Nature Environment and Pollution Technology
An International Quarterly Scientific Journal

p-ISSN: 0972-6268
(Print copies up to 2016)
e-ISSN: 2395-3454

Vol. 22 | No. 4 | pp. 1951-1960 | 2023

Original Research Paper

https://doi.org/10.46488/NEPT.2023.v22i04.021

Open Access Journal

Biodiesel from *Dunaliella salina* Microalgae Using Base Catalyzed Transesterification – An Assessment through GC/MS, FTIR and NMR Studies

V. Hariram*†, M. Janarthanan*, R. Christu Paul, A. Sivasankar*, M. Wasim Akram*, E. Sangeethkumar**, V. Ramanathan**, P. Sajid Khan* and S. Manikanta Reddy***

*Department of Mechanical Engineering, Hindustan Institute of Technology and Science, Padur, Chennai-603103, Tamil Nadu, India

**Department of Automobile Engineering, Hindustan Institute of Technology and Science, Padur, Chennai-603103, Tamil Nadu, India

†Corresponding author: V. Hariram; connect2hariram@gmail.com

Nat. Env. & Poll. Tech.
Website: www.neptjournal.com

Received: 14-03-2023
Revised: 24-04-2023
Accepted: 27-04-2023

Key Words:
Dunaliella salina
Phytochemical analysis
Transesterification
Ultrasonic extraction
FTIR

ABSTRACT

Algal biofuels are a promising renewable feedstock to produce energy that can supplement future energy demands greatly. The present study aims to utilize Dunaliella salina, a hypersaline, unicellular greenish-orange micro-algae, to produce bio-oil. F/2 nutrient media and trace metal and vitamin solution under carbon-dioxide-rich conditions were used to cultivate the microalgae. Ultrasonic extraction method at 60 Hz for 90 min isolated 650 mL of bio-oil. A single-stage based-catalyzed transesterification process with methanol and sodium hydroxide yielded 380 mL of Pure *Dunaliella salina* biodiesel at % an extraction efficiency of 87%. The Phytochemical screening on the cultivated *Dunaliella* sp. was performed to understand its feasibility to be used as a fuel for IC engines. Furthermore, the obtained biodiesel was characterized using Fourier Transform Infrared Spectrometer (FTIR), Gas Chromatography Mass Spectrometer (GCMS), and Nuclear Magnetic Resonance (NMR) spectral analysis.

INTRODUCTION

Global warming and environmental pollution concerns have arisen as a universal problem mainly due to non-renewable petroleum reserves. Continuous growing population, impulsive scarcity of energy, food, unpredictable weather, and insufficiency of cultivable land threaten global economic development (Hariram et al. 2017). Improbability in the availability of petroleum reserves acts up to the above factor leading to economic crises. Notable biomedical, biotechnology, and biological improvements triggered the researchers to reconnoiter feedstocks for new and renewable sources, thereby replacing the petroleum reserves. Among the feedstocks for deriving the biofuel, micro-algal sources gained importance in recent times due to their scalable adaptation Algal omic approach in the abiotic and biotic adaptation environment to understand a micro-algal growth response in a diversified condition created a new pathway to generate algal feedstocks for biofuel preparation (John et al. 2018).

Among the available microalgae, marine algal strain is a prominent sustainable choice for bio-oil production. As it requires primary seawater with a nominal quantity of micro and macronutrients for large-scale production, the further marine micro-algal feedstock is more advantageous than terrestrial vegetable feedstock due to their higher liquid biomass production rate, Superior photosynthesis and lesser requirement of arable land. Marine micro-algal biofuel production focuses on the design of a photobioreactor, micro macronutrients for liquid and biomass enhancement, Techniques for harvesting the biomass, and finally, liquid extraction. Further, the transformation of the extracted bio-oil into its fatty acid methyl ester places a thorough transesterification technique along with Sodium hydroxide and Methanol place a role. Difficulties in estimating the free fatty acid content lead to unsuitable esterification technique selection and partial transesterification leading to soapy sludge development. The transesterification efficiency also depends upon various operational parameters like molar ratio, reaction temperature, reaction time, and catalyst concentration. The resultant biodiesel is also subjected to a few spectroscopic characterization studies like Gas chromatography Mass Spectrometry (GC-MS) analysis, Fourier Transform Infrared Spectrometry (FT-IR) analysis,

and Nuclear Magnetic Resonance (NMR) Spectrometry to understand its feasibility to be used as a substitute fuel in internal combustion engines.

Sarpal et al. (2016) investigated the potential of biomass productivity from *Spirulina* sp., *Chlorella* sp., and *Tetraselmis sp*. They employed the GC-MS and NMR techniques to identify its fatty acid content. Modified RM6, F/2, and WC micro nutrient mediums were employed in microalgae cultivation with nitrogen and phosphate-rich conditions. The laboratory scale cultivation used an ultrasonicator to extract the liquids and was esterified. The GC-MS analysis identified the presence of polar and neutral fatty acids, and NMR analysis revealed the presence of polyunsaturated fatty acids (C18 to C22). Mathimani et al. (2015) asserted an efficient methodology to transesterify chlorella sp. (marine microalgae) using homogenized acidic catalytic transesterification. A maximum of 60% biodiesel yield was noticed at a reaction time of 2.5 h, and 3.5% of sulphuric acid Prominent proportion of Oleic acid, Palmitic acid, and Palmitoleic acid were found in the biodiesel using the Gas Chromatogram. Chisti (2007) has thoroughly reviewed extracting biodiesel from microalgae. He has demonstrated that an increase in global need would be met only with the continuous supply of renewable biodiesel from microalgae. It was also evidenced that algae production from micro-algal feedstock was found to be many folds than the best oil-yielding vegetable crop.

