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INDIGENOUS MICROALGAE STRAINS CHARACTERIZATION FOR A SUSTAINABLE BIODIESEL PRODUCTION

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Running title: Indigenous microalgae strains

Abbreviations: **CFPP:** Cold filter plugging point; **CR:** *Chlorella vulgaris* CCAP211/11B ;**CN:** Cetane Number ; **DW:** Dry Weight; **FAs:** Fatty Acids; **FAMEs:** Fatty Acid Methyl Esters; **GC/FID:** Gas Chromatography Coupled with a Flame Ionization Detector; **HHV:** High Heat Value; **IV:** Iodine Value; **LCN:** Local isolated *Chlorella vulgaris*; **LCS:** Local *Coelastrella thermophila var. globulina*; **SFA :** Saturated Fatty Acids; **MUFA :** Monounsaturated Fatty Acids; **OS:** Oxidative Stability; **PUFA:** Polyunsaturated Fatty Acids; **SN:** Saponification Number; **η :** Kinematic viscosity **ρ :** Density; **μ :** Specific growth rate (day⁻¹)

Abstract

Microalgae have been widely recognized as a promising feedstock for sustainable biofuels production to tackle global warming and pollution issues related to fossil fuels uses. This study identified and analyzed indigenous microalgae strains for biodiesel production, specifically *Chlorella vulgaris* and *Coelastrella thermophila* var. *globulina*, from two distinct locations in Algeria. Molecular identification confirmed their identity, and the microalgae exhibited notable growth characteristics. Local *Chlorella vulgaris* and *Coelastrella thermophila* var. *globulina* showed a good growth and high biomass yield, compared to *Chlorella vulgaris* CCAP211/11B reaching a weight of 1.48 g/L, 1.95g/L and 2.10 g/L, respectively. Lipids content of local *Chlorella vulgaris*, *Coelastrella thermophila* var. *globulina*, and *Chlorella vulgaris* CCAP211/11B, were found to be $31.39 \pm 3.3\%$, $17 \pm 2.26\%$, and $19 \pm 0.64\%$, respectively. *Chlorella vulgaris* stood out as a candidate for biodiesel production due to its equilibrium between SFA and PUFA (43.24 % and 45.27 %). FAs are predominated by SFA and MUFA for *Coelastrella thermophila* var. *globulina* with value of 81.49% (SFA+MUFA). Predicted biodiesel qualities are complying with ASTM6751 and EN14214 standards.

Studied microalgae have therefore a promising potential for biodiesel production. However, optimising cultivation conditions is necessary to enhance biomass and lipids yield at large scale.

Keywords: Microalgae, *Chlorella vulgaris*, *Coelastrella thermophila*, Biodiesel, FAMES.

1- Introduction

Biofuels are becoming a relevant substitute for scarce fossil fuels to tackle its related global warming and pollution impacts facing the modern societies and affecting ecosystems global balance, at an alarming rate ^[1]. Microalgae are considered as an alternative to produce a wide range of value-added chemicals with different applications including biofuels, cosmetics, pharmaceuticals, food, feed ^{[2], [3]}.

Microalgae are prokaryotic or eukaryotic photosynthetic microorganisms with a simple cell structure characterized by high sun-based power conversion into biomass (Ten times higher than plants and macroalgae) due to proficient access to water, CO₂ and nutrients ^[4]. Microalgae are able to use wastewater and flue gases for its growth, which represents a sustainable approach to mitigate GHG emissions, reduce pollution, and generate bioenergy products ^{[5] [6]}.

In developed and developing countries, because of energy security basis, environmental and socio-economic concerns, biofuels are considered relevant to meet energy needs ^[7]. The use of first generation feedstock for biodiesel production is deemed unsustainable due to economic, social and environmental concerns ^[8]. Microalgae third-generation biofuels have the most potential to substitute petroleum derived fuels without the drawbacks of first and second-generation biofuels ^[9]. One of the great biological challenges associated with algal biofuels is the identification and bringing into culture of species with a favorable combination of the following characteristics: high growth rate, high lipid content and ease of harvest and extraction ^[10]. The selection of the microalgae strain is crucial for producing biofuel and high-value co-products to maximize revenue. Suitable microalgae species are selected based on their carbohydrate, lipid and protein contents, which are important for producing high quality biofuels ^[2]. This chemical composition allows microalgae to take advantage of biorefinery concept opportunities. Nevertheless, microalgal commercial viability is highly dependent on evaluation and improved integration of most possible waste bioconversion and valorisation patterns ^[11].

