Experimental Studies on Green-Algae and its by-Products as an Energy Source – Biofuels, Bioenergy, and Biogas Perspectives.

Idris, M. N. * and Bello, O. A.

Energy and Process Refining Technology, Department of Chemical Engineering, University of Maiduguri, Nigeria.

Email: idrismn@hotmail.com

The link to this publication is https://ajoeer.org.ng/otn/ajoeer/qtr-2/2021/04.pdf

C.Engr. Prof. M. N. Idris is a professor in the Department of Chemical Engineering, University of Maiduguri, Nigeria. He is an expert in oil and gas (upstream, midstream and downstream), CFD modelling and simulation, fluidization, nanotechnology (nanosynthesis), process economies, renewable and sustainable energy development. He holds PhD in Chemical Engineering from the University of Leeds, United Kingdom.

His research interests are on applications of computational fluid dynamics (CFD) in modelling and simulation of process systems and design, reactions engineering, nanotechnology and biotechnology etc. He is a full registered and a senior member of American Institute of Chemical Engineers (MAIChE), a chartered member of the Institution of Engineers (C.Eng., UK), a chartered member of the Institution of Chemical Engineers (MIChemE, UK), Nigerian Society of Chemical Engineers (MNSChE), Nigerian Society of Engineers (MNSE), Council for the Regulations of Engineering in Nigeria (MCOREN) and Professional member of Materials Science and Technology Society of Nigeria (MMSN). He is a fellow of the institute of management consultant (FIMC CWC Global) and a fellow of the institute of management specialists (FIMS UK). For further enquiries or information with regard to this book, please contact the author on email: idrismn@hotmail.com and/or phone: +234 70 5877 1096.

Experimental Studies on Green-Algae and its by-Products as an Energy Source – Biofuels, Bioenergy, and Biogas Perspectives.

Idris, M. N. * and Bello, O. A.

Energy and Process Refining Technology, Department of Chemical Engineering, University of Maiduguri, Nigeria.

Email: idrismn@hotmail.com

ABSTRACT

The technology of biofuels is no longer a doubted process of developing renewable energy alternatives to petroleum-based fuels resources. This sustainable technology has increased tremendously with decrease in world-crude oil reserves while the price of energy is on the increase. Biofuels are renewable and less toxic and are readily biodegradable form of fuels from biological sources. Fossil fuel has been a major source of energy for household and industrial use, but concerns about shortage of fossil fuels, increasing crude oil price, energy security and accumulation of greenhouse gases (GHG) in the atmosphere that result to global warming (GW) have led to growing worldwide interest in renewable energy source. In this study, an alga was used instead of traditional agro-based raw materials, because it does not compete with food or fodder and is abundantly available in fresh water or marine ecosystem. The experimental studies conducted show that algal species can be used to produce biodiesel (biofuels). The results reveal that algae are fast growing organism and can be grown artificially. Inducement of fertilizer and other growth media can be used to increase the growth rate of biofuels production. Oil produced from algae oil was esterified using methanol as catalyst. The properties of the biodiesel produced clearly show that biodiesel produced from green algae can be used to run engine. The experimental results show that the Flash point was 135^0C*, Pour point was* 17^0C *and Cloud point was* 18^0C *and are all in agreement with the standard of biodiesel (biofuels).*

Keywords: Green-algae, biofuels, and sustainable energy

1.0 INTRODUCTION

Energy is a very important aspect of life that man cannot neglect, because we need energy to run our day-to-day activities. Generation of energy has taken the centre stage and has been the interest of individuals, organizations and countries. Man has since been generating energy from many sources: natural to man-made complex processes, renewable and non-renewable sources. Fossil fuel has been a major source of energy for household and industrial use, but concerns about shortage of fossil fuels, increasing crude oil price, energy security and accumulation of greenhouse gases (GHG) in the atmosphere that bring about global warming have led to growing worldwide interest in renewable energy source.

GHG contributes not only to global warming (GW); it also has other impacts on the environment and human life. Approximately one-third of the CO_2 emitted each year by human activities is absorbed by oceans and as its levels increase in the atmosphere, the amount dissolved in oceans will also increase turning the water pH gradually to more acidic. This pH decrease may cause the quick loss of coral reefs and of marine ecosystem biodiversity with huge implications in ocean life and consequently in earth life [1].

With the urgent need to reduce carbon emissions, and the dwindling reserves of crude oil, liquid fuels derived from plant material (also termed biofuels) appear to be an attractive alternative source of energy. Compared with other forms of renewable energy (e.g. wind, tidal and solar), biofuels allow energy to be chemically stored, and can also be used in existing engines and transportation infrastructures after blending to various degrees with petroleum diesel [2].

