

Evaluation of heterotrophic chlorella protothecoides microalgae as a most suitable good quality biofuel

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Abstract - The depleting conventional fossil fuel resources and increasing environmental distress has considerably increased research curiosity in biofuels for internal combustion engines. Biofuels and bioproducts produced from plant biomass would reduce global warming. The sourcing of feedstocks, including the impact it may have on biodiversity, land use and competition with food crops, algae have recently received a lot of attention as a new biomass source for the production of renewable energy to provide a new range of third generation biofuels. Accordingly, in this research work oil was extracted from Heterotrophic Chlorella Protothecoides Microalgae biomass by solvent extraction method with soxhlet apparatus, using n-hexane as solvent. An oil yield of 52% by weight was observed with this solvent extraction method. Biodiesel was produced from Heterotrophic Chlorella Protothecoides Microalgae oil by alkali catalyzed transesterification with biodiesel yield of 92 % by volume. Physio-chemical characterization of Microalgae biodiesel was carried out and most of the results obtained were comparable with biodiesel properties and ASTM biodiesel standard. FAMES content of the Heterotrophic Chlorella Protothecoides Microalgae biodiesel was analysed by Gas Chromatography. The results from this analysis shows that Heterotrophic Chlorella Protothecoides Microalgae according to its fatty acid content is an ideal biomass source for biodiesel production.

Key words: Algaeoil, Biodiesel, Transesterification, Heterotrophic, FAMES.

1) INTRODUCTION

Microalgae as future biofuel

Algae biomass can play an important role in solving the problem between the production of food and that of biofuels in the near future. Microalgae appear to be one of the most important and promising biofuel source that is capable of meeting the global demand for transport fuels. Microalgae and Macroalgae have emerged as one of the most promising biofuel source.

Microalgae are more suitable as biofuel than the Macroalgae because

- 1) The oil content in the Microalgae are more compared to the Macroalgae
- 2) Microalgae can grow in all environments.
- 2) Their lipid content could be adjusted through changing growth medium composition.
- 3) Salty or wastewater could be used for growing Microalgae.
- 4) Atmospheric carbon dioxide is the carbon source for growth of Microalgae and producing 100 tons of algal biomass fixes roughly 183 tons of carbon dioxide.
- 5) Microalgae can be fertilized with sewage and waste water.

6) Microalgae sugars can be fermented to make ethanol

7) Microalgae are producing feed for fish and livestock from waste biomass.

2 MATERIALS AND METHODS.

2.1 Proper algae species selection

To select proper algal strains for biofuel production, there are several important factors needed. The lipid content is regarded as one of the most significant aspects. Microalgae contains oil levels between 20 to 80 % by dry weight. [Y. Chisti, et al., 2007 and L.Gouveia et.al., 2009]

The species used in this study is the green Microalgae Chlorella that may be grown both autotrophically and heterotrophically. Further, green Microalgae Chlorella is one of the most understood species in research field compared with other various algae strains. Heterotrophic Chlorella Protothecoides Microalgae is selected for the present work because it contains the crude lipid content of 55.2%. [Demirbas et al.2011].

2.2 Algae oil extraction

Dried sample of Heterotrophic Chlorella Protothecoides Microalgae was collected from Soley Institute, Turkey. The solvent extraction method can be applied to any low oil content materials because solvent extraction method recovers higher percentage

of oils. Solvent extraction method with soxhlet apparatus and hexane solvent was employed for oil extraction. Extracted Microalgae oil was taken to determine the oil content in biomass by using the formula

$$\begin{aligned} \% \text{ Oil Content} &= \frac{\text{Mass of Oil Obtained}}{\text{Mass of Dried Algal Biomass}} \times 100 \\ &= (26/50) \times 100 \\ &= 52\% \end{aligned}$$

2.3. Transesterification Process

Alkali-catalyzed transesterification of vegetable oils proceeds faster than the acid catalyzed transesterification [A. Demirbas, 2009]. So, alkali(NaOH)-catalyzed transesterification was used in the present research work with an yield of 92% by volume. For transesterification process the Heterotrophic *Chlorella Protothecoides* Microalgae oil was collected from Soley Institute, Turkey.

2.4 Physio-chemical characterization of Microalgae biodiesel

Microalgae biodiesel produced after transesterification of algae oil was subjected to basic characterization analysis. Major physio-chemical properties of microalgae biodiesel Viscosity, Density, Calorific value, Acid value, Flash point and Cloud point were determined. Fatty acid profile of Microalgae biodiesel was also obtained by Gas Chromatography Method.