Chailleux et al. (2013) carried out an algo-route investigation to identify various compounds present in microalgae and their rheological characterization. Thermal-dependent and chemical-dependent behavior was noticed in the microalgae characterized through Gas Chromatography, Mass spectrometry, Nuclear Magnetic resonance, and Infrared analysis. Shah and Veses (2016) derived biofuel from *Spirogyra* sp., a freshwater microalgae. The high-density energy fuel (bio-oil) was extracted through pyrolysis techniques (A thermal process without oxygen) in multi-steps. The operating temperature was increased from 25° Centigrade to 650° Centigrade at equal internal time, and the resultant product was characterized using GC-MS and FT-IR analysis. 2, 3, 5 Trimethyl Pyrazole, a long-chain hydrocarbon, was found in a notable proportion up to 20.086%. Yasir Al-Shikaili et al. (2022) used Fourier transform Infrared Spectrometry to analyze the quantity and quality of neutral liquids in Nannochloropsis Salina microalgae. Micro oven drying of 40% centigrade for 12 h and processing the algal sample with potassium bromide transformed the algal feedstock into the fillets. The neutral liquid was identified in the FT-IR spectrum due to its characteristic absorption band between 1742 cm^{-1} and 2920 cm^{-1}. Sarpal et al. (2015) monitored the liquid content of the microalgal biomass by employing the Nuclear Magnetic Resonance technique throughout the cultivation and biodiesel production face.

Laboratorial strains of *Scenedesmus ecornis* and *Chlorella vulgaris* were employed in this study to assess polar and neutral liquids and fatty acid profiles in the microalgal biomass. The ^{13}C and 'H NMR analyses were very effective in monitoring the enhancement in liquid productivity of the micro-algal samples. The fatty acid content using GC-MS analysis revealed a similar hydrocarbon structure as that of fish and vegetable oil with enhanced neutral liquid content in the micro-algal strains. Bisht et al. (2021) adopted the nuclear Magnetic Resonance approach to assess metabolic capabilities like lipidomics and metabolomics activities. Wahlen et al. (2013) derived biodiesel from oleaginous microalgae through the transesterification process from Bacteria, Yeast, and Microalgae. The feasibility of using biodiesel in a C. I engine was performed, and its effect on the exhaust emissions was evaluated.

Most of the reported literature above has analyzed the potential of micro-algal oil using various spectroscopic studies towards bio-oil yielding capability. In the present investigation *Dunaliella* sp., a marine single-cell algae, was cultivated in a controlled environment using an F/2 nutrient medium, and its liquid content was isolated using centrifugation. The bio-oil was expelled by employing an ultrasonicator. Characterization and phytochemical analyses were carried out on the expelled bio-oil and further trans-esterified. The feasibility of the derived biodiesel to be used as a substitute fuel in an IC engine was carried out by employing GC-MS, FT-IR, and NMR Spectral techniques.

MATERIALS AND METHODS

Dunaliella salina is a unicellular greenish-orange halophile microalgae found abundant in the salina environment. *Dunaliella salina* belongs to the Division of Chlorophyta, class of Chlorophyceae, Order of Chlamydomonas, family of Dunaliellaceae Genes of Dunaliella, and Species of *Dunaliella salina* (Fig. 1). The biomass of *Dunaliella salina* was reported to comprise carotenoids and lipids in prominent proportions.

The National Institute of Ocean Technology, Chennai, Tamilnadu, India, provided the algal strain of Dunaliella Salina. The biomass cultivation of the *Dunaliella* sp. was organized in a lab scale model using three sets of Erlenmeyer flasks under acidic conditions. Each Erlenmeyer flask was filled with 200 mL of F/2 nutrient medium under nitrogen-deficient conditions. The F/2 nutrient medium was prepared

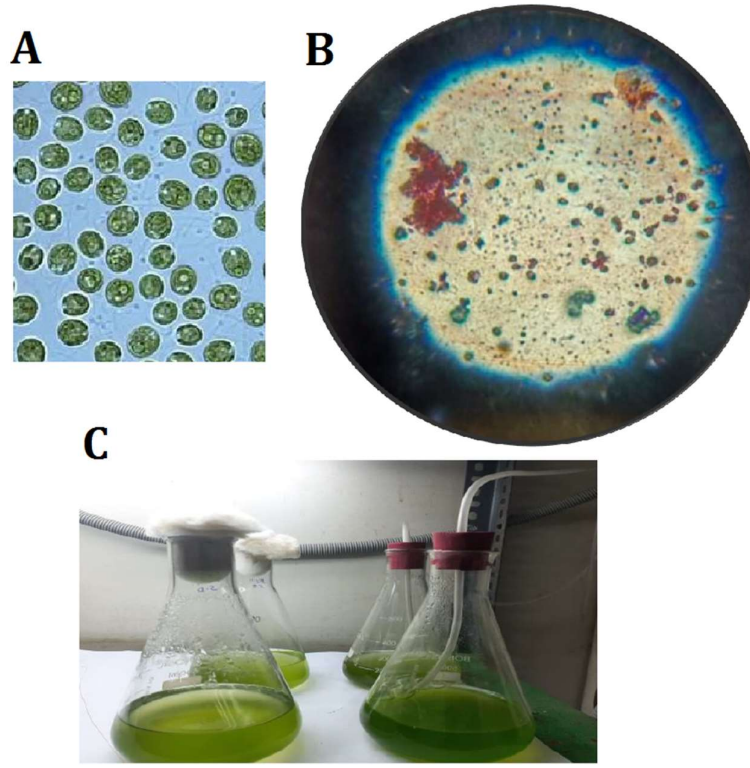

Fig. 1: *Dunaliella salina* – Morphology (A and B) and Growth (C).

