Physiochemical Characterization of Algae Oil from Microalgae of Nike Lake Enugu

Ejim, I. F.[1] and Kamen, F.L.[2]
[1]Department of Chemical Engineering, Institute of Management and Technology Enugu State.
[2]Department of Chemical Engineering, Federal University of Technology, Owerri, Imo State.
E-mail: ijkeejim@ yahoo.com, felyleby@yahoo.com

ABSTRACT: *Algae have emerged as one of the most promising sources of oil when grown in $C0_2$ enriched environment. In this experiment, algae oil extract from microalgae samples collected from Nike Lake was subjected to basic characterization analysis. The physicochemical properties of the produced oil were measured and compared to the properties of soybean oil and other oil seeds. Results showed that saturated fatty acid; oleic gave higher percentage (36%) while other properties obtained were density (0.892 g/cm^3), pH (4.6-5.0). refractive index (1.5), saponification value (200g), acid value (1.9g), free fatty acid value (0.96%), iodine value (100-120), cloud point (10^{0}C), pour point (5-8^{0}C), flash point (110^{0}C) and moisture content (0.85%) freezing points (31-40^{0}C) and greenish yellow in colour. Many of the properties of the algae oil are comparable with those of soybean oil and other oil seeds.*

Keywords: Microalgae, Algae Oil, Nike Lake, and Physiochemical Analysis
Received for publication on 26 July 2013 and accepted in final form 4 August 2013

INTRODUCTION

Algae or singular alga, Latin for "Seaweed" are a large and diverse group of simple, typically auto torphic organisms, ranging from unicellular to multicellular forms, such as the giant kelps that grow to 65 meters in length (**Webster's Third New International Dictionary, 1986)** . They are photosynthetic like plants and "Simple" because their tissues are not organized into the many distinct organs found in land plants. Algae lack the various structures that characterize land plants, such as phyllids (leaves) and rhizoids in non-vascular plants, or leaves, roots, and organs that are found in tracheophytes (Vascular Plants), many are photoautotorphic, although same groups contain members that are mixotrophic, deriving energy both from photosynthesis and uptake of organic carbon either by osmotrophy, myzotrophy or phagotrophy **(Nabors and Murray, 2004)**.

Research into algae for the mass-production of oil is mainly focused on microalgae, organisms capable of photosynthesis that are less than 0.4mm in diameter, including the diatoms and cyanobacteria; as opposed to macroalgae, such as seaweed **(MNRE, 2010)**. The preference towards microalgae is due largely to its less complex structure, fast growth rate, and high oil content. However, researches are in progress for using seaweeds for biofuels, probably due to the high availability of this resource seaweed **(Chetri *et al.*, 2008)**.

Nike Lake is one of the Lakes in the city of Enugu in Enugu State of Nigeria. It is located in south eastern Nigeria with coordinates of 6°30'32.4360"N and 7°30'141, 2560"E. A wide range of algae species grow naturally in Nike lake ranging from diatoms known for their high lipid content and green algae known for its ability

to store energy in the form of either starch or lipids **(Vasudev G, 2010)**.

All the agricultural runoff in the city contains significant amounts of nutrients (from fertilizers) which can result in the available excess nitrogen and phosphorus in the lake and favours algae blooms which use these macro-nutrients for its growth. The urgent need for diversion from fossil fuel to biofuels has made the study of algae oil production of great importance since it has been shown that microalgae oil yield (136,900lit/hect.) is more than twice greater than palm oil yield (5,950lit/hect.) and more than fifty times higher than soybean (446lit/hect.) **(Kumar, *et al;* 2011)**

METHODOLOGY

Characterization of Algae Oil

Algae oil extracted from microalgae samples collected from Nike Lake during June and August (rainy season) of 2011 and 2012 was subjected to basic characterization analysis. The physicochemical properties as well as the fatty acid profile of the algae oil obtained were determined with the view of characterizing the oil. The major properties determined include; Fatty acid profile, density, iodine value, acid value, moisture content, ash content, saponification value, flash point, cloud point, freezing, point pour point, colour, pH and refractive index.