Currently, the most widely available form of biodiesel comes from such oil crops as palm, oilseed rape and soybean. However, several concerns have been raised about sustainability of this mode of production: to produce 2,500 billion liters of biodiesel from oilseed rape (i.e. the current demand of petroleum diesel in the whole UK), 17.5 Mha would be required for plantation – i.e. more than half the land area of UK itself! Moreover, the overall savings in energy and greenhouse gas emissions if the lifecycle of biofuel is considered as a whole are typically below what is normally anticipated; e.g. for biodiesel from oilseed rape or soya [3], a lifecycle assessment indicates that 50% of the energy contained in the fuel will be spent in biodiesel processing itself [3].

The advantages of microalgae over higher plants as a source of transportation biofuels are:

1. Oil yield per area of microalgae cultures could greatly exceed the yield of the best oilseed crops.
2. Microalgae grow in an aquatic medium, but need less water than terrestrial crops
3. Microalgae can be cultivated in seawater or brackish water on non-arable land, and do not compete for resources with conventional agriculture
4. Microalgae biomass production may be combined with direct bio-fixation of CO_2)
5. Fertilizers for microalgae cultivation (especially nitrogen and phosphorus) can be obtained from wastewaters
6. Algae cultivation will not need herbicide or pesticides.
7. The residual Algae biomass after oil extraction may be used as fertilizer
8. The biochemical composition of the algal biomass can be modulated by varying growth conditions and the oil content can be highly enhanced [4].

1.1 Problem Statement

Combustion of fossil fuel releases GHG which causes global warming, acid rain and decrease in the pH of oceans. Attempts to combat these effects lead to the uses of various renewable source of energy. Fuel oil from food crops (Soya beans, rape seed etc.) can be used to produce biodiesel, but other need for food crop is more pressing and also the availability of land to cultivate the crops are reasons why further research on biofuel was carried out. Applying algae to produce biofuel helps to conquer the challenge posed by food crop.

1.2 Significance of the Study

The significance of this research work is that power generation can be achieved using algae as an alternative source to produce biodiesel, bioethanol, and hydrogen. However, the production of hydrogen is complex and requires robust and expert capabilities to achieving the desired objectives.

2.0 BACKGROUND LITERATURE

2.1 Microalgae for biodiesel production

Microalgae are prokaryotic or eukaryotic photosynthetic microorganisms that can grow rapidly and live-in harsh conditions due to their unicellular or simple multicellular structure. Examples of prokaryotic microorganisms are Cyanobacteria (Cyanophyceae) and eukaryotic microalgae are for example green algae (Chlorophyta) and diatoms (Bacillariophyta) [5]. A more description of microalgae is presented by Richmond [6].

Microalgae, recognized as one of the oldest living organisms, are thallophytes (plants lacking roots, stems, and leaves) have chlorophyll as their primary photosynthetic pigment and lack a sterile covering of cells around the reproductive cells [7]. While the mechanism of photosynthesis in these microorganisms is similar to that of higher plants, they 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 [8].

Traditionally microalgae have been classified according to their colour and this characteristic continues to be of importance. The current systems of classification of microalgae are based on the following main criteria: kinds of pigments, chemical nature of storage products and cell wall constituents. Additional criteria take into consideration the following cytological and morphological characters: occurrence of flagellate cells, structure of the flagella, scheme and path of nuclear and cell division, presence of an envelope of endoplasmic reticulum around the chloroplast, and possible connection between the endoplasmic reticulum and the nuclear membrane [9]. There are two basic types of cells in the algae, prokaryotic and eukaryotic. Prokaryotic cells lack membrane-bounded organelles (plastids, mitochondria, nuclei, Golgi bodies, and flagella) and occur in the cyanobacteria. The remainder of the algae is eukaryotic and has organelles [10].

2.2 Potential of Microalgal biodiesel

There are several ways to convert Microalgal biomass to energy sources, which can be classified into biochemical conversion, chemical reaction, direct combustion, and thermochemical conversion as shown in Figure 1. Thus, microalgae can provide feedstock for renewable liquid fuels such as biodiesel and bioethanol [11].

Algae were once considered to be ‘aquatic plants’ but are now classified separately because they lack true roots, stems, leaves, and embryos. While we refer to algae as feedstocks for biofuels, the definition includes all unicellular and simple multi-cellular microorganisms, including both prokaryotic microalgae, e.g., cyanobacteria (Chloroxybacteria), and eukaryotic microalgae, e.g. green algae (Chlorophyta), red algae (Rhodophyta) and diatoms (Bacillariophta) [11]. The main advantages of microalgae derived biofuels over the first- and second-generation biofuels are presented in Figure 1.

