INDIAN JOURNAL OF SCIENCE AND TECHNOLOGY

RESEARCH ARTICLE

Biodiesel (fatty acid methyl ester) from Chlorococcalean *Chlorella vulgaris* by single step *in-situ* transesterification

Sanjaykumar Dalvi[1]*, Swati Sonawane[2]

1 Head of Physics Department, S. N. Arts, D.J. M. Commerce & B. N. S. Science College (Autonomous), 422605, Sangamner, India
2 Assistant Professor, Department of Chemistry, H. P. T. Arts and R. Y. K. Science College, 422005, Nashik, India

OPEN ACCESS

Received: 07.02.2022
Accepted: 21.02.2022
Published: 25.03.2022

Citation: Dalvi S, Sonawane S (2022) Biodiesel (fatty acid methyl ester) from Chlorococcalean *Chlorella vulgaris* by single step *in-situ* transesterification. Indian Journal of Science and Technology 15(12): 535-541. https://doi.org/10.17485/IJST/v15i12.312

* **Corresponding author**.

dalvi@sangamnercollege.edu.in

Funding: None

Competing Interests: None

Published By Indian Society for Education and Environment (iSee)

ISSN
Print: 0974-6846
Electronic: 0974-5645

Abstract

Objectives: To study the single step in-situ transesterification process for synthesis of fatty acid methyl ester (biodiesel) from microalgae biomass Chlorococcalean *Chlorella vulgaris*. **Methods:** The growth and lipid productivity of an isolated microalgae *C. vulgaris* were studied under Chu's 10 modified media under phototrophic cultivation conditions. *In-situ* transesterification of this dry biomass with methanol in presence of base catalyst at 60 ° C was carried out to investigate the fatty acid methyl ester's biochemical composition by FTIR & analysis performed using GCMS technique. The cetane number was also calculated. **Findings:** The biodiesel fraction from *C. vulgaris* biomass was found to be 85.58%. The 43.40% lipid fraction was obtained from the biomass of *C. vulgaris*. In the in-situ transesterification process, the optimal concentration of potassium hydroxide (KOH) and methanol yields an 85.58 percent biodiesel fraction. The presence of lipid compounds and the biochemical composition of fatty acid methyl ester were verified with the help of FTIR spectral analysis. The gas chromatography-mass spectrometry analysis explores the fatty acid methyl ester profile, which comprises 29.05% and 56.54 percent saturated and unsaturated FAMEs. Through GCMS, the fatty acid methyl ester composition of *C. vulgaris* microalgae species was revealed through six types of fatty acid methyl esters, of which 54.95%, 11-octadecenoic acid methyl ester, is the dominant one. The cetane number of *C. vulgaris* biodiesel was determined to be 67.726; this is comparable to diesel fuel according to ASTM-D613, and the presence of a higher fatty acid methyl ester composition indicates favourable biofuel qualities. **Novelty:** The calculation of cetane number by theoretical method and affirmation of ecofriendly single step in-situ transesterification method for biodiesel production.

Keywords: Chlorococcalean Chlorella vulgaris; Biodiesel; GCMS; FTIR; Cetane Number