Determination of Fatty Acid Profile of Algae Oil

An aliquot of 10mg of oil was weighed and mixed with 2ml of hexane. The mixture was vortexed for 2minutes at room temperature, and centrifuged, then an aliquot (2 micro liters) of the hexane layer was collected for GC analysis. Shimadzu's GC-FID system, used for the qualitative and quantitative analysis of fatty acids of the algae oil, consists of a GC – 17A, a flame ionization detector, and a DB –WAX capillary column (60m x 0.25mm, thickness = 0.25µm; J & W Scientific). The initial temperature for oven was set at 180°C and held for 2min. Then the temperature increased from 180°C to 250°C at the ramp of 5°C/min and held at 250°C for 30min. The injector and detector were maintained at 200°C and 220°C, respectively. Helium was used as a carrier gas, and its flow rate was kept at 1.5ml/min. The area percentages were recorded with a standard HP chemstation data system.

Determination of Dry Weight and Moisture Content

The dry weight analysis is based on the simple drying concept. A known amount of algae oil sample is weighed accurately in aluminum weighing dish and is dried to constant weight in a closed oven at 40-45°C, the sample is weighed after every 5 hours. After 50 hours the difference in weights was within 0.01±0.1g, then the sample was considered dry. The weight of the dried algae oil sample was then measured. Moisture content is thus calculated as:

Initial weight of the algae oil sample (wet) = X gm,
Final weight of the algae oil sample (dry) = Y gm,

The dry weight percent = (Y/X)*100 …. …. 2.1
Moisture content (Z %) = (1-(Y/X))*100 … 2.2

Determination of Density of Algae Oil

The weight of a 100ml empty bottle was determined using an electronic weighing balance. The bottle was filled to the brim with the oil and the weight of the bottle and oil determined. The density was calculated using the formula below.

Density (ρ) = $\frac{W2-W1}{V}$ ……..... 2.3

W_2 = weight of bottle and oil,
W_1 = weight of bottle,
V = volume of oil.

Determination of Saponification Value (S.V) of Algae Oil

2g of oil sample was placed in a 250ml conical flask and 25ml of 0.5M ethanol potassium hydroxide solution added. A reflux condenser was attached and the flask content refluxed for 30min on a water bath with continuous swirling until it simmered. The excess potassium hydroxide was titrated with 0.5M hydrochloric acid using phenolphthalein indicator while still hot. A blank determination was carried out using distilled water under the same condition and the S.V calculated as thus:

$$\textbf{Saponification value (S.V)} = \frac{(B-R)*28.05}{Weight\ of\ oil} \quad \ldots\ldots\ldots \textbf{2.4}$$

Where B = Titre value of blank (distilled water), R= Titre value of algae oil sample.

Determination of Free Fatty Acid (FFA) of Algae Oil

The method used for the determination is that of British standard institute No.684. 4g of the oil was placed in a 250ml conical flask and warmed. 25ml methanol was added with thorough stirring followed by two drops of phenolphthalein indicator and a drop of 0.14M sodium hydroxide solution. The content was then titrated with 0.14M sodium hydroxide solution while shaking vigorously until a permanent light pink colour, which persisted for 1 minute, was seen. The end point was recorded and used to calculate the FFA value as thus:

$$\textbf{\% FFA (as oleic)} = \frac{Titre\ x\ N\ x\ 28.2}{Weight\ of\ sample} \quad \ldots\ldots \textbf{2.5}$$

Where N = molarities of base

Determination of Acid Value (A.V)

The method used for the determination of free fatty acid (FFA) of algae oil was repeated for acid value (A.V) as indicated below;

Acid value = %FFA (as oleic) x 1.99 … 2.6

Determination of Iodine Value (IV) of Algae Oil

The oil sample (1g) was placed in a 250ml conical flask stopper and placed in the drawer for exactly 30 minutes. Potassium iodine solution (10ml of 15% W/V) was added to the flask washing down any iodine that may be found on the stopper. This was titrated against 0.14M Na_2SO_3 until the sodium become light yellow. Starch indicator (1%, 2ml) was added and the titration continued until the blue colour just disappeared. A blank (distilled water) determination was carried out under the same conditions. The titre value was recorded and used to calculate the I.V as indicated below.

$$\textbf{Iodine value} = \frac{(B-R)\ x\ molarities\ of\ Na2SO3\ x\ 12.69}{Weight\ of\ sample} \quad \ldots\ \textbf{2.7}$$

B = Titre value of Blank (distilled water),
R = Titre value for oil sample determination.