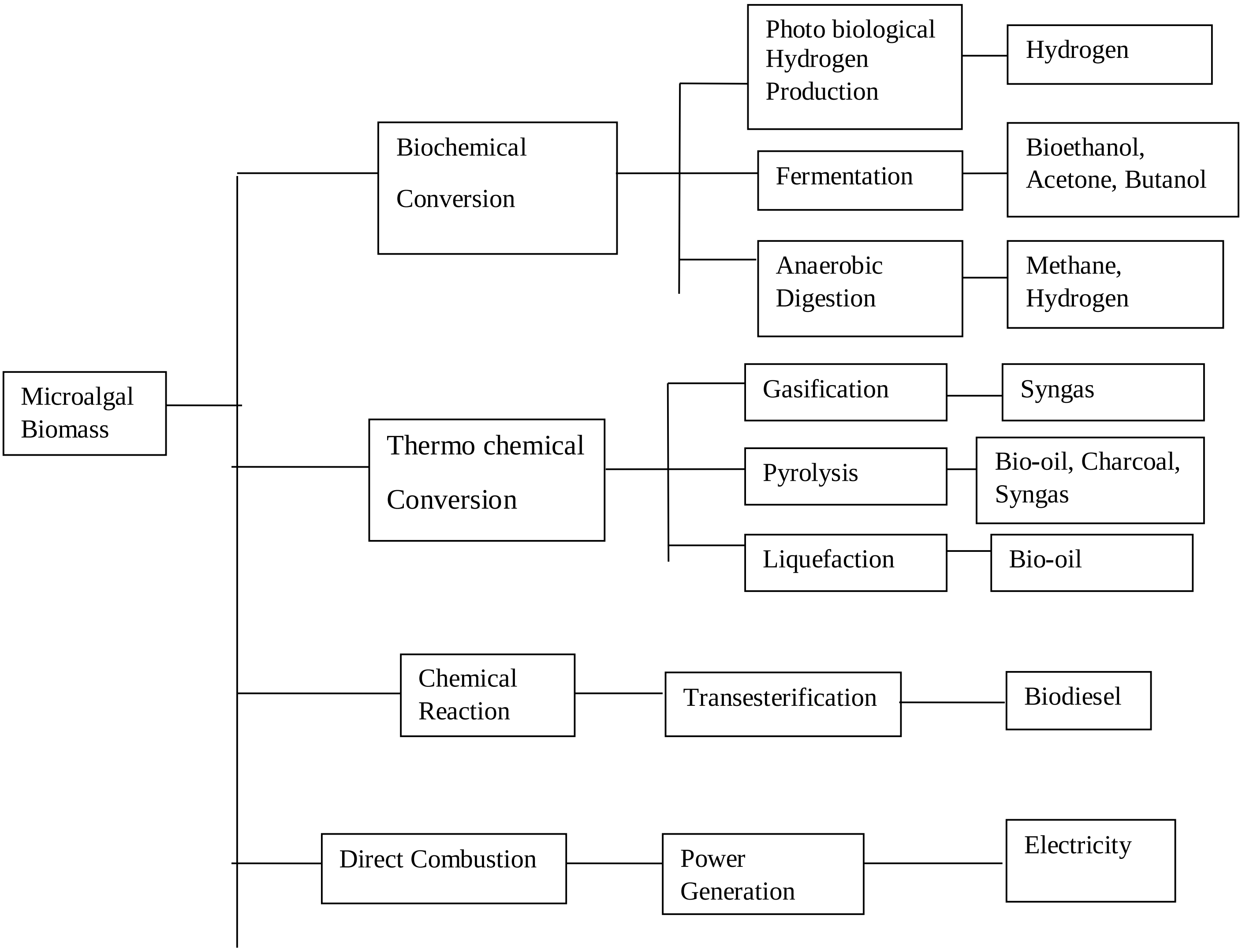

Figure 1: Conversion processes for biofuel production from Microalgal biomass

The microalgae can be produced all year round and the quantity of oil production exceeds the yield of the best oilseed crops, e.g., biodiesel yield of 58,700 litres /hactre for microalgae containing only 30% oil by weight compared with 1190 litres/hactre for rapeseed or Canola [12], 1892 litres/hactre for Jatropha [13], and 2590 litres /hactre [14].

Typical oil yields from various sources are listed in the table 1. The rapid growth potential and numerous species of microalgae with oil content in the range of 20–50% dry weight of biomass is another advantage for its choice as a potential biomass. The exponential growth rates can double their biomass in periods as short as 3.50 hrs [15].

Table 1 Typical oil yields from the various biomass sources in ascending order

S/No.	Crop	Oil yield (Litre/hactre)
1	Corn	172
2	Soybean	446
3	Peanut	1,059
4	Canola	1,190
5	Rapeseed	1,190
6	Jatropha	1,892
7	Karanj (Pongamia pinnata)	2,590
8	Coconut	2,689
9	Oil palm	5,950
10	Microalgae (70% oil by wt.)	136,900
11	Microalgae (30% oil by wt.)	11 58,700

Data sources: Chisti [3]; Lele [4]

A significant advantage to environment is that algae cultivation does not require herbicides or pesticides application [16]. In addition, these can also produce valuable co-products such as proteins and residual biomass after oil extraction, which may be used as feedstock or fertilizer [20] or fermented to produce bioethanol or biomethane [17]. Also, the microalgae are capable of photo-biological production of 'bio-hydrogen' [18].

It therefore becomes rather imperative that the combination of potential biofuel production, CO_2 fixation, bio-hydrogen production, and bio-treatment of wastewater; as summarised above, accentuates the potential utilization of microalgae [11].

