RESEARCH ARTICLE

Fuel Properties of Fatty Acid Methyl Esters (FAME) Produced with Fats of Gamma - Irradiated Mutants of Oleaginous Microalga - *Chlorella* sp. KM504965

Dhandapani Senthamilselvi and Thangavel Kalaiselvi*

*Department of Agricultural Microbiology, Tamil Nadu Agricultural University, Coimbatore- 641 003

Corresponding author: tkalaiselvi@tnau.ac.in

ABSTRACT

In the current study, biodiesel properties of fatty acid methyl ester (FAME) derived from fats of four gamma-irradiated mutants (CI801, CI802 CI803 & CI804) and wild type (CIWT) of *Chlorella* sp. KM504965 were determined and compared with American Society for Testing and Materials (ASTM), European Norm (EN) and Indian standards (IS). Maximum concentration of saturated fatty acids (62.69%), monounsaturated fatty acids (36.29%) and polyunsaturated fatty acids (15.49%) recorded in CIWT, CI801 and CI803 respectively. The kinematic viscosity of CI801 (4.88 mm^2s^{-1}), CI802 (4.90 mm^2s^{-1}), CI803 (4.73 mm^2s^{-1}), CI804 (4.88 mm^2s^{-1}) and CIWT (4.92 mm^2s^{-1}) fits with ASTM, EN and IS. The density of CI801 (0.83 gcm^{-3}), CI802 (0.84 gcm^{-3}), CI803 (0.78 gcm^{-3}), CI804 (0.80 gcm^{-3}) and CIWT (0.81 gcm^{-3}) was slightly lower than the standard limits (0.86 – 0.90 gcm^{-3}). The high heating value of CI801 (37.62 $MJKg^{-1}$), CI802 (38.15 $MJKg^{-1}$), CI803 (34.93 $MJKg^{-1}$), CI804 (36.44 $MJKg^{-1}$) and CIWT (36.99 $MJKg^{-1}$) were in accordance with ASTM standards. The iodine value was 47.38 mgI_2 $100g^{-1}$ (CI801), 52.18 mgI_2 $100g^{-1}$

(Cl802), 50.87 mgI_2100g^{-1} (Cl803), 47.04 mgI_2100g^{-1} (Cl804), and 27.45 mgI_2100g^{-1} (ClWT). The oxidative stability was 10.78 h (Cl801), 6.60 h (Cl802), 6.44 h (Cl803), 9.06 h (Cl804) and 39.83 h (ClWT). The cetane number of Cl801 (63.3), Cl802 (61.80), Cl803 (62.16), Cl804 (64.99) and ClWT (68.7) was greater than the standard values (61 to 65). It is concluded that the biodiesel from mutants could comply IS, ASTM, and EN and equivalent with biodiesel from vegetable oil.

Keywords: *Chlorella*; *Mutants*; *Fats*; *Biodiesel property*; *Viscosity*; *Density*

INTRODUCTION: The increasing trend of global warming and the imminent depletion of natural energy reserves necessitate the development of cleaner and renewable alternate fuels like biofuel technologies. "Due to raging 'food versus fuel' debates, first-generation biofuels based on food crops were discouraged. Second-generation biofuels rely on lignocellulosic biomass yet involve costly pre-treatment methods and sophisticated technologies to convert biomass into biofuels. Owing to several advantages microalgae was recognized as a potential feedstock for third-generation biofuels production (Ghosh *et al*., 2016). Microalgae are unicellular algal species capable of growing fast and thriving in wastewaters. In addition, their productivity is comparatively higher than plants. They are also capable of utilizing CO_2 or flue gases and thus help mitigate greenhouse gas emissions (Mondal *et al*., 2017). Despite these advantages, microalgae-based biofuels are not commercially feasible due to lower productivity and high cost (Chu *et al*., 2017). Most natural strains isolated could not meet the industrial standards such as fast growth rate, robustness, high lipid content, ability to grow in low-cost media, thus necessitating efforts for strain improvement (Zhu *et al*., 2016, Aratboni *et al*., 2019, 12, Arora *et al*., 2020). Saxena *et al*. (2015) have briefed various development strategies focused on strain improvement with desired key characteristics. Among various strategies, random mutagenesis using gamma radiation has been found to yield stable mutants.