in three stages. Stage one comprises the stock solution followed by trace metal and vitamin solutions. The stock solution comprises five different chemical mixtures that are 75 $g.L^{-1}$ of $NaNO_3$ in distilled H_2O, 5 $g.L^{-1}$ of NaH_2PO_4 in distilled H_2O, 30 $g.L^{-1}$ of Na_2CO_3 in distilled H_2O along with trace metal & vitamin solution. The trace metal solution is prepared by amalgamating the following trace metals with 950 mL of distilled water. 3.15 g of $FeCl_3$, 4.36 g of Na_2 $(EDTA)_2$, 9.8 $g.L^{-1}$ $CuSO_4$ in $DH_{2,6.3}$ $g.L^{-1}$ Na_2 MoO_4 22.0 g $ZnSO_4$, 10:8 $CoCl_2$ and 180 $g.L^{-1}$ of $MnCl_2$ in dH_2O. The F/12 vitamin solution is prepared once again by mixing 950ml of dH_20 with thiamine and the stock solution further by adding 200 mg of thiamine HCL, 10 mL of Biotin as 0.1 $g.L^{-1}$ in dH_20 and 1 mL of Cyanocobalamin as 1 $g.L^{-1}$ in dH_2O. The Biomass cultivation was initiated in the laboratory scale with an illuminated fluorescent light intensity between 70µ $mol.m^{-2}s^{-1}$ and 90µ $mol.m^{-2}s^{-1}$. The Carbon dioxide was allowed to recirculate continuously with the cultivation temperature between 27±2° Centigrade. The growth face of *Dunaliella salina* was monitored continuously for up to 72 h, beyond which stationary growth face was attained. The wet biomass of *Dunaliella salina* was carefully harvested and subjected to freezing at less than 6°C in a refrigerator for pelleting and lyophilization.

Extraction of Bio-Oil from *Dunaliella salina*

An ultrasonic Bio-oil extraction process was adopted to derive algal oil from harvested *Dunaliella salina* biomass. 100 g of *Dunaliella salina* biomass was mixed with 30 mL of a mixture containing methanol, chloroform, and double distilled water in the ratio of 1:2:0.4 to improve the extraction efficiency of the ultra-sonication process. The ultrasonicator was allowed to operate at 60 Hz for 90 min, during which the cell wall membranes of the *Dunaliella salina* were ruptured, thereby expelling out the algal oil. The extracted oil was then transferred into a beaker along with the sediments, and 10 mL of methanol was further added and thoroughly mixed. The Whatman filter paper was employed to remove the unwanted particles and isolate the algal oil. The resultant algal oil was treated with 6 mL acetone at an elevated temperature to remove the traces of H_2O in the algal oil. This procedure was repeated 17 times to isolate 650 mL of algal oil from *Dunaliella salina*.

Transesterification of Algal Oil

Since the free fatty acid content in the *Dunaliella salina* bio-oil was estimated as 1.82 single-stage transesterification process with sodium hydroxide methanol was adopted to

convert a tri-glyceride into its mono alkyl methyl esters. The transesterification conditions were set to the optimistic levels based on the literature as molar ratio 1:6, 0.6% by weight of NaOH as a catalyst, 70°C, and 120 min as reaction time, respectively. Initially, 1.2 g of Sodium hydroxide pellets were dissolved in 100 mL of methanol Solution in a beaker for 20 min and 450 rpm agitation speed for the formation of sodium methoxide solution. As stated earlier, the molar ratio between algal bio-oil and methanol was maintained at 1:6 by mixing 40 mL of sodium methoxide solution with 120 mL of algal oil in a single batch base catalyze transesterification process. The entire mixture was transferred into a flat bottom conical flask and maintained at 70°C for 120 min to initiate the transesterification reaction. A cooling period of 180 min was allowed after transferring the entire content into a separating funnel. A ring formation distinction between the glycerol and algal biodiesel as the lower and upper layers conformed to the completion of the transesterification process. The rotating knob of the separating funnel was turned on to remove the glycerol. Thereby, algal oil was isolated. The obtained algal oil was heated up to 60°C for 2 min after the addition of 5 mL acetone for the removal of trace elements and water molecules (John et al. 2017). This batch process was repeated 6 times, yielding 380 mL of pure Dunaliella salina biodiesel at a transesterification efficiency of 87%.

Gas Chromatography-Mass Spectrometer (GCMS)

To identify the various Fatty Acid Methyl Esters in esterified bio-oil extracted from the *Dunaliella sp*. marine algae. The single quadruped 5977B mass spectrometer (8890) Agilent GC system was employed to identify the various FAME's through thermal stabilization. The GC system was equipped with an SSL injector and capillary columns with an inlet split ratio of 7500:1. The over temperature of the GC system can be raised to 450°C. A pre-heated monolithic hyperbolic quadrupole mass filter was employed in the chemical and electron impact ionization mode. The quadruple temperature and the ion source temperature were varied between 106°C -200°C and 150°C - 350°C respectively. HP5 MSUI and DB-WAX type of polar capillary column were used to identify the various FAME's.

Fourier Transform Infrared Spectrometer (FTIR)

The phenomenon of total internal reflection was utilized to understand the transmittance of *Dunaliella sp*. biodiesel. A single reflection module by Bruker Alpha Platinum Total Attenuated Internal Reflectance FT-IR instrument was employed in this study. A transmittance vibration signal was obtained when an infrared beam was allowed to pass through the biodiesel sample and the crystal where the total internal reflection takes place, producing the angle of incidence. The spectral range of the Bruker Alpha Platinum spectrometer was between 500 cm^{-1} and 4000 cm^{-1} with a resolution of 2 cm^{-1}. One mL of the biodiesel sample was injected into the rock-solid Michelson Interferometer equipped with diamond and brazed Tung Carbide crystal and Deuterated triglycine Sulphate (DTGS) as the detector.

Nuclear Magnetic Resonance (NMR)

Bruker AVANCE 3 HD 500 FT-NMR spectrometer was used to analyze the presence of Carbon and Proton observation and decoupling in the biodiesel sample. The spectrometer is equipped with ASCEND 500 MHz high-performance actively shielded Superconducting magnet with an operational field at 11.746 Tesla and 54 mm Standard bore. The lock Control unit (Shim) system has 36 orthogonal shim gradients and Bruker Smart Magnetic Control System. The electronic control system compresses a bi-directional connection with a compact AQS-IPSO 3 frequency channel and 1 gradient channel. Advanced pulse generating system with a time resolution of 12.5 ns and frequency generating System of 2 RF channels with a frequency range. 6-643 MHz was used to synchronize the frequency and amplitude. A 6-365 MHz ATR transmitter with 500 w pulse power, high dynamic, multi-nuclear, linear amplifier was used as the transmitter. 4 mm broadband cross-polarization Magic Angel Spinning (MAS) multi-nuclear probe was used to observe the decoupling of ^{1}H nuclei, whereas a 1.7 mm Triple Inverse Probe (TIP) with gradient was used to observe the decoupling cross-polarization of ^{13}C nuclei in the spectrometer.