2.3 Biodiesel Production

Transesterification of algal oil with simple alcohol has long been the preferred method for producing biodiesel The Transesterification process is most widely use all over the world. The overall Transesterification reaction is given by three consecutive and reversible equations as shown in equations 1 and 2:

Triglyceride + ROH = Diglyceride + RCOOR (1)

Diglyceride + ROH = Monoglyceride + RCOOR (2)

In the first reaction, the conversion of triglycerides to diglycerides is, followed by the conversion of triglycerides to monoglycerides, and of monoglycerides to glycerol, yielding one methyl ester molecule per mole of glyceride at each step [19].

The complete chemical reaction mechanisms of the transesterification process are as shown in Figure 2:

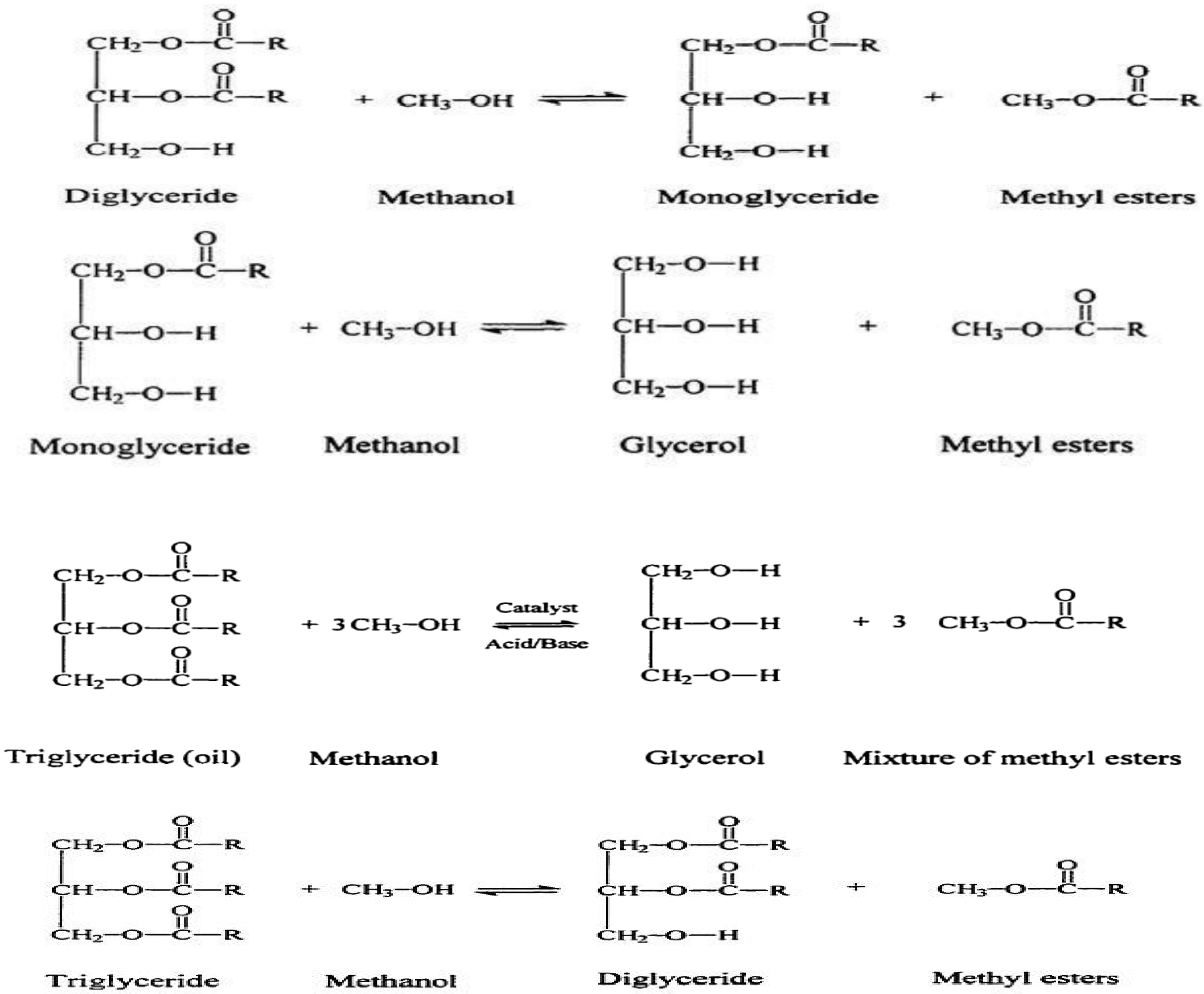

Figure 2: complete chemical reaction mechanisms of the transesterification process

2.4 Species of algae used for biofuel production

Many species of algae have been researched with the intention of using these species as a potential feedstock for biodiesel. Of these species, Botryococcusbraunii has appeared in literature as a laboratory favourite, although it has not been commercially cultivated on an industrial scale. The following table lists some species of algae and their associated lipid content, but the table is not intended by any means to be comprehensive. In addition to the four species provided, there has also been a certain amount of interest in other algal species such as *Scenedesmusdimorphus*, *Euglenagracilis*, *Tetraselmischui*, various *Spirulina* species, and many others that have been profiled as part of the ASP.