Furthermore, strains generated via mutagenesis do not fall under the strict regulatory limitations of genetically engineered microorganisms, requiring the least legal and regulatory permit before deployment at commercial scale (Baek *et al*., 2016, Ottenheim *et al*., 2018). Another advantage with microalgae is that they can be grown in either autotrophic, heterotrophic, or mixotrophic modes depending on the availability of resources. The commonly studied process algal biomass production is outdoor photoautotrophic cultivation due to the simplicity of scalability, photosynthetic character and the additional benefit of CO_2 sequestration (Kim *et al*., 2019). Microalgae also utilize organic molecules as primary energy and carbon source through heterotrophic and mixotrophic nutritional modes. This facilitates greater biomass productivity, which provides economic feasibility for large-scale production using effluents rich in carbon sources (Zhang *et al*., 2014). Biodiesel standards, namely IS (Indian standards), ASTM D6751 (American Society for Testing and Materials), and EN 14214 (European Norms), were designed to confirm rich product quality and to develop consumer's confidence in biodiesel (Knothe *et al*., 2005). Biodiesel is an assortment of fatty acid alkyl esters produced by trans-esterification of fats from any biological sources, including animal fats and vegetable oil. Biodiesel is advantageous over conventional fuels because it possesses lower emission profiles, non-toxic and biodegradable (Ganapathy et al., 2011). Despite several advantages of microalgal biodiesel, it faces many economic and technical issues (Mata *et al*., 2010). Although microalgae-derived lipids can be converted to methyl esters and alkanes for use in spark-ignition engines, compression ignition engines, and aircraft gas turbine engines, the suitability of algal biodiesel, however, depends on the fatty acid (FA) composition (Bucy *et al*., 2012). The FAME composition of biodiesel determines several properties of the biofuel, such as viscosity, oxidative stability, calorific value (CV), cetane number (CN), saponification value (SV), iodine value (IV), degree of unsaturation (DU), cold filter plugging point (CFPP) and long-chain saturation factor (LCSF), (Kalayasiri *et al*., 1996, Ramos *et al*., 2009 and Islam *et al*., 2013). With this background, the current study was carried out to evaluate the biodiesel properties of FAME derived from gamma-irradiated mutants of lipid accumulating *Chlorella* sp. KM504965 (Cl801, Cl802, Cl803, and Cl804) and wild type (ClWT) in terms of viscosity, oxidative stability, density, cetane number, iodine value, saponification value, degree of unsaturation, cold flow plugging point, and long-chain saturation factor.

MATERIAL AND METHODS

Microalgae strains and culture conditions

Oleaginous gamma-irradiated mutants (CI801, CI802, CI803, and CI804) and wild type (CIWT) of *Chlorella* sp. KM504965 maintained in the Algal Culture Collection unit of the Department of Agricultural Microbiology, Tamil Nadu Agriculture University, Coimbatore, were selected to investigate their fuel properties under phototrophic cultivation. The purity of culture was maintained by repeated plating and by regular observation under a light microscope.

Cultivation of oleaginous gamma-irradiated mutants

Four gamma-irradiated mutants (CI801, CI802, CI803, and CI804) and wild type (CIWT) were cultured in a 500 mL Erlenmeyer flask containing 250 mL modified BG-11 medium (Stanier *et al*., 1971). The medium composed of $NaHCO_3$ (2.0 g L^{-1}), $NaNO_3$ (1.5 g L^{-1}), $K_2HPO_4.3H_2O$ (40 mg L^{-1}), $MgSO_4.7H_2O$ (75 mg L^{-1}), $CaCl_2.2H^2O$ (36 mg L^{-1}), $FeCl_3.6H_2O$ (3.15 mg L^{-1}), Citric acid (6.0 mg L^{-1}), EDTA $Na_2.2H_2O$ (4.36 mg L^{-1}), H_3BO_3(2.86 mg L^{-1}), $MnCl_2.4H_2O$ (1.18 mg L^{-1}), $ZnSO_4.7H_2O$ (0.22 mg L^{-1}), $Na_2MoO_4.2H_2O$ (0.39 mg L^{-1}), $Co(NO_3)2.6H_2O$ (0.05 mg L^{-1}) and $CuSO_4.5H_2O$ (0.08 mg L^{-1}). The cultures were incubated at 24±1ºC under a cool white fluorescent light intensity of 40 µmol photon m^{-2} s^{-1} with 16:8 h light-dark cycle interval for 20-25 days and shaken manually 3-6 times a day.

