

Biodiesel Production from Selected Microalgae Strains and Determination of its Properties and Combustion Specific Characteristics

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Abstract

Biofuels are gaining importance as significant substitutes for the depleting fossil fuels. Recent focus is on microalgae as the third generation feedstock. In the present research work, two indigenous fresh water and two marine Chlorophyte strains have been cultivated successfully under laboratory conditions using commercial fertilizer (Nutrileaf 30-10-10, initial concentration=70 g/m³) as nutrient source. Gas chromatographic analysis data showed that microalgae biodiesel obtained from Chlorophyte strains biomass were composed of fatty acid methyl esters. The produced microalgae biodiesel achieved a range of 2.2 - 10.6 % total lipid content and an unsaturated FAME content between 49 mol% and 59 mol%. The iodine value, the cetane number, the cold filter plugging point, the oxidative stability as well as combustion specific characteristics of the final biodiesels were determined based on the compositions of the four microalgae strains. The calculated biodiesel properties compared then with the corresponding properties of biodiesel from known vegetable oils, from other algae strains and with the specifications in the EU (EN 14214) and US (ASTM D6751) standards. The derived biodiesels from indigenous Chlorophyte algae were significantly comparable in quality with other biodiesels.

Keywords: Microalgae, chlorophytes, FAME compositions, empirical models, biodiesel properties

1. Introduction

The demand for alternative fuels has increased in the past several years. Nowadays, biofuels play a leading role in the substitution of the depleting fossil fuels [1,2]. Biofuels derived from biological materials, and this is the main differentiation from other non-fossil fuel energy sources (e.g. wind and wave energy). Biological-based fuels can be solid (e.g. wastes), gaseous (e.g. methane, dimethyl ether, hydrogen) and liquid (e.g. biobutanol, biodiesel). The biggest advantage of biofuels is the environmentally friendliness that they have over fossil fuels. Particularly, biodiesel (from the Greek word βίος: life + diesel from Rudolf Diesel) is a sustainable and renewable fuel, as a result of solar energy conversion into chemical energy. It is biodegradable (degrades about four times faster than petrodiesel) [3], non-toxic and basically free of sulfur and aromatics. The emissions produced by burning of biodiesel are less reactive with sunlight than those produced by burning gasoline; it emits generally less carbon dioxide, carbon monoxide, particulate matter, hydrocarbons, smoke, noxious fumes and odors than mineral diesel fuel. Furthermore, comparing biodiesel with petrodiesel, the former shows higher combustion efficiency, higher cetane number, higher flash point, and inherent lubricity (about 66% better than petrodiesel) [3-5].

On the other hand, biodiesel exhibits higher viscosity, lower energy content, higher cloud point, higher pour point, decreased oxidative stability and slightly increased nitrogen oxide (NOx) emissions compared with petroleum-based diesel fuel [3]. In addition, it is worthy of remark that the first produced biodiesel derived from edible oil seed crops (first generation feedstocks) is lurking a serious risk of disturbing the overall worldwide balance of food reserves and safety. The second generation feedstocks for biodiesel production are obtained from non-edible oil seed crops, waste cooking oil, animal fats, etc. However, these feedstocks are not sufficient to cover the present energy needs. Therefore, recent worldwide attention is on algae, mainly microalgae, as the third generation feedstock [6].

Microalgae are microscopic photosynthetic organisms that are found in both marine and freshwater environments. Bacillariophyceae (diatoms), Chlorophyceae (green algae), and Chrysophyceae (golden algae) are the three most important classes of microalgae in terms of abundance. They do not compete for land; instead they can grow in salty (sea), brackish (lagoons) and fresh (lakes) water. These photosynthetic organisms sequester the atmospheric CO₂ and release oxygen. In spite of the fact that the photosynthetic mechanism of microalgae is similar to land-based plants, the primitive simple cellular structure of microalgae, their large surface-to-volume-body ratio and their efficient access to water, CO₂, and other nutrients provide them with the ability to grow much faster than terrestrial crops by converting more efficient solar energy into biomass [7,8]. The fact that microalgae can complete an entire growing cycle every few days offer them the potential to produce more oil per acre than any other feedstock being

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used to make biodiesel [9]. According to Becker (1994) [10], there are algae types that are comprised of up to 40% of their overall mass by fatty acids. The producing algal fuels can give 30 to 100 times more energy per hectare compared to biofuels produced from terrestrial crops [9].

