EXTRACTION OF OIL FROM MICROALGAE AND AQUATIC PLANTS HARVESTED FROM FISH REARING STRUCTURES

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Abstract

Continued reliance on land crops for biofuel production may lead to destruction of forestlands and also contributes to the increase in emission of greenhouse gases. Oil extraction was carried out on microalgae and two aquatic plants to evaluate potentials for industrial and domestic uses in serving as substitute for conventional oils and non-renewable fossil fuels. The aquatic plants Elodea and Lemna were sieved from the surface of an earthen fish pond using a hand net, while the microalgae samples (MCA) were scrapped from the walls and water surface of a concrete fish tank. Soxhlet extraction method was used to extract the oils from the samples. The microalgae sample yielded 7.2ml of oil per 100g sample; 5.0ml per 100g sample was extracted from Lemna, while 3.2ml per 100g sample was obtained from Elodea. After drying of the oils, the fats weighed 2.25g, 2.10g and 1.80g respectively. Percentage fat contents were 2.25%, 2.10% and 1.80%.Microalgae and aquatic plants could serve as sources of oil to substitute conventional oils and non-renewable biofuels; with microalgae yielding more oil than aquatic plants.

Key Words: Biofuels, Elodea, Lemna, Microalgae, Soxhlet Extractor

1.0 Introduction

Escalating fuel prices, the emerging concern about global warming that is associated with burning fossil fuels, quest for economic growth, fighting poverty and the growing demand for petroleum products have spurred new interest in the search for alternative sources of natural oil for fuel (Abubakar *et al*., 2012). Biofuels have been at the forefront of the discussion. A number of sources for the production of biofuels have been considered. Biofuels are fuels that are produced from living organisms or from metabolic byproducts. Algae are members of a group of predominantly aquatic, photosynthetic organisms of the kingdom Protista. They range in size from the tiny flagellate *Micromonas* that is 1 micrometre in diameter to giant kelps that reach 60 metres in length. The majority of algae that are intentionally cultivated fall into the category of microalgae (also referred to as phytoplankton, microphytes, or planktonic algae. Algae have multiple advantages over traditional energy crops. They have a higher growth rate than other crops, a shorter maturity rate, a higher biomass production rate than other cash crops, as well as using far less land than conventional crops (Lee *et al*., 2009). Furthermore, with algae there is no competition for land space that could be used for food crops. For example, using corn as a feedstock for making ethanol creates a negative competition between human and animal consumption and also fuel production (Huang *et al*., 2010).The United States Department of Energy estimates that if algae fuel replaced all the petroleum fuel in the United States, it would require 39,000 km^2 which is only 0.42% of the U.S. map, or about half of the land area of Maine. This is less than 14.2% the area of corn harvested in the United States in 2000 (Dyer, 2008).
Commercial and industrial algae cultivation has numerous uses, including production of food ingredients such as omega-3 fatty acids or natural food colourants and dyes, food, fertilizer, bio-plastics, chemical feedstock, pharmaceuticals, and algal fuel, and can also be used as a means of pollution control. Algae have a number of important characteristics; they can be grown with minimal impact on fresh water resources (Yang *et al*., 2010; Cornell, 2008).They can also be produced using ocean and wastewater and algal fuel are biodegradable and relatively harmless to the environment if spilled (Eastern Daily Press, 2008).

Algae have the potential to counteract a portion of the greenhouse effect and water pollution, because through photosynthesis, some algae have the ability to fix carbon dioxide produced by industrial plants. Some other algae also fix nitrogen and absorb other contaminants such as heavy metals and phosphorous (Gouveia and Oliveira, 2009). One of the main obstacles

to fully taking advantage of lipid-producing algae is the ability to successfully and efficiently extract oil from the cell biomass; additionally, there is the concern of extracting the oil in the safest and most environmentally sustainable manner.

Algae, like higher plants, produce storage lipids in the form of triacyglycerols (TAGs). Algae are known to accumulate more lipids in nutrient deficient conditions. Researchers identified the most dramatic increases in the lipid content of the cultures during nutrient-deficient conditions.