Determination of Pour Point of Algae Oil

Using ASTM D5949, the algae oil sample was heated and then cooled by a Peltier device at a rate of 1.5 +/- 0.1°C/min. At 3°C intervals, a pressurized pulse of compressed gas was imparted onto the surface of the oil sample. Multiple optical detectors continuously monitor the algae oil sample for movement. The lowest temperature at which movement was detected on the sample surface was determined to be the pour point.

Determination of Cloud Point of Algae Oil

Using ASTM D5773, the test oil was cooled by a Peltier device at a constant rate of 1.5 +/- 0.1°C/min. During this period, the oil sample was continuously illuminated by a light source. An array of optical detectors continuously monitors the algae oil sample for the first appearance of a cloud of wax crystals. The temperature at which the first appearance of wax crystals was detected in the oil sample was determined to be the cloud point.

Determination of Flash Point of Algae Oil

The method used here is by Cleveland Open Cup (COC). The oil sample was contained in an open cup which was heated and at intervals a flame was brought over the surface. The measured flash point will actually vary with the height of the flame above the oil surface, and at sufficient height the measured flash point temperature was noted.

Determination of Freezing Point of Algae Oil

The differential Scanning Calorimeter was used to determine the enthalpy of fusion of the algae oil. The result was noted.

Determination of pH of Algae Oil

Indicators were used to measure pH of algae oil, by making use of the fact that their colour changes with pH. Visual comparison of the colour with a standard colour chart provided a means to measure pH of the algae oil. Universal indicator consists of a mixture of indicators such that there is a continuous colour change from about pH 2 to pH 10.

Determination of Refractive Index of Algae Oil

A thin layer of the oil to be measured was placed between two prisms. Light is shone through the liquid at incidence angles all the way up to 90°, i.e., light rays parallel to the surface. The second prism has an index of refraction higher than that of the oil, so that light only enters the prism at angles smaller than the critical angle for total reflection. This angle was measured by looking through a telescope, placed in the focal plane of a lens. The refractive index of the oil was then calculated from the maximum transmission angle θ as,

$$\eta = \eta_G \sin \theta \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad (2.8)$$

Where η_G is the refractive index of the prism

RESULTS AND DISCUSSION

Table 1: Physiochemical Properties and Fatty Acid Compositions of Algae Oil from Nike Lake

Properties	Value	Composition	Weight percent
Density (g/cm3)	0.892	Oleic acid	36
pH	4.6 – 5.0	Palmitic acid	15
Refractive index	1.5	Stearic acid	11
Saponification value	200	Linoleic acid	7.4
Acid value	1.9	ISO	8.4
Iodine value	100 – 120	Free fatty acid	0.96
Flash point (^oC)	110	Moisture content	0.85
Freezing point(^oC)	31 – 40		
Pour point(^oC)	5 – 8		
Cloud point(^oC)	10		
Cetane Number	+7		
Appearance	Greenish yellow		

From the fatty acid profile for microalgae present in Table 1, the result showed percentage of palmitic acid 15%, oleic acid 36%, stearic acid 11%, iso-8.4% and linoleic acid 7.4%. The fatty acids of microalgae oil were compared with that of soybean oil and it showed significant difference in quantity of polyunsaturated fatty acid than monounsaturated and saturated fatty acids of both oil.

The acid value of algae oil is 1.9, this low acid value shows the effect of solvent extraction over mechanical extraction as the acid content of algae oil has been reduced during the solvent extraction (**Kumar *et al.*, 1981**). This acid value shows the extent to which constituent glycerides can be decomposed by lipase action (**Trabi *et al.*, 1997**).

