Korean Journal of Chemical Engineering (2024) 41:2567–2580
https://doi.org/10.1007/s11814-024-00157-7

ORIGINAL ARTICLE

Potential of Algal Biomass and Their Cultivation for Biofuels Production as Plausible Bio-resource for Economic Sustainability

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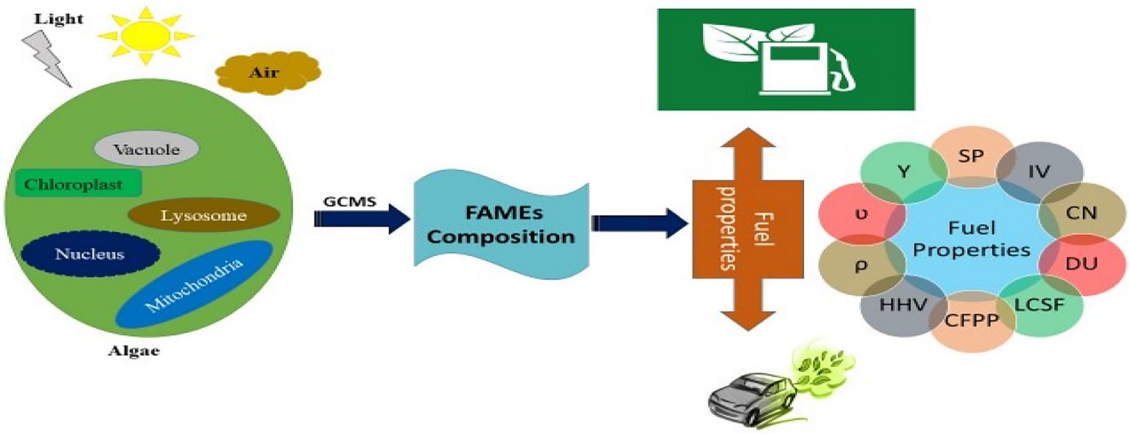
Received: 4 February 2024 / Revised: 8 March 2024 / Accepted: 15 March 2024 / Published online: 21 March 2024

Abstract

Depletion of conventional energy resources necessitates the exploration of new alternative raw materials for sustainable biofuel production to improve socio-economic development. This research focuses on the cultivation of specific algae varieties, biomass quantification, fatty acid profiling, and their potential application in biodiesel production. The study examines diverse emerging algal species, including *Ulothrix*, *Stigeoclonium*, *Chlorella vulgaris*, *Cladophora*, *Oedogonium*, *Oscillatoria*, *Spirogyra*, and *H. reticulatum*. Over a 4-week cultivation period, all species demonstrated increased dry biomass, with *Ulothrix* exhibiting the maximum growth (19 g) and *Stigeoclonium* the minimum (5 g). Lipid composition analysis by microwave-assisted extraction (MAE) indicated varying percentages (% DW) among strains, with *Ulothrix* sp. displaying the highest lipid content (62.4%). Lipid yields, crucial for biodiesel, followed the order: *Ulothrix* > *Stigeoclonium* > *C. vulgaris* > *Cladophora* sp. > *Oedogonium* > *Oscillatoria* > *Spirogyra* sp. > *H. reticulatum*. Further analysis of fatty acid methyl esters (FAMEs) composition using GC–MS revealed 24 detected FAMEs, with percentage ranges for specific fatty acids. The total FAMEs yield reached approximately 98% (w/w) from algal biodiesel, showcasing variations in saturated, monounsaturated, and polyunsaturated FAMEs content among strains. Fatty acid profiles, emphasizing linoleic, stearic, and oleic acids, were analyzed, identifying *Cladophora* sp., *Chlorella vulgaris*, and *Stigeoclonium* sp. as promising biodiesel candidates based on growth rates and fuel properties. All algal strains met or exceeded specifications, except for *H. reticulatum* in cetane number. Despite highlighting challenges in large-scale algal cultivation for cost-effective biomass production, this study underscores the potential of expanding the algae biorefinery value chain to include advanced biofuels and valuable co-products, presenting a significant global impact on the gross domestic product.

Graphical abstract



Keywords Algal fuel · FAMEs · Fuel parameters · Oil · Algae

Extended author information available on the last page of the article

Introduction

With the global upturn of energy utilization, the subsequent decrease in energy assets and the cumulative costs towards energy security, there is an urgent global requirement to seek new or alternate renewable energy commodities to replace or supplement conventional fuel resources whilst minimizing the effect on the environment [1]. However, biodiesel production from edible crops is unfeasible because > 50% of edible oil crops are used as a global food source. Nevertheless, macroalgae is an alternative renewable energy source [2]. Algae have the ability to grow in non-arable or limited land, have a high growth rate in 24–48 h, fast carbon dioxide fixation [3, 4] and can grow on wastewater. Several algal species can tolerate and complete their life cycle under brackish and hypersaline conditions. They can yield about 250 times more oil per acre as compared to soybeans and can produce 7–31 times more oil than palm oil. Algae has the ability to produce 25–220 times more lipids [5]. The green algae were chosen in this investigation as they are all fast growing and their lipid composition ranges from 20 to 65% with the majority of fatty acids similar to that present in vegetable oils [6].

Chlorella vulgaris is a freshwater green alga having thick cell wall providing mechanical and chemical protection against heavy metals and therefore, is proposed as a proficient, waste removing algal species in polluted and contaminated soil. *C. vulgaris* has relatively small cells but it is able to adapt under changing physicochemical conditions. *C. vulgaris* often accumulates high lipid content offering its cultivation on wastewater for the generation of energy [7]. *Cladophora* sp. *Stigeoclonium* sp. and *Ulothrix* sp. can grow in fresh water as well as brackish lakes, also withstanding alkaline streams with 40–80 mg/L salinity resistance. The biomass of *Cladophora* sp. improves due to enrichment of nitrogen (N_i, urea) and its relatively high optimization of light for photosynthesis as previously reported in the algae, (*Ulothrix* sp.) or *Oedogonium*, *Spirogyra* sp. and *H. reticulatum* which accumulates large amounts of biomass [8]. *Hydrodictyon reticulatum* is a commonly found large algae which can survive in wide temperature ranges and is easily harvestable than other algal species. It is highly adaptable with fast reproduction rates and growth processes. *H. reticulatum* absorbs ammonia, nitrogen, nitrate and inorganic phosphorus to maintain optimum growth and its large appearance and dominance in the pond habitat. *H. reticulatum* has shown to have an unusually high heavy metal composition and absorption capacity like copper, iron, manganese, cadmium, chromium and lead [9]. *Oedogonium* sp. and *Oscillatoria* sp. tolerates desiccation with good biomass productivity due to its thick mats like stature that shield algal cells in dehydrated condition. Several investigations have previously demonstrated that energy production from the biomass *Spirogyra* sp. is feasible to produce biogas, biodiesel, bioethanol and hydrogen [10].