RESULTS AND DISCUSSION

Qualitative Analysis and Phytochemical Analysis

The presence of alkaloids, carbohydrates, protein amino acids, flavonoids, phenolic compounds, tannin, carotenoid carboxylic acid, volatile oils, and fixed oil was identified by employing the phytochemical screening approach. Dragendroff's reagent and Barfoed's reagents were primarily used in a phytochemical screening process.

The Dragendroff's reagent is prepared in two stages: a stock solution and a working Solution. The stock solution is composed of Sodium iodide (4 g), Bismuth carbonate (5.2 g), and Glacial acidic acid (50 mL). The mixture was boiled for 20 min, and a 12 h cooling period was allowed, during which sodium acetate crystal precipitated 40 mL of the filtrate were was thoroughly mixed with ethyl acetate (160 mL) in 1 L of distilled water. The working solution (Dragendroff's reagent) was prepared by amalgamating 10 mL of stock solution, 20 mL of acidic acid, and 70 mL of double distilled water. Similarly, Barfoed's reagent was prepared by thoroughly mixing copper acetate (30.5 g) and

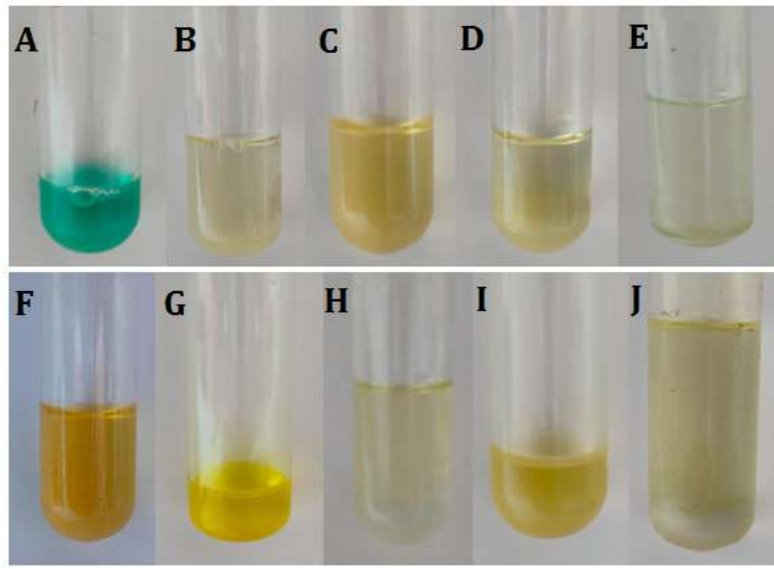

Fig. 2: *Dunaliella salina* – Phytochemical screening and analysis.

glacial acidic acid (1.8 mL) in 100 mL of distilled water (Shaikh & Patil 2020).

Carotenoids Detection

Carr-Price reaction was used to detect the presence of Carotenoid in the *Dunaliella sp*. sample. In a glass plate, 10 mL of *Dunaliella* sp. algal extract was evaporated to dryness. A saturated chloroform and antimony dichloride solution was dropped on the glass plate. A transformation of bluish-green color to bright red confirmed the presence of carotenoids (Fig. 2A).

Alkaloid's Detection

The Dragendroff's reagent was used to identify the presence of alkaloids. 2 mL of *Dunaliella sp*. sample extract was mixed with 2 mL of Dragendroff's reagent in a test tube under an aseptic condition. The appearance of radish brown indicated the presence of alkaloids (Fig. 2B).

Carbohydrate Detection

1 mL of centrifuged *Dunaliella* sp. extract is mixed with 2 mL of Barfoed's reagent in a test tube and heated for 3 min. The disappearance of red precipitate confirms the absence of monosaccharide carbohydrates (Fig. 2C).

Proteins and Amino Acid Detection

Biuret test with the *Dunaliella* sp. extract was employed to detect the amino acids and proteins. The test was performed with 2 mL of *Dunaliella* sp. sample extract, a drop of copper sulfate solution (2%), and 1 mL of ethanol and potassium hydroxide fillets. The non-appearance of the pink-colored ethanolic layer indicated the absence of amino acids and proteins (Fig. 2D).

Flavonoids Detection

A two-step alkaline reagent test was adopted to detect the presence of flavonoids. In the primary steps, 2% sodium hydroxide solution (2 mL) and 3 drops of dilute hydrochloric acid were mixed with 1 mL of *Dunaliella* sp. algal extract in a test tube. The solution's color transformation from, for instance, yellow to transparent and colorless liquid during dilute hydrochloric acid addition indicates the presence of flavonoids. The secondary step involved adding Ammonium hydroxide solution (10%) to the *Dunaliella* sp. algal extract turning the solution into a fluorescent yellow color liquid, confirming the presence of flavonoids (Fig. 2F).

Phenolic Compounds Detection

The iodine test was adopted to identify the presence of Phenolic compounds. A few drops of dilute Iodine solution were mixed thoroughly with 1 mL of *Dunaliella* sp. algal extract. The color non-transformation into dark red confirmed the absence of phenolic compounds (Fig. 2E)

Tannin Detection

A gelatin test was adopted to detect the presence of tannin. The base solution was prepared using 1% gelatin solution with 10% sodium chloride. The centrifuged *Dunaliella* sp.

algal extract was dissolved in 5 mL of distilled water and further thoroughly amalgamated with the base solution. A white precipitate formation after 10 min indicated the presence of Tannin in the algal strain.

Carboxylic Acid Detection

A simplified effervescence test was used to identify the carboxylic acid. After a few minutes of mixing, 1 mL of sodium bicarbonate solution was mixed with an equal amount of centrifuged Dunaliella sp. extract in the effervescence, indicating the presence of carboxylic acid, as shown in Fig. 2J.

