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CONTENT AND COMPOSITION OF LIPID PRODUCED BY *CHLORELLA VULGARIS* FOR BIODIESEL PRODUCTION

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Abstract.

This study aims at investigating the lipid profile of *Chlorella vulgaris* to determine its suitability as an alternative bio-fuel source. *Chlorella* vulgaris (stock culture UTEX 259) was sub-cultured at laboratory scale. The cells were kept in 250ml, 500ml, and 1000ml Erlenmeyer flasks with 200ml, 300ml, and 600ml of medium respectively and shaken occasionally in Bold Basal Medium with initial pH value 7 at the temperature of 22± 3^0c with constant light intensity for the culture medium which was not more than 2500 lux on a 16:8 light to dark cycle for 7 weeks. The *Chlorella vulgaris* cells were harvested and the oil extracted. The percentage lipid content was determined by soxhlet extraction and was shown to be 25%. The GC-MS analysis of the transesterified oil showed this lipid profile; nonanoic acid, decanoic acid, palmiltic acid, stearic acid ,oleic acid, linoleic acid with this chain length C9:0,10:0,16:0,18:0,18:1,18:2 respectively. The percentage unsaturated fatty acid for nitrogen rich media and nitrogen deprived media were 79.22% and 74.2% respectively while the percentage saturated fatty acids were 20.2% and 25.8% respectively. This study shows that *Chlorella vulgaris* is a suitable candidate for biodiesel production because the lipid profile, lipid composition and level of unsaturation meets requirement of oil suitable for biodiesel production.

Key words: *Chlorella vulgaris* , lipids, oleic acid ,bio-fuel.

INTRODUCTION

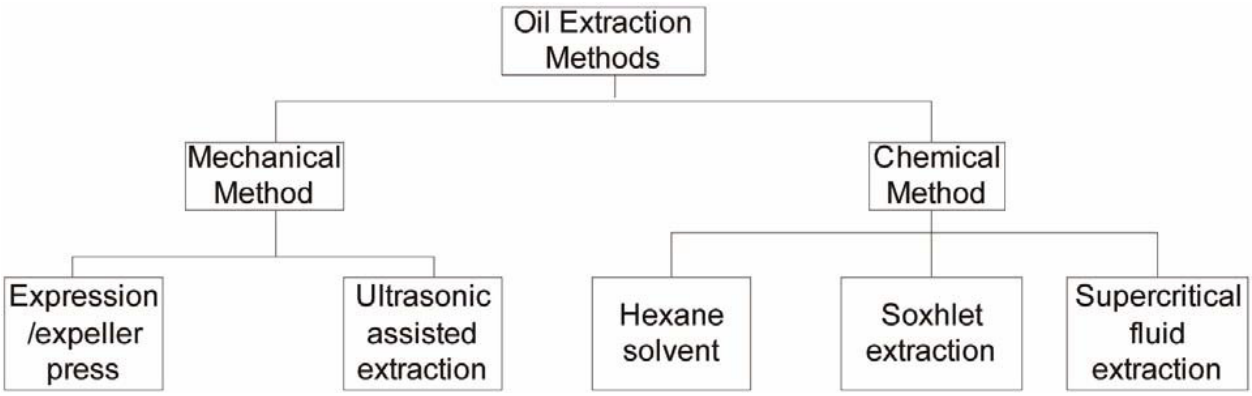
Microalgae are currently being investigated by scientists in the U.S. and abroad as agents for the fixation of CO2 from power plants. The U.S. Department of Energy (through the National Energy Technology Laboratory) is supporting several projects in this area. Recently an "International Network for Bio-fixation of CO_2 and Greenhouse Gas Abatement with Microalgae" was formed through the International Energy Agency Greenhouse Gas R&D Programme, with support from the U.S. DOE, to coordinate and channel R&D activities in this field (Pedroni et al., 2002).Over 80% of the energy we use comes from three fossil fuels: petroleum, coal, and natural gas. About 98%of carbon emissions result from fossil fuel combustion. Reducing the use of fossil fuels would significantly reduce the amount of carbon dioxide and other pollutants produced. This can be achieved by either using less energy altogether or by replacing fossil fuel by renewable fuels. Renewable energy is a promising alternative solution because it fixes CO_2 in the atmosphere through photosynthesis. They also produce lower or negligible levels of greenhouse gases and other pollutants when compared with the fossil energy sources they replace.