Generally there is a need to promote the currently underrated agricultural sector. Algae oil which is a renewable form of fuel could suitably substitute the non-renewable forms of fossil fuels. Continued reliance on land crops for biofuel production may lead to destruction of forestlands and also contributes to the increase in emission of greenhouse gases by the destruction of forests.

Non-food sources of biofuel and other form of oils (such as edible oil, fish oil substitutes etc) are better recommended than their food sources counterparts (Sexton *et al*., 2008), as the world faces an impending ecological disaster if fuel production is restricted to food crops such as corn, sugar beets, sugar cane etc. Examples of non-food sources of biofuels include Jatropha and Algae.

The growth of most algae, which are usually considered as weeds and unwanted, actually requires marginal lands when compared to their food crop counterparts. So it is only reasonable that these organisms are exploited and utilized for the production of oils which could be beneficial for a host of functions. This paper reports findings of an investigation into the extraction of the oil content of two aquatic plants and microalgae samples taken from fish rearing facilities in order to compare their yields.

2.0 Materials And Methods

Selected plants:

Elodea is a genus of aquatic plants often called the waterweeds. It survives best in freshwaters. The introduction of some species of *Elodea* into waterways in parts of Europe, Australia, Africa, Asia, and New Zealand has created a significant problem and it is now considered a noxious weed. Elodea has lent itself as a biofuel source in recent times. Substantial amounts of lipids have been extracted from the aquatic weed.

Duckweed, plants of the Lemnaceae family, has the distinction of being the smallest angiosperms in the world with the fastest doubling time. Duckweeds are excellent laboratory

models for fundamental biology as well as field applications such as environmental bio-monitoring and wastewater remediation. Recently, there has been an increasing effort to maximize the beneficial properties of duckweeds (such as its lipid content) and to utilize it in other areas of the world.

Microalgae, like higher plants, produce storage lipids in the form of triacyglycerols (TAGs). Comparatively algae produce more oil than any other oilseeds which are currently in use.

2.1 Collection and Preparation of Samples

Samples of Elodea (ELO) and Lemna (LEM) were collected from an earthen pond (36m length by 28m width by 1.5m height) by the use of hand nets in Sanusi Fish Farm, Olodo, Oyo State. Their weights were measured using the Camry Emperors Scale; their respective wet weights were: 3kg and 2kg. Microalgae sample (MCA) (1kg wet weight - also measured using the Camry Emperors Scale) was collected from the walls and water surface of a concrete fish tank; (7m length by 3m width by 1.5m height) in the same Farm. The tank and pond were not in operation hence fish species in them could not be accounted for; though glimpses of some unidentified fish species were observed to jump intermittently. The pond and tank had been left in this state for more than a month allowing for the observed blooms. Plates 1, 2 and 3 shows the Lemna, Elodea and Microalgae sampled in the fish rearing structures before harvest.

Plate 1: Earthen pond bloomed with Lemna

Plate 2: Earthen pond showing submerged Elodea

Plate 3: Concrete tank showing microalgae agglomeration on the surface

Samples of the aquatic plants and microalgae were transported to the department of Aquaculture and Fisheries laboratory in the University of Ibadan, Nigeria for identification in line with the procedure of Bellinger (1992).

The samples were sundried daily between 27th February and 1st March, 2013; until the weights of the samples subsequently remained constant. Minimum and maximum temperatures of the days were 23 and 31^{o}C; 21 and 29^{o}C; 23 and 33^{o}C respectively, while relative humidity recorded were 73%, 82% and 70% respectively for the three days. The samples were then packed in plastic containers ready for extraction of oils.

The samples were chopped and blended using a 400watts capacity Sonik® milling machine .This was done so as to increase the surface area through which the extraction solvents can act in order to improve yields.