Fixed Oil and Fat Detection

Stain/Spot tests were adopted to identify the presence of fat and fixed oils. A major quantity of centrifuged *Dunaliella* sp. algal extract was placed between two filter papers and pressed. Upon evaporation, the presence of an oil stain on the filter paper indicated its presence.

Volatile Oil Detection

Fluorescence was used to detect the volatile oil presence in *Dunaliella* sp. Sample. 10 mL of the *Dunaliella* sp. algal extract was filtered till the saturation level and subjected to UV light rays. The non-observant of bright pinkish fluorescence indicated the absence of Volatile oils.

Comparison of Physio-Chemical Properties – Diesel, *Dunaliella salina* Algal Oil and its Biodiesel

The physicochemical properties of *Dunaliella salina* algal oil and its biodiesel were compared with commercial diesel in Table 1. The density of *Dunaliella salina* algal biodiesel was slightly increased by 0.545% but was found to be within limits. The gross calorific value showed significant appreciation up to 24.89%, along with a notable increase in oxygen content. The transesterification process considerably reduced the kinematic viscosity from 22.9875 cSt to 2.657 cSt, thus making it more suitable for CI engine usage (Hariram et al. 2018).

Fourier Transform Infrared Spectrometer (FTIR)

The bending and stretching Vibration seen between 547.17 cm^{-1} and 3008.32 cm^{-1} in the FTIR spectrum of *Dunaliella* sp. biodiesel is showcased in Fig. 3, a strong signal compressing of stretching vibration at 1741.22 cm^{-1} indicates the transformation of bio-oil from *Dunaliella* sp. into its fatty acid methyl ester. A group bending-stretching vibration was noticed between 1019.14 cm^{-1} and 1460.99 $cm^{-1,}$ indicating long-chain hydrocarbon (C=H). Several weak signals were seen at 844.85 cm^{-1}, 914.1 $cm^{-1,}$ and 1437.12 $cm^{-1,}$ indicating a carboxylic group's presence. Strong signals were seen at 2853.91 cm^{-1} and 2923.46 $cm^{-1,}$ along with the weak trailing signal 3008.32 $cm^{-1,}$ confirming the complete transformation of the bio-oil and its FAME's. No signal formation between 1750 cm^{-1} and 2800 cm^{-1} is one of the characteristic features of biodiesel obtained through a single state-based transesterification process (Hariram et al. 2017).

Gas Chromatography-Mass Spectrometer (GCMS)

A single-stage base-catalyzed transesterification process with sodium hydroxide and methanol converted the bio-oil extracted from the *Dunaliella* sp. marine algae into its biodiesel. Gas Chromatography-Mass Spectrometer analysis was conducted on an esterified biodiesel sample to understand its transesterification efficiency and identify the presence of various fatty acid methyl esters. The mass chromatogram of the biodiesel reviewed the presence of nine different FAME's at retention time (RT) between 19.93 and 30.48 min (Fig. 4). A characteristic base Peak was noticed at m/z 74 in all the distinct fragmentation patterns to identify fatty acid methyl ester. It was noticed that the mass chromatogram and the fragmentation patterns of the algal bio-oil and the esterified biodiesel were in line with the McLafferty rearrangement process. Several mass

Table 1: Physio-chemical properties of *Dunaliella salina* algal oil, its biodiesel, and diesel.

Property	*Dunaliella salina* algal oil	*Dunaliella salina* algal biodiesel	Diesel
Molecular formula	$C_{13} - C_{24}$	-	$C_{12}H_{22}$
Gross calorific value [$kJ.kg^{-1}$]	30547	40547	42700
Density [$kg.m^{-3}$]	834.786	860	842
Kinematic Viscosity [cSt]	22.9875	2.657	2.82
Sulfur content [% vol]	0.34	0.20	0.04
Flashpoint [^oC]	270-280	48	69
Cetane number	36	45	48.5
Oxygen content [% wt]	5.678	9.871	0
Ash content	1.315	0.479	0

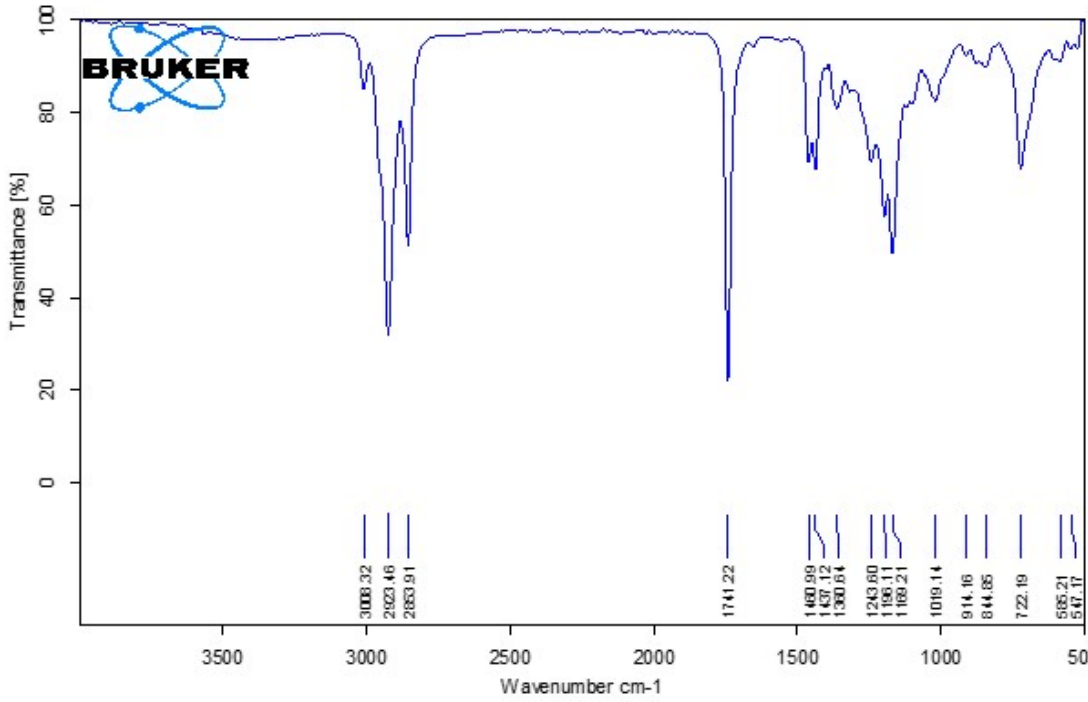

Fig. 3: *Dunaliella salina* biodiesel – FTIR Transmittance.