Preparation and analysis of Fatty Acid Methyl Ester (FAME)

For FAME preparation, the cultures were grown in 500 mL flasks containing 250 mL BG11 medium with 1.8 x 10^6 cells/mL as inoculum for 21 days. At late log phase, the biomass was harvested by centrifugation at 6000 rpm for 10 min and then, the obtained pellets were washed twice with distilled water. It was dried in an oven at 60 °C until it reached a constant weight. 100 mg dried algal biomass was mixed with 10 mL solution containing methanol (4.25 mL), concentrated sulphuric acid (0.75 mL), and chloroform (50 mL). The mixture was kept in a water bath at 90°C for 2 h. After cooling, 1 mL of distilled water was added, mixed thoroughly, and centrifuged at 5000 rpm for 5 min. The lower phase containing FAME was collected, and its composition was analyzed through gas chromatography with flame ionization detector (FID) (PerkinElmer Clarus 680, USA) and Elite-5 column (30 m × 0.25 mm × 0.25 µm). The following conditions were used for the study, injection temperature - 220 °C; initial column temperature - 160 °C; final temperature - 190 °C at a rate of 3 °C min^{-1}; detector temperature - 270 °C. The carrier gas (helium) flow rate was maintained at 1.3 mL min^{-1}. Standard FAME mix (FAME mix C4-C24, Catalog No: LRAC0565, Sigma) was used as a standard to determine the FAME composition of lipids by comparing with the retention time and sample peak area (Thangavelu *et al*., 2020).

Estimation of biodiesel fuel properties

The biodiesel properties of FAME were obtained from gamma-irradiated *Chlorella* sp. KM504965 (CI801, CI802, CI803, and CI804) and wild type (CIWT) were estimated in terms of kinematic viscosity (KV), density (ρ), high heating value (HHV), saponification value (SV), iodine value (IV), cetane number (CN), degree of unsaturation (DU) long-chain saturation factor (LCSF), cold filter plugging point (CFPP), and oxidative stability (OS) using the following analytical equations.

Kinematic viscosity (KV)

Kinematic viscosity is one of the fuel properties specified in the biodiesel standards. It was determined using the formula given by Ramirez *et al*., 2012.

Kinematic viscosity (υ, $mm^2\ s^{-1}$) at 40 °C, density (ρ, $g\ cm^{-3}$) at 20 °C, and HHV of the biodiesel were assessed using the following equations

$$\ln(\upsilon) = \sum N_i\,(-12.503 + (2.496 \times \ln Mw_i) - 0.178 \times D_i) \quad (1)$$

$$\rho = \sum N_i\,(0.8463 + (4.9/Mw_i) + 0.0118 \times D_i) \quad (2)$$

$$HHV = \sum N_i\,(46.19 - (1794/Mw_i) - 0.21 \times D_i) \quad (3)$$

Wherein 'Mwi' is the molecular weight, 'Ni' is the percentage and 'Di' is the number of double bonds in the given fatty acid.

The saponification value (SV), iodine value (IV), and cetane number (CN) were calculated using the following equations (Knothe, 2016)

$$SV = \sum (560 \times N)/M \quad (4)$$

$$IV = \sum (254 \times DN)/M \quad (5)$$

$$CN = 46.3 + (5458/SV) - (0.225 \times IV) \quad (6)$$

Wherein' D' is number of double bonds in the fatty ester; 'M' is molecular mass of fatty ester, and 'N' is percentage of particular fatty acid.

Oxidative stability (OS)

The oxidative stability measures the formation of volatile acids. The predictive oxidative stability was calculated based on C18:2 and C18:3 contents as suggested by Islam and Park (Islam *et al*., 2013, Park *et al*., 2008) following equation (7).

$$OS = 117.9295/(wt\%\ C18{:}2 + wt\%\ C18{:}3 + 2.5905) \quad (7)$$

Degree of unsaturation (DU)

The DU of biodiesel has a profound effect on oxidative stability. It was estimated using the formula (8) (Patel *et al*., 2017).

$$DU = MUFA + (2 \times PUFA) \quad (8)$$

Wherein' MUFA' is monounsaturated fatty acid and 'PUFA' is polyunsaturated fatty acid

Long-chain saturation factor (LCSF)

LCSF is also an important property which determines the behavior of biodiesel at lower temperatures. It was estimated using equation (9) (Sarin *et al*., 2009)

$$LCSF = (0.1 \times C16{:}\,0) + (0.5 \times C18{:}\,0) + (1 \times C20{:}\,0) + (1.5 \times C22{:}\,0) \quad (9)$$