Adequate species selection based on oil yields and lipids composition quality are among the principal challenges in microalgae biofuel production and its industrial feasibility ^[12]. Despite the fact that several strains of microalgae were employed in the biodiesel production, only limited numbers of strains were able to show high potential in the accumulation of lipid in terms of its quality and quantity ^[9]. Screening native algae for species with desirable traits gives a robust biological platform for bioresource production given their pre-adaptation to the prevailing regional abiotic and biotic factors ^[13]. Bioprospecting and utilizing of indigenous strains is benefited by local microalgae species

isolating and selecting, regarding their physiological characteristics, as they are highly adapted to the local climatic environment, unused domestic biological resources utilization, and foreign species biological contamination prevention, when cultivated for large scale operations ^[14].

This study identified and analyzed indigenous microalgae strains isolated from two distinct regions in Algeria for biomass and biodiesel production. Experiments were performed for both native strains with a reference strain from CCAP culture collection of algae and protozoa. They were cultivated in batch mode using a torus photo bioreactor under autotrophic conditions in order to evaluate their potential as candidate for biomass and lipids generation for biodiesel production. In this respect, the characteristics of algal growth, biomass, biochemical and fatty acids composition, and derived biodiesel quality were investigated.

2. Materials and methods

2.1 Sample collection, Isolation, and identification of microalgae

The freshwater microalgae were collected from Oued-Hamlil Spring located in Zeboudja municipality, in the North of Algeria (LCN) (36°23'33.6"N 1°31'41.7"E) and the Tamentit foggara located in ADRAR, in the south of Algeria (LCS) (27°45'52.8"N 0°15'56.5"W). BG11 medium was used for isolation (24 h light, 4000 lux, 25 °C, 7 days) and serial dilution was used for purification ^[15].

FastDNA™ SPIN Kit for Soil from MP Biomedicals was used for DNA extraction of local *Chlorella vulgaris* (LCN). The 18s rDNA genomic region was amplified with Primers Euk1A Fw and Euk516r ^[16]. The PCR amplification was carried out according to ^[16] with a varied conditions using 5 min for initial denaturation at 94 °C, followed by 25 cycles as follows: 30 s for DNA_denaturation at 94 °C, 1 min for primers annealing at 63 °C and 1.5 min for amplification at 72 °C, then by a final period of 10 min at 72 °C for amplification. PCR products were sequenced by Secugen (Spain) employing the Sanger method ^[16].

The DNA of local *Coelastrrella thermophila var. globulina* was extracted using a NucleoSpin Plant II commercial kit (Macherey-Nagel, Germany). The ITS rDNA genomic region was amplified with

Primers ITS Fw and Rv ^[17]. The PCR amplification was carried out using 5 min for initial denaturation at 95 °C, followed by 35 cycles as follows: 30 s for DNA denaturation at 95°C, 30s min for annealing of primers at 55 °C and 45s for amplification at 72 °C, followed by a final period of 7 min at 72 °C for amplification. The purified fragments were subjected to the Sanger sequencing method using the Applied Biosystems BigDye v3.1 kit and PCR primers that were used to amplify the fragments of interest.

The sequencing results were assembled using the Geneious (version 11.1.5) software package. To assign identities to the studied strains, two different molecular methods were used, Basic Local Alignment Search Tool (BLAST) and Tree Building. For Tree Building, the study strains sequences and a selection of sequences gathered from the GenBank database, were aligned using the ClustalW algorithm^[18].

Phylogenetic trees were inferred from the aligned sequence data using the maximum likelihood (ML) method implemented in RAxML (8.2.12) on the Cyberinfrastructure for Phylogenetic Research Portal (CIPRES) (http://www.phylo.org/sub_sections/portal) ^[19]. The evolutionary model used was the default GTR+ Γ . A total of 1000 rounds of bootstraps were conducted. Trees were drawn with the aid of the iTOL tool (<https://itol.embl.de>) ^[20].

2.1. Microalgae strains cultivation

Two isolated microalgae strains were cultivated in batch mode under autotrophic conditions in a torus photobioreactor of 1.3 L effective volume. *Chlorella vulgaris* CCAP211/11B was cultivated as reference strain (CR). All strains were maintained in culture using sterilised BG11 medium (120 °C, 20 min). Local strains cultivation was carried out in triplicate using BG11 medium under the following conditions: Temperature 25 °C, light intensity 7000 Lux (LED lamps), Cells density 10^6 cells/mL, Aeration 50 mL/min (air flowmeter Borkshort), agitation 150 rpm/min. The three microalgae cultures were lasted for 18 days with initial pH set at 7.5 without control during the culture.