During the past few years, we have been thoroughly examining the partial hydrogenation of polyunsaturated methyl esters of sunflower oil to monounsaturated methyl esters in order to improve the final quality of biodiesel [11]. Then, we studied the optimisation of cotton seed biodiesel quality, through the modification of its fatty acid methyl ester (FAME) composition and those hydrogenation conditions that could supply it [12]. In the present research work, two indigenous fresh water and two marine Chlorophyte microalgae strains have been cultivated successfully under laboratory conditions. The extracted oil from the algal biomass was transesterified to biodiesel. Various biodiesel properties and combustion characteristics were determined based on existing empirical models of biodiesel properties.

2. Experimental details

2.1 Materials

Hydrogen, nitrogen, argon, zero air and helium 5.0N were purchased from Linde Hellas and Axarlis. NaOH, CH₃OH, HCl (4M), BF₃, iso-octane and petroleum ether were purchased from Sigma-Aldrich. Na₂SO₄ was used for drying and was obtained from Panreac.

2.2 Analytical Method

The method of gas chromatography with flame ionization detector (FID) was used for qualitative and quantitative analysis of the fatty acid methyl esters. Hewlett Packard 5890-Series II GC/FID equipped with a BPX 70 capillary column (50 m x 0.22 mm I.D. x 0.25 µm film thickness) of SGE Analytical Science (Trajan) was used for FAME analysis. Column temperature was kept initially constant at 177 °C for 18 min. Then, it was raised at 210 °C at a rate of 2.3 °C/min and remained there for 23 min. The temperature of the split injector was 200 °C and the temperature of the detector was 250 °C. The split ratio was 1:60 and the injected volume was 2 µL. Helium and nitrogen at 36 mL/min were used as carrier gas and make up gas, respectively. The flow rates of hydrogen and zero air were 30 mL/min and 330 mL/min, respectively. Solutions of standards compounds were used for peak identification.

2.3 Typical procedure of algal biodiesel production

Figure 1 illustrates the process flow diagram of a typical algal biodiesel production starting from the cultivation of Chlorophyte strains till the prediction of the final biodiesel properties. The whole work was carried out in three well equipped laboratories:

- i) The Fisheries Research Institute of the Hellenic Agricultural Organization-DEMETER with expertise in eco-physiology, land-based algae cultivation and chemical analysis.
- ii) The Biofuels Laboratory of the Department of Petroleum and Mechanical Engineering at Eastern Macedonia and Thrace Institute of Technology specialised in biofuels production, analysis, catalysis and applications.

- iii) The Chemical Process Modelling and Simulations Laboratory of the Department of Petroleum and Mechanical Engineering at Eastern Macedonia and Thrace Institute of Technology specialised in scientific modelling and simulations of process engineering, where the prediction of the final biodiesel properties occurred.

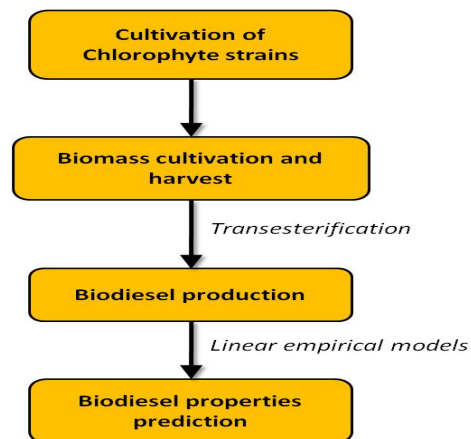


Fig. 1. Typical procedure of algal biodiesel production.