Cold flow plugging point (CFPP)

The cold flow plugging point of a fuel is the temperature at which wax crystals begin to form as it is cooled. It precipitates in the solid phase, giving a cloudy appearance to the fuel. The cloud point in the petroleum industry refers to the temperature below which the waxes present in diesel or bio wax in biodiesel make the fuel appear cloudy. The thickened fuel with solidified wax clogs the engine's fuel filter. Therefore, cloud point indicates the tendency of the oil to plug filters in cold climates (Abdulvahitoglu 2019). It was estimated using the equation (10)

$$CFPP = (3.1417 \times LCSF) - 16.477 \quad (10)$$

RESULTS AND DISCUSSION

FAME profile of gamma-irradiated mutants (CI801, CI802, CI803, and CI804) and wild type (CIWT) of *Chlorella* sp. KM504965

The FAME profiles of the organisms play a crucial role in the selection of the candidate for quality biodiesel production (Thangavel *et al*., 2018). To evaluate the gamma-irradiated mutants (CI801, CI802, CI803, and CI804) and wild type (CIWT) of *Chlorella* sp. KM504965 as a biodiesel feedstock, its fatty acid composition was determined by GC with FID. The FAME composition of lipids was determined by comparing the retention time and samples peak area with the standard FAME mix. Based on the length, fatty acids are categorized as short-chain fatty acids (up to 5 carbons), medium-chain fatty acids (6 to 12 carbons), long chain fatty acids (13-21 carbons), and very long-chain fatty acids (22 or more carbons) (Vyas and Chhabra, 2017). The fatty acid chain length and degree of unsaturation are significant in determining their biodiesel properties (Thangavel *et al*., 2018). In the current study, the FAME profiles of gamma-irradiated mutants (CI801, CI802, CI803 and CI804) and wild type (CIWT) of *Chlorella* sp. KM504965 showed a wide range from short-chain to very-long-chain fatty acids. It includes saturated and unsaturated forms of up to 24 carbon chain lengths. The similarity between microbial oils and vegetable oils paves the way for utilizing this microbial biomass as a new generation feedstock for biodiesel production.

In the present study, gamma-irradiated mutants produced almost all primary fatty acids as reported in vegetable oils such as capric acid (C10:0), lauric acid (C12:0), myristic acid (C14:0), palmitic acid (C16:0), stearic acid (C18:0), behenic acid (C22:0), myristoleic acid (C14:1), cis-10-pentadecanoic acid (C15:1), palmitoleic acid (C16:1), cis-10-heptadecenoic acid (C17:1), oleic acid (C18:1) linoleic acid (C18:2), linolenic acid (C18:3) and cis-11,14-eicosadienoic acid (C20:2) (Table 1). Among the mutants and the wild type, the higher content of palmitic acid (34.31 %) and oleic acid (31.92 %) found in CI804 are the most suitable fractions to indicate biodiesel quality. High quantity of butyric (5.11 %) and linoleic acid (15.57 %) in CI803 mutant reveals its usage as a substitute fuel to emulate the combustion properties. Besides, they are essential fatty acids for human beings (Simopoulus, 2008), thereby providing feasibility for extraction to be used in the human diet. The fatty acids profile of CI802 mutant with lauric acid (9.72 %), myristic acid (1.86 %), cis-10-pentadecanoic acid (7.26 %), and arachidic acid (3.67 %) indicates its use as a replacement for cupuacu butter in cosmetics (Cohen and Jackix, 2009). The mutant CI801 exhibited tridecanoic acid (0.90 %), myristoleic acid (0.23 %), palmitoleic acid (6.25 %), stearic acid (5.09 %) and behenic acid (1.88 %). Whereas, wild type (CIWT) recorded higher capric (4.32 %), stearic (8.29 %), behenic (4.98 %) and lignoceric acid (3.39 %). Compared to jatropha (14.66 %) and soybean (9.88 %) (Rangaswamy *et al*., 2017) all the gamma-irradiated mutants accumulated more palmitic acid. However, the major fatty acids comprise myristic, palmitic, stearic, oleic, and linoleic acid responsible for biodiesel production in sufficient quantities in gamma-irradiated mutants. Hence, these gamma-irradiated mutants can be used as a potent feedstock for biodiesel generation.