Physico-chemical properties of Heterotrophic *Chlorella Protothecoides* Microalgae biodiesel are presented in the table 1

Properties	Heterotrophic <i>Chlorella Protothecoides</i> Microalgae biodiesel	Method/Device for Physio-chemical characterization
Density (kg·L ⁻¹) at 15°C	0.864	Pycnometer
Viscosity (mm ² ·s ⁻¹ , cP at 40°C)	2.84	Redwood viscometer.
Flash Point (°C)	125	Pensky Martin's 'closed' tester.
Solidifying point (°C)	-12	Cloud and Pour Point apparatus
Acid value (mg KOH·g ⁻¹)	0.273	KOH Titration method
Heating value (MJ·kg ⁻¹)	40.833	Adiabatic bomb calorimeter

Table 1: Physico-chemical properties of Heterotrophic *Chlorella Protothecoides* Microalgae biodiesel.

2.5 Determination of Fatty acid profile of Microalgae biodiesel by Gas Chromatography Method.

A systematic analysis of the FAME composition and comparative fuel properties is very important for species selection for biodiesel production. The fatty acid composition present in microalgae biodiesel was determined by Gas Chromatogram (GC). This is generally achieved by open tubular column gas chromatography through methanolic transesterification of the lipidic matrix. This is a well established conventional GC method that produces effective results.

Procedure: The fatty acid profile of Microalgae biodiesel was quantified and qualified using CHEMITO GC 8610 instrument with a flame ionization detector. The column was packed with BPX-70 phase (50% cyanopropyl and 50% methylsiloxane). The injection port was maintained at 250°C and the detector port was maintained at 260°C. The temperature of the oven was initially 160°C and was increased by 7.5°C per minute to a final oven temperature of 240°C. The carrier gas flow rate used here was 0.3 ml min⁻¹ (Nitrogen) and 15 ml min⁻¹ make up gas (Nitrogen). Hydrogen and oxygen were used as flame gas at 35 and 350 ml min⁻¹ respectively. A 1 µL syringe from Hamilton Co. was employed for injection. The Chromatogram obtained after GC analysis of Microalgae FAMES is given in fig 1.