2.2 Water Sampling and Quality Assesment

Physico-chemical parameters; Temperature, pH, Dissolved Oxygen, Alkalinity, Phosphate, Nitrite and Nitrate were analysed. Hanna test kit was used in determining alkalinity, pH, ammonia, nitrite and nitrate parameters. Water samples from the earthen pond and concrete tank were collected in separate bottles. Winklers Titrimetric Method was used to determine dissolved oxygen. Two bottles each were dipped into the earthen pond and concrete tank separately; they were filled to the brim taking all precautions to prevent escape of oxygen. Absorption Spectrophotometer was used in determining phosphate levels.

2.3 Extraction of Oil from Samples

The dry mass of the samples (Elodea, Lemna and Microalgae; 100g each) were transported to Multi-environmental Management Consultants Ltd, Ikorodu, Lagos, for oil extraction and characterization.Crude lipid extraction was carried out (Plate 4), using the Soxhlet extraction method (AOAC, 2006). 250ml capacity extracting flask was dried in the oven at 105^{o}C, transferred to the desicator to cool to laboratory temperature and the weight of the flask was measured. The sample (100g) was weighed into the porous thimble.

200ml of petroleum ether was measured and then added to the dried 250ml capacity flask. The covered porous thimble with the sample was placed in the condenser of the Soxhlet extractor arrangement that has been assembled. The sample was extracted for five (5) hours.

The porous thimble was removed with care and the petroleum ether in the top container (tube) was collected for the recycling for reuse. The extraction flask was removed from the heating mantle arrangement when it was almost free of petroleum ether. The extraction flask with the oil was oven dried at 105^{o}C for the period of one (1) hour. The flask containing the dried oil was cooled in the desicator and the weight of the dried oil was measured.

Plate 4: Extraction of oils using the Soxhlet Extractor

Percentage extracted was determined using the formula:

$$\textbf{\% Fat content} = \frac{\textbf{weight of lipid in grams}}{\textbf{weight of sample in grams}} \times 100$$

3.0 Results And Discussion

Water Quality Parameters in Sampled Facilities

Temperature is one of the most important factors that controls the behavior and distribution of organisms. Mean temperatures of 28±1.00^{o}C and 29±0.00^{o}C were recorded for the earthen pond and concrete tank respectively. Optimal temperature for phytoplankton culture is generally between 20 and 24^{o}C (FAO, 1996), though this varies with different factors such as the species involved, composition of the culture medium. Microalgae are known to tolerate temperature ranges between 16 and 27^{o}C (FAO, 1996). Padmavathi and Durga (2007) also observed 27.16 – 32.48^{o}C as optimum temperature range. Adebisi (1981) recommended (26.5 - 32.5^{o}C) for tropical rivers. Temperatures lower than these acceptable range retards growth and higher temperatures are lethal. Temperature could also be affected by phytoplankton blooming; the more the bloom the higher the temperature as a result of accumulation of more organisms and also other biological activities. Due to the fact that optimal temperature for growth is species-specific, the obtained values can be technically accepted considering these specific species.

pH is a very important factor in biotic communities because most plants and animals can survive within a narrow range. Mean values of 7.0±0.20 and 7.5±0.30 were recorded in earthen pond and concrete tank respectively. pH range for most cultured algal species is between 7 and 9 (FAO, 1996) . From the measured values, it shows that the pH is within recommended values (6.09 – 8.45) given by Ugwumba *et al.,* (2011), Ramaraj *et al.,* (2010) and Atobatele *et al.* (2005). Boyd and Linchtkoppler (1979) also reported a recommended pH of 6.5 – 8.5 which also happens to be in accordance with findings from this work.

Dissolved Oxygen (DO) which is very important for sustenance of aquatic life was recorded at 3.8±0.10 and 4.1±0.00mg/L (mean value) in earthen pond and concrete tank respectively. Ramaraj *et al.,* (2010) gave 3.3- 8.2 mg/L, as recommended range for algal culture. The values were also in line with the findings (2.99 – 5.49mg/L,)of Padmavathi and Durga (2007).

Phosphate values of 9.79±0.62mg/L and 10.50±0.56mg/L for the earthen pond and concrete tank respectively were recorded. Phosphate are essential for the growth of phytoplankton and it germs primary productivity of a water body. When occurring in the excess concentrations, it causes algal blooms which deplete oxygen as observed. Reports have stated that when algae are starved of phosphate they tend to produce more lipids (Khozin-Goldberg and

Cohen, 2005). Phosphate values were much higher than findings from Padmavathi and Durga (2007) that observed 0.2 - 1.2 mg/L.

