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Research Paper

CHARACTERIZATION OF ALGAE OIL (OILGAE) AND ITS POTENTIAL AS BIOFUEL IN KENYA

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Abstract: A survey of microalgae biodiversity in three Kenyan Rift valley lakes identified high oil yielding species abundantly distributed naturally. The species were cultured in BBM and BG-11 media to obtain pure clones and lipids (oil) extracted by the Bligh and Dyer method. The peak lipid content ranged from 1.5 – 10.5% of algal biomass with *Chlorella* species showing the highest yields (10.5%), followed by *Euglena* acus (5.88), *Nitzschia* (3.68%), *Ankistrodesmus falcatus* (1.58%) and *Scenedesmus acuminatus* (1.56 %). The algae oil extracted from *Chlorella* spp contained significantly higher polyunsaturated long chain fatty acids with docosatetraenoate (C22:4) and octadecatetraenoate (C18:4) to be the major components. On the other hand, algae oil from *Euglena* spp exhibited higher mono-unsaturated long chain fatty acids with erucate (C22:1) and eicosenoate (18:1) being the major components. This implies that algae oil from *Euglena* spp has a much higher degree of oxidative stability compared to *Chlorella* spp and has can be cultivated for biofuel.

Keywords: Microalgae, algae oil, biofuel, in Kenya

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. In the United States for example, biodiesel is produced currently from soybeans, canola

oil, animal fat, palm oil, corn oil, waste cooking oil and jatropha oil [1, 2, 3]. However, this is likely to change as several companies are attempting to commercialize biofuels from microalgae due to the food-fuel conflict of using food crops for biofuel production.

Both macro algae (commonly known as seaweed), and microalgae (also referred as phytoplankton), are much more diverse than other marine and freshwater plants and a host of species colonize the East African aquatic systems [4]. One of the key functions of microalgae is that they act as efficient converters of solar energy because of their simple cellular structure and are a vital part of many food webs. Incidentally, because the cells grow in aqueous suspension, they have more efficient access to water, CO_2 and other nutrients. For instance, one acre of algae absorbs 2 million tons of CO_2 per year. Consequently, algae are capable of producing 30 times the amount of oil per unit area of land, compared to terrestrial oilseed crops [5, 6].

Recent studies have shown that the microalgae generally produce more of the right kinds of natural oils for bio-fuel than macroalgae [7]. In addition, microalgae are completely renewable, and are therefore a sustainable source of biofuel. This renewed interest in algae as a source of fuel (also referred as oilgae), has unfortunately, not been appreciated in our region. Given the call for improved fuel production, and knowing that microalgae are currently the prime target for such enterprise, there is a need to identify the microalgae species with high bio-fuel content in our aquatic environments. In this study, we characterized high oil yielding microalgae present in Kenyan aquatic environment and examined their potential for bio-fuel production.

MATERIALS AND METHODS

Materials

The solvents and chemicals used in this study were of analytical grade and obtained from Genscript (Piscataway, NJ, USA). The culture media was prepared by chemicals from Sigma (MO, USA). Silica gel 60 TLC plates were obtained from Merck (Darmstadt, Germany). Qualmix fish S standards were kindly donated by Dr. Betty Mbatia from Kenya Polytechnic University College.

Sample collection and analysis

Water samples for microalgae (phytoplankton) analysis were collected at subsurface using a 2 micron phytoplankton net and transported to the laboratory for analysis. Species identification, composition and abundance was quantified under an inverted microscope at 400x magnification (Zeiss Axioinvert 35) using standardard keys for East African plankton [8, 9].