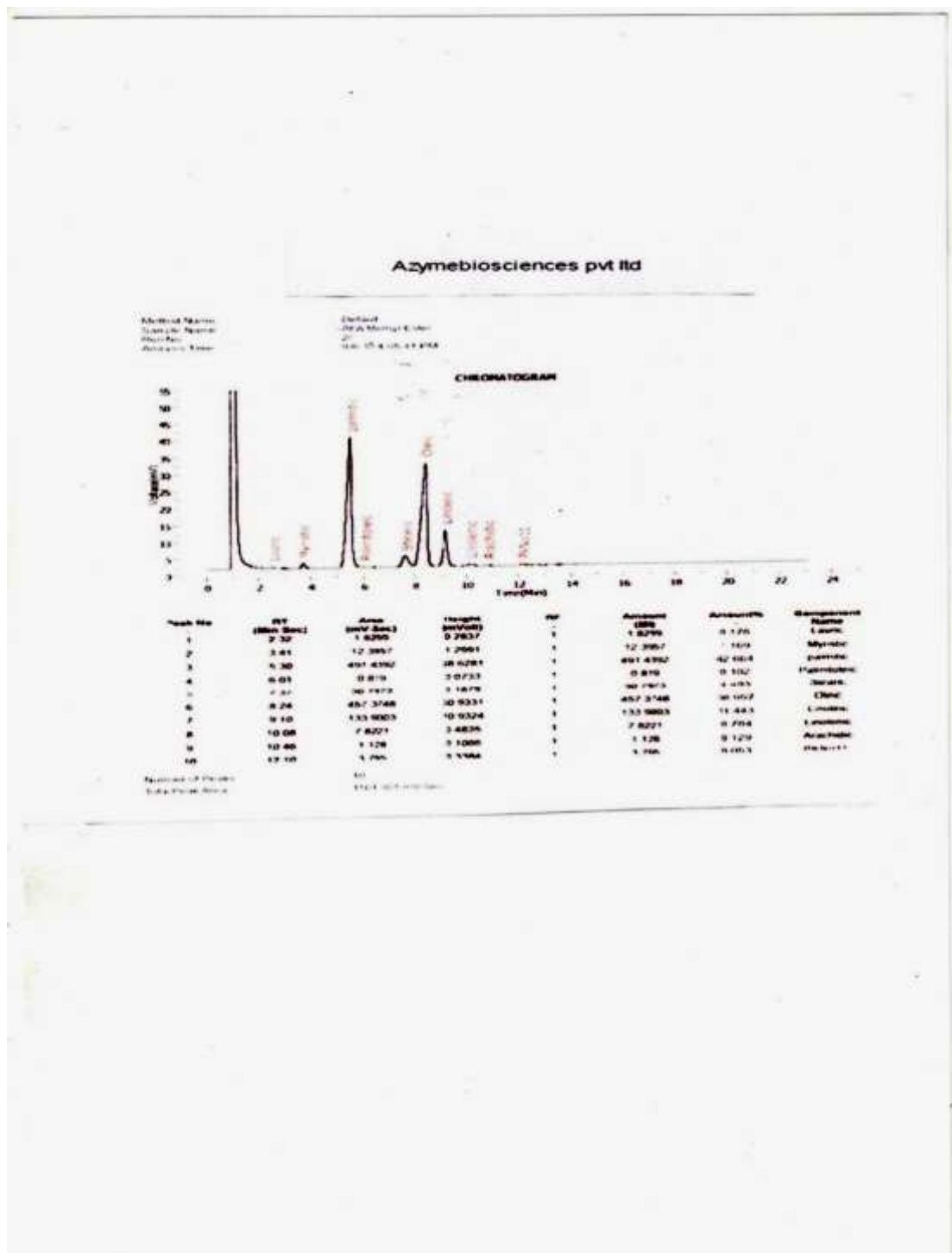


Fig 1): The Chromatogram obtained for the FAMES content of the biodiesel obtained from alkali catalysed transesterification of Heterotrophic Chlorella Protothecoides Microalgae oil.

3. RESULTS AND DISCUSSIONS

3.1 Microalgae oil extraction and its efficiency

The lipid content in the Heterotrophic Chlorella Protothecoides Microalgae biomass was determined by solvent extraction with Soxhlet apparatus using hexane as solvent. And lipid content was found to be 52% by dry weight of biomass.

3.2 Transesterification of Microalgae oil and biodiesel conversion efficiency

The alkali (NaOH) catalysed transesterification of Heterotrophic Chlorella Protothecoides Microalgae oil resulted in 92% by volume of Microalgae biodiesel yield.

3.3 Physio-chemical properties of Microalgae biodiesel

Physico-chemical properties of Heterotrophic Chlorella Protothecoides Microalgae biodiesel are presented in the table 2.

Properties	Biodiesel from Heterotrophic Chlorella Protothecoides Microalgae biodiesel
Density (kg·L ⁻¹) at 15 ⁰ C	0.864

Viscosity ($\text{mm}^2 \cdot \text{s}^{-1}$, cP at 40°C)	3.84
Flash Point ($^\circ \text{C}$)	125
Solidifying point ($^\circ \text{C}$)	-12
Acid value ($\text{mg KOH} \cdot \text{g}^{-1}$)	0.273
Heating value ($\text{MJ} \cdot \text{kg}^{-1}$)	40.833

Table 2: Physico-chemical properties of Heterotrophic *Chlorella Protothecoides* Microalgae biodiesel.

These properties are compared with Diesel fuel and ASTM biodiesel standard and are presented in table 3.

Properties	Biodiesel from Heterotrophic <i>Chlorella Protothecoides</i> Microalgae biodiesel	Diesel fuel	ASTM biodiesel standard
Density ($\text{kg} \cdot \text{L}^{-1}$) at 15°C	0.864	0.838	0.84 – 0.90
Viscosity ($\text{mm}^2 \cdot \text{s}^{-1}$, cP at 40°C)	2.84	1.9 – 4.1	3.5 – 5.0
Flash Point ($^\circ \text{C}$)	125	60	Min 100
Solidifying point ($^\circ \text{C}$)	-12	-50 to 10	-
Acid value ($\text{mg KOH} \cdot \text{g}^{-1}$)	0.273	Max 0.5	Max 0.5
Heating value ($\text{MJ} \cdot \text{kg}^{-1}$)	40.833	40 – 45	-

Table 3: Physico-chemical properties of Heterotrophic *Chlorella Protothecoides* Microalgae Biodiesel compared with Diesel fuel and ASTM biodiesel standard.

Most of the properties are comparable with Diesel fuel and ASTM biodiesel standard. So the produced biodiesel is suitable for IC engines.

3.4 FAMES Content analysis of the Microalgae biodiesel by Gas Chromatography.

FAMES content of the Heterotrophic *Chlorella Protothecoides* Microalgae biodiesel was analysed by Gas Chromatography and results are presented in the table 4. and also represented graphically in fig 2.