Biodiesel production from algal biomass is predominantly processed by hydrothermal conversion and gasification. Although these methods generate fuel and fulfill the requirements of energy demands, these methods are expensive, energy consuming and require the input of dried, algal biomass as an additional energy cost [11]. To date, biodiesel is derived from the transesterification of fatty acids, however, this process faces several hurdles such as the high energy consumption in the drying of biomass, restricted storage because of oxidative instability and polyunsaturated fatty acid (PUFAs) affect both cold filter plugging point (CFPP) and iodine values (IV). These shortcomings can be reduced by selecting an appropriate algal strain [12]. Moreover, the quality of biodiesel demands must conform to the optimum cetane number (CN) standard, appropriate CFPP, density (ρ) and viscosity (υ) [13].

Along with biomass yield and fatty acid composition are the critical considerations for selecting suitable algae for the biodiesel generation. Fatty acid ethyl esters (FAME) and lipid composition can vary even in the same algal strain [14]. Therefore, the selection of suitable algal species is the critical step for the large-scale biodiesel generation [15]. The essential biodiesel properties of are the CN, degree of unsaturation (DU), saponification value (SV), CFPP, long chain saturation factor (LCSF), IV, oxidation stability (Y), υ, ρ and high heating value (HHV_i). Apart from that, some properties like high density, viscosity and acid value along with low cloud and pour points preclude their use in compression ignition (CI) engines as these can cause serious damage to the parts of the engine and reduce engine life [16]. The FAME composition is used to compute the iodine and saponification values which is further used to calculate the cetane number [17]. Iodine values increase with increases in the degree of unsaturation of the fatty acid compositions. The cetane number is the indicator of fuel combustion ability, and increases with the increasing chain length and degrees of saturation of fatty acids [18]. The filter plugging point is unswervingly related to the degrees of unsaturation and also increases with increases in the degrees of unsaturation of fatty acids. The higher heating value is referred to as the heat of combustion and increases with increasing chain length and degrees of saturation of fatty acids [19]. The higher the density the higher the energy. Viscosity upturns with amassed chain length but declines with rises in the number of double bonds [20]. Oxidation stability is associated with large scale fuel generation and storage [21]. The key challenge is the need to identify and isolate microalgae species that not only exhibit a high growth rate

but also maintain a lipid composition suitable for efficient biodiesel production. Overcoming this challenge is essential for improving the viability and sustainability of microalgae biodiesel as an alternative energy source. There are limited studies available to screen algal resources for fatty acid and lipid composition in natural and local areas of Pakistan [22, 23]. The production of biodiesel local, non-food algal biomass can reduce the cost of fuel rises and protect the environment from degradation. The large-scale production of biofuel from algae has enormous potential, yet they will require significant and innovative strategies in order to compare the quantities with other algal-based feedstock. This research primarily aims at screening algae with suitable fatty acid composition to produce biofuel from Pakistan. Preliminary studies of test species would assist in assessing their potential for optimum biofuel yield under suboptimal conditions. Recent research in microalgae has been directed towards increasing the lipid content in these organisms. This is being achieved through the optimization of growth conditions and the systematic screening of freshwater microalgae to identify the most suitable species. The ultimate goal is to enhance the efficiency and feasibility of using microalgae for biodiesel production.

The study explores the biodiesel potential of the non-food algae, showcasing a novel avenue for their utilization for industrial application. Characterizing fatty acid methyl ester (FAME) produced from the studied algal oil using standard methods adds a technical dimension to the research and to reduce energy crises in developing countries.

Materials and Methods

Sampling, Identification, and Culturing of Algae

Various species of algae were collected from different areas in Punjab, Pakistan. Algal strains were chosen based on their morphology, and further identification was carried out by matching the 18SrDNA and ITS regions. DNA extraction was performed on the selected algal strains. The 18SrDNA gene was then amplified using specific primers listed in Supplementary Table S1. The amplified DNA was sent to a sequencing service provider, Macrogen. The obtained DNA sequences were utilized to construct a phylogenetic tree using MEGA6 software. 5 g of each algal strain were batch-cultivated using Blue Green medium 11 (referenced in Supplementary Table S2). Cultivation was carried out at a pH of 7, temperature at 25 °C, and a 16 h light/ 8 h dark cycle for a total period of 30 days (Fig. 1). After the cultivation period, algal biomass was harvested using centrifugation. The harvested biomass was used to extract lipids, which are likely of interest for their potential use in biofuel production or other applications. The primers used for the molecular analysis, specifically for amplifying the 18SrDNA gene, are listed in Supplementary Table S1 [24].

Lipid Extraction from Algae

A dried algae sample weighing 1 g added in a 1:2 ratio of methanol/chloroform (50 ml) for extraction. The extraction is carried out at 800 watts power for 120 s in microwave oven (Haier HGN—36100EGS). An additional 20 ml of chloroform was added, and a second 120 s extraction step was performed. The extracted lipids were obtained from the algae sample after the microwave-assisted extraction. The extracted lipids were then filtered using filter paper. The filtered lipids were dried through evaporation. The dried and filtered lipids were subjected to transesterification [25].

The lipid contents for each algal strain were determined using a method established by [26]. The weights of the extracted algal oil were utilized to quantify the lipid content. Lipid contents were calculated based on the weights of the extracted algal oil. The extraction efficiency was expressed as a percentage, representing the proportion of lipid content relative to the initial algal biomass. The calculated lipid content was expressed as a percentage.

$$\text{Lipid content (\% DCW)} = (\text{weight of lipids}/\text{weight of sample}) \times 100. \quad (1)$$

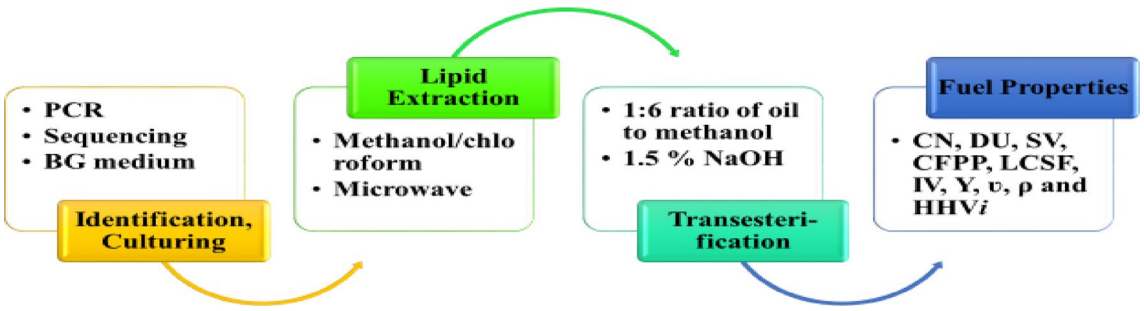


Fig. 1 A schematic illustration of the experimental setup to assess the potential of algal biomass and their cultivation for biofuels production