In the beginning [13-15], two indigenous fresh water (ChlorF1, ChlorF2) and two marine (ChlorM1, ChlorM2) Chlorophyte microalgae strains from Northern Greece have been selected and cultivated successfully under laboratory conditions (Figure 2). Commercial fertilizer (Nutrileaf 30-10-10, initial concentration=70 g/m³) was used as nutrient source. Land-based facilities (Figure 3) at the greenhouse of Fisheries Research Institute were used for the mass cultivation and harvest. The cultivation took place during spring to autumn. Subsequently, the samples were dried in a Christ freeze dryer and the algal biomass was prepared for oil extraction. Lipids were extracted from the homogenized fresh edible portion by the method of Bligh and Dyer (1959) [16] and were further prepared for fatty acid analysis according to the procedure of Kates (1972) [17]. Methyl esters were prepared by saponification with 0.5 N NaOH and methylation with 14% boron trifluoride-methanol [18].

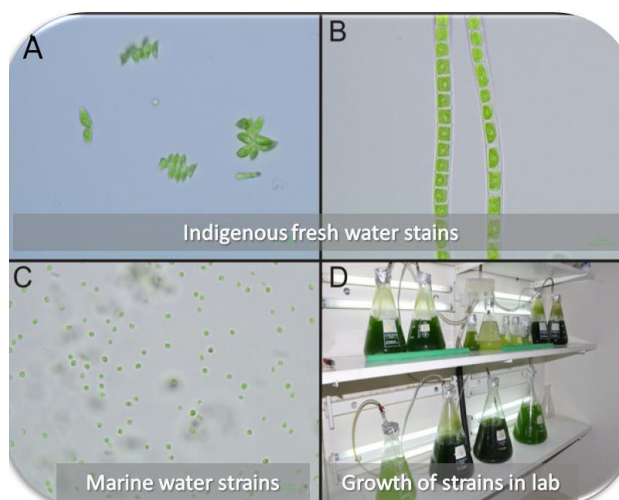


Fig. 2. Chlorophyte strains cultivated under laboratory conditions using commercial fertilizer (Nutrileaf 30-10-10, initial concentration=70 g/m³) as nutrient source: A. B) indigenous fresh water stains. C) a marine water strain of Chlorella. D) growth of strains under laboratory conditions.

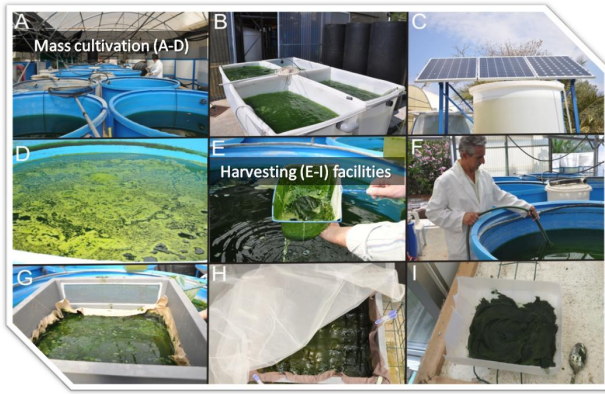


Fig. 3. Mass cultivation (A-D) and harvesting (E-I) facilities of indigenous fresh water Chlorophyte strains at the greenhouse of Fisheries Research Institute, Greece.