Microalgae culture and lipid extraction

Dominant algae species were isolated and inoculated in 50 ml Bold basal media or BG-11 media in 75cm^2 vented culture flasks and maintained under 12h:12h light: dark photoperiod at 27 ^oC. Algal biomass was monitored at 680 nm using DU 640B spectrophotometer (Beckman, CA, USA) for exponential growth. After a period of two weeks, the algal cells were harvested by centrifugation (7000 rpm, 10min) and screened for lipid/oil content by a procedure adapted from [10] and modified by [11]. Briefly, 200 ml of the algal culture was centrifuged (7,000 rpm, 10 min, 27^oC) to form an algae pellet. Lipids were extracted by adding 50ml of chloroform/methanol (2:1), homogenized briefly using a Virtis homogenizer (Gardiner, USA) and the extract placed in an orbital shaker overnight at room temperature. The mixture was then filtered through glass wool and the filtrate transferred into separating funnel. Resultant residue was recovered and lipids re-extracted with 50ml of chloroform/methanol (2:1). The combined filtrate was washed by addition

of 0.2 volumes of 0.5% sodium chloride, shaken well and left to stand for 1 hour for phase separation. The lower phase (chloroform phase) containing lipids, was recovered and placed in a dry, pre-weighed volumetric flask. Chloroform was evaporated in an oven at 55^{o}C and weight of the lipids obtained by subtracting the weight of the empty flask from the weight of flask with lipids.

The percentage lipid content was determined as;

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

Thin layer Chromatography

To determine the lipid composition of the algae, a thin layer chromatography was carried out. Briefly, lipid samples were diluted in extraction solvent (chloroform: methanol- 2:1) and separated on silica gel 60 TLC plates using the mobile phase Hexane/Diethyl ether/Acetic acid (50:50:1). The lipids and fatty acids were visualized with iodine and the bands identified using response factor (RF) values.

Determination of fatty acid profile

In order to determine fatty acid profile of the extracted oil, a sample was transesterified into fatty acid methyl esters (FAME) as described by [12]. Briefly, 20 mg of lipid sample was mixed with 2ml of toluene, followed by addition 2ml of 1.5% of sulphuric acid in dry methanol. After mixing well, the mixture was incubated at 55^{o}C overnight. 4 ml of saturated NaCl solution was added, vortexed and 2 ml of hexane (HPLC grade) added followed by 3ml of sodium hydrogen carbonate (2% $NaHCO_3$). The mixture was vortexed and 180 µl of the upper phase taken for gas chromatography analysis. Prior to GC analysis, a thin layer chromatography was run to confirm that derivatization had occurred. An internal standard (C 13:0) was added before running the sample on GC machine to have a final concentration of 0.5 mM.

Gas chromatography analysis

Fatty acid methyl esters (FAME) were separated and quantified using gas chromatography (Varian chrompack CP 3800 GC) system equipped with a flame ionization detector (FID). SupelcowaxTM 10 fused silica capillary column (60 m x 0.32 mm x 0.25 µm film thickness; Supelco, Bellefonte, PA, USA) was used to separate FAME. The carrier gas was helium at 550kpa. The temperature programme for separation was as follows: initial column oven temperature of 35^{o}C was held for 3 min, increased to 240 at 10^{o}C/min and held for 35 min. The detector temperature was kept constant at 300^{o}C.

The instrument was calibrated with a one-point calibration method, using a standard mixture of fish oil FAME of known proportions (Qualmix fish S). The response factors of the different methyl esters were obtained from analysis of the standard mixture and were used to calculate the relative amounts of different fatty acids in a sample based on mol %. These data were compared with the internal standard (methyl tridecanoate) to determine the absolute amount (µmol) of the fatty acids in the sample. GC data provided are based on duplicate measurements.

RESULTS AND DISCUSSION

The survey identified five major wild microalgae species with high lipid content abundantly distributed in the Kenyan fresh and saline-alkaline lakes. Laboratory monocultures of *Scenedesmus spp*, *Chlorella spp, Ankistrodesmus spp, Euglena acus*, and *Nitzschia spp* were