Transesterification and FAMEs Analysis by GC–MS

The transesterification was conducted at a temperature of 60 °C. The process was carried out for 120 min. A water bath (Electrical Thermostatic) was used for maintaining the specified temperature. 1.5% NaOH was added in the 1:6 ratio of oil to methanol, then heated this mixture in the water bath. After the reaction, the mixture was cooled using an ice bath. The top methyl layer was removed. This separation was likely facilitated by the differences in density and properties of the two layers. The separation process was enhanced by centrifugation for 10 min at 13,000×*g* at room temperature. This step likely helps in further separating the different components of the reaction mixture. The biodiesel product was washed with deionized water. This washing step was likely intended to purify the biodiesel by removing any remaining water-soluble impurities or residues from the reaction then upper layer of fatty acid was isolated and injected into GCMS for fatty acid profiling [27]. The recovered FAMEs were analyzed by GCMS for their suitability as an energy source. A DB-5 column was used for chromatography, with specific dimensions (length = 3 cm and thickness = 0.5 µm). The injector temperature was set at 220 °C. The oven temperature was raised from 110 to 280 °C at a rate of 10 °C/min. The carrier gas used was helium, with a flow rate of 1 ml/min. The ionization voltage for mass spectrometry was set at 70 eV. Mass spectrometry was performed in scan mode over a range of 40–950 m/z (mass-to-charge ratio). Each sample, consisting of the recovered FAMEs, was injected in a volume of 1 µl.

Fuel Quality

Fuel quality parameters such as CN, DU, SV, CFPP, LCSF, IV, *Y*, υ, ρ and HHV_i were calculated using following equation as previously mentioned in [28].

$$\mathrm{IV} = \sum \left(254 \times D_i \times N_i/\text{Molecular weight of ith fatty acid}\right), \tag{2}$$

where D_i is the number of double bonds of *i*th FAME and N_i is the percentage of each FAME (Table 1). Saponification value (SV) was calculated by using following equation:

$$\mathrm{SV} = \sum \left(560 \times N_i/\text{Molecular weight of ith fatty acid}\right), \tag{3}$$

where N_i is the percentage of each FAME (Table 1).

Cetane number (CN) was calculated by using following equation:

$$\mathrm{CN} = 46.3 + (5458/\text{saponification value}) - (0.225 \times \text{Iodine value}), \tag{4}$$

where CN is the cetane number.

Degree of unsaturation (DU) was calculated by using following equation:

$$\mathrm{DU} = \sum \mathrm{MUFA} + (2 \times \mathrm{PUFA}), \tag{5}$$

where MUFA is mass fraction of mono-unsaturated fatty acids and PUFA is the mass fraction of poly-unsaturated fatty acids.

Long chain saturation factor (LCSF) was calculated by using following equation:

$$\begin{aligned}\mathrm{LCSF} =& (0.1 \times \mathrm{C16:0}) + (0.5 \times \mathrm{C18:0}) \\ &+ (1 \times \mathrm{C20:0}) + (2 \times \mathrm{C24:0}).\end{aligned} \tag{6}$$

Cold filter plugging point (CFPP) was calculated by using following equation:

$$\mathrm{CFPP} = (3.1417 \times \mathrm{LCSF}) - 16.477, \tag{8}$$

where LCSF is Long chain saturation factor.

Higher heating value (HHV) was calculated by using following equation:

$$\mathrm{HHV}_i = 46.19 - 1794/M_i - 0.21 \times N, \tag{8}$$

where M_i is the molecular weight of *i*th FAME and *N* is the number of double bond of *i*th FAME.

Density (ρ) was calculated by using following equation:

$$\rho = 0.8463 + 4.9/M_i + 0.0118 \times N, \tag{9}$$

where M_i is the molecular weight of *i*th FAME and *N* is the number of double bond of *i*th FAME.

Kinematic viscosity (υ) was calculated by using following equation:

$$ln(\upsilon i) = -12.503 + 2.496 \times \ln\left(M_i\right) - 0.178 \times N, \tag{10}$$

where M_i is the molecular weight of *i*th FAME and *N* is the number of double bond of *i*th FAME.

Predictive Oxidation stability (*Y*) was calculated by using following equation:

$$Y = 117.9295/X + 2.5905(0 < 100), \tag{11}$$

where *X* is the content percentage of linoleic and linolenic acids ($0 < X < 100$) and *Y* is Oxidation stability in hours.

Statistical Analysis

The results were presented in terms of mean values along with the standard error. One-way Analysis of Variance (ANOVA) was used for the comparison analysis using SPSS 17. To further investigate specific differences, post hoc tests were conducted using the Least Significant Differences (LSD) procedure. The data were analyzed using

Table 1 FAME composition (%) from the 8 algae species

Fatty acids	C:D	*C. vulgaris*	*Cladophora* sp.	*H. reticulatum*	*Oedogonium* sp.	*Oscillatoria* sp.	*Spirogyra* sp.	*Stigeoclonium* sp.	*Ulothrix* sp.
Myristoleic acid	14:1	–	0.3	02	0.1	–	0.1	0.2	–
Myristic acid	14:0	0.2	0.1	0.1	0.3	0.3	0.05	0.5	0.1
Hexadecadienoic	16:2	–	–	0.2	0.4	–	2.2	1.0	–
Palmitoleic acid	16:1	0.1	–	0.1	2.9	0.3	0.3	1.1	–
Palmitic acid	16:0	1.0	0.4	3.7	1.1	3.0	0.1	0.6	0.2
Linolenic acid	18:3	–	–	–	0.4	–	0.6	0.1	–
Linoleic acid	18:2	0.5	2.0	–	0.3	2.0	1.0	0.5	–
Oleic acid	18:1	0.1	–	–	3.1	2.0	0.3	3.4	0.4
Stearic acid	18:0	–	–	1.5	1.3	3.0	0.06	1.2	–
Gondoic acid	20:1	–	–	–	–	–	0.1	0.5	–
Arachidic acid	20:0	–	–	0.4	–	–	0.1	0.8	–
Erucic acid	22:1	0.1	0.1	–	0.4	–	–	–	–
Caprylate	8:0	–	0.3	2.1	–	0.7	–	0.2	0.3
Caprate	10:0	–	0.1	3.5	–	1.3	–	0.1	0.6
Laurate	12:0	–	0.6	16.2	–	8.5	–	0.4	0.2
Methyl Myristate	14:0	2.2	3.3	4.9	4.5	–	2.1	1.0	3.3
Methyl palmitoleate	16:1	3.1	3.2	22.5	3.1	10.0	5.9	1.9	5.1
Methyl palmitate	16:0	40.0	34.3	8.1	46.4	12.0	49.0	43.3	24.4
Linolenate	18:3	0.8	7.6	0.3	2.3	9.0	1.0	2.1	2.6
Linoleate	18:2	2.1	5.4	10.4	2.5	3.0	0.5	1.0	9.0
Methyl oleate	18:1	42.5	42.0	14.0	28.0	16	29.1	34.6	44.2
Methyl stearate	18:0	3.9	–	4.6	2.1	27.0	5.2	2.0	3.8
Arachidate	21:0	1.0	–	2.8	–	0.6	1.1	–	2.4
Erucate	22:1	1.0	–	0.1	–	–	0.5	0.1	2.2

Turkey's method for making pairwise comparisons. The significance level chosen for determining statistical significance is $P < 0.05$.