1 Introduction

Continuous global population expansion, fast industrialization, urbanisation, and economic growth all compel a rise in fossil fuel used to fulfill rising energy demand [1]. Using alternative fuels is the most practical approach to fulfill rising energy demand. Biofuel from algal biomass is an alternative fuel to petroleum that has a lot of potential. Algal biomasses are promising alternative fuels due to their high growth rate, high photosynthetic activity, large marine farming scale for mass cultivation, small land requirements, high biomass conversion rate and ease of handling, and reduced, potentially net-zero CO2 emissions [2]. The purity of the culture was confirmed by repeated plating and by regular observation under a microscope. Microalgae harvested using centrifugation and drying of algae at 60°C for vaporization of water. The biochemical composition of dry *C. vulgaris* biomass determined by FTIR. Algal biomass can also be used to make biodiesel. Trans esterifying triglycerides (TGs) or esterifying free fatty acids (FFAs) with light alcohol in the presence of a base, acid, or enzyme catalyst produce biodiesel fuel [3,4]. Because microalgae-based biofuel has advantages over traditional fossil fuels, there is a growing global recognition that they are both environmentally beneficial and economically viable solutions [5]. Quick growth, high lipid content, and excellent yield make algae a substitute for biofuel production in contrast with non-edible and edible biofuels [6]. Biodiesel can be formed from micro emulsification, pyrolysis, thermal cracking, and transesterification processes [7]. As per among the all biodiesel production processes; transesterification is the most suitable process, because it produces biodiesel of high yield, comparable properties with diesel. This process is also feasible as per economic point of view. The energy demand of future can be met by the blending of different generation oil feedstocks [1]. The fatty acid composition and the functional groups present in oil and biodiesel were determined by Gas Chromatography Mass Spectrometry (GC-MS) and Fourier Transform Infrared Spectrometry (FTIR) techniques [8]. Moreover, the physico-chemical attributes of the resultant biodiesel must be determined to examine its suitability for application in diesel engines and check its compliance with the established standards like the American Society for Testing and Materials (ASTM) and the European norms (EN); cetane number (CN) is one of the most important physico-chemical property of biodiesel induced mainly by its parent feedstock and controlled partly by the production and refining processes [9]. The performance of diesel fuel is measured using cetane rating or also well-known as cetane number. The greater the value, the enhanced the rate at which fuel burns in an engine of an automobile [10].

In this article, the development of monoculture of isolated microalgae, growth study of microalgae *C. vulgaris* under Chu's 10 modified media, in-situ transesterification of microalgae biomass, determination of the biological composition of microalgae by FTIR, fatty acid methyl ester profile by GC-MS and CN of prepared bio-fuel are discussed. The objective of this work is to reduce the cost of biofuel production by in-situ transesterification of microalgae Chlorcoccalean *Chlorella vulgaris* (*C. vulgaris*) and to maximize the fatty acid methyl ester (biodiesel) production.

2 Materials and Methods

2.1 Chemicals

Methanol (99.8%), Potassium hydroxide (pellets) purchased from Merck were analytical grade reagents.

2.2 Algae Assessment

Microscopic algae were collected with a mesh net 25-30 μm pores by simply scooping a jar through the fresh water river Godavari, Kopargaon (M.S.) India. The water sample was observed under a research microscope to define the morphological characteristics of microalgae by using standard literature and monographs. The growth study of *C. vulgaris* was carried out under phototropic cultivation conditions. The Chu's10 modified media consisted of trace elements in fresh water solution (mg/lit): Ca (NO3)2 (0.04); K2HPO4 (0.01); MgSO4.7H2O (0.0250); Na2CO3 (0.02); Na2SiO3 (0.025); FeCl3 (0.008). The water samples of 10 ml were transferred to a 500 ml conical flask containing 200 ml of sterilized Chu s10 modified media and then incubated on a rotary shaker at 27 °C and 150 rpm under continuous illumination using white fluorescent light at intensity of 40μmol/m^2/s. Every two days, the flasks were examined for algal growth using an optical microscope, with serial dilutions being made in Chu s 10 modified media from flasks showing growth. Subcultures were made by inoculating 50 μl culture solution onto petri plates containing Chu's10 modified media solidified with 1.5% (w/v) of bacteriological agar. These procedures were repeated for each of the original flasks. The Petri plates were incubated at 27 °C under continuous illumination for two weeks. Repeated plating and regular observation under a microscope confirmed the purity of the culture. Microalgae were extracted by centrifugation and dried at 60°C for water vaporization. FTIR was used to evaluate the biochemical composition of dry *C. vulgaris* biomass.

2.3 In-situ transesterification of *C. vulgaris* Microalgae

A two-necked round bottom flask with a thermometer and condenser to collect fine dry *C. vulgaris* powder biomass. The 40 mg of microalgae powder was mixed with 7 ml of methanol and 0.05 M of potassium methoxide. The reaction mixture was heated continuously with constant stirring at 60 °C for 60 minutes at 400 rpm.