Biodiesel properties

The chemical constituents of the feedstock determine the biodiesel properties. The chain length and the degree of unsaturation of fatty acid profile define the biodiesel characteristics (Thangavel *et al*., 2018). The estimated and measured biodiesel values must meet the requirements established by national (IS 15607-05) and international standards (ASTM 6751-3 and EN 14214) (Atadashi *et al*., 2010) (Table 2. Thus, the fuel characteristics like kinematic viscosity, density, cetane number, saponification value, high heating value, iodine value, degree of unsaturation and oxidative stability were assessed for vehicular and fuel quality and also for effective engine efficiency. Once the FAME meets the above-mentioned performance parameters, particular FAME can be directly used in unmodified engines as biodiesel (Patel *et al*., 2016). Therefore, in this study, the most important properties of the biodiesel obtained from the fats of gamma-irradiated mutants and the wild type of *Chlorella* sp. KM504965 grown under phototrophic conditions were estimated theoretically (Srinivasan *et al*., 2021). The biofuel properties of the mutants and the wild type will be validated by comparing with the values derived through analysis under laboratory conditions.

The fuel properties like KV, D, HHV, CN, SV, IV, DS and OS were evaluated and given in Table 2. The determined values were compared with IS, ASTM, and EN standards. Among 4 mutants, maximum concentration of saturated fatty acids (53.09 %), monounsaturated fatty acids (36.29 %), and polyunsaturated fatty acids (15.49 %) was recorded in CI802, CI801, and CI803, respectively, whereas the wild type had saturated fatty acids (62.69 %), monounsaturated fatty acids (30.4 %), and polyunsaturated fatty acids (0.38 %). The gamma irradiated mutants had a DU of 52 to 58 %.

Kinematic viscosity (KV)

Biodiesel's kinematic viscosity is one of the main properties that play a crucial role in the combustion of fuel (Mondal *et al*., 2019). The higher the viscosity, the lower will be the flow rate at low temperatures. The viscosity increases with an increase in saturated fatty acid content and length of fatty acid. The kinematic viscosity of algal biodiesel has been reported to be 10-15 % higher than the standard diesel fuels due to its larger molecular structure and weight (Balat 2011, Agarwal 2007, Ahmad *et al*., 2011). The specification as per ASTM D6751 and EN ISO 3104 for KV ranges from 1.9 to 6.0 mm^2 s^{-1} and 3.5 to 5.0 mm^2 s^{-1} respectively and from 2.5 to 6.0 mm^2 s^{-1} by IS 15607. The KV of gamma-irradiated mutants and wild type ranged between 4.7 and 4.92 mm^2 s^{-1} and fits with ASTM, EN, and IS standards. This implies that biodiesel derived from these mutants may atomize and combust completely which may contribute to better engine life (Karmaker *et al*., 2018). The higher limit of viscosity creates performance-related issues, particularly at low temperature, while the lower limit causes fine fuel particles with high velocity and low mass and thus the viscosity is greatly affected by temperature (Knothe *et al*., 2005, Yuan *et al*., 2009).

Density

The gamma irradiated mutants and wild type of *Chlorella* sp. KM504965 recorded density in the range of 0.78 – 0.84 g cm^{-3} which is slightly lower than the standard limits (0.86 – 0.90 g cm^{-3}) set by ASTM EN and IS. Similarly, FAME profile-derived density values of *Chlorella vulgaris* and *Botryococcus terribilis* had a range of 0.82 and 0.84 g cm^{-3} (Nascimento *et al*., 2013). On the contrary, *Scenedesmus dimorphus* recorded a higher value of 0.91 g cm^{-3} (Muhammad *et al*., 2013). The other fuel properties like HHV and cetane number also depend on the density of FAME.

Higher heating value (HHV)

Calorific value implies the value of the heat of combustion (Knothe *et al*., 2005), which is the energy released during fuel combustion. Fuels with HHV are more efficient for smaller engines. The HHV values of FAME of gamma-irradiated mutants and wild type in the range of 35 – 38.15 MJ Kg^{-1} were found to be analogous with values reported for other microalgal species like *Chlorella vulgaris* (38.1 MJ Kg^{-1}) and *Scenedesmus obliquus* (37.5 MJ Kg^{-1}) (Nascimento *et al*., 2013, Muhammad *et al*., 2013). The HHV rises with carbon number in FAME and increases with carbon and hydrogen ratio to oxygen and nitrogen (Ramirez verduzco *et al*., 2012).