Results

Molecular Identification of Algae

Eight algal strains were chosen based on several criteria such as abundance in the environment, large lipid content, rapid growth and productivity (Fig. 2). The 18S rRNA or ITS sequences of the selected algal strains were analyzed. Basic Local Alignment Search Tool (BLAST) and CLUSTALW were used to assemble and compare the sequences.

Figure 3 illustrates the phylogenetic tree, depicting the evolutionary relationships among the selected algal strains. Each algal strain is associated with a specific accession number. The identification percentages represent the similarity of each algal strain's genetic sequence to known sequences in the database.

Algal Growth in Blue Green Medium

Algal growth was observed on BG11 medium, the growth measurements were taken at the 4th week of cultivation. The growth is quantified in grams of dry weight (DW) (Fig. 4). The dry weight measurements for each of the following algal strains: 14 g of DW in *C. vulgaris*, *Cladophora* sp. (7 g), *H. reticulatum* (5 g), *Oedogonium* sp. (8 g), *Oscillatoria* sp. (11 g), *Spirogyra* sp. (6 g), *Stigeoclonium* sp. (4 g) and *Ulothrix* sp. (19 g). The maximum growth among the tested algal strains was observed in *Ulothrix* sp., reaching 19 g of dry weight. *Stigeoclonium* sp. exhibited the lowest growth among the tested strains, with 4 g of dry weight. The dry weight measurements highlight significant variations in growth rates among the different algal strains.

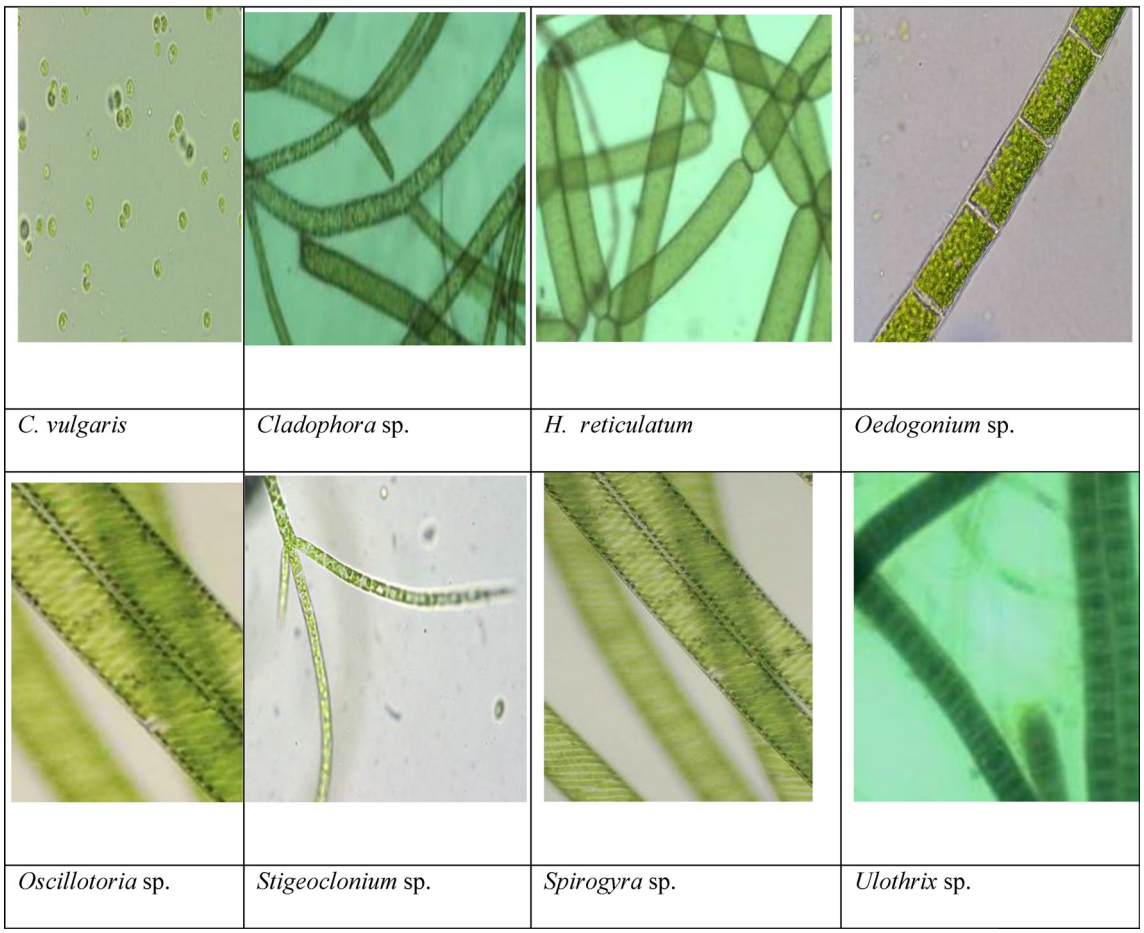


Fig. 2 Microscopic images of potential biodiesel algal strains were studied for their biofuel potential

Lipid Composition of Algal Strains

The results of the lipid composition (% DW) by MAE as illustrated in Fig. 5 were: *C. vulgaris* (45.7%), *Cladophora* sp. (38.2%), *H. reticulatum* (20.4%), *Oedogonium* sp. (34.6%), *Oscillatoria* sp. (32.7%), *Spirogyra* sp. (27.6%), *Stigeoclonium* sp. (56.2%) and *Ulothrix* sp. (62.4%). This unit indicates the proportion of lipids relative to the dry weight of the algal biomass. The lipid compositions were obtained using Microwave-Assisted Extraction (MAE). Each percentage value represents the proportion of lipids in the dry weight of the respective algal strain. For example, *Ulothrix* sp. has the highest lipid composition at 62.4% of its dry weight, while *H. reticulatum* has a lower lipid composition at 20.4%. The results show variations in lipid composition among the different algal strains. Some strains, such as *Ulothrix* sp. and *Stigeoclonium* sp., have relatively high lipid contents, while others, like *H. reticulatum* and *Spirogyra* sp., have lower lipid contents. The results were likely visualized in Fig. 5, which likely provide a graphical representation of the lipid composition data, facilitating a quick and visual understanding of the differences among the algal strains.

