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Evaluation of biodiesel production from microalgae collected from fresh water habitat

Sudip Shah[1]*, Prakash Lokesh[2]

[1]Department of Biochemistry, Padmashree Institute of Management and Sciences, Bangaluru-60, India

[2]Department of Biochemistry, Oxford College of Science, Bangaluru– 560102, India

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Abstract

Background: Algae are the fastest growing in the world. About 50% of their weight is oil. This lipid can be used to make biodiesel for cars, trucks and airplanes. Algae will someday be competitive as a source of biofuel. Continuous use of petroleum sourced fuels is now widely recognized as unsustainable because of depleting supplies and the contribution of these fuels to the accumulation of carbondioxide. **Methodology**: In this study, we tried to evaluate the physico-chemical properties of algal oil. A naturally occurring algal sample was collected from Kommaghatta lake, Bangalore. Algae were identified as Spirogyra sps. Oil was extracted from the dried algal samples using chloroform: methanol as a solvent system. Fatty acid analysis was done in Indian Institute of Horticultural Research, Bangalore. Physico-chemical properties of algal oil such as density, lipid content, pH were estimated. **Results:** Gas chromatographic analysis revealed higher percentage of methyl palmitate, methyl oleate, methyl linoleate. The physico-chemical properties of algal oil meet the properties of the standard fuel. **Conclusion:** It is concluded that the algal oil can be used as a potential biofuel.

***Keywords**:* MMP; Physio-chemical analysis; MSA; SOPMA; I-Mutant; Polyphen

1. Introduction

The need to reduce the dependency on the fossil fuel as well as to tackle the global warming has led to stringent study for the sustainable alternative fuel. The environmental and economic sustainability can be achieved when fuel production process are not only renewable but also able to sequester the atmospheric carbondioxide (CO_2). Taken this under consideration, biofuels can be the unquestionable in order to mitigate the gaseous emission (eg, CO_2, N_2, SO_X). Biodiesel is non toxic and biodegradable alternative fuel that is obtained from renewable sources[1]. At present, the main source for the biodiesel is the consumable oleaginous crops. In contrast, producing biodiesel from microalgae can be the suitable source for the next generation biofuels and also appear to represent the only current renewable source of oil that could meet the global demand[2-4].

Biodiesel has attracted attention from the past few years as a renewable and environmental friendly fuel because of the diminished petroleum reserve and the deleterious environmental consequences of exhaust gases from petroleum diesel. Biodiesel (fatty acid alkyl esters) is an alternative diesel fuel derived from the transesterification of vegetable oils or lipids or alcohol with or without the presence of a catalyst[5,6]. Oleaginous crops (eg. rapeseed, soybean, sunflower and palm), has been taken as the main crops for the source of biodiesel[7-9]. But biodiesel production from crops can lead to severe consequences for the global food supply. Also an increase in price of food-grade oils causing the cost of biodiesel to increase and preventing its usage even if it has advantages comparing with diesel fuel.

But recent research has clearly proven that oil production from algae is clearly superior to that of terrestrial plants and has the potential to completely displace fossil fuel[10,11]. Algae are simple aquatic organisms that

*Corresponding author
Full Address :
Department of Biochemistry, Padmashree Institute of Management and Sciences, Bangaluru-60, India
Phone no. +447448552265
E-mail: ss_ss42@hotmail.com

photosynthesize. It can utilize salt and waste water streams, thereby greatly reducing freshwater use[12]. Important advantages of microalgae are, unlike other oil crops, they can double their biomass within 24hr. Their production is not seasonable and they can be harvested daily. They are capable of synthesizing more oil per acre than the terrestrial plants (**Table I**) which are currently used for the fabrication of biofuels[11,13]. The per unit area yield of oil from algae is estimated to be from 20,000 to 80,000 L per acre, per year, this is 7-31 times greater than the next best crop palm oil[14].

A key consideration is the choice of algal strain. Much current research work is focused on small number of fast -growing microalgae species which have been found to accumulate substantial quantities of lipids. Within the green algae, typical species include *Chlamydomonas reinharditii, Dunaliella salina* and various *Chlorella species*, as well as slow growing can contain over 60 wt% lipid, much of which is secreted into the cell wall. Other important algal groups include the diatoms *Phaeodactylum tricornutum and Thalassiosira pseudonana*[15]. Microalgae have convenient fatty acid profile allowing biodiesel production with high oxidation stability. The physico-chemical properties of biodiesel from microalgal oil in general (density, pH, viscosity, acid value, heating value, etc) are comparable to those of fuel biodiesel[16].