The reaction mixture was cooled to room temperature and then separation of the algal residue and product was done. The algal residue was mixed with petroleum ether to collect the remaining product. The petroleum ether mixed with methanol to separate unreacted oil from algae powder. The petroleum ether layer containing the fatty acid methyl ester was washed with distilled water to remove impurities like catalyst, glycerol etc. The mixture of product and water was kept for 8 hours.

The impurities were decanted and repeated water washes were given till the pH of the decanted water nearly became the same as distilled water. After water washing, the final product was heated to 80 °C. Finally, the prepared FAME product was preserved in an airtight container and analyzed by gas chromatography mass spectrometry. Figure 1 shows the single-step in-situ transesterification process.

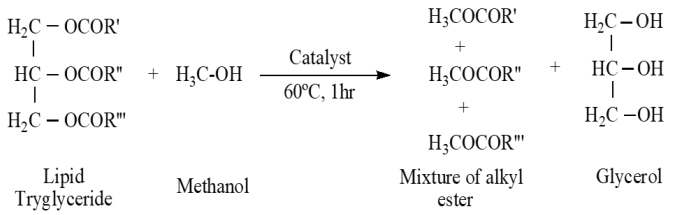

Fig 1. In-situ transesterification of microalgae lipidtriglyceride to (alkyl esters) biodiesel

2.4 Fourier Transforms Infrared Spectroscopy Analysis

The functional groups of *C. vulgaris* microalgae biomass and its fatty acid methyl ester (biodiesel) were studied using FTIR. The FTIR spectra were done on an FTIR-8400, Shimadzu's Pvt. Ltd. The dried algal cells were mixed with KBr pellet powder prior to scanning. The spectrum was collected in the mid-IR range (64 scans) from 4000 to 800 cm-1. Each spectral curve's peak was meticulously observed.

2.5 Gas Chromatography Mass Spectroscopy Analysis

The analysis of the fatty acid methyl ester profile of *C. vulgaris* microalgae was carried out by GC-MS. The Shimadzu's gas chromatography system with a flame ionization detector is installed with a capillary polysiloxane glycol column (30 m × 0.25 mm = 0.25 μm thickness, BPX 70, 0.25 μm film). The 0.2 μl of the sample was injected with a split ratio of 1:60. The oven's initial temperature was 180 °C, which remained constant for 3 minutes. Then the temperature was increased from 180 °C to 260 °C at an incremental rate of 4°C/min and was kept constant at 260 °C for 15 minutes. The injector temperature was 220°C and the detector was set at 240°C, respectively. Nitrogen was used as the carrier gas, and the column flow rate was 1 ml/min.

2.6 Cetane Number estimation

Cetane number (CN) is the capacity of fuel to light rapidly in the wake of being infused. Higher the CN esteem, the better the start nature of the fuel. The CN is one of the significant boundaries, which are considered during the determination of unsaturated fat methyl ester (Acclaim) as biodiesel. It was determined by utilizing condition (1) as follows:

$$Cetane\ Number\ (CN) = 46.3 + 5458/SN - 0.225 \times IV \quad (1) \tag{1}$$

The saponification number (SN) is the quantity of milligram of potassium hydroxide expected to saponify 1 gram of fat under the predefined conditions. It is the proportion of the normal atomic load of the relative multitude of unsaturated fats present. It was determined by utilizing condition (2).

$$SN = \sum 560 \times Ai/MWi \tag{2}$$

The iodine value (IV) is the measure of the amount of unsaturated content present in fatty acids. The iodine value is directly proportional to the number of C=C. The higher the unsaturation represents, the higher the iodine value. It was calculated by

using equation (3).

$$IV = \sum 254 \times D \times Ai/MWi \tag{3}$$

Where,

- Ai : Acid percentage
- MWi : Molecular mass of each component
- D : The number of double bond

3 Results and Discussion

The growth and lipid productivity of an isolated microalgae, Chlorococcalean *C. vulgaris*, were studied under Chu's 10 modified media under continuous illumination using white fluorescent light at intensities of 40 μmol/m2/s. The biomass productivity of *C. vulgaris* was observed to be 0.629 gl-1 d-1 and the lipid extracted from biomass was 0.273 gl-1 d-1. The obtained lipid content was 43.40%. The cell growth of *C. vulgaris* was dependent on light intensity [11] ; the statement is verified.