2.5 Advantages of algal biodiesel

Biodiesel produced using Algae as feed stock has certain advantage over other feed stock. Its advantages are:

1. Algae have rapid growth rates compared to oil crops.
2. It grows practically anywhere.
3. A high per-acre yield (7–31 times greater than the next best crop e.g. palm oil)
4. A certain species of algae can be harvested daily.
5. Algae biofuel contains no sulphur contents
6. Algae biofuel is non-toxic fuel.
7. Algae biofuel is highly biodegradable.
8. Algae oil extracts can be used as livestock feed and even processed into ethanol.
9. Can reduce carbon (C) emissions based on where it's grown.

3.0 MATERIALS AND METHODS

3.1 Sample collection

Algal samples were collected from two points at Alaraham fishpond Maiduguri, using a clean container. Replicate samples were collected from the two sources. The samples collected served as starter cultures for large biomass generation. The samples were immediately taken to the laboratory for cultivation as shown in Figure 3.

Figure 3: Algae pond displayed samples

3.2 Media preparation

The modified Bristol media was used in this experiment for algal cultivation. The medium is composed of the following compositions as stated in Table 2.

Table 2 : Media composition of algae cultivations

S/No.	Chemical composition	Mass weight

		grams (g)	milligrams (mg)
1.	$NaNO_3$	0.25	-
2.	$CaCl_2$	0.025	-
3.	$MgSO_4 \cdot 7H_2O$	0.075	-
4.	K_2HPO_4	0.075	-
5.	KH_2PO_4	0.018	-
6.	NaCl	0.25	-
7.	FeCl	-	0.5

Figure 4 and 5 respectively present the algae growth media from the specimen samples and spectrophotometer device used in the experimental analysis.

Figure 4: Algae growth media

Figure 5: Spectrophotometer device

3.3.1 Biomass Determination

l0 ml sample was removed from the culture and centrifuged at low speed for 5 minutes. The pellet was suspended in 1 ml deionized water and transferred to a pre-weighed test tube. Three washes were performed following which the sample was spun at high speed for l0 minutes, all the supernatant was removed, and the pellet was weighed as shown in Figure 6.

Figure 6: Collected algae biomass

3.3.2 Chlorophyll Content Determination

To determine chlorophyll content, 5m1 of the algal sample was centrifuged for 5 minutes at 13000rpm and re-suspended in methanol, after 30 minutes, the sample was spun for another 5 minutes at the same speed. When the pellet was white, absorbance was read at 650nm and 665nm using spectrophotometer. When the pellet was still green, the volume of methanol was increased until the pellet was white.

Chlorophyll content of the sample was calculated using the following formulae

Chlorophyll (µg/ml) = 25.5 X (A_{650}&) + 4 X (A_{665}).

Where;

A_{650} = absorbance at 650nm

A_{665} = absorbance at 665nm

The spectrophotometer was blanked with methanol, and correction was made to allow for different sample and methanol volumes.

3.4 Harvesting of biomass

The algal biomass was harvested using a sieve with a pore size of 0.01 mm. the liquid phase was trapped into a small bowl after passing through the sieve while the algal cells were collected from the surface of the sieve.

3.5 Lipid Extraction

To the sample containing 1 ml water (1ml cell suspension), 3.75m1 mixture of chloroform/methanol (1/2) was added and vortex for 10-15 minutes. Then, 1.25ml chloroform was added by mixing for 1minute and followed with 1.25ml water mixing for another minute before centrifugation. The upper phase was discarded, and the lower phase collected separately [20].

Figure 7: Extracted Algae Lipid

3.7 Biodiesel production processes

Production of biodiesel in the study followed the method of (Boccard *et al.* 2008) as outlined below:

3.7.1 Mixing of catalyst and methanol.

0.25 g NaOH was mixed with 24 ml methanol and stirred properly for 20 min.

3.7.2 Transesterification of algal oil

Micro algal biomass was concentrated via centrifugation at 1000 rpm for 15 minutes. Then methanol/ethanol was added to the biomass concentrates and was vigorously shaken for 10 minutes. The mixture was centrifuged at 1000 rpm for 5 minutes to separate the lipid from residue biomass. The lipid was aspirated using pasture pipette and kept in clean sample bottle. About 0.02g of NAOH per 5ml of lipid was added to esterify the lipid to diesel.