successfully grown in BG-11 medium under illumination of 40 watts full spectrum fluorescent bulbs (100-1300 lux) operated on a 12:12h dark:light cycles. The peak lipid content ranged from 1.5 – 10.5% of algal biomass (Fig. 1). *Chlorella* species showed the highest oil yields (10.5%) followed by *Euglena acus* (5.78%), *Nitzschia* (3.68%), *Ankistrodesmus falcatus* (1.58%) and *Scenedesmus acuminatus* (1.58%). Previous study done on *Chlorella protothecoides* spp under nitrogen limitation showed a 46.1% lipid content [13]. However, [14] observed that nitrogen deprivation did not have a great impact on fresh water microalgae species like *Chlorella* and *Scenedesmus* spp which had lipid content of 19.6% and 21.1%, respectively. Similar results have been observed from algae grown in municipal waste waters in California, USA [15]. In comparison, total lipid content of pure *Scenedesmus* and *Chlorella* cultures have been reported to range from 12-45% [16]. Nonetheless, much higher algal lipid productivities were envisioned than observed in this study.

When the lipid extracts were separated by thin layer chromatography, at least four bands appeared (Fig. 2). The Retardation factors (Rfs) of the bands indicated the presence of triacylglycerols, free fatty acids, diacylglycerol and monoacylglycerol, when compared with the standard Rfs values of neutral lipids.

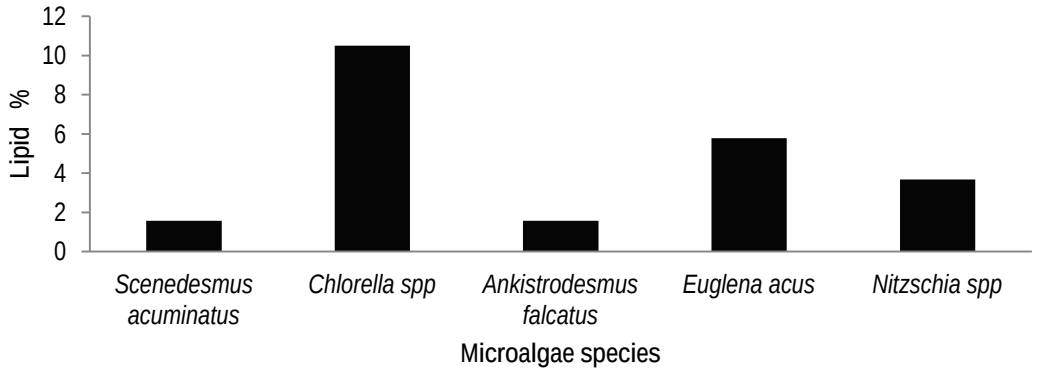

Fig. 1: Percentage of lipid productivities of five selected microalgae species

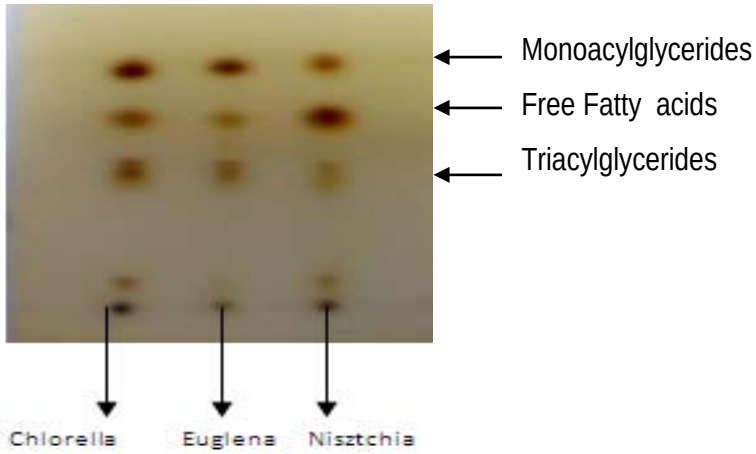

Fig. 2: Thin layer chromatogram showing triacylglycerols, free fatty acids, diacylglycerol and monoacylglycerol present in *Nitszshia*, *Chlorella* and *Euglena* species

Typical process of production of biofuels [called trans-esterification (alcoholysis)] produces esters of fatty acids and glycerol. In the present study, trans-esterification of algae oil yielded significantly high long carbon-chain fatty acids (Table 1). The major components in *Chlorella* spp were docosatetraenoate (C22:4)(26.2%) and octadecatetraenoate (C18:4) (18.9%), in *Euglena* spp, were erucate (C22:1)(37.1%) and eicosenuate (C20:1)(18.6%), while *Ankistrodesmus* spp had docosatetraenoate (C22:4)(19.6%) and erucate (C22:1)(17.8%) (Fig. 3, 4, 5).