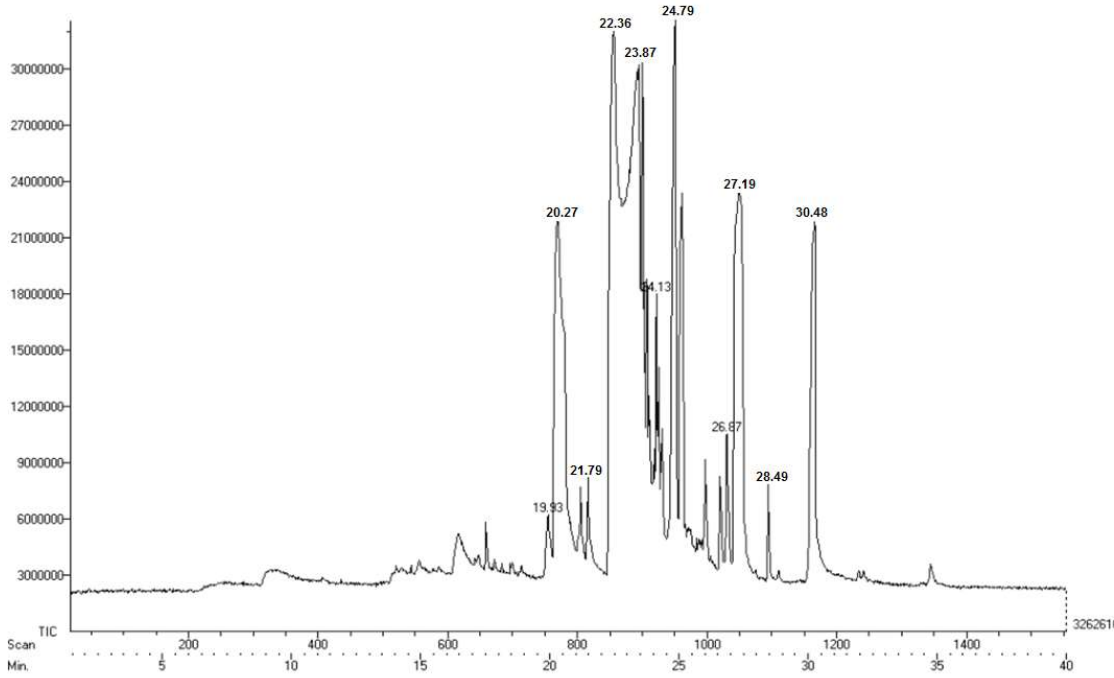

Fig. 4: *Dunaliella salina* biodiesel – GCMS chromatogram.

fragmentation patterns of the FAME showcase the loss of carbo-methoxy ions due to β cleavage. Multiple profusions were also noticed in a few of the mass fragmentation patterns, which may be due to the loss of the methoxy group and the rearrangement of carbon and hydrogen atoms during the transesterification process. The presence of unsaturated

fatty acids like 14, 17-Octadecadienoic acid methyl ester at RT 22.36 min is due to the repositioning and regrouping of the hydrogen ion in the Carbonyl group (Sarpal et al. 2016).

The various fatty acid methyl ester presenting the biodiesel sample was found to be 9-hexadecenoic acid methyl ester at RT 19.93, hexadecanoic acid methyl ester at RT 20.27, heptadecanoic acid methyl ester at RT 21.79, 14, 17-octadecadienoic acid methyl ester at RT 22.36, eicosanoic acid methyl ester at RT 24.79, 13-docosenoic acid methyl ester at RT 26.87, docosanoic acid methyl ester at RT 27.19, tricosanoic acid methyl ester at RT 28.49, and tetracosanoic acid methyl ester at RT 30.48.

Nuclear Magnetic Resonance (NMR)

Proton NMR (^{1}H NMR): ^{1}H NMR solution was prepared by dissolving 7.5 mL of *Dunaliella salina* algal biodiesel with 0.8 mL of Deuterated methanol as a Solvent. The relaxation delay and the pulse during the Injection of the sample were maintained at 10 sec and 90°, respectively. The Proton NMR Spectrum of the *Dunaliella salina* biodiesel compresses mainly fatty acid esters along with alkaloids, alkanes, and steroids in minor proportions. The characteristic peak in the ^{1}H NMR spectrum of *Dunaliella salina* biodiesel spectrum was noticed at 3.669 ppm, which is due to the functional group of fatty acid esters (Fig. 5). A prominent signal corresponding to the carbonyl functional group is seen at 5.387 ppm. Clustered peaks indicative of unsaturated long-chain hydrocarbons are evident in the range of 3.330 ppm to 2.108 ppm. Signals at 5.397 ppm and 5.408 ppm were apportioned to OCH and OCH_2 ester groups due to their transformation from triglyceride to mono-alkyl fatty acid ester. The ^{1}H NMR spectrum also exhibited a faint doublet peak at 3.336 ppm, possibly arising from alkenes in the excipient. The existence of the triplet peak at 1.377 ppm confirmed the presence of polyunsaturated fatty acid in the *Dunaliella salina* biodiesel Sample (Bisht et al. 2021).