Microalgae biodiesel are regarded as a promising alternative fuel for IC engines [Singh, J et al., 2010; Mustafa, B.2011].One method of harnessing microalgae is by producing biodiesel from its oil. Biodiesel fuel gives a comparable engine performance and emission to petroleum diesel [Al-lwayzy, S.H et al., 2012]. Biodiesel from microalgae oil has received significant attention recently as it is renewable, environmentally friendly and represents the ability to convert CO2 to oil [Hossain et al., 2008]. Microalgae oil contains high values of palmitic acid, and the concentration of linoleic acid met the requirements of the European legislation for biodiesel [Converti, A et al.,2000].Microalgae biofuels are non-toxic, highly bio-degradable, contain no sulphur and the leftover materials (after extracting the oil) can be used for ethanol production or as soil fertilizer [Demirbas, A et al., 2011]. The natural biodiesel resources such as oil crops and waste cooking oil are not sufficient to cover the global transportation fuel demand [Chisti, Y.2007]. Therefore, exploring other potential sources for alternative fuels is a necessity [Yusaf, T et al.,2013].

Oil extraction from algae is one of the costly processes that can determine the sustainability of algae-based bio- diesel. Oil extraction methods can be broadly classified as shown below.

Each of these methods has drawbacks: The mechanical press generally requires drying of the algae, which is an energy intensive step. The use of chemical solvents poses safety and health issues. Supercritical extraction requires high pressure equipment that is both expensive and energy intensive. Table 2 compares oil

yields of microalgae with other oil feedstock. It is seen from that there are significant variations in biomass productivity, oil yield and biodiesel productivity. Microalgae are more advantageous due to higher biomass productivity, oil and biodiesel yield. The table shows that low, medium and high oil content micro-algae have high oil yield/ha/year and hence higher biodiesel productivities (l/ha/yr) which is much more than the productivities of oil seed crops. This is one of the most important reasons that microalgae have attracted the attention of researchers in India to scientifically grow, harvest, extract oil and convert it to biodiesel.



Micro-algal oil content

Table 1. Oil from different microalgal species

Type of microalgae	Oil content (% dry wt. basis)
Botryococcus braunii	25 - 75
Chlorella sp.	28 - 32
Crypthecodinium cohnii	20
Cylindrotheca sp.	16 - 37
Dunaliella primolecta	23
Isochrysis sp.	25 - 33
Monallanthus salina	>20
Nannochloris sp.	20 - 35
Nannochloropsis sp.	31 - 68
Neochloris oleoabundans	35 - 54
Nitzschia sp.	45 - 47
Phaeodactylum tricornutum	20 - 30
Schizochytrium sp.	50 - 77
Tetraselmis suecica	15 - 23

Table 1. Oil contents of some microalgae strains (Demirbas et al., 2010)

RESULT AND DISCUSSION

Materials and Methods

Microalgae Strain and Medium

The stock culture of *Chlorella vulgaris* UTEX 259 was obtained from National Research institute for chemical technology (NARICT) Zaria, Kaduna

The Bold Basal Medium was used and the stock solutions were prepared from the chemicals presented below.