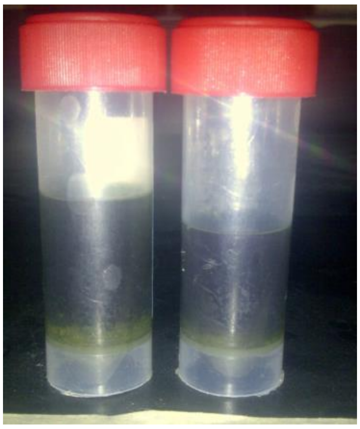

Figure 8: Biodiesel Oil

CH_2 — $OCOCR_1$			CH_2— OH R_2—$COOCH_3$
|		Catalyst	|
CH — $OCOR_2$ + $3HOCH_3$		⇄	CH — OH+R_2 — $COOCH_3$
CH_2 — $OCOR_3$			CH_2— OH R_3 COOCH3
Triglyceride	Methanol		Glycerol Methyl esters
(Parent oil)	**(Alcohol)**		**(Biodiesel)**

3.8.3 Settling: After shaking, the solution was kept for 16 h to settle the biodiesel and sediment layers clearly.

3.8.4 Washing: Biodiesel was washed by 5% water until it has become clean.

3.8.5 Drying: Biodiesel was dried by using dryer and finally kept under the running fan for 2h.

4.0 RESULT S AND DISCUSSIONS

The algae sample collected were able to grow luxuriantly for the period of two weeks (about 336 hours). Growth parameters in terms of biomass and chlorophyll were monitored for the two weeks period. Growth patterns of the algae collected from the two places are presented in Table 3. The growth was shown to increase from day 2 to the last day especially in sample A. For sample B however, there is increase also from the second day to the tenth day but subsequently followed by decrease to the fourteenth day. Highest rate of growth was recorded between day 4 to 6 and day 8 to 10 with an increase of 0.22g for two days in sample A. Similarly, 0.17g was recorded as the highest increase in biomass in sample B. It occurred between days 2 to 4 and 6 to 8 [21].

Table 3: Algal biomass

Sample	Algal growth (mg/ml) after a period (days)							
	0	2	4	6	8	10	12	14
A	0.02	0.18	0.31	0.53	0.63	0.85	0.92	0.96
B	0.02	0.11	0.28	0.40	0.57	0.59	0.55	0.52

Figure 9 is a graphical profile of algae biomass plotted against the days the measurement was taken. The graph clearly show the increasing biomass of sample A and the decrease in biomass growth of sample B. We can clearly state that sample A production is sustainable compared to sample B.

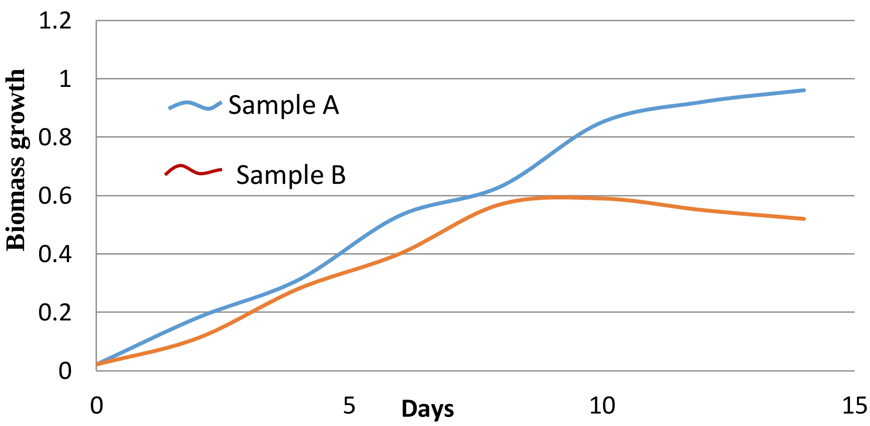

Figure 9 : Graph of biomass growth against days of algae growth

The result for the chlorophyll contents of the algal samples was determined and presented in Table 4. An average of 5.11 *u*g/ml chlorophyll was obtained in sample A. Highest (9.14 µg/ml) chlorophyll content was observed after 8 days of cultivation. There was gradual increase in the chlorophyll contents from the start of the cultivation until after day 8 when a decrease was observed.

However, lower chlorophyll contents were observed in sample B with an average of 3.66 μg/ml. The gradual increase in chlorophyll contents was observed from the start of the algal culture until day 8 when the highest (6.44 µg/ml) content was recorded whereas decrease was observed in the latter days.

Table 4 : Chlorophyll contents of the algae

	Chlorophyll contents (µg/ml) after a period (days)							
Sample	0	2	4	6	8	10	12	14
A	1.36	1.49	1.71	4.78	9.14	8.21	7.72	6.49
B	1.25	1.11	1.22	2.32	6.44	6.21	5.17	5.57

Figure 10 is a graphical profile of chlorophyl content of the 2 samples plotted against the days.

