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ORIGINAL ARTICLE

Physico-Chemical Characterization of Algal oil: a Potential Biofuel

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ABSTRACT

A total of six naturally occurring algal biomass bulk samples were collected from different localities of north India. Algae were identified one blue-green alga Tolypothryx and rest five were green algae Pithophora, Spirogyra, Hydrodictyon, Rhizoclonium and Cladophora. Oil was extracted from the dried algal samples and fatty acid analysis was done. Physico-chemical properties of algal oils such as density, viscosity, lipid content, pH and non-saponifiable fats were estimated. Gas chromatographic analysis revealed higher percentage of methyl palmitate, methyl stearate, methyloleate and methyl linoleate. The physico-chemical properties of algal oil meet all the properties given by American society for testing and materials (ASTM) D6751, ISO 15607and EN14214- Europe. It is concluded that the algal oil can be used as a potential biofuel.

Key words: *Algae, biofuel, fatty acid, transesterification, gas chromatography.*

INTRODUCTION

The need of energy is increasing constantly, because of increase in industrialization and population explosion. The basic sources of energy are fossil fuels (petroleum, coal and natural gas), hydro and nuclear [1] however, fossil sources are limited and will be exhausted by near future [2]. Biodiesel is a biofuel consisting of monoalkyl esters that are derived from organic oils, plant or animal, through the process of transesterification [3]. It is also biodegradable, nontoxic and has low emission profile as compared to petroleum diesel [4].Shay (1993) reported that algae are one of the best sources of biodiesel. In fact algae are the highest yielding feedstock for biodiesel. It can produce 250 times more than the amount of oil per acre as soybeans. In fact biodiesel from algae may be the only way to produce enough automobile fuels to replace current gasoline usage. Algae produce 7 to 31 time greater oil than palm oil [5]. The best algae for biodiesel would be microalgae [6]. Microalgae have much more oil than macroalgae and it is much faster and easier to grow and harvest [7].

The use of microalgae can be a suitable alternative because algae are the most efficient biological producer of oil on the planet and a versatile biomass source and may soon be one of the Earth's most important renewable fuel crops [8]. Higher photosynthetic efficiency, higher biomass production, a faster growth rate than higher plants, highest CO_2 fixation and O_2 production, growing in liquid medium which can be handled easily make the algae to stand high in front of other oil seed crops. Their production is not seasonal and can be harvested throughout the year [9, 10]. As a matter of fact, average oil yield from microalgae can be 10 to 20 times higher than the yield obtained from oleaginous seeds and/or vegetable oils (Table 1) [9, 11]. Different types of biofuels can be derived from microalgae. These include methane produced by anaerobic digestion of algal biomass [12], biodiesel derived from microalgal oil [13-15] and photo-biologically produced bio-hydrogen [16, 17] etc.

Table-1 Comparison of some sources of biodiesel [9]

Crop	Oil yield(L ha^{-1})
Soybean	446
Canola	1,190
Jatropha	1,892
Palm	5,950
Microalgae	136,900

Algae has a great ability to fix CO_2, thus it is an interesting method for the removal of gases emitted from power plants and can be used to reduce greenhouse gases with higher production of microalgal biomass and consequently higher biodiesel yield [18,19]. Few microalgae have convenient fatty acid profile and unsaponifiable fraction allowing biodiesel production with high oxidation stability [20-23]. The physical and fuel properties of biodiesel from microalgal oil in general (e.g. density, viscosity, acid value, heating value etc.) are comparable to those of fuel diesel [24-26]. Therefore, the present investigation was carried out to test the algal oil content and their properties to use it as biofuel.

MATERIALS AND METHODS

Algal Samples

A total of six fresh water algae samples were collected in bulk from different localities of north India. Algal biomass was handpicked from fresh water bodies and immediately after collection; samples were brought to the laboratory, air dried for two days later on dried at 40^0C in an oven for 2-3 days till the dry weight was constant. A part of fresh algae samples were observed for identification and preserved in 4% formaldehyde and deposited at Phycology Laboratory of National Botanical Research Institute, Lucknow.