Carbon NMR (^{13}C NMR)

Similar to the sample injection Proton NMR, ^{13}C NMR analysis was also initiated with Deuterated methanol of solvent for profiling the unsaturated fatty acid composition. Since ^{13}C NMR possesses a relatively larger chemical shift range between 0 ppm to 200 ppm, sharper and distinctive peaks of monoglycerides, diglycerides, triglycerides, and epoxy ester were identified (Fig. 6). In the ^{13}C NMR spectrum of *Dunaliella salina* biodiesel, a characteristic singlet peak was noticed at 47.967 ppm due to the existence of mono-alkyl long-chain hydrocarbon. A cluster peak

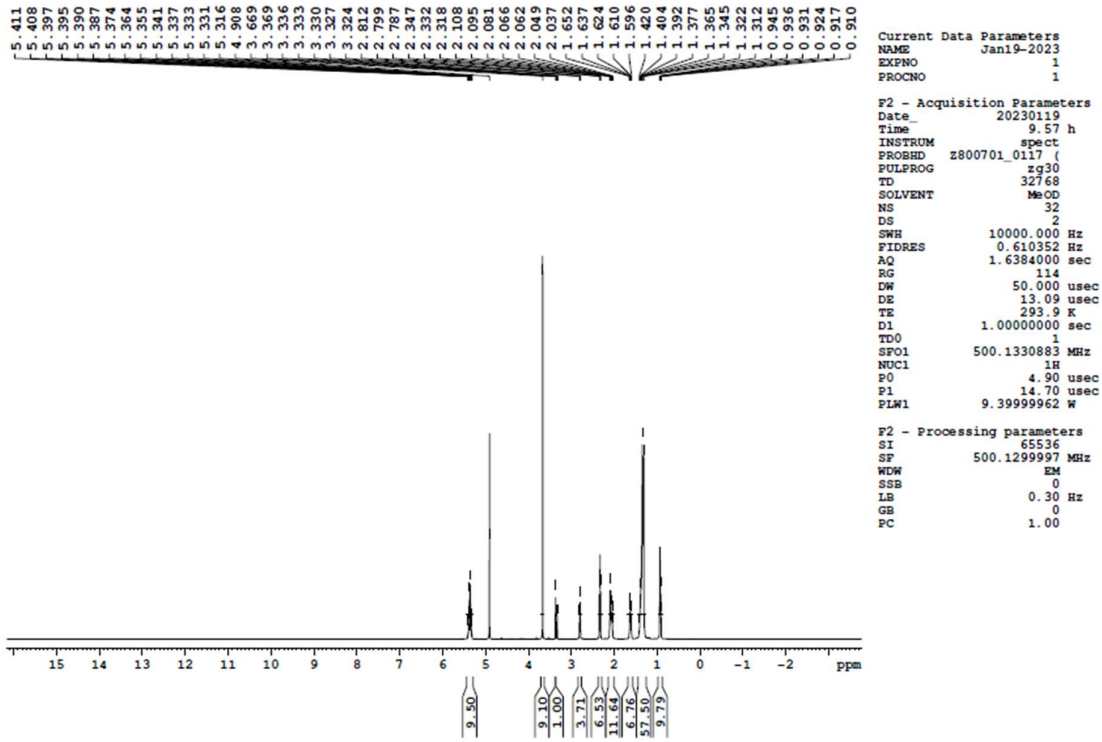

Fig. 5: *Dunaliella salina* biodiesel – ^{1}H NMR spectrum.

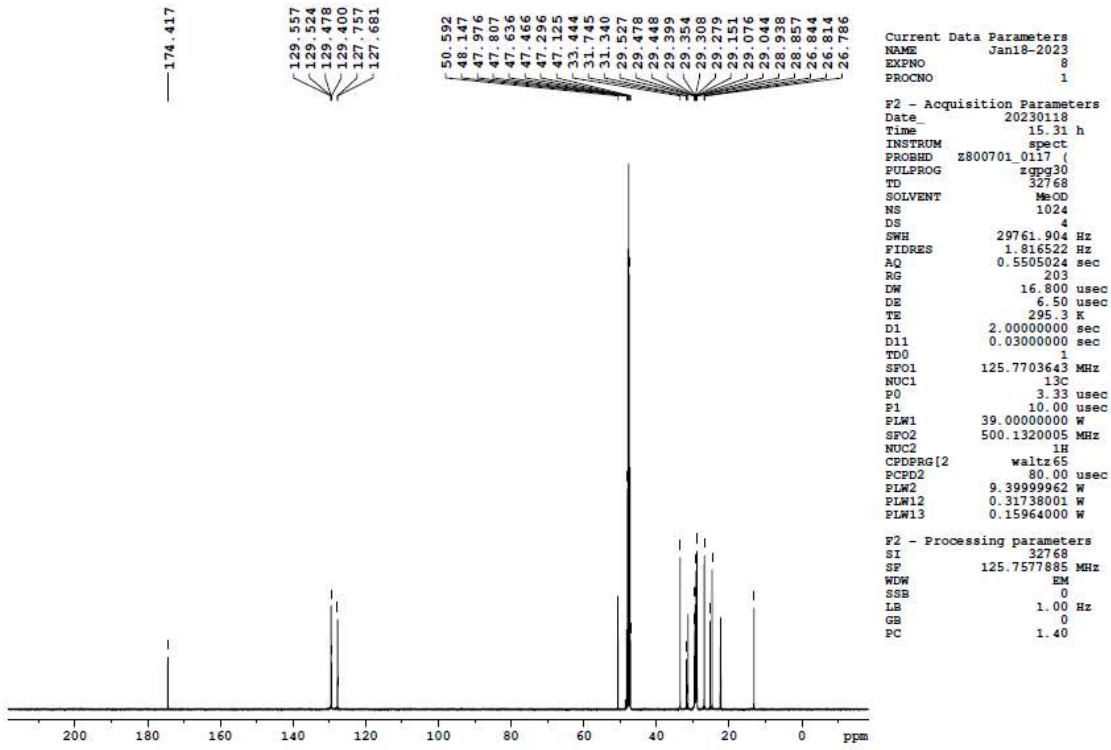

Fig. 6: *Dunaliella salina* biodiesel – ^{13}C NMR spectrum.

between 127.681 ppm and 129.557 ppm showed the strong existence of carbonyl group unsaturated esters. A terminal peak at 174.417 ppm evidenced the completion of the transesterification reaction with the presence of long-chain hydrocarbons. A large group cluster peaks between 26.814 ppm and 33.444 ppm showed the presence of a carboxylic group. The presence of monoglycerides was also evidenced by a weak signal at 16. 442 ppm in the ^{13}C NMR spectrum of *Dunaliella salina* biodiesel.