Similarly, 44–47% lipid content was obtained at 50 and 100 mol photons m-2s-1, whereas 35–40% was obtained at 20–400 mol photons m-2s-1; thus, our results for *C. vulgaris* in Chu's 10 modified media were comparable to the referred literature. Chemical conversion of *C. Vulgaris* microalgae biomass through an in-situ transesterification process yields a mixture of fatty acid methyl esters.

The FTIR characteristic spectral signatures (4000–400 cm^{-1}) of lipid from *C. vulgaris* microalgae species are presented in Figure 2.

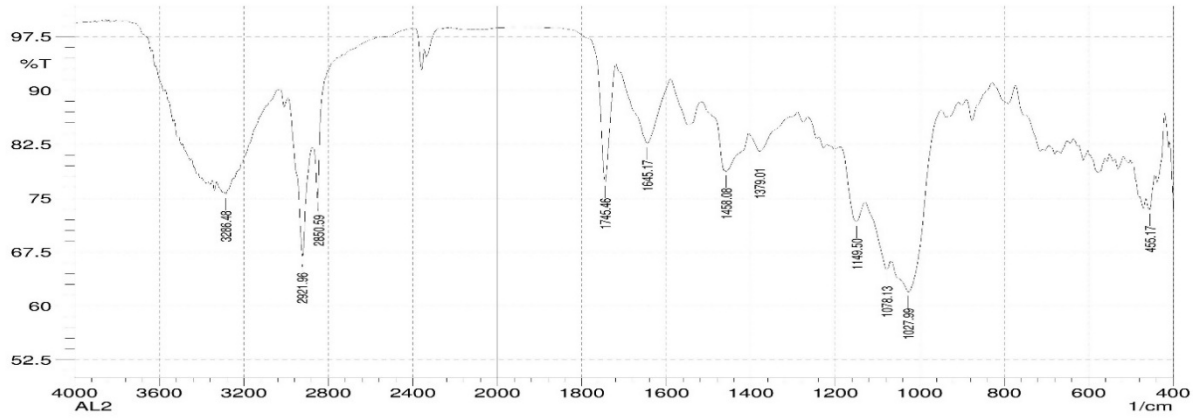

Fig 2. FTIR Spectrum of *C. vulgaris* microalgae biomass.

The absorption bands in the FTIR spectrum are indicative of the presence of hydroxyl (-OH), carbonyl (C=O), aromatic C, alkane, alkene, and amide groups in the algae species. The band at 3286 cm-1 is due to O-H stretching vibrations in the algal species. A band with a tall peak at 2921.96 cm-1 can be attributable to asymmetric C –H vibrations, mostly due to methylene groups of lipids. The prominent bands present at ~1745.46–1645.17 cm-1 are due to the presence of C=O of ester or fatty acids. A small peak at ~1458.08–1379.01 cm-1 is due to the aromatic C=C bond associated with ester. A prominent band at ~ 1027.99–1149.50 cm-1 in the studied algal species was due to the C–O–C stretching in polysaccharides. The small bands found 800.00–455.17 cm-1 are attributed to aliphatic and aromatic C–H bending. FTIR spectra showed different absorption bands were assigned to specific functional groups following biochemical standards and published literature [12]. The FTIR spectrogram of *C. vulgaris* microalgae species has a specific spectral signature in terms of lipids and other biochemical compositions.

The FTIR characteristic spectral signatures of the fatty acid methyl ester of *C. vulgaris* (4000–400 cm^{-1}) were obtained after an in-situ transesterification process (Figure 3).