The saponification value of algae oil is 200, this implies that the triglycerides of algae oil have low molecular weight of fatty acids (saturated and unsaturated). The result obtained compared favourably with that of soybean oil (193). The free fatty acid of algae oil gives 0.9 showing that the oil is unstable and can easily degrade and form soap when reacted with alkaline and form stable emulsion and thus prevent separation of biodiesel from glycerol during transesterification (**Demibas, 2003**).

The iodine value of algae oil (100-120) is comparable to those of sunflower oil (124) and sesame seed oil (115) (Talkit, et al; 2012). In general, the greater the iodine value, the higher the degree of unsaturation and the higher the tendency of the oil to undergo oxidative rancidity (**Pearson, 1981**).

Also from Table 1, the pour point of algae oil is between 5°C to 8°C while soybean oil gave -12^0C to -16^oC. This gives an idea of the temperature at which the oil becomes semi solid and losses its flow characteristics. Similarly the cloud point which gives 10°C for algae is higher than soybean -9^0C. This

shows the temperature at which the oil is no longer completely soluble, precipitating as a second phase giving the oil a cloudy appearance. The flash point of the oil was seen to be 110°C for algae oil which is the lowest temperature at which it can vaporize to form an ignitable mixture in air.

CONCLUSION

The focus of this research work was to characterized algae oil from microalgae collected from Nike Lake, Enugu. The properties of the algae oil were determined and compared with soybean and other oil seeds. It was discovered that algae oil if properly obtained from its biomass and can serve as oil suitable for biodiesel production.

REFERENCES

Chetri, A.B; Tauo, M.S; Budge, S.M; Watts, K.C; Islam, M.R.. (2008) 'Non-edible Plant Oils as new Sources for Biodiesel Production'. International Journal Molecular Sciences; Vol. 9: Pp169-180.

Dermibas, A. (2005) 'Biodiesel Production from Vegetable Oils Via Catalytic and Non-catalytic Supercritical Methanol Transesterification Methods'. *Progressive Energy Combustion*, Vol.31, 466-487.

Kumar, P; Ruseela, M.R; Toppo, K. (2011) 'Physiochemical Characterization of Algae oil and Comparison with some sources of Biofuel' *Asian Journal of Biological Sciences.* Volume 2, Number 3. Pp 493 497.

Kumar, T.A., Kumar A, and Raheman, H.; (2007) 'Biodiesel Production from Jatropha Oil *(Jatropha curcas)* with high free Fatty Acids: an Optimized Process'. *Biomass and Bioenergy*; Vol.31: 569–75.

Ministry of New and Renewable Energy (MNRE) (2010) 'Annual Report 2009-10' Chapter 3: Renewable Energy for Rural Applications.

Nabors, S; Murray W. (2004); '*Introduction to Botany*' San Francisco, CA: Pearson Education, Inc.

Pearson, D. (1981); The Chemical Analysis of Food, 7th edition, Churchhill Livingstone, Edinburgh. Pp505-530.

Talkit, K; Mahajan, D.T; Masand, V.H. (2012) 'Physiochemical Properties of Soybean Oil and their Blends' *Journal of Chemical, Biological and Physical Sciences*. Vol.3, No.1. Pp 490 – 497.

Trabi, M. Gubitz G.M., Steiner W., Foidl N, (1997); 'Toxicity of *Jatropha curcas* Seeds'. Proceedings of the symposium Jatropha 97 (Biofuels and Industrial Products from *Jatropha curcas*, Managua, Nicaragua.

Vasudev, G. (2010) 'Evaluation of Lake Erie Algae as Bio-fuel Feedstock' Master of Science in Chemical Engineering Thesis. University of Toledo

Webster's Dictionary (1986) "Alga, Algae". *Webster's Third New International Dictionary of the English Language Unabridged with Seven Language Dictionary*. Encyclopedia Britannica, Inc.

Reference to this paper should be made as follows: Ejim, I. F. and Kamen, F.L. (2013), Physiochemical Characterization of Algae Oil from Microalgae of Nike Lake Enugu, *J. of Engineering and Applied Science, Vol.5, No.1, Pp. 181-187.*