The algal samples collected and analyzed were identified as *Tolypothrix*- a blue green alga and rest were green algae *Pithophora, Spirogyra, Hydrodictyon, Rhizoclonium* and *Cladophora*. Similar dry weights (100g) of all samples were taken for further experiments and analysis.

Oil Extraction

Dried algal biomass (5g) was taken in solvent mixture (100 ml) of chloroform and methanol (2:1, vol./vol.) and the content were refluxed for 4 hrs. After the extraction, the contents were cooled and filtered (or centrifuged) to separate the biomass and washed the biomass with 25 ml of chloroform twice to extract the residual lipids present in the biomass. The extracts were pooled and taken in a separating funnel and washed with 1% aqueous sodium chloride solution (50 ml) 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. If the biomass is available in smaller quantities, the content may be reduced accordingly.

The physico-chemical parameters of oil such as pH, viscosity, density were analyzed by standard methods of analysis (AOAC, 1995) and algal oil characters were compared with biofuel standards contained in ISO15607and EN14214.

Isolation of Unsaponifiables

2g algal oil dissolved in 200 ml of ethanol potassium hydroxide (2M) was refluxed for 1h. The reaction mixture was diluted to 200 ml with distilled water and transferred to a separating funnel. The unsaponifiables were extracted three times with 100 ml of dietheylether. The ether extracts were first washed with 100 ml of aqueous solution of potassium hydroxide (0.5 M) to remove any residual free fatty acids. Further washing and cleaning was carried out five times with 100ml distilled water, and the ether layer removed in a rotary evaporator. The value was expressed in weight percent (w/w).

Transesterification of Algal Oils

The algae oil (400 mg) and 15ml methanolic sulphuric acid [2% sulphuric acid dissolved in methanol (wt/vol)] were

taken into round bottom flask and refluxed for 4 hrs. The reaction was monitored by thin layer chromatography (TLC) with the solvent system, Hexane and Ethyl acetate at the ratio of 9:1. The reaction was continued till the oil spot was disappeared on TLC plate. After the completion of reaction (2-4 hr), the contents were transferred to separating funnel and 25ml water was added to it. The aqueous layer was extracted twice with ethyl acetate (25 ml each) and pooled the ethyl acetate layer. The extract was dried over anhydrous Na_2SO_4 and concentrated under vacuum. The dried fatty acid methyl esters (FAME) were analysed using gas chromatography.

Gas Chromatographic Analysis

The fatty acid composition of algal fatty acid methyl esters were determined by gas chromatographic analysis using agilent 6890 gas chromatograph unit equipped with FID at IICT at Hyderabad. A fused silica capillary column DB-225 (30 x 25 mm I.d. x 0.25 µm, film thickness) was used for the analysis of FAME. The oven temperature is programmed at 160^0C for 2 min. The flow rate of career gas (N_2) was 1.5 ml/min. The injector and detector temperature were maintained at 220^0C and 255^0C respectively. The area percentages are recorded with a standard HP chemstation data system.

RESULTS AND DISCUSSION

The oil extraction was done by several methods and using different solvents, but the results showed that the best procedure is soxhlet with solvent mixture of chloroform and methanol (2:1, vol/vol) and for microalgal cell disruption the ultrasonic method is best. Here the results are shown only with soxhlet extraction method using above solvent. (Table-2).

Table-2 Algal oil percentage and physico-chemical properties.

Samples	Oil percentage (w/w)	pH	Density g/cm^3	Viscosity at 40^{o}C(mm^2/sec)	Non-saponifiable fat (%)
Tolypothrix	12.78	7	0.857	4.1	0.137
Pithophora	10.37	7	0.873	4.2	0.181
Spirogyra	14.82	7	0.884	4.4	0.232
Hydrodictyon	13.58	6	0.868	3.9	0.231
Rhizoclonium	11.64	7	0.889	4.3	0.237
Cladophora	11.76	6	0.892	3.8	0.244