3. Results and discussion

The produced biodiesel from the microalgae biomass achieved an unsaturated FAME content between 49 mol% and 59 mol% and a range of 2.2 - 10.6 % total lipid content. For the calculation of the total lipid content, approximately 5g of the sample was mixed with 5 g celite and 100 mL hydrochloric acid (4 M) and hydrolysed (Büchi Hydrolysis Unit B-411) at 100 °C, for 30 minutes. Then, the sample was dried in a common microwave oven and extracted via an automated Soxhlet apparatus with petroleum benzin 40-60 (Büchi Extraction System B-811). Right after the solvent evaporation, the beakers were weighted and the total lipid content was calculated according to Equation 1:

$$\% \text{ Fat} = \left(\frac{B_1 - B_2}{\text{Sample Weight}} \right) \times 100 \quad (1)$$

Where:

B₁: Weight of the beaker with the sample

B₂: Weight of the weighted beaker without the sample

Gas chromatographic analysis data revealed that algal biodiesels obtained from Chlorophyte biomass were composed of fatty acid methyl esters (Figure 4). The compositions of the produced biodiesels by the four microalgae strains, two indigenous fresh water (ChlorF1, ChlorF2) and two marine (ChlorM1, ChlorM2) Chlorophyte microalgae strains, are summarised in Table 1. Also, it can be noticed that biodiesels derived from the specific marine Chlorophyte microalgae strains do not contain C22:0.

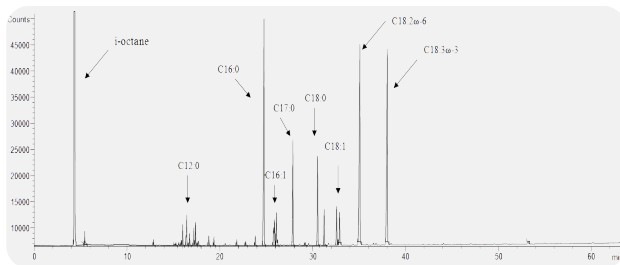


Fig. 4. Typical GC chromatograph of biodiesel from Chlorophyte strains.

Based on the biodiesel compositions derived from microalgae strains (Table 1), the iodine value, the cetane

number, the cold filter plugging point and the oxidative stability of the ultimate biodiesels were determined. Most of the literature in the field of empirical modelling and prediction of biodiesel properties employs the generic linear Equation 2. According to Equation 2, the biodiesel (mixture of FAMES) is regarded as near-ideal solutions of pure FAMES and its properties can be derived from a simple linear equation of the known specific properties of the individual pure FAMES.

$$P_{\text{Biodiesel}} = \sum_{i=1}^N P_{\text{FAME}_i} \cdot C_{\text{FAME}_i} \quad (2)$$

Where:

$P_{\text{Biodiesel}}$: Property of biodiesel as a mixture of FAMES

P_{FAME_i} : Specific property of a pure FAME

C_{FAME_i} : Concentration of a pure FAME in the mixture

Table 1. Composition of biodiesels derived from four Chlorophyte microalgae strains (fresh water: ChlorF1, ChlorF2 and marine: ChlorM1, ChlorM2).

Biodiesel composition (wt.%)				
FAME	ChlorF1	ChlorF2	ChlorM1	ChlorM2
C12:0	1.85	1.25	2.17	2.70
C16:0	38.62	22.32	26.33	23.38
C16:1	10.43	4.05	3.75	3.32
C17:0	2.49	4.70	1.97	9.38
C18:0	5.22	8.15	11.49	8.59
C18:1	16.33	5.72	4.02	3.61
C18:2	11.89	14.75	14.87	24.92
C18:3	12.57	36.69	35.40	24.11
C22:0	0.59	2.38	0.00	0.00

3.1 Determination of iodine value and cetane number of algal biodiesel

The iodine value (IV) is a measure of the total unsaturation within a FAME mixture. Particularly, IV expresses the grams of iodine which will react and will finally be added to the double bonds of 100 g biodiesel. Moreover, the IV indicates the tendency of the oil or fat to polymerize and form engine deposits. Despite unsaturated fatty acid components in FAMES are required, due to the restriction of FAME solidification, higher degree of unsaturation leads to the unwanted event of FAME polymerization.