Table 1: Fatty acid composition (in µmoles) of oil recovered from *Chlorella, Euglena* and *Ankistrodesmus* microalgae species

	Fatty acid	*Chlorella* spp	*Euglena* spp	*Ankistrodesmus* spp
Tetradecanoate	C14:0	0.195	0.422	0.778
Pentadecanoate	C 15:0	3.572	4.144	1.13
Hexadecanoate	C 16:0	0.131	0.176	0.134
Palmitoloate	C 16:1	0.044	0.103	0.181
Stearate	C 18:0	3.155	3.151	0.701
Oleate	C 18:1	0.089	0.133	0.142
Linoleate	C 18:2	1.289	1.21	1.561
Linolenate	C 18:3	0.871	1.283	2.047
Octadecatetraenoate	C 18:4	17.465	0	11.193
Eicosenoate	C 20:1	7.892	13.515	10.118
Arachdidonate	C 20:4	6.359	2.69	2.805
Eicosapentaenoate	C 20:5	3.816	4.408	5.504
Erucate	C 22:1	11.601	27.01	11.828
Docosatetraenoate	C 22:4	24.222	10.168	13.033
Docosapentaenoate	C 22:5	3.992	2.788	3.607
Dososahexaenoate	C 22:6	7.825	1.533	1.738

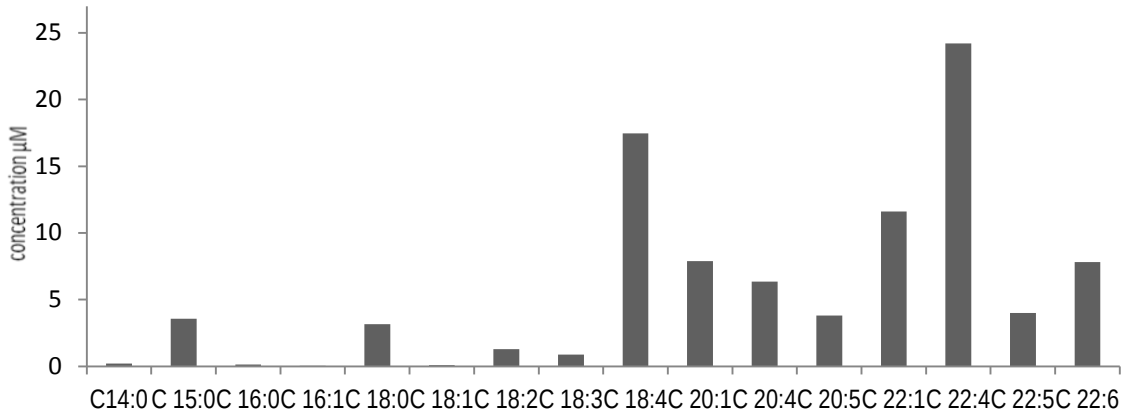

Fig. 3: Profiles showing the different fatty acids methyl esters (FAME) present in algae oil extracted from *Chlorella* species and analyzed by gas chromatography