Components of the Bold Basal medium

Stock solutions Concentration per litre of distilled water (g·L−1) 10ml 25g/l $NaNo_3$, 10ml 2.5g/l $CaCl_2.2H_2O$, 10ml 7.5g/l $MgSO_4.7H_2O$,10ml 7.5g/l K_2HPO_4 ,10ml 17.5KH_2PO_4,10ml 2.5NaCl,1ml 50.0 EDTA,1ml 31.0 KOH, 1ml 4.98,10ml CaCO3 $FeSO_4.7_2O$,1drop H_2SO_4,1ml 11.42 H_3BO_3,1ml 8.82 $ZnSO_4.7H_2O$,1ml 1.44 $MnCl_2.4H_2O$,1ml 0.71M_OO3,10ml .$NaHCO_3$

To prepare MBL medium, one mL of each stock solution (1–11) was added to one litre of Milli-Q water. The pH was adjusted to 7.0 using hydrochloric acid. The media was autoclaved at 121 °C (15 PSI) for 15 min.

Culture Conditions

The *Chlorella vulgaris* was grown at the Algae laboratory in National Research institute for chemical technology, zaria Kaduna state using sterilized Bold Basal Medium under aseptic conditions. The culture temperature was fixed at 22 ± 3 °C. Fluorescent light was used to supply constant light intensity for the culture which was not less than 2500 lux on a 16:8 light to dark cycle. Cells were kept in 250ml, 500ml, 1000ml Erlenmeyer flasks with 200ml, 300ml, 600ml of medium respectively and shaken occasionally. In other to obtain larger quantities of biomass, cells were transferred into 1500ml Erlenmeyer with 1000ml of medium.

Microalgae Harvesting

The microalgae cells were harvested using a Beckman Avanti J-251 high speed centrifuge (Beckman Coulter, chaska, MN, USA) at 8000 rpm for 10 min. The samples were then transferred to pre-weighed Petri dishes. In order to determine the dry weigh of the chlorella vulgaris cells the resulting biomasses were oven dried. Lyophilised cells were stored until the time of oil extraction.

Lipid Content

Lipid Content (% by weight of algae) = Weight of lipid plus flask- Weight of flask/Weight of algae input.

Soxhlet Extraction of Total Lipids

0.35g of algae was placed in a soxhlet and extracted for about 3 hour using 205 milliliter of chloroform methanol 2:1(v/v). After the extraction was completed, the solvent was recovered and the oil was weighed. The weight of the oil was calculated in grams.

Lipid productivity

Daily lipid productivity was calculated using the equation:

Daily lipid production (mg lipid l^{-1} day^{-1}) = DW x (lipid /100/day) x 1,000

Where, DW = algal dry weight (g l^{-1}), lipid = g 100 g^{-1} DW, and day = growth period.

Table 2. Fatty acid profile of *Chlorella vulgaris* grown in nitrogen deprived media.

Fatty acids	Molecular weight (g/mol) [a]	Distribution in sample (%)[b]	a× b/100
Tridecanoic acid	228	1.22	2.78
Palmitic acid	256	24.58	62.93
Linoleic acid	298	1.86	5.54
Oleic acid	282	60.98	181.70
Decanoic acid	160	2.52	4.03
Octadecanoic acid	144	2.09	3.01
Octadecadienoic acid	266	6.75	17.95
Average molecular weight			277.9

Table 2 shows the results of the lipid profile, molecular weight, distribution in sample and average molecular weight of fatty acids of Chlorella vulgaris grown in a nitrogen deprived media analyzed by GCMS

Table 3. Fatty acid profile of *Chlorella vulgaris* grown in nitrogen enriched media.

Fatty acids	Molecular weight (g/mol)[a]	Distribution in sample (%)[b]	a×b/100
Palmitic acid	256	18.82	48.17
Oleic acid	282	73.22	206.48
Nanoic acid	176	1.96	3.44
Octadecenoic acid	266	6.00	15.96
Average molecular weight			274.05

Table 3 shows the results of the lipid profile, molecular weight, distribution in sample and average molecular weight of fatty acids of *Chlorella vulgaris* grown in a nitrogen deprived media analyzed by GCMS

LIPID CONTENT DETERMINATION

The result of the soxhlet extraction shows that the percentage lipid(oil) content of the extracted algae cells is 25% which is in accordance to some literature that says that the lipid content of chlorella vulgaris is in the range of 20-32%.The lipid content is calculated below.