Alkalinity levels observed in the earthen pond and concrete tank were 108±2.65mg/L and 112±2.65mg/L respectively. This was in accordance with findings from Tucker and Hargreaves (2004) who recommended a range of 50 - 250mg/L.

Mean Nitrite (NO^-_2) values of 0.66±0.03mg/L and 0.52±0.02mg/L were observed in the earthen pond and concrete tank. Cline (2012), reported a convenient range of 0 - 0.6mg/L indicating that values observed were close to the upper limits of the recommended range. These high levels could be attributed to the feeding regime and high organic activities in the concrete tank and earthen pond respectively.

Mean Nitrate (NO^-_3) values in the earthen pond and concrete tank were 34.3±3.06mg/L and 31.1±1.87mg/L respectively. Nitrate levels in water are attributed to the although, ammonia and nitrate are less harmful to aquatic organism life decomposition of organic effluent and waste water released into the body (Ugwumba. *et al* 2011), (Atobatele *et al.,* 2005).

A comprehensive data on the sampled water quality parameters is given in table 1.

Table 1: Values of selected water quality parameters in the fish culture structures

Parameters	**Earthen pond**	**Concrete Tank**
pH	7.0 ± 0.20*	7.5 ± 0.30
Phosphate (mg/L)	9.79 ± 0.62	10.50 ± 0.56
Alkalinity (mg/L)	108.00 ± 2.65	112.00 ± 2.65
Nitrite (mg/L)	0.66 ± 0.03*****	0.523 ± 0.02
Nitrate (mg/L)	34.30 ± 3.06	31.10 ± 1.87

Temperature (^{o}C)	28.00 ± 1.00	29.00 ± 0.00
Dissolved Oxygen (mg/L)	3.80 ± 0.10	4.10 ± 0.00

*Values in the table are Means ± Standard deviation of three samples

3.1 Gravimetric and Volumetric Analyses of the extracted oils

After complete drying of the samples; Elodea (ELO) weighed 606g, Lemna (LEM) weighed 323g while Microalgae (MCA) weighed 110g. Percentage moisture content was calculated to be 79.80%, 83.85% and 89.00% respectively. Under the same soxhlet extraction method, the volumes of the extracts were recorded at 3.9ml for ELO, 5ml for LEM and 7.2ml for MCA. Volumes extracted from the aquatic plants were lower than that of the microalgae extract. This conflicts with reports that microalgae are high producers of high amounts of algae oil (Solix Biofuels, 2008), the results obtained here were very low and this could be as a result of agglomeration of various species in the microalgae sample or the presence of unfavourable algal species. These species tend to inhibit the tendency of the oil-producing algae species to significantly release their oil contents. The extracted oils were not viscous and all had a golden yellowish colour. Plate 5 shows the extracts from the samples. The oils were also dried before the gas chromatography analysis and their weights were 1.8g for ELO, 2.1g for LEM and 2.25g for MCA. Percentage fat contents were also calculated for each of the samples, the values were 1.8%, 2.10% and 2.25% for ELO, LEM and MCA respectively. A comprehensive detail of gravimetric and volumetric values of the samples is shown in table 2. Results from this works indicates the possibility of obtaining a litre of oil from about 20kg of dry algae depending on the species type, percentage lipid/fat composition and also availability of optimum culture conditions/media. Even though microalgae are still the leading primary source of oilgae, the aquatic plant/macroalgae could be tapped considering their ease of harvest and relative abundance as unwanted weeds in our environment.