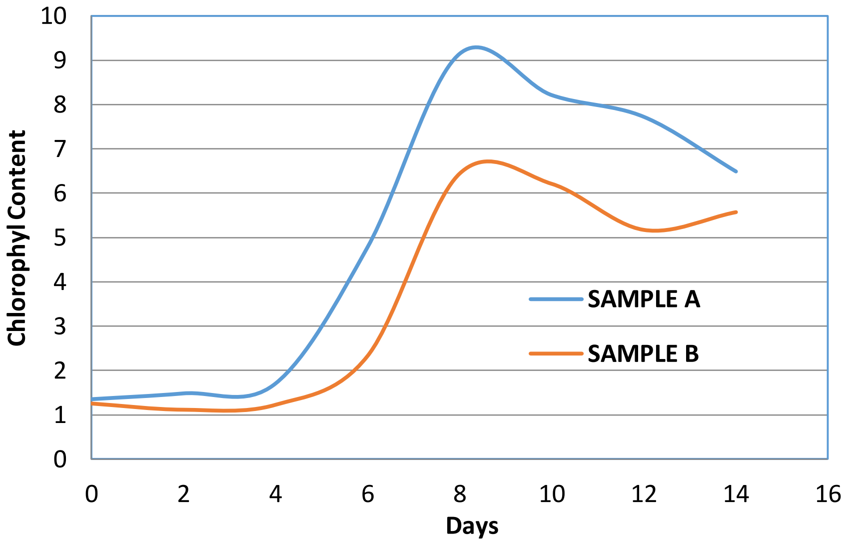

Figure 10 : Chlorophyl Content mesurement profile

Table 5 : Comparative analysis of biofuel production of samples A and B.

Time (Days)	**Production from Samples**	
	A	**B**
0	1.11	1.11
2	1.49	1.11
4	1.71	1.22
6	4.78	2.32
8	9.14	6.44
10	8.21	6.21
12	7.72	5.17
14	6.49	5.57

Table 5 represents the production comparison of samples A with B. It is evident that sample A has a higher yield. This observation is in relations to the previous explanation as found in Table 3 and Figure 9.

4.1 Determination of Algae Biodiesel Properties

The properties of the Biodiesel produced was determined; properties tested were flash point, pour point and cloud point. All properties determined were found to be within the range of standard biodiesel. It is significant to determine the cloud and pour point of the biodiesel as higher values of these properties indicate that biodiesel will form gel at higher temperature causing engine damage. The flash point of Alage Biodiesel was found to be 135 ^{o}C which is close to that suggested in the literature. The cloud point was found to be 18 ^{o}C and it also has a pour point of 17 ^{o}C. The properties of the produced biodiesel tally with that suggested by literaure [22].

Table 6: Properties of alage biodiesel in comparison with standards and petroleum diesel

Property	Algae Biodiesel	ASTM D751	Petroleum Diesel
Flash Point	135	126	74
Cloud Point	18	-	-12
Pour Point	17	14	-16

4.2 Growth and Production of Biofuel

The biomass and chlorophyll contents monitored depicted a major source of rate of algal growth. In both sample A and B, rapid increase in the algal cells was observed two days after inoculation. This was attributed to the availability of adequate nutritional requirements present in the medium as against the sampling sites. Nutrient availability has been a key to rapid growth of microbial cells. This agreed with the findings of [21], who reported that active algal growth was subject to adequate nutrients, sunlight, and aeration. [22] Also stated that a major advantage of microalgae is their very fast growth rates; they can double in numbers in outdoor mass cultures in a day, or, after inoculation, even several times per day.

The pattern of growth in both the samples was similar to that of normal microbial growth, in which a log, stationery and lag phases were observed. In sample B for instance, there was continuous increase in the algal growth from day 0 to day 10, followed by steady decrease from day 10 to day 12 and further decrease by day 14. The same pattern was observed also in sample A, although the log phase lasted longer than that of sample B.

The decrease in growth rate might be due to depletion of nutrients or accumulation of secondary metabolites that may be lethal to the cells. This agreed with many findings reported elsewhere in the literature [23].

Similarly, the results of the chlorophyll content were in consonant with that of the algal growth. The amount of the chlorophyll was increased until after the tenth and eighth day for sample A and B respectively. Thus, the chlorophyll decreased with decrease in the growth rate [24] and [25].

The Property of Algae Biodiesel was determined; it is close to the standards that are found in the literature. The cloud Point was found to be 135^{0}C which agrees with the literature. The high flash point was due to the presence of alcohol which was not removed during washing, efficient washing technique could remove this. The Cloud point and pour point were found to be 18^{0}C and 17^{0}C respectively. Thus biodiesel derived from green alage could serve as good alternative to petroleum diesel [23, 24, 25].

5.0: CONCLUSION

The laboratory studies conducted show that algal species can be used to produce biodiesel (bioethanol). The results reveal that algae are fast growing organism and can be grown artificially. Fertilizer can be used as an enzyme and/or other growth media can be used to increase the growth rate of bio-diesel production. Oil produced from algae oil was esterified using methanol as catalyst. The Properties of the biodiesel produced clearly show that biodiesel produced from green algae can be used to run engine. The various parameters like the flash point, pour point and cloud point etc., are all in agreement with standard biodiesel.