CONCLUSION

This investigation aims at producing biodiesel from *Dunaliella salina* microalgae. The following conclusion was made at the outset.

- *Dunaliella salina,* hypersaline unicellular microalgae, was cultivated in an aseptic environment under carbon dioxide-rich conditions with F/2 as a nutrient medium.
- Ultrasonic-assisted bio-oil extraction process with the combination of methanol, chloroform, and double distilled water in ratios of 1:2:0.4 operating at 60 Hz for 90 min isolated 650 mL of *Dunaliella salina* bio-oil.
- Single-stage base-catalyzed transesterification process with a 1:6 molar ratio, 0.6% by weight of NaOH, 70°C reaction temperature, and 120 min reaction time yielded 380 mL of *Dunaliella salina* biodiesel at a transesterification efficiency of 87.2%.
- The phytochemical screening process with Dragendroff's and Barfoed's reagents revealed the presence of carotenoids, alkaloids, flavonoids, and carboxylic acid.
- The Fourier Transform Infrared transmittance evidenced the transformation of bio-oil into its FAME's by strong stretching vibrations at 1741 cm^{-1} and 2853.91 cm^{-1}.
- The Gas Chromatography-Mass Spectrometry analysis revealed the presence of Octadecadienoic acid methyl ester at RT 22.36 in notable proportions.
- The Proton NMR (^1H NMR) with a characteristic peak at 3.669 ppm confirmed the presence of fatty acid esters. Further, signals at 5.397 ppm and 5.048 ppm apportioned to OCH and OCH_2 resulted from the transformation of mono-alkyl esters to FAME. The Carbon NMR (^{13}C NMR) spectrum also revealed long-chain hydrocarbon resulting from the transesterification reaction by cluster peaks between 127.681 ppm and 129.557 ppm.

Thus, with the above qualitative outcomes, it can be concluded that biodiesel derived from *Dunaliella*

salina could be a promising substitute for petro-diesel feedstock.

REFERENCES

Bisht, B., Kumar, V., Gururani, P., Tomar, M.S., Nanda, M., Vlaskin, M.S., Kumar, S. and Kurbatova, A. 2021. The potential of nuclear magnetic resonance (NMR) in metabolomics and lipidomics of microalgae: A review. Arch. Biochem. Biophys., 710: 108987. https://doi.org/10.1016/j.abb.2021.108987

Chailleux, E., Audo, M., Bujoli, B., Queffelec, C., Legrand, J. and Lepine, O. 2013. Alternative binder from microalgae: Algoroute project. HAL, 11: 7-14.

Chisti, Y. 2007. Biodiesel from microalgae. Biotechnol. Adv., 25(3): 294-306. https://doi.org/10.1016/j.biotechadv.2007.02.001

Hariram, V., John, G. and Seralathan, S. 2017. Spectrometric analysis of algal biodiesel as a fuel derived through base-catalyzed transesterification. Int. J. Ambient Energy, 40(2): 195-202.

Hariram, V., Prakash, S., Seralathan, S. and Micha Premkumar, T. 2018. Data set on optimized biodiesel production and formulation of emulsified *Eucalyptus teriticornisis* biodiesel for usage in compression ignition engine. Data Brief, 20: 6-13. https://doi.org/10.1016/j.dib.2018.07.053

John, G., Hariram, V. and Seralathan, S. 2018. Emission reduction using improved fuel properties of algal oil biodiesel and its blends. Energy Sour. Part A, 40(1): 45-53.

John, G., Hariram, V., Seralathan, S. and Jaganathan, R. 2017. Effect of oxygenate on emission and performance parameters of a CI engine fuelled with blends of diesel-algal biodiesel. Int. J. Renew. Energy Res., 7(4): 2041-2047.

Mathimani, T., Uma, L. and Prabaharan, D. 2015. Homogeneous acid-catalyzed transesterification of marine microalga *Chlorella* sp. BDUG 91771 lipid e– an efficient biodiesel yield and its characterization. Renew. Energy, 81: 523-533. https://doi.org/10.1016/j.renene.2015.03.059

Sarpal, A.S., Costa, I.C.R., Teixeira, C.M.L.L., Filocomo, D., Candido, R., Silva, P.R., Cunha, V.S. and Daroda, R.J. 2016. Investigation of biodiesel potential of biomasses of microalgae *Chlorella*, *Spirulina*, and *Tetraselmis* by NMR and GC-MS techniques. J. Biotechnol. Biomater., 6(1): 1-15.

Sarpal, A.S., Teixeira, C.M.L.L., Silva, P.R.M., Vieira da Costa Monteiro, T., Itacolomy da Silva, J., Smarcaro da Cunha, V. and Daroda, R.J. 2015. NMR techniques for determination of lipid content in microalgal biomass and their use in monitoring the cultivation with biodiesel potential. Appl. Microbiol. Biotechnol., 1-15.

Shah, Z. and Veses, R.C. 2016. GC-MS and FTIR analysis of bio-oil obtained from freshwater algae (spirogyra) collected from Freshwater. Int. J. Environ. Agric. Res., 2(2): 134-141.

Shaikh, J.R. and Patil, M.K. 2020. Qualitative tests for preliminary phytochemical screening: An overview. Int. J. Chem. Stud., 8(2): 603-608. https://doi.org/10.22271/chemi.2020.v8.i2i.8834

Wahlen, B.D., Morgan, M.R., McCurdy, A.T., Willis, R.M., Morgan, M.D., Dye, D.J., Bugbee, B., Wood, B.D. and Seefeldt, L.C. 2013. Biodiesel from microalgae, yeast, and bacteria: engine performance and exhaust emissions. Energy & Fuels, 27(1): 220-228.

Yasir Al-Shikaili, T., Thomasson, J.A., Ge, Y., Brown, L. and Brown, Jola. 2022. FTIR transmission spectroscopy for measurement of algae-neutral lipids. Agriculturists Eng. Int., CIGR J., 24(4): 111-118.