Table 2: Gravimetric and Volumetric Values of the samples

	Elodea	Lemna	Microalgae
Weight of wet sample (g)	3000	2000	1000
Weight of dry sample (g)	606	323	110
Moisture Content (%)	79.80	83.85	89.00
Weight of sample used for Gas Chromatography analysis (g)	100	100	100
Volume of oil Extracted from 100g of samples (ml)	3.9	5.0	7.2
Weight of dried oil sample (g)	1.80	2.10	2.25
Percentage Fat Content (%)	1.80	2.10	2.25

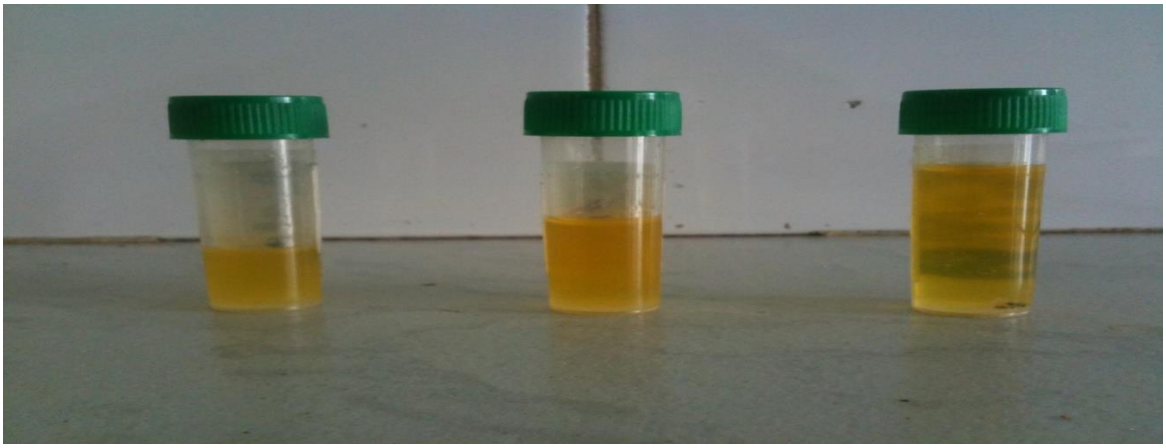

Plate 5: Oil Extracted from Elodea, Lemna and Microalgae samples respectivel

4.0 Conclusion

Findings in this study reveals the feasibility of obtaining oils extracts from aquatic plants and microalgae, though, these values were low. Production of algae oil has a vast and cogent prospect and for its viable actualization on larger scales, it should be supported and encouraged

by any affected parastatals. The poor yield of the microalgae could be the agglomeration of different species which could hinder the growth of favourable species or inhibit their lipid production

5.0 References

Abubakar, L.U., Mutie, A.M., Kenya, E.U. and Muhoho A. 2012. Charaterization of Algae Oil (Oilgae) and its Potential as Biofuel in Kenya. *J. Applied phytotechnology in Environmental Sanitation*. Volume 1, Number 4: 147 – 153

Adebisi, A.A. 1981. The physic-chemical; hydrology of a tropical seasonal river upper Ogun River, *Hydrobiologia*, 79: 159-167.

Alternate Energy. 2012. Biofuels: News and Information about Biofuel and Biomass Fuel Technology. Retrieved from *http://www.alternative-energy-news.info/technology/biofuels/* on 15 January 2013.

Amaro, H.M., Guedes, A.C. and Malcata, F.X. 2011. Advances and Perspectives in Using Microalgae to Produce Biodiesel. *Applied Energy*, vol 88, no, pp. 3402-3410.

Atobatele, O.E., Morenikeji, O.A. and Ugwumba, O.A. 2005. Spatial variation in physical, chemical parameters and benthic macro-invertebrate fauna of river Ogunpa, Ibadan. *The Zoologist* 3:58-67.

Becker E.W. 1994. Microalgae: Biotechnology and Microbiology. NY: Cambridge University Press, p.178

Bellinger, E.G. 1992. *A Key to Common Algae, Freshwater and Some Coastal Species*, 4th Edition. Institute of Water and Environmental Management, London

Boyd, C.E. and Linchtkoppler, F.R. 1979. Water quality management in fish pond culture. *International centre for Aquaculture, Agric experimental station*, Auburn University. pp 20.