In summary, this research provides insights into the lipid composition of various algal strains obtained through Microwave-Assisted Extraction. This information is crucial for assessing the potential of these strains for lipid-based applications, such as biofuel production, where a high lipid content is desirable.

FAMES Analysis of Algal Species

Table 1 presents the FAMEs composition of all eight tested algal strains. The composition of FAMEs was determined using GC–MS analysis. A total of 24 FAMEs were detected in the analysis. Percentage ranges with C16:0 (21–50%), C14:0 (1–5%), C18:1 (20–45%), C18:0 (2–17%), C12:0 (0.2–0.6%) (Fig. 6). C16:0 is the predominant saturated fatty acid. C18:1 (Oleic Acid) is the predominant monounsaturated fatty acid (MUFA). C18:2 and C18:3 are among the detected polyunsaturated fatty acids (PUFAs). The total FAMEs yield was calculated as

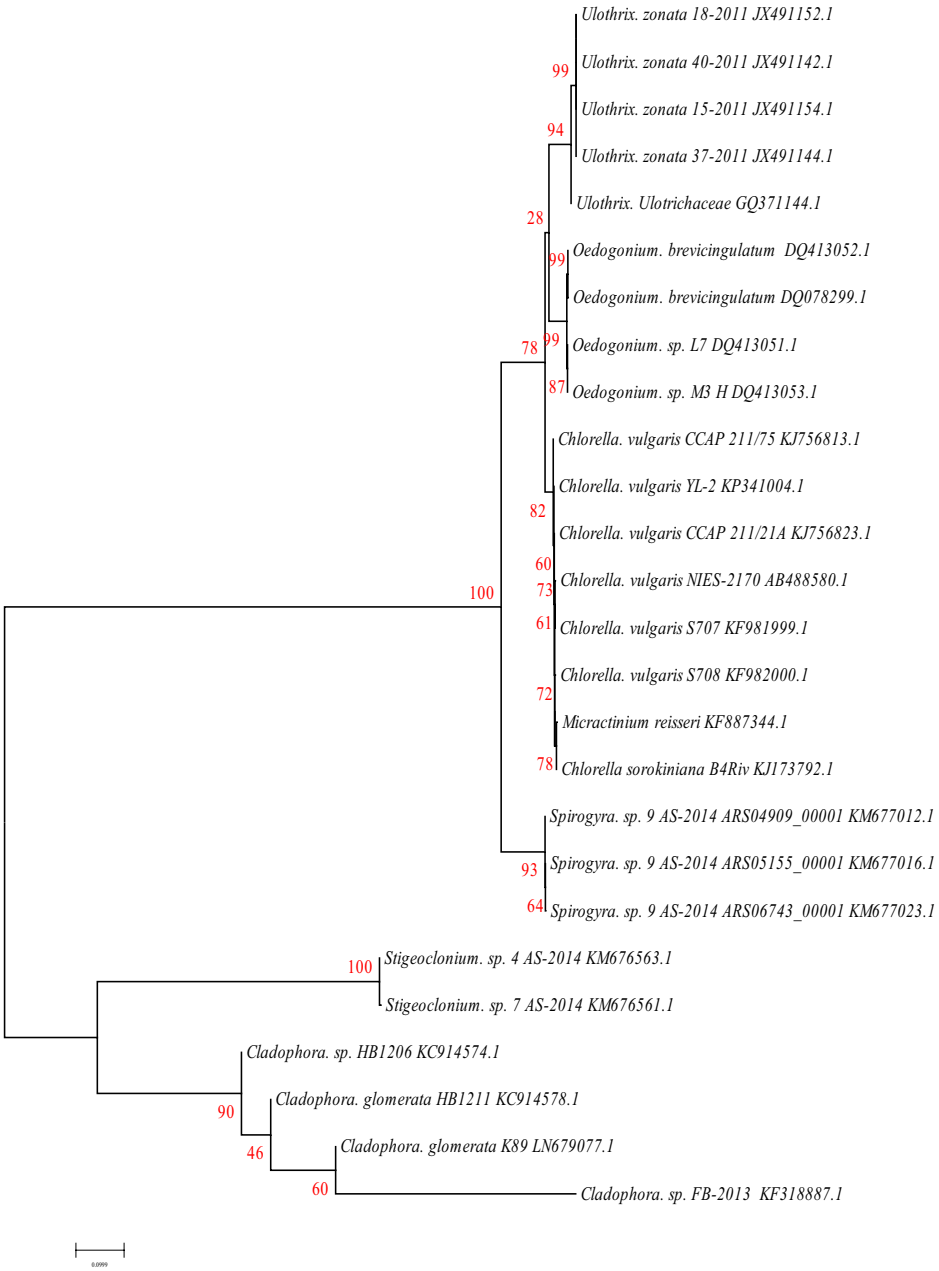


Fig. 3 Phylogenetic tree constructed using nucleotide substitution models, specifically the Kimura 2-parameter model, through the neighbor-joining method. The construction involved 1000 rounds of bootstrap resampling to assess the reliability of the tree's branches

approximately 98% (w/w) from algal biodiesel. The calculation involves determining the fractions of discrete methyl esters in the FAMEs composition. The highest saturated FAMEs content (55.4%) is observed in *Spirogyra* sp. and *Ulothrix* sp. *Ulothrix* sp. also yields the highest monounsaturated FAMEs (52.8%). *Cladophora* sp. shows the highest content of polyunsaturated FAMEs (15.7%).

The results provide detailed information on the types and percentages of foremost fatty acids in tested algae strains. This information is essential for evaluating the suitability of these algal strains for biodiesel production, as different fatty acids have different properties that can impact the quality and characteristics of the biodiesel produced.

Fuel Properties from Fatty Acid Profile of Different Algae Strains

The composition of FAME varied significantly between each algae species inadvertently influencing fuel properties. The characterization of oil was conducted using ASTM and EN standards to assess the quality of the resulting methyl esters. Subsequently, various critical fuel properties were determined in accordance with these standards. The values

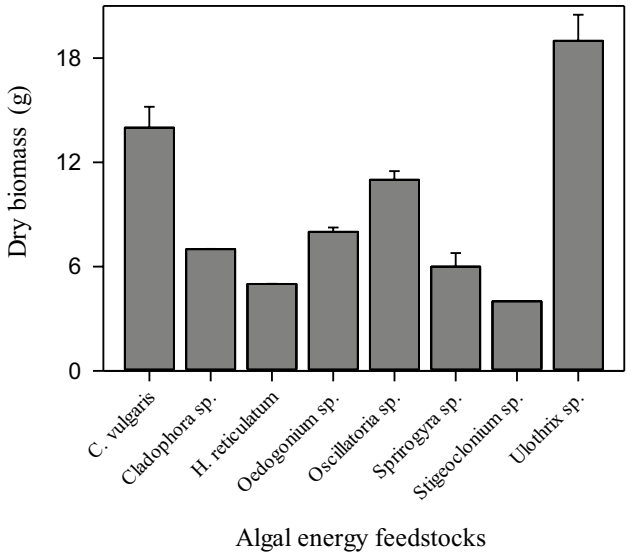


Fig. 4 Biomass productivity at 4th week of cultivation in different algal strains suitable for biodiesel productions for cultivation