Weight of algae = 0.353g
Weight of empty flask = 103.433g
Weight of flask containing oil extracted = 103.520g

$$\text{Percentage(\%) lipid content} = \frac{\text{weight of flask containing oil - weight of empty flask}}{\text{Weight of dry algae}} \times 100$$

$$\frac{103.520 - 103.433}{0.353} \times 100$$

$$= 25\%$$

BIOMASS PRODUCTIVITY

Daily biomass productivity was calculated using the equation
Daily biomass production (mg biomass l^{-1} day^{-1}) = DW/SV/day × 1000
Where DW=dry weight (gl^{-1}),SV= sample volume, day=growth period..

LIPID PRODUCTIVITY

Daily lipid productivity was calculated using the equation:
Daily lipid production (mg lipid l^{-1} day^{-1}) = DW x (lipid /100/day) x 1,000
Where, DW = algal dry weight (g l^{-1}), lipid = g 100 g^{-1} DW, and day = growth period.
Estimation of lipid productivity.
Lipid productivity (mg/lipid/day) = DW × lipid/100/day × 1000

$$0.353 \times 0.087/100/82 \times 1000$$

$$= 3.75\text{mg/lipid/day. (DW =algae dry weight)}$$

FAME COMPOSITION (GC-MS ANALYSIS)

Fatty acid profile is a major key in biodiesel production. Fatty acid composition is very important factor for biodiesel production because it is strongly influenced in the properties of biodiesel. To investigate the suitability of algae oil for biodiesel production, the fatty acid profile of *Chlorella vulgaris* grown in the culture media was analyzed .

Table 4 .FAME & Lipid Composition of *Chlorella vulgaris*.

Fatty acid profile	Fatty acid name	Molecular weight	Distribution in sample
C9:0	Nonanoic acid	176	1.96
C10:0	Decanoic acid	160	2.52
C13:0	Tridecanoic acid	228	1.22
C16:0	Hexadecanoic acid	256	24.58
C18:0	Octadecanoic acid	144	2.09
C18:1	Octadecenoic acid	282	60.98
C18:2	Octadecadienoic acid	298	1.86

The lipid content result from the soxhlet extraction showed that the percentage lipid is 25%.Benedict et al., 2013 reported lipid content of 24.85 – 36.45%.Ignacio et al., 2015 reported 52 – 58%.Yanna et al., 2009 reported 38% lipid content. Teresa et al., 2013 reported lipid content of 33.5%.

The fatty acid profile was analyzed by GCMS and it was found that extracted oil contained both saturated and unsaturated fatty acids. The most abundant chain length being C9:0, 10:0, 13:0, 16:0, 18:0, 8:1 and 18:2 with C18:1 dominating. The percentage unsaturated fatty acid for nitrogen rich media and nitrogen deprived media were 79.22% and 74.2% respectively while the percentage saturated fatty acids were 20.2% and 25.8% respectively. This result shows that the percentage unsaturated fatty acid is higher than the percentage saturated fatty acid.

This agrees with the following research findings. Chinnasamy et al., (2010) also observed similar fatty acid profile in crude algal oil using GC. Gouveia & Oliveira, (2009) reported that microalgal lipids derived from

Chlorella vulgaris,Scenedesmus maxima, Nannochloropsis oleabundans,Scenedesmus obliquus and Dunaliella tertiolecta weremainly composed of unsaturated fatty acids (50-65%).Farooq et al.,(2013) reported similar fatty acid profile with percentage unsaturated(77.85%) and percentage saturated (21.5%). Chattip et al.,2012 also reported similar fatty acid profile with percentage unsaturated(65.3%) and percentage saturated(34.7%).the average molecular weight of the fatty acids of this study(277.9 & 274.04) is similar to that of similar of Chattip et al.,2012(273.8). Christain L.,2008 had similar results in an Msc thesis presented at university of Idaho,2008.the oil content(25%) is within range as Mata T.,(2010) and Feinberg D et al.,1984 reported oil content of 30% stating the range to be (5-40%).

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