Iodine value (IV)

This value determines the double bond present in unsaturated fatty acids which helps in deciding the oxidative stability of the fuel (Mondal *et al*., 2019). The EN 14214 sets a maximum value of 120 mg I^2 100 g^{-1} for IV but ASTM D6751 doesn't define the limits of IV. High IV results in glyceride polymerization and heating that causes the formation of gum. Low IV indicated the fuel's non-corrosive nature. IV of gamma-irradiated mutants and wild type's biodiesel was in the range of 27.45 - 52.18 mg I_2 100 g^{-1} which showed their better quality and falls below the standard EN 14214 maximum set values.

Oxidative stability (OS)

The oxidative stability is expressed in terms of hours. The OS of gamma-irradiated mutants and wild type was in the range of 6.44 h to 10.78 h and 39.83 h respectively; but the value postulated by ASTM and EN were a minimum of 3 and 6 h respectively. The higher value may be due to the presence of more double bonds or unsaturated fatty acids (C18:2 and C18:3) in the biodiesel. Stansella *et al.*, 2012 reported that the greater the OS, the biodiesel's shelf life will be more. The double bond position and number impact the biodiesel properties, which affect the normal combustion of diesel engines. The saponification value of obtained gamma-irradiated mutants and wild type's biodiesel was in the range of 186 to 200 mg g^{-1}.

Cetane number (CN)

The calculated CN value of gamma-irradiated mutants and wild type was greater than the values (61 to 69) specified by ASTM, EN, and IS, and could serve as a better alternative fuel. The optimum concentration of saturated and long straight-chain fatty esters could have been attributed to the higher CN values. The CN has both lower and higher limits. At lower limits, it is responsible for creating problem engine start in cold weather or environmental conditions and might generate noise and gaseous emission without sufficient combustion of biodiesel, while higher CN provides greater biodiesel ignition, better starting behavior in cold conditions, smooth running of the engine and proper combustion resulting in decreased particulate and gaseous emissions (Hoekman *et al*., 2012).

Long-chain saturation factor (LCSF) and Cold flow plugging point (CFPP)

LCSF and CFPP reveal the biodiesel flow performance at low temperatures (Nascimento *et al*., 2013). The wild type (CIWT) exhibited the highest amount of long-chain saturated fatty acids (14.86 %), CFPP (30.21 °C) followed by gamma-irradiated mutants CI802 showed LCSF (10.50 %) and CFPP (16.5 °C) while CI803 showed the lowest amount of LCSF (5.86 %) and CFPP (1.9 °C).

CONCLUSION

Microalgal biodiesel is considered as one of the alternative fuels for future energy requirements. In this context, it is essential to assess the fuel properties of the biodiesel produced using microalgal fat. The results of the present study indicate the theoretical biodiesel properties of the FAME derived from gamma-irradiated *Chlorella* sp. KM504965 mutants. Since, the calculated values satisfy the national and international standards of biodiesel, in the future, FAME/biodiesel from these mutants can be produced on a larger scale and their empirical biodiesel properties can be studied under laboratory conditions. Thus, the study revealed that these gamma-irradiated mutants could benefit biodiesel production at a large-scale level in the future.

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Ethics statement

This article does not contain any studies with human participants or animals performed by any of the authors.

Consent for publication

All the authors agreed to publish the content.

Competing Interests

The authors declare no conflict of interest in publication of this content.

Author contributions

Idea conceptualization-DS and TK, Experiments - DS ,Guidance - TK, Writing original draft – DS and TK, Writing- reviewing & editing - DS and TK.

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Table 1. Comparative analysis of FAME profiles of mutants and wild type of *Chlorella* sp. KM504965 with vegetable oils used in biodiesel production (a. Soybean oil, b. Rapeseed oil) (Srinivasan *et al*., 2021)

Fatty Acid Methyl Ester		FAME profiles of gamma irradiated mutants at late log phase					Vegetable oil (%)	
		CI801	CI802	CI803	CI804	CIWT	a	b
C4:0	Butyric Acid Methyl Ester	-	-	5.11	-	0.1	-	-
C6:0	Caproic Acid Methyl Ester	0.16	0.01	0.06	0.01	0.01	-	-
C8:0	Caprylic Acid Methyl Ester	0.24	0.03	0.03	0.01	0.01	-	-