The absorption band in the FTIR spectrum of fatty acid methyl esters is indicative of the presence of carbonyl (C = O), alkoxy (C-O), alkane (C = C) and aliphatic (C–H) functional groups. A band with a tall peak at ~ 2922.24 – 2853.62 cm-1 can be attributable to asymmetric alkane (C – H) vibration stretching due to the methylene group of the fatty acid methyl ester. The characteristic sharp band present at ~ 1743.18 cm-1 is due to the presence of the carbonyl (C = O) group of an ester. The

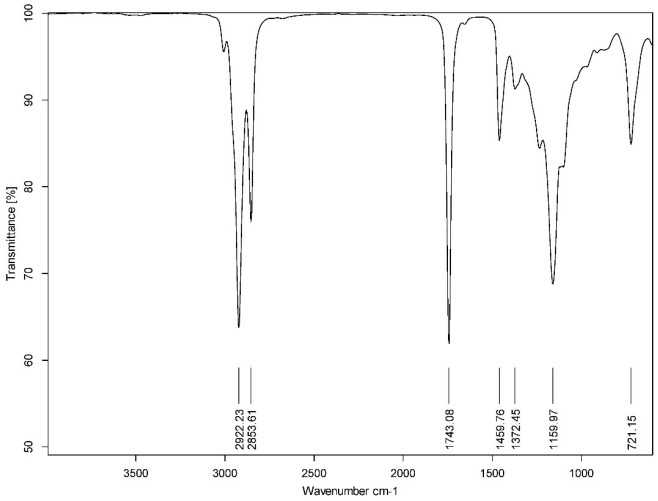

Fig 3. FTIR Spectrum of *C. vulgaris* fatty acid methyl ester (Biodiesel)

small peak at ~ 1459.75–1372.60 cm-1 is due to the alkene (C = C) bond associated with ester. The prominent peak at ~ 1160.60 cm-1 is due to alkoxy (C–O) stretching vibration. The small pointed peak at ~ 721.40 cm-1 is attributed to aliphatic (C–H) bending. The FTIR analysis shows that the spectrograms of *C. vulgaris* biomass and its fatty acid methyl ester showed different peak intensities. The hydroxyl band (-OH) frequency~3286.00 cm-1 appears in the *C. vulgaris* biomass spectrogram while it is not observed in the fatty acid methyl ester spectrum after the in-situ transesterification process. Through GCMS, the fatty acid methyl ester composition of *C. vulgaris* microalgae species was revealed through 6 types of fatty acid methyl esters.

Fatty acid methyl esters (FAME) are obtained at retention times (min) of 8.914, 10.58, 11.94, 12.11, 13.33, and 13.47. The four components of biodiesel at retention times of 8.914, 10.58, 12.11, and 13.47 show the base peak at m/z 74.05. This characteristic peak occurs due to McLafferty rearrangement; the one component at 11.94 shows a base peak at m/z 81.5 and the other component, at 13.33, shows a base peak at m/z 55. Table 1 shows the peak obtained with various retention times, percentage areas, names of the obtained compounds, and molecular formulae. Figure 4 shows the molecular structures of FAMEs of the obtained fatty acid methyl ester (biodiesel) product.