This work aimed to investigate, estimate the algal oil content and their properties to use it as biofuel.

2. Materials and methods

2.1 Algal Sample

Algae (*Spirogyra sps*) was collected in bulk from the Kommaghatta Lake, Bangalore, a fresh water pond. Algal biomass was handpicked and immediately after collection; brought to the laboratory, air dried for two days. Later on dried at 40°C in a hot air oven for two days till the dry weight was constant.

Table 1. Comparison of some sources of biodiesel[11]

Crops	Oil yield (Lha^{-1})
Soybean	446
Canola	1,190
Jatropha	1,892
Palm	5,590
Microalgae	136,900

2.2 Oil extraction[17]

Oil extraction from microalgal biomass was performed in a Soxhlet apparatus using Chloroform: methanol (2:1) solvent system after cell disruption by sonication for 20min. After extraction, the contents were cooled and filtered to separate the biomass and washed the biomass with 25ml of chloroform twice to extract the residual lipids present in the biomass. The extracts were pooled and taken in separating funnel and washed with 1% aqueous sodium chloride solution twice. The solvent layer was passed through anhydrous sodium sulphate (sodium sulphate was taken in a glass funnel with cotton plug) and removed the solvent using rota-evaporator under vacuum to get the algal oil. The weight of algal oil was taken to determine the oil content in biomass.

The physico-chemical parameters of oil such as pH, density were analyzed.

2.3 Oil characterization [18,19]

2.3.1 Transesterification of Algal Oils

The extracted lipid was methylated by dissolving in methanol and heating for 10 min at 70°C, followed by combining with 14% borum trifluoride (BF_3) in methanol reagent and heating for 30 min at 70°C by attaching air condenser[19]. Methyl esters of lipids were subsequently extracted in heptane. Heptane extract was subsequently dried on anhydrous sodium sulphate (Na_2SO_4) and filter through 0.2µm nylon filter prior to inject in gas chromatography (GC) and GC-MS/MS.

2.3.2 Gas Chromatographic Analysis

The fatty acid composition of algal fatty acid methyl esters (FAME) were quantified by gaseous chromatography using a Varian gas chromatograph (model 3800) equipped with FID by using a 30 m × 0.25 mm with 0.25µm film thickness fused silica capillary column (VF-5 Factor Four, Lake Forest, CA, USA). The temperature programmes for the column was, it ran isothermally at 100°C for 4 minute then allowed to reach to 220°C at the rate of 3°C per minute then hold for 4 minute followed by at the rate of 5°C per minute to 260°C and hold for 10 minutes. Injector and detector temperatures were maintained at 250°C and 260°C respectively. Helium was used as the carrier gas at a flow rate of 1 ml/min. Flow rates were 20 ml/min for the H_2 and 250 ml/min for breathing air at FID detector.

Chromatograms were recorded in the data station by using Varian WS soft ware. Fatty acids were identified by comparing the relative retention times of FAME peaks from samples with those of standards (reference standard received from Sigma-Aldrich, USA) and also comparing the spectra available with two spectral libraries using Wiley and NIST-2007. The total FAME was estimated by the sum of all GC-FID peak areas in the chromatogram and individual compounds was quantified by comparing the known individual FAME procured as standard. All the data were analyzed by using three individual run.

2.4 Statistical Analysis

All the data of cases and controls were entered into Microsoft Excel (Microsoft office 2007) and Statistical Package for Social Service (SPSS for Window version; SPSS 17.0, Inc., Chicago, IL). Data were expressed as Mean ± SD.

3. Results

The results of extraction methods, from previous study, indicated that the best procedure is soxhlet[20,21]. As in comparison our study revealed the use of soxhlet for better oil extraction preceded by sonication. Also, for microalgal disruption, the ultrasonic method is more efficient than vortex and homogenizer[22].

Results in **table II** showed the lipid amounts extracted from the algal species by the extraction methods described in the experimental section. Biodiesel production (FAME) was found in the sample. Density of algal oil (**Table III**) matches the density ranges of a biofuels given by EN 14214 (European Standard EN 14214., 2008)[23] and ISO 15607 (0.86-0.90g/cm^3).