Cornell, C.B. 2008. "First Algae Biodiesel Plant Goes Online: 1 April 2008". Gas 2.0. *http://gas2.org/2008/03/29/first-algae-biodiesel-plant-goes-online-april-1-2008/*. Retrieved 16 January 2013.

Cline, D. 2012. Water Quality in Aquaculture. Alabama Cooperative Extension System, Auburn University. Retrieved from www.extension.org/pages/58707/water-quality-in-aquaculture on 17

October 2012

Dyer, G. 2008. "A Replacement for Oil". The Chatham Daily News. *http://www.chathamdailynews.ca/ArticleDisplay.aspx?e=1075914.* Retrieved on 18 April 2013.

Eastern Daily Press. 2008. Green Dream. Backed by MP's. Eastern Daily Press. January 2008. Archived from the original on 22 June 2008. *http://www.globeco.co.uk/Bio diesel_news_0007.html.* Retrieved 10 March 2013.

FAO. 1996. Manual on the Production and Use of Live Food for Aquaculture. Fisheries and Aquaculture Department. Edited by P. Lavens and P. Sorgeloos. Laboratory of Aquaculture and Artemia Reference Center University of Ghent, Belgium. *FAO Fisheries Technical Paper* 361

Gouveia, L. and Oliveira, A. C. 2009. Microalgae as a Raw Material for Biofuel Production. J. *Ind. Microbiol. Boit*. 2009, 36, 269-274.

Huang, G., Chen, F., Wei, D., Zhang, X. and Chen, G. 2010. Biodiesel production by microalgal biotechnology. *Appl.Energ*. 2010, 87, 38-46.

Khozin-Goldberg, I. and Cohen, Z. 2005. The effect of phosphate starvation on the lipid and fatty acid composition of the fresh water *Eustigmatophyte monodus Subterraneus.* Received 18 April 2005; received in revised form 5 January 2006 Available online 23 February 2006

Lee, J.Y., Yoo, C., Jun, S.Y., Ahn, C.Y. and Oh, H.M. 2009. Comparison of several methods for effective lipid extraction from microalgae. *Biores. Technol*. 2009, 101, S75-S77.

Padmavathi, P. And. Durga P.M.K. 2007. Studies on Algal Bloom Disasters in Ponds Carp Culture. *AcharyaNagarjuna University* Nagarjun Nagar India. – 522 510 Andhra Pradesh, India

Ramaraj, R., David, D.W., Paris, T. and Chen, H. 2010. Algae Growth in Natural Water Resources. Doctoral Research Fellow, Professor, Department of Soil and Water Conservation, National Chung-Hsing University, Taichung 402, Taiwan, R.O.C.

Sexton S., Rajagopal, D., Zilberman, D. and Hochman, G. 2008. Food Versus Fuel: How Biofuels Make Food More Costly and Gasoline Cheaper Agricultural and Resource economics update. *Giannini Foundation of Agricultural Economics*. University of California V. 12 no. 1

Solix Biofuels 2008. Why algae? Retrieved from *http://www.solixbiofuels.com/html/why_alage.html on 10 October 2012.*

Tredici, M.R. 2010. Photobiology of microalgae mass cultures: understanding the tools for the next green revolution. *Biofuels*, vol. 1, pp. 143-162.

Tucker, C.S. and Hargreaves, J.A. 2004. Biology and Culture of Channel Catfish, 1st Edition Volume 34: 11 Dec 2004 Imprint: *Elsevier Science* ISBN: 9780080472201 Pages: 262

Ugwumba, A.A.A., Ogidiaka, E. and Esenowo, I.K. 2011. Physico-chemical parameters and Benthic macro-invertebrates of Ogunpa river. Bodija, Ibadan, Oyo state. *The Zoologist* 5:61-72.

Yang, J., Ming, X., Xuezhi Z., Qiang H., Sommerfeld, M. and Yongshen, C. 2010. Life-cycle analysis on biodiesel production from microalgae: Water footprint and nutrients balance. *Bioresources Technology* 10: 1016. *http://www.personal.umich.edu/ mingxu/files/papers/Algae.pdf*.