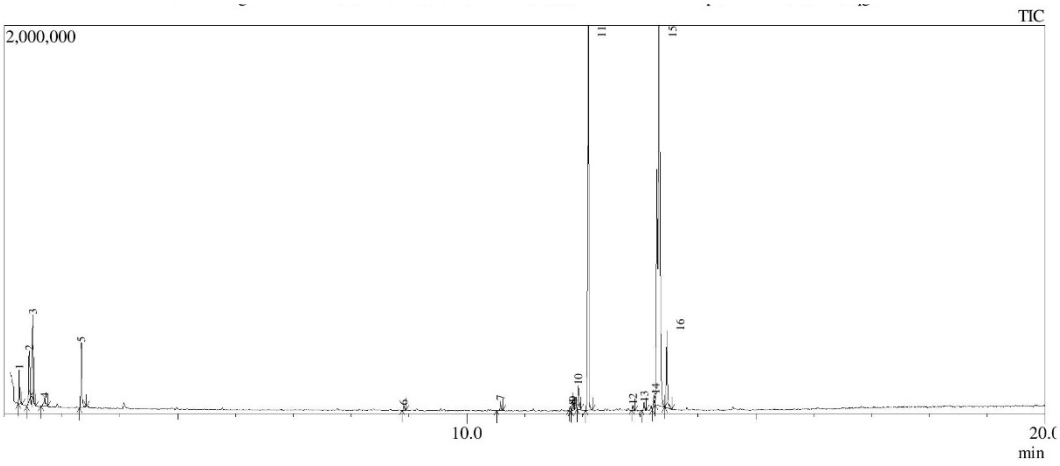

Fig 4. Gas chromatogram of *C. vulgaris* microalgae FAME's.

The *C. vulgaris* FAME (Biodiesel) has a higher content of 11-Octadecenoic acid, methyl ester (54.95%), Pentadecanoic acid, 14-methyl-, methyl ester (23.75%), Octadecanoic acid, methyl ester (4.59%), 5,8-Octadecadienoic acid, methyl ester (1.59%), Decanoic acid, methyl. The *C. vulgaris* biodiesel contains 29.05% saturated FAMEs and 56.54 percent unsaturated FAMEs. The obtained FAMEs ranged from C9 to C19. FAME analysis revealed a large quantity of polyunsaturated fatty acids.

Table 1. Fatty Acid Methyl Esters profile of *C. vulgaris* Microalgae

Peak	Retention Time	Area%	Name of the Compound	Molecular Formula
1	8.914	0.26	Octanoic acid, methyl ester	$C_9H_{18}O_2$
2	10.58	0.45	Decanoic acid, methyl ester	$C_{11}H_{22}O_2$
3	11.94	1.59	5,8-Octadecadienoic acid, methyl ester	$C_{19}H_{34}O_2$
4	12.11	23.75	Pentadecanoic acid, 14-methyl-, methyl ester	$C_{17}H_{34}O_2$
5	13.33	54.95	11-Octadecenoic acid, methyl ester	$C_{19}H_{36}O_2$
6	13.47	4.59	Octadecanoic acid, methyl ester	$C_{19}H_{38}O_2$

As per our calculations, the CN of *C. vulgaris* was 67.726. As per European specifications EN 14214:2008, the minimum biodiesel CN is 51, whereas in the U.S. (ASTM D6751), the minimum acceptable value is 47. Our results clearly indicate that the CN of prepared biodiesel fuel satisfies both the standards. The determination of CN experimentally is difficult and time-consuming too. Therefore, the theoretical models are quite reasonable and predictive, as in this case. The fatty acid methyl ester composition provides a good balance for oxidation for longer storage and a decreased cold filter plugging point for use in cold places, as well as good biofuel properties. Hence, it gives confirmation to the previous work done that the *C. vulgaris* microalgae can be utilized as a decent discretionary possibility for energy fuel, which can be obtained by the single-stage in-situ transesterification process.

4 Conclusion

The present study highlights the affirmation of a single-step in-situ transesterification process for the synthesis of fatty acid methyl ester (biodiesel) from microalgae *C.vulgaris*. The optimal concentration of potassium hydroxide (KOH) and methanol in the in-situ transesterification process yields a good percentage of biodiesel fraction. The FTIR and GCMS have proved to be one of the confirmative tools for the characterization of biodiesel as it enables faster prediction of FAMEs production. The theoretical CN calculation method is also a satisfactory way to minimize the time for the CN value of prepared fuel. The presence of a higher composition of fatty acid methyl ester exhibits good biofuel properties such as oxidative stability, lubricity, and quality ignition. The study revealed that the single step of in-situ transesterification reduces the process cost. The harmful organic solvent, hexane, is not utilized at all in this method. Hence, fatty acid methyl ester synthesis through in-situ transesterification is eco-friendly. The profile of fatty acid methyl esters produced by Chlorococcalean *C. vulgaris* indicates it is an optimal fuel source for combustion. It contributes as a key solution to issues such as energy security, economic development, climate change, and energy security.