2.2. Monitoring culture and growth measurement

The culture was sampled regularly to monitor different parameters of growth such as cells concentration, optical density, and dry biomass. Biomass was estimated by optical density at 680 nm wavelength using an ultraviolet/visible spectrophotometer (PerkinElmer life and Analytical, UK) in 1 cm test tubes. Specific growth rate (μ day⁻¹) was calculated according to the equation $\mu = \ln(X_2 / X_1) / (t_2 - t_1)$, where X is the biomass yield (g/L) and t is the time (day). Cells concentration was estimated using Malassez Counting chambers where cells total number was calculated using Eq.1. After 18 days, the cultures were harvested by centrifugation at 4000 rpm for 10 min and washed twice with distilled water. Wet biomass was dried in an oven at 70°C for 24 h [21].

Cells Density (Cells/mL) = Total number of cells counted x 10⁵ x DF (DF: Dilution factor) (1)

2.3 Biochemical composition determination

Microalgae biochemical composition for total carbohydrate and proteins analysis were carried out by the phenol-sulphuric acid and Lowry methods as described by [22], respectively. Ash content was determined according to [21]. The total lipids content was estimated by subtracting the percentage of ashes, carbohydrates and crude proteins out of 100%.

2.4. Lipids analysis and biodiesel predicted qualities

Crude oils from microalgae were extracted by a Bligh and Dyer method [23]. Aliquots of crude lipid were acid-catalyzed transmethylated to FAMES by reaction with H₂SO₄ (5%) (analytical grade), then heated for 90 min at 85 °C. Once the mixture reached room temperature, 1.5 mL of NaCl (0.9%, w/v) and 1 mL of hexane (analytical grade) were added to facilitate phase separation. The mixture was mixed vigorously and the hexane phase containing fatty acids was recovered. Identification and quantification of different fatty acids was performed using gas chromatography (Agilent, Kyoto, Japan) equipped with FID detector and BD-EN14103 capillary column (30 m x 320 μ m x 0.25 μ m). The velocity of the carrier gas (Helium) was maintained at 33 cm/s. The sample injection volume was 2 μ L and was executed in a split mode (1:20 ratio). The back-detector temperature was 260 °C. The

oven temperature profile was incrementally brought from 50 °C to 230 °C with varying holding time. The relative quantity of different fatty acids was calculated based on area under the curve and analysis was carried out in triplicates.

Biodiesel qualities derived from fatty acids composition such as Saponification number (SN) and Iodine value (IV), Cetane number (CN), Oxidative stability (OS), Cold filter plugging point (CFPP), were estimated using empirical correlations according to ^[24]. High Heat Value (HHV) was calculated according to ^[25]. Kinematic viscosity (η) and density (ρ) were calculated according to ^[26].

3-Results and discussion

3.1 molecular identification

Indigenous microalgae were isolated from two different locations in Algeria for their high growth and lipids potential for biodiesel production. Molecular identification was carried out to confirm the identity of the isolated microalgae strains according to the 18S rDNA and ITS region amplification. Isolated strains were found 99 % and 100 % identity with that of previously sequenced *Chlorella vulgaris* (North of Algeria) and *Coelastrella thermophila var globulina* (South of Algeria) in the NCBI database, respectively. Molecular identification was also supported by the results from the phylogenetic analysis of the 18S rDNA and ITS region sequences (Figure 1).

Figure 1:

3.1 Microalgae strains growth

Figure 2 presents microalgae growth expressed by cell density/ml. The highest densities were 35.71×10^6 cell/ml and 21.19×10^6 cell/mL for CR and LCN strains, respectively. Highest cells density for LCS was 2.75×10^6 cells/ml. LCS cells density remained very low throughout culture if compared to both CR and LCN. Maximum growth rates were 0.35 d^{-1} and 0.59 d^{-1} at day 3 for LCN, and CR, respectively. LCS growth rate was found to be 0.38 d^{-1} at day 3 and 0.53 d^{-1} at day12. These results

were close to those reported in literature about LCN and LCS on BG11 medium, which fluctuated around $0.231\text{-}1.1\text{ d}^{-1}$ throughout the exponential growth phase, and then decreased in the stationary growth phase ^[27].

Figure 2:

Several parameters affect microalgae growth, which can be categorized into nutritional factors (e.g., carbon, nitrogen, phosphorus, silicon, metals, vitamins) and environmental factors (e.g., pH, temperature, light intensity, aeration). pH is crucial for microalgae growth. While most species are pH-sensitive, *Chlorella vulgaris* can grow within a wide range of pH values from 4 to 10 ^[28].

The highest growth rate and biomass productivities were observed at pH 9-10 ^[29]. In this study, initial pH for the three strains (LCS, LCN, CR) was 7.5, which increased to 10.84 after 3 days for LCN, to 10.89 for LCS, and to 10.19 at day 10 for CR. CR growth declined after day 11, caused probably by the high pH of 10.94 (figures 2 and 3).