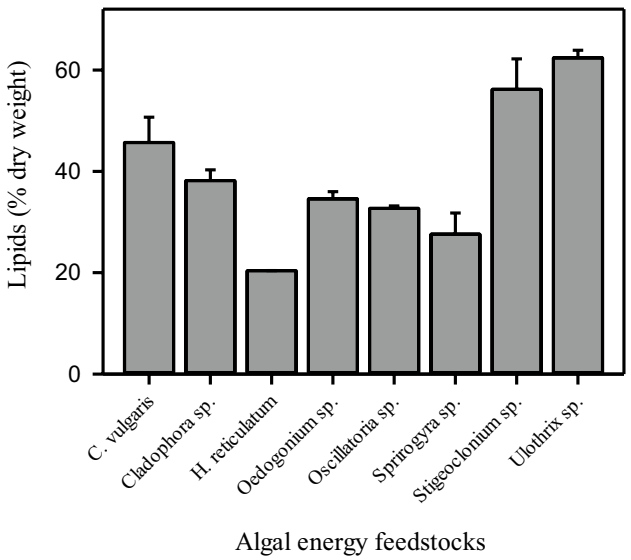


Fig. 5 Lipid productivity in different algal strains suitable for biodiesel productions at 4th week of cultivation

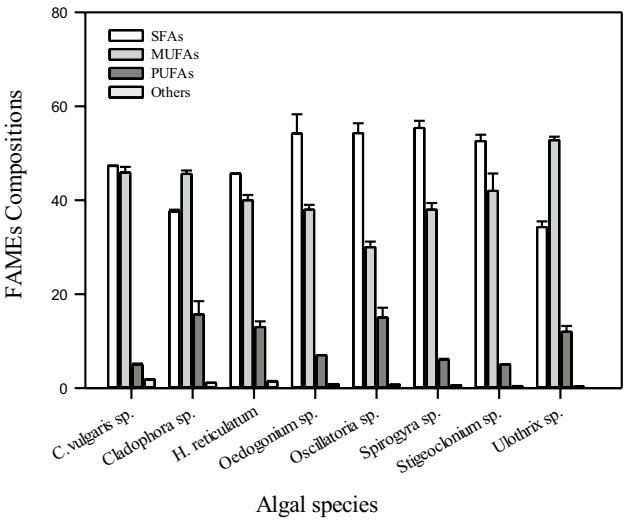


Fig. 6 Fatty acid methyl ester (FAMEs) composition (% dry weight) of selected algal strains at 4th week of cultivation

obtained for these fuel properties are presented in Table 2, allowing for a direct comparison with the standard values.

The density of the biodiesel falls within the range of 0.87–0.89 g cm^{-3}, meeting the defined limit by the EN standard. This is crucial for economical fuel consumption; as lower density aligns with international standards. The kinematic viscosity of the produced biodiesel ranges from 3.6 to 4 mm^{-2}/s, satisfying both ASTM and EN standards. Controlling viscosity is vital for engine efficiency, as higher viscosity may affect atomization during combustion. The reported viscosity values indicate suitable combustion and engine efficiency. This study addresses a major concern in biodiesel commercialization, which is its poor low-temperature properties, including cloud point, pour point, and cold filter plugging point (CFPP). The biodiesel from this research exhibits favorable low-temperature properties with a CFPP ranging from − 14 to 1.4 °C. This surpasses the EN 14214 specification, which requires a CFPP of ≤ 5/ ≤ − 20 °C. The biodiesel's low CFPP implies improved performance in colder regions, making it suitable for use without any issues related to low-temperature conditions [29]. ASTM D6751 specifies a minimum cetane number of 47, while EN 14214 specifies a range of 47–51. The experiment found that the cetane number in all algal strains was greater than 47, except for *H. reticulatum* [25]. The experiment found that the iodine values were within the specified range. No specifications for SV are reported in standards. The experiment found that SV ranged between 194 and 314 mg KOH/g in all eight algae strains. No specifications for DU are reported in standards. DU ranged between 51 and 76 in the studied algae strains. ASTM standard specifies that HHV for biodiesel should be greater than 35 MJ/kg, while there is no standard in EN 14214. The experiment found that the values of HHV were greater than 35 MJ/kg in all eight algal strains. EN 14214 specifies that the oxidation stability should be ≥ 6 h at 110 °C, while there is no specification in ASTM D6751. The overall oxidation stability of the biodiesel for all eight algae strains was within the specified range, with the highest value being 237 h for *C. vulgaris*. In summary, the results from the investigation indicate that the biodiesel produced from the studied algal strains generally meets or exceeds the specified standards.

Algal biomass is a feasible option for biodiesel as it produces 250 times more oil compared to other sources and

Table 2 Fuel characteristics

Species	IV (g $I_2$100/g fat)	SV (mg KOH/g)	CN	DU	LCSF	CFPP (°C)	HHV_i (MJ/kg)	ρ (g cm^{-3})	υ (mm^2 /s)	Y (hour)
EN 14214	≤120	–	≥51	–	–	≤5/≤− 20	NA	0.86–0.9	3.5–5	≥6
ASTM D6751-02	–	–	≥47	–	–	–	≥35	–	1.9–6	–
Ulothrix sp.	85	194	51	76	2.1	− 9.81	38	0.88	3.6	120
C. vulgaris	95	196	52	55	2.0	− 14.21	38	0.87	3.7	237
Cladophora sp.	119	205	51	77	3.4	− 5.7	38	0.87	3.6	61
Oedogonium sp.	107	197	52	51	5.2	0.1	38	0.87	4	171
Spirogyra sp.	99	200	52	51	2.7	− 7.9	40	0.88	3.9	76
Oscillatoria sp.	113	201	49	60	3.9	− 4.2	41	0.88	3.9	77
Stigeoclonium sp.	100	192	52	53	5.7	1.5	36	0.89	4.1	199
H. reticulatum	168	314	25	66	3.5	− 5.4	41	0.87	3.6	120