The result showed 10-15% amount of oil in all the algal samples. Green alga *Spirogyra* showed the maximum amount of oil 14.82%, where as *Pithophora* revealed 10.37% oil. Density of all algal oils matches the density ranges of a biofuels given by EN 14214 and ISO 15607 (0.86-0.90g/cm^3), only the *Tolypothrix* oil sample showed a little lower value that is 0.857. The viscosity range given by EN 14214 and ISO 15607 is 3.5-5.0 mm^2/s, thus our results are compatible with these standards. Fatty acid profile for all microalgae is presented in table-3. From the results it was clear that the main components of oil obtained from our samples were palmitic acid (16:0), oleic acid (18:1), linoleic acid (18:2), palmitoliec acid (16:1), and myristic acid (14:0). All the microalgal lipids were mainly composed of 50-60% unsaturated fatty acids. A significant percentage of palmitic acid (C16:0) was 25-35% and that of oleic acid (C18:1) was 18-35%. ISO 15607and EN14214 specifies a limit of linolenic acids (C18:3) andpolyunsaturated ($\geqslant$4 double bonds) fatty acid contents to be 1% respectively, for a quality biodiese.

Table-3 Fatty acid profile of tested algal oils.

Fatty acid	*Tolypothrix*	*Pithophora*	*Spirogyra*	*Hydrodictyon*	*Rhizoclonium*	*Cladophora*
12:0	0.7	1.3	1.1	0.9	1.4	1.2
13:0	0.2	0.4	0.3	0.1	0.3	0.7
14:0	5.8	5.5	6.4	7.0	6.2	9.7
14:1	1.9	0.9	1.1	0.3	2.1	1.2
15:0	-	0.8	0.6	0.4	0.6	-
16:0	31.8	29.7	25.2	26.9	30.4	27.5
16:1	4.7	6.2	5.4	3.8	4.8	5.4
16:2	2.4	3.6	3.8	2.8	1.6	2.3
17:0	2.8	0.4	0.3	0.4	1.7	0.8
18:0	2.7	3.9	4.5	2.6	6.1	4.3
18:1	23.4	28.4	33.3	34.8	18.9	22.9
18:2	8.6	11.2	10.8	9.3	9.7	8.6
18:3	8.4	0.4	0.7	2.5	9.6	6.1
20:0	1.4	1.2	1.3	1.1	1.9	1.6
20:1	0.6	0.9	0.7	1.2	0.4	0.8
20:2	0.4	0.1	-	0.2	-	0.3
20:3	0.6	1.2	0.6	2.1	0.6	0.6
20:4	0.1	-	0.1	-	-	-
20:5	-	-	-	-	-	0.2
21:0	0.6	0.1	-	0.2	-	0.8
22:0	0.9	1.6	1.4	0.9	1.2	1.3
22:1	0.1	-	0.2	0.1		
22:3	0.2	0.3	0.2	0.4	0.6	0.4
22:3	1.4	1.7	1.4	1.5	1.9	3.2
24:1	0.3	0.2	0.6	0.5		0.1

It can be seen from table-3 the oils of all our samples revealed the presence of linolenic acid within the range and polyunsaturated ($\geqslant$4 double bonds) fatty acids in most of the samples were absent. In *Spirogyra* and *Tolypothrix* only arachidonic acid (eicosatetraenoic acid, 20:4) was 0.1% and in *Cladophora,* timnodonic acid (eicosapentaenoic acid, 20:5) was 0.2%. Thus all the analyzed microalgae oils can be used for good quality biodiesel.

Many of the specifications directly depend upon the fatty acid composition of the biofuel. Many of the specifications like cetane number, kinematic viscosity, oxidative stability and cold flow properties 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 Thus methyl palmitate and methyl stearate have good ignition property. Heat of combustion and melting point increases with the increase in number of carbon atoms and decreases with an increase in an unsaturation. Cold flow properties of a fuel such as cloud point, pour point and cold filter plugging point are determined by the amount of higher melting saturated esters or other higher melting minor components regardless of nature of the unsaturated esters..

Unsaturated fatty esters are oxidatively unstable in comparison to the saturated fatty esters because double bonds impart to fatty acids are susceptible to reaction with oxygen. Especially, fatty acid chains with methylene-interrupted double bonds (linoleic and linolenic acids) are susceptible to oxidation. Methyl linoleate is 41 times more susceptible to oxidation in comparison to the methyl oleate [27]. Thus only a very small amount of antioxidant is required to improve the oxidative stability of algal biodiesel.

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