The IV was determined by using the widely accepted method of EN 14214 standard (Annex B) [19]. The IVs of the four algal biodiesel were calculated according to Equation 3.

$$\begin{aligned} IV_1 &= 0.95 \cdot C16 : 1 \%w/w + 0.86 \cdot C18 \\ &\quad : 1 \%w/w + 1.732 \cdot C18 \\ &\quad : 2 \%w/w + 2.616 \cdot C18 \\ &\quad : 3 \%w/w + 0.785 \cdot C20 \\ &\quad : 1 \%w/w + 0.723 \cdot C22 \\ &\quad : 1 \%w/w \end{aligned} \quad (3)$$

Table 3 revealed that the produced algal biodiesel derived from ChlorF1 surpassed in IV other selected microalgae strains and also Coelastrum [20]. ChlorF2 feedstock offered an IV equal to sunflower feedstock [21]. However, ChlorF2, ChlorM1 and also soybean biodiesels [21] are out of the specifications of EN 14214 (Table 2).

Table 2. Predicted biodiesel properties comparing with the specifications in the EU and US standards.

Property	Units	ASTM D6751	EN 14214	ChlorF 1	ChlorF 2	ChlorM 1	ChlorM 2
Iodine Value	g I ₂ / 100 g BD	-	max. 120	78	132	125	112
Cetane Number	-	min. 47	min. 51	56	45	46	49
CFPP	° C	-	-*	7	15	10	4
Oxidative Stability	h; 110 ° C	min. 3	min. 6	7	5	5	5

* Country specific CFPP (Greece) according to ELOT EN 14214: +5 ° C / -5 ° C

Table 3. Predicted biodiesel properties comparing with the corresponding properties of biodiesel from known vegetable oils and from other algae strain.

Property	Units	Chlor F1	Chlor F2	Chlor M1	Chlor M2	Coelastu m[20]	Soybean [21]	Sunflower [21]	Palm[21]
Iodine Value	g I ₂ / 100 g BD	78	132	125	112	88	128	132	57
Cetane Number	-	56	45	46	49	57	49	50	61
CFPP	° C	7	15	10	4	-5	-5	-3	10
Oxidative Stability	h; 110 ° C	7	5	5	5	4	1	1	4

* Country specific CFPP (Greece) according to ELOT EN 14214: +5 ° C / -5 ° C

Cetane number (CN) affects a number of engine performance parameters such as combustion, stability, driveability, white smoke, noise, and emissions of CO and hydrocarbons. The higher the cetane number is, the better it is in its ignition properties (shorter the ignition delay time) and combustion characteristics. The CN of biodiesel, as a mixture of FAMES, depends strongly on compound structure. Generally, CN increases with increasing chain length and degree of saturation. Although FAMES with higher CN (such as CN of methyl stearate ca. 100 and CN of methyl palmitate ca. 85) are favored for use as biodiesel [22], the increase of CN means also reduction of biodiesel degree of unsaturation, which leads to the undesirable solidification of FAMES at higher temperature. Therefore, the upper limit of CN (65) has been specified in US biodiesel standard (ASTM PS 121–99).

In the current research work, the cetane numbers of the four algal biodiesel were obtained using the empirical method expressed by Equation 4 [12,23,24]. For the determination of saponification number (SN) and iodine value (IV₂), which this empirical method demands in advance, Equation 5 and Equation 6 were employed, respectively [12,18,24]. Apparently, the calculated CN is negatively correlated to IV₂ (Equation 4), due to the fact that CN decreases with increasing biodiesel degree of unsaturation.

$$CN = 46.3 + \frac{5458}{SN} - 0.225 \cdot IV_2 \quad (4)$$

Where:

CN: Cetane number

SN: Saponification number (Equation 5)

IV₂: Iodine value (Equation 6)

$$SN = \sum \frac{(560 \cdot A_i)}{M_i} \quad (5)$$

Where:

SN: Saponification number

A_i: % w/w of each fatty acid methyl ester

M_i: Molecular mass of each fatty acid methyl ester

$$IV_2 = \sum \frac{(253.81 \cdot D \cdot A_i)}{M_i} \quad (6)$$

Where:

IV₂: Iodine value

D: Number of double bonds of each fatty acid methyl ester

A_i: % w/w of each fatty acid methyl ester

M_i: Molecular mass of each fatty acid methyl ester

According to Table 2, algal biodiesel derived from ChlorF1 strain emerged the best CN among the four selected Chlorophyte biodiesel feedstocks. In addition, ChlorF1 offered a better CN to the ultimate biodiesel than biodiesel derived from soybean and sunflower feedstocks [21]; and almost equal CN to the produced biodiesel from Coelastrium feedstock [20].