5.1: RECOMMENDATION

Within the limit of the experimental studies, it is recommended that more efforts should be geared toward the production of biodiesel due to its environmental friendliness to reduce the effect of CO_2 in the depletion of the ozone layer. Since it has been determined that biodiesel produced from green algae can perform excellently well in compression ignition engines. Also, production of biogas from green algae should be carried out in large scale. Further work should be done to determine the algal species with the highest yield of oil and biodiesel. Further studies can help to explore more useful products from algae.

6.0: REFERENCES

Ormerod W. G, Freund P, Smith A, Davison J. (2002), *Ocean storage of CO_2*. IEA greenhouse gas R & D programme UK: International Energy Agency, London England, UK

Singh J, Gu S. (2010). *Commercialization potential of microalgae for biofuels production*. Renew Sustain Energy Rev. **14**:2596–610.

Hill J, Nelson E, Tilman D, Polasky S, Tiffany D (2006). *Environmental economic and energetic costs and benefits of biodiesel and ethanol biofuels*. Proc Nat'l Acad. Sci. USA. **103**:11206–10.

Liliana Rodolfi, Graziella Chini Zittelli, Niccolo` Bassi, Giulia Padovani, Natascia iondi, Gimena Bonini, Mario R. Tredici (2008). *Microalgae for Oil: Strain Selection, Induction of Lipid Synthesis and Outdoor Mass Cultivation in a Low-Cost Photobioreactor*. Wiley InterScience.

Li Y, Wang B, Wu N, Lan C. Q, (2008). *Effects of nitrogen sources on cell growth and lipid production of Neochloris oleoabundans*. Applied Microbiology and Biotechnology. **81**(4):629–36.

Richmond A. (2004). *Handbook of Microalgal culture: biotechnology and applied phycology*. Blackwell Science Ltd, UK.

Brennan L, Owende P. (2010). *Biofuels from microalgae--A review of technologies for production, processing, and extractions of biofuels and co-products*. Renewable and Sustainable Energy Reviews **14**:557-577.

Chisti Y. (2007). *Biodiesel from microalgae*. Biotechnology Advances **25**:294-306.

Tomaselli L. (2004). *The Microalgal cell. In: Richmond A, eds. Handbook of Microalgal Culture: Biotechnology and Applied Phycology*. Oxford: Blackwell Publishing Ltd.:3-19.

Lee R. E (2008). *Phycology*. 4th ed. Cambridge Cambridge University Press; 2008

Jasvinder Singh and Sai Gu (2010). *Commercialization potential of microalgae for biofuels production*; Renewable and Sustainable Energy Reviews 2010.

Schenk P, Thomas-Hall S, Stephens E, Marx U, Mussgnug J, Posten C, (2018). *Second generation biofuels: high-efficiency microalgae for biodiesel production*. BioEnergy. **1**(1):20–43.

Chisti Y. *Biodiesel from microalgae*. Biotechnol Adv. 2007;25(3):294–306.

Lele S. (2017). *Indian Green Energy Awareness Center*, http://www.svlele.com/karanj.htm.

Metting F. B (1996). *Biodiversity and application of microalgae*. J Ind. Microbiol. **17**(5–6):477–89.

Rodolfi L, Zittelli GC, Bassi N, Padovani G, Biondi N, and Bonini G, (2018). *Microalgae for oil: strain selection, induction of lipid synthesis and outdoor mass cultivation in a low-cost photobioreactor*. Biotechnol Bioeng. **102**(1):100–12

Spolaore P, Joannis-Cassan C, Duran E, and Isambert A (2006). *Commercial applications of microalgae*. J Biosci. Bioeng. **101**(2):87–96.

Hirano A, Ueda R, Hirayama S, and Ogushi Y (1997). *CO_2 fixation and ethanol production with Microalgal photosynthesis and intracellular anaerobic fermentation*. Energy. **22**(2–3):137–42.
Qin J. (2015). *Bio-hydrocarbons from algae—impacts of temperature, light and salinity on algae growth*. Barton, Australia: Rural Industries Research and Development Corporation; 2005
Olaizola M. (2003). *Commercial development of Microalgal biotechnology: from the test tube to the marketplace*. Bio molecular Engineering. **20**:459-466.
Chaumont D. (1993) *Biotechnology of algal biomass production: a review of systems for outdoor mass culture*. Journal of Applied Phycology. **5**:593-604.
Pulz O. (2001). *Photobioreactors: production systems for phototrophic microorganisms. Applied Microbiology and Biotechnology*. **57**:287-293.
Nigam PS, Singh A. (2010). *Production of liquid biofuels from renewable resources. Progress in Energy and Combustion Science*. In press. DOI: 10.1016/j.pecs.2010.01.003.
Mata T. M, Martins A. A, Caetano N. S (2010). Microalgae for biodiesel production and other applications: A review. Renewable and Sustainable Energy Reviews. **14**:217-232.
Harun R, Singh M, Forde G. M, Danquah M. K (2010). Bioprocess engineering of microalgae to produce a variety of consumer products. Renewable and Sustainable Energy Reviews. **14**:1037-1047.