trend in fatty acid content of lipids extracted from *Nannachloropsis* sp. using sun-drying. Both are acceptable as ASTM recommendation for biodiesel.

High saponification values were observed in both sun dried and oven dried biomass oil. A high saponification value from algal oil indicates that it can be used as efficient feedstock for biodiesel synthesis. The high saponification and high acid values found in algal oil are common to most non edible oils used for biodiesel production. This is in consistency with the previous study by Veljkovic et al. and Zhang and Jiang, [31,32].

The produced biodiesel was subjected to performance requirement properties. The properties include Kinematic viscosity, Flash point and Cetane number.

Kinematic viscosity is one of the most important fuel quality parameter. Sun dried biomass oil has higher viscosity than oven dried biomass oil. This could be due to higher degree of saturated fatty acids in the sundried oil. Kelaiya, et al. [30] got similar results in their study of fuel properties of *Chlorella* sp. for biodiesel. Biodiesel normally possessed superior kinematic viscosity than fossil diesel. Findings show that Kinematic viscosity increases with degree of saturations and carbon length and decreases with the degree of unsaturation [30].

The flash point and cetane number of the sundried and oven dried *Chlorella* biomass biodiesel were studied. Oven dried biomass biodiesel has a relatively higher flash point than sun dried biomass biodiesel. However, there is no significant difference between the two observed. Fuels above flash point of 66°C are considered safe fuel and are suitable for all climatic conditions [27].

The cetane number of the sundried biomass biodiesel was slightly higher than the cetane number of oven dried biomass biodiesel. The significant dropped of the oven dried biomass biodiesel cetane number could be due to effects of unsaturated fatty acid chains. The cetane number decreases with unsaturation [33]. This result obtained was comparable with the previous study by Bello et al. [34]. The fuel

performance requirement properties obtained are in agreement with the ASTM biodiesel standard.

Fatty acids composition has a profound effect on the fuel property of biodiesel.

In this study, fatty acid profile result had shown that there was variation in composition of saturated fatty acids and mono-unsaturated fatty acids depending upon the drying technique. Sun dried biomass oil had shown the higher composition of saturated fatty acids than oven dried biomass oil which possessed a balance of both saturated and unsaturated fatty acids. The high composition of saturated fatty acids in the sundried biomass oil could be as a result of oxidation of unsaturated fatty acids by sunlight or can be attributed to the desaturation effect by enzyme degradation as indicated by Abhishek et al. [35]. The balanced for saturated and unsaturated fatty acids confirms high quality product [35]. According to Knothe [5], the most common fatty acid esters in biodiesel are C16:0, C18:0, C18:1, C18:2 and C18:3. This is true for biodiesel feedstocks such as soybean, sunflower, rapeseed, palm and peanut oils [36]. These findings were in agreement with those reported by Pratoomyot et al. [37]. Isik et al. [38] and Tan et al. [39] reported C16:0, C18:0 +1 and C18:3 as the main fatty acid components in *Chlorella vulgaris* and *Scenedesmus abundans* respectively.

A higher content of saturated fatty acids are desirable for better oxidation stability of biodiesel. This is beneficial for industry as biodiesel could be stored for a longer period. On the other hand, a higher content of unsaturated fatty acids is beneficial for cold flow properties of biodiesel. This will lead to a possible usage of the fuel even in cold countries and during the cold months. It is desirable that there is a mixture of both saturated and unsaturated fatty acids in the oil so that both the oxidation stability and cold flow property strike a balance [35].

5. CONCLUSION

Microalgae isolated from Kaduna State University fish pond was authenticated to be *Chlorella vulgaris*.

The study showed that Sun and Oven drying techniques has no significant effect on the quantity of oil produced from biomass of *Chlorella vulgaris.* Sun drying technique at the temperature of 25-35ºC for 72 hours has an effect on the Acid value (0.414), Kinematic viscosity (5.200), Cetane number (54.00) and composition of saturated and unsaturated fatty acids, although both the sun and oven drying techniques quality value were within the standard ASTM values recommended. Oven dried biomass oil was found to be of high quality because of the balanced in saturated and unsaturated fatty acid compositions (C14:0, C16:0, C19:0, C11:1, C18:1 cis9 and C22:1ω9).

ACKNOWLEDGEMENTS

I would like to thank Dr. M.S. Abdussalami, Dr Y. Umar and the entire Biological Sciences lecturers and visiting lecturers, Nigerian Defence Academy, Kaduna.

My gratitude also goes to everyone at Chemistry laboratory, Kaduna State University, Kaduna, in particular to Mrs. Khadijah for her guidance, support, assistance, advice and for allowing me to use their facilities to perform a significant portion of the research to make sure this work is a success.

My gratitude also goes to DNA laboratory staffs especially Mrs Ramlat for the help and advice in carrying out some aspect of the research.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Peer-review history:
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http://www.sciencedomain.org/review-history/26988