FAMES	Wt%
C16:0 Palmitic acid	42.664
C18:0 Stearic acid	4.493
C18:1 Oleic acid	38.997
C18:2 Linoleic acid	11.433
C18:3 Linolenic acid	0.704
Others	1.809

Table 4: FAMES content of the Heterotrophic *Chlorella Protothecoides* microalgae biodiesel

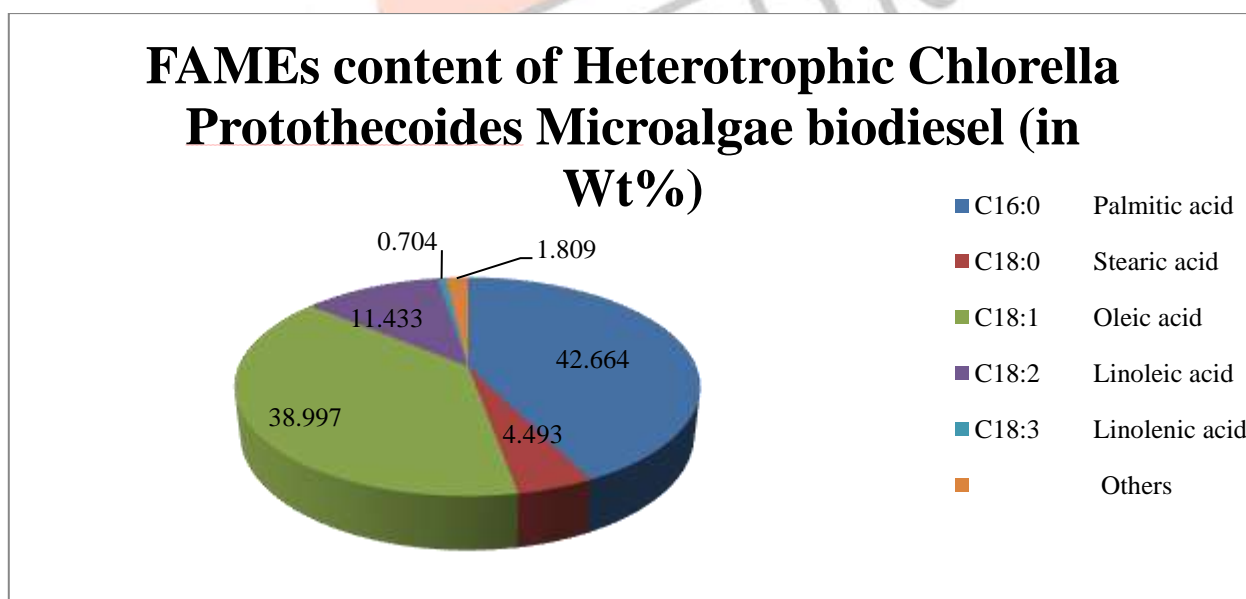


Fig2: FAMES content of the Heterotrophic *Chlorella Protothecoides* Microalgae biodiesel

The GC analysis results from the table shows that almost all the common FAMES content are present in the micro algae biodiesel. The most common fatty acids of microalgae are Palmitic-(hexadecanoic-C16:0), Stearic-(octadecanoic-C18:0),Oleic(octadecenoic-C18:1), Linoleic (octadecadienoic -C18:2) and Linolenic-(octadecatrienoic-C18:3) acids [Knothe,

G.2009]. Higher oleic acid content decreases the Cold Filter Plugging Point (CPPF) for use in cold regions [Stournas et. Al 1995] and increases oxidative stability for longer storage [Knothe, G.2005]. So higher Oleic Acid content of Heterotrophic *Chlorella Protothecoides* Microalgae biodiesel indicates that it is most suitable good quality biodiesel for IC engines.

4. CONCLUSION

Currently the fossil resources are not regarded as sustainable and questionable from the economical, ecological and environmental point of views. Because of the many advantages over the conventional energy resources, the production of biodiesel has attracted much attention in recent years. The production of liquid transportation fuels from Microalgae biomass at laboratory scale is technically feasible with an oil yield of 52 % by dry weight, using solvent extraction method and n-hexane as solvent. Alkali catalysed transesterification found to be suitable for reduction of viscosity Microalgae oil with 92% conversion efficiency. Transesterified Microalgae biodiesel properties are comparable with biodiesel properties and ASTM biodiesel standards. The GC analysis results shows that Heterotrophic *Chlorella Protothecoides* Microalgae biodiesel contains almost all the common FAMES content. And contains higher oleic acid content which increases oxidative stability of the biodiesel for longer storage and decreases the Cold Filter Plugging Point (CPPF) of biodiesel for use in cold regions. So these properties make it most suitable good quality Microalgae biodiesel. Further Microalgae biodiesel and diesel blends are to be prepared and to be tested in DI Diesel engine for checking the suitability of biodiesel as DI diesel engine fuel and also for testing DI diesel engine performance.

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