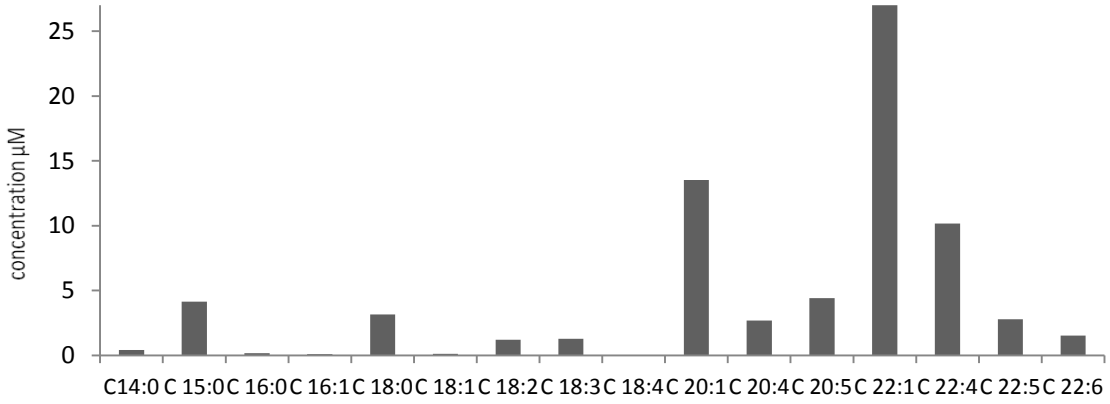

Fig. 4: Profiles showing the different fatty acids methyl esters (FAME) present in algae oil extracted from *Euglena* species and analyzed by gas chromatography

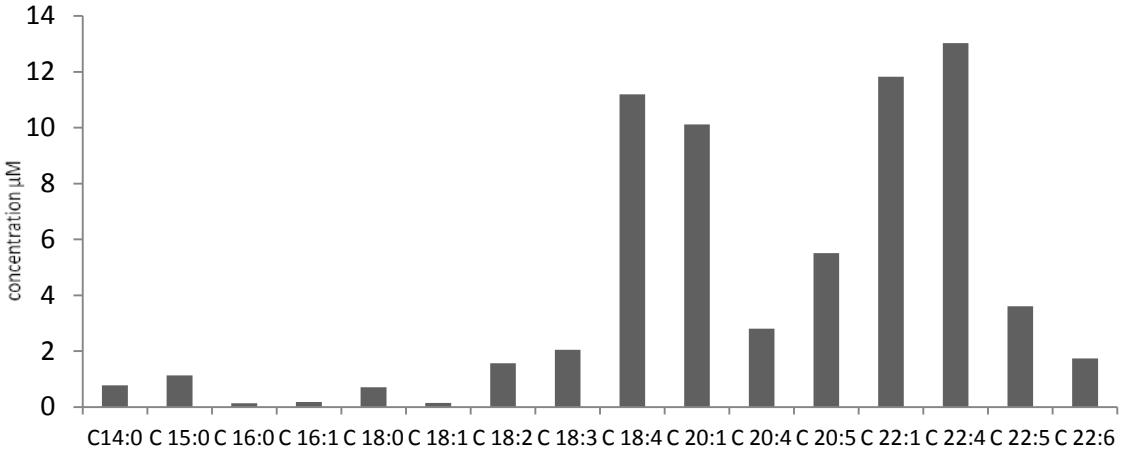

Fig. 5: Profiles showing the different fatty acids methyl esters (FAME) present in algae oil extracted from *Ankistrodesmus* species and analyzed by gas chromatography

These results show that algae oil from *Euglena* spp contains significantly fewer poly-unsaturated fatty acids compared to *Chlorella* and *Ankistrodesmus* spp. Generally, the properties of the triglyceride and biodiesel fuel are determined by the amounts of each fatty acid that are present [17]. Biodiesel with a larger weight fraction of polyunsaturated fatty acids with more than three double bonds appears to exhibit inferior oxidative and thermal stability. Hence, it can be concluded that the biodiesel produced from *Euglena* spp in this study has greater oxidative and thermal stability, as it contains significantly fewer poly-unsaturated fatty acids.

CONCLUSIONS

This research has provided a proof- of-concept that microalgae present in the Kenyan aquatic environment can be a potential source of bio-fuel. Lipid content ranged from 1.5- 10.5%. While this lipid productivity is lower than that of terrestrial oil plants, higher productivity is a goal of continued research. The oil can successfully be converted to biodiesel using a single step, acid catalyzed trans-esterification method. In addition, the biofuel produced from *Euglena* oil was found to have more favourable fuel properties. Overall, regional cultivation of the microalgae and

processing into bio-fuels can provide economic benefits to the Kenyan rural communities and save a large proportion of foreign exchange used on importing fossil fuels.

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