Fatty acid profile was determined for the microalgae and the results are presented in **table IV**. All microalgae lipids are mainly composed of unsaturated fatty acids and a significant percentage of palmitic acid (C16:0) was also present. Among the unsaturated fatty acids special attention should be given to the linolenic (C18:3) and polyunsaturated (≥4 double bonds) contents, due to the EN 14214 (2004) that specifies a limit of 12 and 1% respectively for a quality biodiesel[24]. It can be seen from table the oils of our sample revealed the presence of linolenic acid within specifications and polyunsaturated (≥4 double bonds) fatty acids in the sample was absent.

Heat of combustion and melting point increases with the increase in number of carbon atoms and decreases with an increase in an unsaturation. Higher levels of polyunsaturated fats lower the cold filter plugging point (CFPP); the temperature at which the fuel starts to form crystals/solidifies and blocks the fuel filters of an engine. It can be seen that the more unsaturated oil is, lower the melting point. Therefore colder climates require a higher unsaturated lipid content to enable the fuel to perform at low temperatures. So the analyzed microalgae oils reveals with the above specifications and it may be used with other oils, or without restrictions as raw material for other biofuels production process.

Hence, lipid quality is an important issue for biodiesel production, as the alkyl ester content dictate the stability and performance of the fuel, and this in the end is an important factor in meeting international fuel standards. Many of the specifications directly depend upon the fatty acid composition of the biofuel. The properties like cetane number, kinetic viscosity, oxidative stability and

Table II Total lipid, biodiesel, biomass of *Spirogyra species* using the extraction solvent system chloroform/ methanol (2:1v/v)

Type	Fresh weight	Dry weight	Extracted oil	Biomass
Spirogyra sps	25 g	13g (52%)	3.1g (12.4%)	4.2g (16.8%)

Table III Physical properties of the algal biodiesel

Type	Density (g/cm^3)	pH
Sample	0.884	7.3

Table IV Fatty acids profile of tested algal oil

Fatty Acids	Carbon Length	Average (µg/mg)	Std. deviation
Lauric	C12:0	0.217	0.019706
Myristic	C14:0	4.158	0.396076
Pentadecanoic	C15:0	0.385	0.042536
Palmitic	C16:0	4.097	15.5998
Palmitoleic	C16:1	160.467	0.519096
Heptadecanoic	C17:0	3.904	0.706406
Stearic	C18:0	57.348	1.88236
Oleic	C18:1	28.698	22.13859
Linoleic	C18:2	158.242	9.117076
Linolenic	C18:3	12.371	8.597298
Nonadecanoic	C19:0	0.131	0.035152
Arachidic	C20:0	0.789	0.260341
Eicosenoic	C20:1	1.123	0.202312
Heneicosenoic	C21:0	0.121	0.026161
Behenic	C22:0	0.090	1.088823
Erucic	C22:1	0.050	0.010773
Docosadienoic	C22:2	4.008	0.040834
Trieicosenoic	C23:0	0.361	0.086777
Tetraeicosenoic	C24:0	2.698	1.105343
Hexaeicosenoic	C26:0	0.140	0.083077
Saturated		190.117	21.02142
Unsaturated		249.305	36.6749

cold flow in form of the cloud point or cold filter plugging point directly depend upon the fatty acid composition of the biofuel.

Ignition quality of a fuel is improved with increase in cetane number and cetane number of a fatty acid increases with the increase in chain length. Cetane number is another measure describing the combustion quality of diesel fuel during compression ignition. In a particular diesel engine, higher cetane fuels have shorter ignition delay periods than lower cetane fuels. Therefore it is important to ensure that the cetane number of biodiesel also meets the engine cetane rating[25].

Finally, to reduce microalgal biomass overall production costs, the biomass cake remaining after oil has been extracted can be used as fertilizer or feed, can undergo anaerobic fermentation to obtain biogas and/or a pyrolysis process, or to extract high value chemical compounds. Similar biorefinery concept is been reported by Danielo[26]. Rana and Spada also, suggest that to make plants accessible to small procedures, such as agricultural farms, in near future, could integrate this concept in order to obtain biofuels, electricity and feed for livestock[27].

3. Conclusion

Algal biofuel can be the potential alternative renewable energy source. The work presented in this report leads to a deeper understanding of the properties of algal biomass and algal oil. With further intensive research, it can be a potential fuel.

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Conflict of interest

The author's declares none.

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