References

1) Singh D, Sharma D, Soni SL, Sharma S, Sharma PK, Jhalani A. A review on feedstocks, production processes, and yield for different generations of biodiesel. *Fuel*. 2020;262:116553–116553. Available from: https://dx.doi.org/10.1016/j.fuel.2019.116553.
2) Khan MI, Shin JH, Kim JD. The promising future of microalgae: current status, challenges, and optimization of a sustainable and renewable industry for biofuels, feed, and other products. *Microbial Cell Factories*. 2018;17(1):1–21. Available from: https://dx.doi.org/10.1186/s12934-018-0879-x.
3) Aghbashlo M, Tabatabaei M, Rastegari H, Ghaziaskar HS, Valijanian E. Exergy-based optimization of a continuous reactor applied to produce value-added chemicals from glycerol through esterification with acetic acid. *Energy*. 2018;150:351–362. Available from: https://dx.doi.org/10.1016/j.energy.2018.02.151.
4) Ziyai MR, Mehrpooya M, Aghbashlo M, Omid M, Alsagri AS, Tabatabaei M. Techno-economic comparison of three biodiesel production scenarios enhanced by glycerol supercritical water reforming process. *International Journal of Hydrogen Energy*. 2019;44(33):17845–17862. Available from: https://dx.doi.org/10.1016/j.ijhydene.2019.05.017.
5) Ray A, Nayak M, Ghosh A. A review on co-culturing of microalgae: A greener strategy towards sustainable biofuels production. *Science of The Total Environment*. 2022;802:149765–149765. Available from: https://dx.doi.org/10.1016/j.scitotenv.2021.149765.
6) Kumar SC, Thakur AK, Aseer JR. Algae Biofuel as a Substitute for Compression Ignition Engine: A Review. *Lecture Notes in Mechanical Engineering*. 2022;I:43–51. Available from: https://doi.org/10.1007/978-981-16-5371-1_5.
7) Ghedini E, Taghavi S, Menegazzo F, Signoretto M. A Review on the Efficient Catalysts for Algae Transesterification to Biodiesel. *Sustainability*. 2021;13(18):10479–10479. Available from: https://dx.doi.org/10.3390/su131810479.
8) John CB, Raja SA, Deepanraj B, Ong HC. Palm stearin biodiesel: preparation, characterization using spectrometric techniques and the assessment of fuel properties. *Biomass Conversion and Biorefinery*. 2021. Available from: https://dx.doi.org/10.1007/s13399-020-01265-0.
9) Aghbashlo M, Peng W, Tabatabaei M, Kalogirou SA, Soltanian S, Hosseinzadeh-Bandbafha H, et al. Machine learning technology in biodiesel research: A review. *Progress in Energy and Combustion Science*. 2021;85:100904–100904. Available from: https://dx.doi.org/10.1016/j.pecs.2021.100904.
10) Kipkorir D, Nturanabo F, Tewo R, Rutto H, Enweremadu C. Properties of waste-distilled engine oil and biodiesel ternary blends. *Heliyon*. 2021;7(8):e07858–e07858. Available from: https://dx.doi.org/10.1016/j.heliyon.2021.e07858.

11) Loh SH, Chen MK, Fauzi NS, Aziz A, Cha TS. Enhanced fatty acid methyl esters recovery through a simple and rapid direct transesterification of freshly harvested biomass of Chlorella vulgaris and Messastrum gracile. *Scientific Reports*. 2021;11(1):1–9. Available from: https://dx.doi.org/10.1038/s41598-021-81609-6.
12) Krisnangkura K. A simple method for estimation of cetane index of vegetable oil methyl esters. *Journal of the American Oil Chemists' Society*. 1986;63(4):552–553. Available from: https://dx.doi.org/10.1007/bf02645752.