has numerous properties that favor it as a biodiesel feedstock [30, 31]. Algal biofuel is renewable, biodegradable, emits low sulfur oxides and could be utilized by engines without major amendment [32]. Moreover, algal biodiesel has significant pollutant control potential in a sustainable mode compared to soybean derived fuel. Biodiesel made from soybean oil releases about 1% nitrogen, 8% phosphorus, and 13% pesticides per net energy gain [33]. In present research, 8 different local strains of the green microalga were selected due to their high lipid productivity, growth rates and commercial biodiesel production. After growing eight algal strains for 28 days, the biomass production of *Ulothrix* sp. was the most prominent (19 g) while *Stigeoclonium* sp. (4 g) showed the lowest biomass under similar conditions. Algal biomass was highest in *Ulothrix* sp. and due to its adaptation in polluted and brackish water. *Ulothrix* sp. regulates the optimum nutrient flux and photosynthetic areas. *Chlorella vulgaris* which is composed of a thick cell wall provides mechanical and chemical protection against heavy metal resistance and is a candidate for removing algal species in polluted and contaminated soil and maintaining the optimum biomass as presented in current research. The high growing frequency of algal biomass also demands the maximum accumulation of lipid yield making them a good source for bioenergy. The lipid composition was 45.7% in *C. vulgaris*, 38.2% in *Cladophora* sp., 20.4% in *H. reticulatum*, 34.6% in *Oedogonium* sp., 32.7% in *Oscillatoria* sp., 27.6% in *Spirogyra* sp., 56.2% in *Stigeoclonium* sp. and 62.4% in *Ulothrix* sp. The highest lipid yield was extracted from *Ulothrix* sp. (62.4%) whilst the lowest extracted lipid yield was in *H. reticulatum* (20.4%). The results from the lipid composition is comparable to other studies such as 38% in *C. vulgaris* [6]. Approximately 38 mg/g lipid was obtained from *Stigeoclonium* sp. while 10% lipids were observed in *Spirogyra* sp. The results of the study reported by Khola and Ghazala in the year 2012, showed that the extracted lipids were 3.5% in *Cladophora* sp., 3.3% in *Spirogyra* sp. and 3.9% in *Oedogonium* sp. [34]. Lipid composition varies from species to species [35]. In one study Vasistha et al. (2020) estimated that the diatom can produce 46 tons oil/hectare/year and algae has ability to produce lipids up to 50% [36], moreover, algae is photosynthetically efficient as they can capture greater amounts of light than conventional plants, ultimately promoting growth [28]. With respect to the FAMEs analysis, results indicated that in all 8 algal species fatty acids identified ranged from C12 to C22 chain lengths. C16, C18, and C20:5 comprised 85% of the total fatty acid pool. Fatty acids are very important as the biodiesel excellence is associated with the degrees of saturation/unsaturation of fatty acids as found in this research with *Stigeoclonium* sp. (5.7) which increased CFPP to 1.5 °C resulting in high density (0.89 g cm^{-3}) and viscosity (4.1 mm^2 s^{-1}). Therefore, biodiesel sourced from *Stigeoclonium* sp. could be recommended for hot arid areas of Pakistan and developing countries that face heat stress regularly. Whereas biodiesel extracted from *C. vulgaris* and *Ulothrix* sp. can be recommended for colder regions due to their lower CFPP − 14 °C and − 9 °C compared to other algal strains. In this study, palmitic acid was identified as the foremost fatty acid (30–35%) which has previously been reported in *Chlorella* sp., and *Scenedesmus* sp. under cultivation in sewage water [37, 38]. Linoleic acid along with oleic acid are the dominant fatty acids in algae [39]. Palmitic acid is reported as key fatty acid in *Tolypothrix* [40], *Pithophora* [41], *Spirogyra*, *Hydrodictyon*, *Rhizoclonium* and *Cladophora* [42, 43] while *Chlorella vulgaris* consists mainly of methyl linoleate and methyl palmitate [44, 45]. Approximately 96 wt % of FAMEs were observed in wet *Chlorella vulgaris* biomass [46].

In the current research, the values of CN were within the range of standards in most of the studied alga except for *H. reticulatum*. A high CN value relates with lower nitrogen oxide emissions that leads to better ignition properties [47]. In this study, IV was less than 120 g I_2/100 g in all algae

except for *H. reticulatum*. Biodiesel with large IV has low oxidation stability (OS) as presented in Table 2 [48]. High Saponification Value in biodiesel indicates a high acid content, which can lead to soap formation. This soap formation has the potential to negatively impact the quality of biodiesel and may have adverse effects on both the combustion properties of biodiesel and the machinery of engines using it. Controlling SV is important to ensure the overall quality and performance of biodiesel [49]. Results indicated that SV and DU were within the acceptable range in all 8 algae strains as shown in Table 3. The higher the DU, the more susceptible the biodiesel is to oxidation. Monitoring and controlling DU is important for ensuring the oxidation stability and overall quality of biodiesel as a fuel [50].

Energy generated by the complete burning of fuel is known as HHV [46]. This implies that as the energy content of the fuel increases (higher HHV), the amount of fuel needed for a given amount of energy decreases, indicating greater fuel efficiency [51]. At low temperatures, biodiesel flow is determined by its CFPP [52]. The results of the analysis revealed that the CFPP values of biodiesel produced from the studied algae strains were within an acceptable range. The CFPP is closely tied to the SFA content, and its determination helps in understanding the low-temperature operability and potential cold-weather performance of the biodiesel. At low temperatures, precipitation of stearic and palmitic acid occurs that can be recovered from blocked biodiesel fuel filters [53]. The kinematic viscosity of a fuel, such as biodiesel or diesel, has a direct impact on the CFPP. Understanding this relationship is crucial for ensuring proper engine operation, particularly in cold weather conditions where fuel flow characteristics become more critical [54]. In the current study, ρ and υ for all 8 algae were amid range. Biodiesel storage is directly related to the OS, the need for biodiesel to have suitable oxidation stability (OS) to prevent autoxidation during storage. The specified requirement is an OS greater than 6 h at 110 °C, indicating a desire for the biodiesel to exhibit high resistance to oxidation under elevated temperature conditions. This is important for maintaining the quality of biodiesel during storage. The values of the oxidation stability of biodiesel were within the ranges of reported standards. Hence, these fatty acids of studied algae are good candidates for biodiesel production and can be blended with petroleum oil for transportation and other applications. During the storage of biodiesel, the autoxidation may result in increases in viscosity, detected as deposits or sludge in biodiesel. In the current research, the OS was within range in all algal strains according to EN 14214. Results indicate that *C. vulgaris* is an appropriate candidate for the production of biodiesel among all tested algal strains.