C10:0	Capric Acid Methyl Ester	1.55	1.84	0.49	0.73	4.32	-	-
C11:0	Undecanoic Acid Methyl Ester	0.75	0.36	0.13	0.02	0.31	-	-
C12:0	Lauric Acid Methyl Ester	5.80	9.72	5.21	6.71	4.76	-	-
C13:0	Tridecanoic Acid Methyl Ester	0.90	0.01	0.02	0.16	0.04	-	-
C14:0	Myristic Acid Methyl Ester	1.80	1.86	1.22	2.05	2	-	-
C14:1	Myristoleic Acid Methyl Ester	0.23	0.02	0.03	0.02	0.66	-	-
C15:0	Pentadecanoic Acid	2.66	2.80	2.92	-	0.52	-	-
C15:1	Cis-10-Pentadecenoic Acid	4.53	7.26	2.78	-	3.7	-	-
C16:0	Palmitic Acid Methyl Ester	28.77	26.56	27.98	34.31	32.35	10-12	2-6
C16:1	Palmitoleic Acid Methyl Ester	6.25	0.24	0.19	0.95	0.54	-	-
C17:0	Heptadecanoic Acid	0.98	1.01	0.55	1.02	1.23	-	-
C17:1	Cis-10-Heptadecenoic Acid	0.90	0.06	0.35	0.44	0.59	-	-
C18:0	Stearic Acid Methyl Ester	5.09	3.62	1.93	-	8.29	3-5	4-6
C18:1	Oleic Acid Methyl Ester	24.38	20.79	23.58	31.92	24.91	18-26	52-65
C18:2	Linoleic Acid Methyl Ester	7.97	14.78	15.47	9.98	0.34	49-57	18-25
C20:0	Arachidic Acid Methyl Ester	-	3.67	0.06	0.01	0.01	-	-
C18:3	ϒ-Linolenic Acid Methyl Ester	0.38	0.49	0.25	0.44	0.03	6-9	10-11
C21:0	Heneicosanoic Acid	-	0.03	0.02	0.05	0.37	-	-
C20:2	Cis-11,14-Eicosadienoic Acid	0.17	0.03	0.02	0.01	0.01	-	-

C22:0	Behenic Acid Methyl Ester	1.88	1.57	1.36	3.24	4.98	-	-
C24:0	Lignoceric Acid Methyl Ester	-	-	-	-	3.39	-	-
Others		4.61	3.24	10.24	7.92	6.53		

Table 2. Biodiesel properties of FAME derived from Cl801, Cl802, Cl803, Cl804 mutant and wild type ClWT

Fuel parameters	Cl801	Cl802	Cl803	Cl804	ClWT	Approved specifications for biodiesel		
						US limits ASTM D6751	Europe limits EN 14214	Indian limits IS 15607
SFA (%)	50.58	53.09	47.09	48.32	62.69	-	-	-
MUFA (%)	36.29	28.37	26.93	33.33	30.4	-	-	-
PUFA (%)	8.52	15.3	15.74	10.43	0.38	-	-	-
Degree of unsaturation	53.33	58.97	58.41	54.19	31.16	-	-	-

Cetane number	63.3	61.80	62.16	64.99	68.7	47 min	51 min	51 min
Iodine Value (mg $I_2$100g^{-1})	47.38	52.18	50.87	47.04	27.45	-	120 max	-
High Heating Value (MJ kg^{-1})	37.62	38.15	34.93	36.44	36.99	>35	-	-
Kinematic Viscosity at 40 °C (mm^2 s^{-1})	4.88	4.90	4.73	4.88	4.92	1.9-6.0	3.5-5	2.5-6.0
Density at 20 °C (g cm^{-3})	0.83	0.84	0.78	0.80	0.81	0.86-0.90	0.86-0.90	0.86-0.90
Saponification Value (mg g^{-1})	197.34	200.35	199.86	186.43	191.03	0.50 max	0.50 min	0.5
Long chain saturation factor (wt %)	8.24	10.50	5.86	8.30	14.86	-	-	-
Cold flow plugging point (°C)	9.4	16.5	1.9	9.6	30.21	-	-5 to -15	-
Oxidation Stability (h)	10.78	6.60	6.44	9.06	39.83	3 h min	6 h min	-
Concentration of C18:3 (%)	<1	<1	<1	<1	<1	-	12 max	-
FAME having ≥4 double bonds	ND	ND	ND	ND	ND	-	1max	-