3.2 Determination of cold filter plugging point and oxidative stability of algal biodiesel

Low-temperature performance is one of the most important properties of biodiesel and it is mainly specified by the cold filter plugging point (CFPP) of the fuel. The CFPP method is accepted nearly worldwide. In fact, CFPP predicts more effectively the limiting fuel temperatures, where start-up or operability problems may be expected after prolonged exposure, than cloud point (CP) and pour point (PP) could achieve. Particularly, CFPP is the temperature at which a fuel causes a filter to plug as a result of the crystallization of the implicating ester structures. Biodiesel fuels with significant amounts of saturated fatty compounds will display higher CFPPs, due to the fact that saturated fatty compounds have significantly higher melting points than unsaturated fatty compounds [4]. Thus, in a mixture, saturated fatty compounds crystallize at higher temperature than the unsaturated ones. Furthermore, the longer the carbon chains in the biodiesel, the worse the low-temperature properties [21].

For the determination of the four algal biodiesel CFPPs, the long chain saturated factor LCSF B (Equation 7) of Ramos et al. (2009) [21] was taken into account in conjunction with Equation 8 (with a reported R²=0.966) [21].

$$LCSF(B) = 0.1 \cdot C16(wt. \%) + 0.5 \cdot C18(wt. \%) + 1 \cdot C20(wt. \%) + 1.5 \cdot C22(wt. \%) + 2 \cdot C24(wt. \%) \quad (7)$$

$$CFPP = 3.1417 \cdot LCSF(B) - 16.477 \quad (8)$$

Though EN 14214, EN 116, ASTM D-6751 and ASTM D-6371 standards include and describe CFPP measurement method, they do not specify any limit, due to its temperature dependency by the varying climate conditions at different

countries. According to ELOT EN 14214 (Table 2), in Greece, CFPP is specified as +5 °C (in summer) / -5 °C (in winter). It can be seen in Table 2 that only ChlorM2 gave a good CFPP value.

Oxidative stability is another important parameter that several standards (such as EN 14214 and ASTM D6751) monitor. Although biodiesel is environmentally compatible, biodiesel fuels are more sensitive to oxidative degradation than fossil diesel fuels. Oxidative stability is strongly affected by the number of FAME molecules having multiple double bonds. Particularly, the oxidative stability decreased with the increase of the contents of polyunsaturated methyl esters. The stability of fatty compounds is influenced, apart from structural features of the compounds themselves, by a variety of other factors such as conditions of storage, temperature, time, presence of oxygen, metal traces and also light [25]. It is worthy of remark that even if appropriate prevention measures are taken, oxidation can be delayed but not completely prevented.

The oxidative stabilities of the current algal biodiesels were determined according to the empirical methodologies of Sarin et al. (2010) [26] and Park et al. (2008) [27]. Almost all oxidative stabilities were within ASTM D6751 specifications, whereas the under comparison soybean and sunflower oils [21] are out of the ASTM specs (Table 2 and Table 3). However, only ChlorF1 is in line with EN 14214 specs. According to Ramos et al. (2009) [21], it is very difficult to meet the minimum limit of Rancimat induction period of 6 h for biodiesel fuels derived from common raw materials, unless antioxidants are added to the biodiesel.