Table 3 shows the biodiesel properties of twelve algae species from literature survey. According to biodiesel standard EN 14214 and ASTM D6751, fuel properties such as IV,

Table 3 Biodiesel properties of different algal strains

Algae	IV (g $I_2$100/g fat)	SV (mg KOH/g)	CN	DU	LCSF	CFPP (°C)	HHV*i* (MJ/kg)	ρ (g cm^{-3})	υ (mm^2/s)	Y (hour)	References
EN 14214	≤ 120	–	≥ 51	–	–	≤ 5/≤ − 20	NA	0.86–0.9	3.5–5.0	≥ 6	[24, 28, 58–60]
ASTM D6751-02	NA	–	≥ 47	–	–	NA	NA	NA	1.9–6.0	–	
Ankistrodesmus falcatus	96	191	53	85	4.38	− 2.7	36.6	0.82	3.6	6.7	
Ankistrodesmus fusiformis	108	189	50	99	3.75	− 4.7	36.9	0.82	3.6	5.6	
Kirchneriella lunaris	130	192	45	111	3.53	− 5.4	38.2	0.85	3.7	5.3	
Chlamydomonas sp.	26	206	66	27	10.8	17.6	36.5	0.81	3.9	20.2	
Chlamydocapsa bacillus	109	187	51	100	3.93	− 4.1	37.1	0.83	3.6	5.6	
Coelastrum microporum	84	195	55	86	4.02	− 3.8	38.8	0.86	4.1	8.6	
Desmodesmus brasiliensis	83	195	55	87	4.43	− 2.6	39.0	0.86	4.1	8.1	
Scenedesmus obliquus	34	204	65	36	8.95	11.6	37.5	0.83	4.0	18.5	
Pseudokirchneriella subcapitata	79	194	56	82	4.23	− 3.2	38.3	0.85	4.1	9.4	
Chlorella vulgaris	50	189	63	56	8.04	8.8	38.1	0.84	4.2	14.3	
Botryococcus braunii	90	188	55	99	1.51	− 11.7	39.2	0.86	4.3	13.8	
Botryococcus terribilis	64	184	61	67	5.08	− 0.5	37.3	0.82	4.1	12.2	

SV, CN were within range in algae species except *Kirchneriella lunaris* [55]. Values of DU, LCSF, HHV, υ and oxidation stability were within range of standards. Values of CFPP were within range of standards except *Chlamydomonas* sp., *Scenedesmus obliquus* and *Chlorella vulgaris*. The density of only *Coelastrum* sp., *Desmodesmus* sp. and *Botryococcus* sp. were within range of standards out of twelve algal strains [56]. During the storage of biodiesel, auto oxidation may increase in viscosity and detected as deposits or sludges in biodiesel. Hence, autoxidation is unsuitable for engine fuels due to blockage of fuel filters and causing fuel pump and injector operational problems [57]. In the present study, biodiesel was produced by transesterification from eight algae strains. Oxidation stability was within range in all algae strain according to EN 14214. The studied algae are presented in the decreasing order of oxidation stability as follows: *C. vulgaris* > *Stigeoclonium* sp. > *Oedogonium* sp. > *Ulothrix* sp. > *H. reticulatum* > *Oscillatoria* sp. > *Spirogyra* sp. > *Cladophora* sp. So, results indicated that *C. vulgaris* qualified as an appropriate algae specie for biodiesel production among all tested algal strains.

Table 4 demonstrate the properties of algal oil, *Jatropha curcas* oil (JCO) and diesel. Algal oil density is greater than the diesel but fewer than the *Jatropha* oil while viscosity of algal oil is greater than both oils. The heating value almost similar to diesel but greater to the JCO [61]. Cetane number is almost similar to diesel but greater to the JCO. The higher value of kinematics viscosity of algal oil indicating that algal oil is extra gooey than both oils. The iodine value of algal oil is greater than the JCO and diesel. The oxidation stability of algal biodiesel is more than JCO but less than to diesel indicating that algal oil can be stowed like diesel [62].

Production of biodiesel especially from the non-food resource is an eco-friendly substitute to the other conservative liquid energies. The biodiesel generation from new resources and their proper adaptation to replace existing energy commodities are still a challenge. Therefore, there is a need to achieve more progress in algal biofuel production for transportation. Now to date, more importance is given to the traditional feedstock which shows the discern non market advantage and issue related to environmental sustainability [66]. However, biofuel production form the discussed alga can reduce land gladding for crop production, greenhouse gas emission and prompt food supply and acceptable cost. Algae biomass offered resource for biofuel production that does not have any food/land controversy but still unable to compete with fossil fuels due to intensive resources and cost of environment maintenance (energy, water, nutrients) [67]. Anyhow there is chances to recycle waste material as inputs in fuel production, moreover algal fuel overcome resource competition and sustain prices and biodiversity.

Conclusions

In conclusion, our study highlighted the cultivation and lipid composition analysis of various new algal strains for potential biodiesel production using suboptimum resources. Algal growth, quantified in dry weight (DW), demonstrated substantial variability among the strains, with *Ulothrix* sp. exhibiting the maximum growth at 19 g DW, while *Stigeoclonium* sp. showed the lowest at 4 g DW. The lipid composition analysis revealed significant differences among the strains, with *Ulothrix* sp. and *Stigeoclonium* sp. displaying notably high lipid contents. Fatty acid methyl ester (FAME) composition analysis through GC–MS identified 24 FAMEs, with variations in saturated, monounsaturated, and polyunsaturated fatty acids among the strains. The biodiesel properties, including cetane number, iodine values, saponification value, density, kinematic viscosity, and oxidation stability, were evaluated against ASTM D6751 and EN 14214 standards. Most algal strains met or exceeded the specified standards, except for *H. reticulatum* in cetane number. Notably, *C. vulgaris* exhibited an impressive oxidation stability of 237 h. Our findings underscore the potential of diverse algal strains for biodiesel production, emphasizing their varied lipid compositions and compliance with established standards. This research contributes to the understanding of algal biofuel potential and offers insights for future bioenergy strategies in Pakistan. In summary, the exploration of algal strains as biodiesel feedstock holds promise for sustainable energy production, with implications for addressing global energy challenges and reducing dependence on conventional fuels. Further research and development in this area can pave the

Table 4 Comparative analysis of algal fuel properties with JC oil and diesel

Fuel properties	Density at 15 °C	Cetane number	Saponification value	Oxidation stability at 110 °C	Iodine value	Heating value	Kinematic viscosity at 40 °C	CFPP	References
Units	Kg m^{-3}	–	mg KOH/g	hours	g I_2/100 g	MJ/kg	mm^2s^{-1}	°C	
Algal oil	919	51	152	8.83	119.1	41	33.06	18	[63]
Jatropha curcas	940	38	198	2.36	94.00	38	24.5	–	[64]
Diesel	836	51	210	17.3	1.35	40–45	3.03	− 6	[65]

way for a more environmentally friendly and economically viable energy future.

Acknowledgements The authors extend their appreciation to the Researchers Supporting Project number (RSPD2024R745), King Saud University, Riyadh, Saudi Arabia and this work was also supported by the 2022 Research Fund of the University of Seoul for Yong Jun Choi.

Data availability All data generated or analyzed during this study are included in this article.

Declarations

Conflict of interest The author declares no conflict of interest on this manuscript.

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