3.3 Determination of combustion specific characteristics of the final algal biodiesels

Equations 9-14 were employed to determine the combustion specific characteristics (CSC) of algal biodiesels. Equation 9 describes the general reaction equation for stoichiometric (complete) combustion of a saturated FAME in oxygen; and Equations 10-12 depict those of unsaturated FAME with one, two and three double bonds (DB), respectively. The oxygen content of FAMES, the required stoichiometric amount of oxygen in the complete combustion of FAMES and the produced stoichiometric amount of carbon dioxide in the complete combustion of FAMES were calculated according to the Equations 9-12, using the molecular weights of FAMES in Table 4.

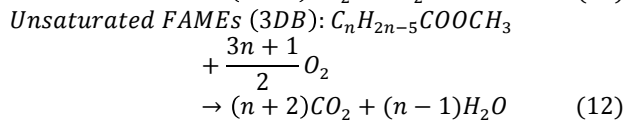
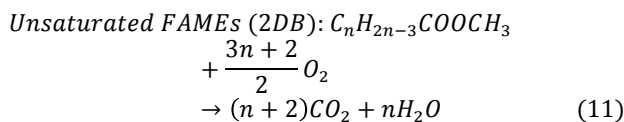
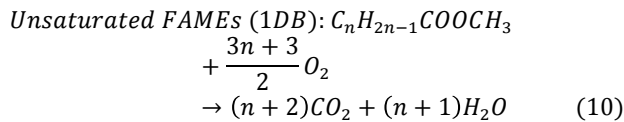
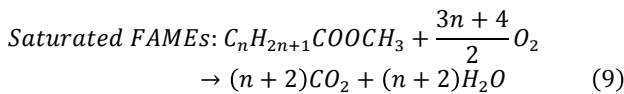


Table 4. Molecular weights of fatty acids (FAs) and their methyl esters (FAMES), where Cn: Number of carbon atoms and DB: Number of double bonds.

Molecular Weight		
Cn:DB	FA	FAME
C12:0	200.3233	214.3503
C16:0	256.4314	270.4585
C16:1	254.4155	268.4425
C17:0	270.4585	284.4855
C18:0	284.4855	298.5126
C18:1	282.4696	296.4966
C18:2	280.4536	294.4807
C18:3	278.4377	292.4647
C22:0	340.5937	354.6207
C24:0	368.6478	382.6748

Table 5. Combustion specific characteristics (CSC) of biodiesels derived from four Chlorophyte microalgae strains (fresh water: ChlorF1, ChlorF2 and marine: ChlorM1, ChlorM2).

CSC	Units	ChlorF1	ChlorF2	ChlorM1	ChlorM2
O ₂ in FAME	g O ₂ /100 g biodiesel	11.41	11.16	11.26	11.27
Required O ₂ in SC*	g O ₂ /100 g biodiesel	288.96	288.18	287.91	288.31
Produced CO ₂ in SC*	g CO ₂ /100 g biodiesel	279.87	281.97	281.52	281.16
Ignition Delay (ID)	° CA	9	10	10	10
Total Combustion Duration (TCD)	° CA	59	56	57	57

* SC: Stoichiometric (complete) combustion

According to Gopinath et al. (2010) [28], the ignition delay (ID) of the fuel and the total combustion duration (TCD) were determined in terms of degree crank angle, by employing Equation 13 and Equation 14, respectively. Table 5 sums up the examined combustion specific characteristics of algal biodiesel.

$$ID = 0.0962 \cdot X + 4.6837 \quad (13)$$

$$TCD = -0.2806 \cdot X + 72.202 \quad (14)$$

4. Conclusion

All selected Chlorophyte microalgae strains from Northern Greece have been cultivated successfully under laboratory conditions. Gas chromatographic analysis data showed that biodiesels obtained from indigenous Chlorophyte algae strains were composed of fatty acid methyl esters. The algal biodiesels achieved a range of 2.2-10.6% total lipid content and an unsaturated FAME content between 49 mol% and 59 mol%. The properties and combustion specific characteristics of the ultimate biodiesels were determined, based on the compositions of the various substrates. The derived biodiesels from indigenous Chlorophyte algae (above all ChlorF1) were significantly comparable in quality with other biodiesels.

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