Figure 3:

3.2 Dry weight Biomass Evolution

Dry weight biomass yield is a critical economic determinant for algal biofuel biotechnology. Figure 4 shows dry weight evolution for the three strains studied. *Chlorella vulgaris* CCAP211/11B and *Chlorella vulgaris* presented a maximum biomass dry weight of 2.10 g/L and 1.95 g/L on day 18, respectively. *Coelastrella thermophila var.globulina* (LCS) achieved a dry weight of about 1.48 g/L.

Results are in agreement with other studies where ^[30] found the highest dry biomass for *Chlorella vulgaris* in BG-11 ($1.64 \pm 0.07\text{ g/L}$). ^[31] have reported that native isolated local *Chlorella vulgaris* from Egypt had the highest biomass in BG11 ($1.86 \pm 0.1\text{ g/L}$). In comparable conditions of high pH, ^[32] have found an equivalent biomass yield in the range of 1.5- 2 g/L for *Chlorella sorokiniana*. This might be due to higher nitrogen favoring biomass growth, as demonstrated by ^[33] for *Chlorella sp* cultivated in BG11 compared to other mediums (BBM, TAP). ^[34] have found that *Chlorella vulgaris*

could thrive in alkaline conditions where a pH of 10.5 gave the most relevant growth in biologically treated water of poultry slaughterhouse. ^[35] have found that cultivation of *Coelastrella rubescens* without CO₂ enrichment resulted in a rapid alkalization of the medium to pH 9.5–10.5, impairing the physiological conditions of the cells. Dissolved inorganic carbon concentration is influenced by pH (e.g., CO₂ and H₂CO₃⁻; pH < 7, HCO₃⁻²; 7 < pH < 9, CO₃⁻²; pH > 9). Since CO₂ and HCO₃ can be utilized by microalgae cells but CO₃⁻² can hardly be assimilated, a method that can efficiently control microalgae suspension pH to 6–10 is useful to improve carbon availability, thus improving CO₂ fixation efficiency ^[36]. This could explain the LCS and LCN growth improvement since the 11th day due to pH decreasing under 10 (figure 1).

3.3 Biochemical composition of the biomass

Biochemical composition of microalgae was carried-out for proteins, lipids, carbohydrates, and ash (figure 4). LCN, LCS, and CR strains showed a protein content of 31 ± 3.30%, 39 ± 2.26%, and 36 ± 0.64%, respectively. Carbohydrate content of 15 ± 0.23 %, 24 ± 1.40%, and 22 ± 1.20 % were obtained for LCN, LCS, and CR, respectively. Lipids content of LCN, LCS, and CR strains were found to be 31.39 ± 3.3%, 17 ± 2.26%, and 19 ± 0.64%, respectively. ^[37] reported that median composition of phytoplankton is 17.3% for lipids, 32.2% for protein, 15% for carbohydrates (% dry weight) under nutrient unlimited conditions. Under the stationary phase of growth, the composition is shifted to 22.5% lipids, 27% protein, and 21.8% carbohydrates.

A remarkably high content of ash of 20–23 % DW was observed. A range of 1.5 to 20 % of ash content was reported for microalgae, which was largely influenced by high minerals salts in BG11 medium ^[38].

Figure 4

Microalgae lipid accumulation is species dependent and is affected by the cultivation parameters, such as light intensity, temperature, salinity, operation times, and nutrients (carbon/nitrogen/phosphorus) ^[39]. ^[2] reported an average of 2 to 24 % of lipid content for *Chlorella vulgaris* strain. However, it

could reach a content of 62.5 wt% in BG11 medium under nitrogen concentration of 62 mgN/L ^[40]. Lipid accumulation is more sensitive to nitrogen N-limitation other than P-limitation and it is suggested to set optimum conditions (e.g. N:15-35 mg/L and P:4-16 mg/L) to balance the lipid accumulation and biomass growth ^[41]. Local isolated *Chlorella vulgaris* showed a lipid content of 18.57% in nitrogen limited digestate effluent (25 mg NH₄-N/L, pH 7.8) at stationary phase (24th day of cultivation), with a FAs composition dominated by the C18:2, C18:1, C16:0, compared to a lipid content of 10% DW for *Chlorella vulgaris* SAG 211/11b ^[42]. Lipids content for *Coelastrella genus* is ranging from 17.02 to 26.47% DW ^[43] and it could reach-up to 50 %DW under N and P starvation, indicating its potential as a lipid-yielding strain ^[44].

The optimal pH range for microalgae growth is strain-specific, while a range from 7.0-9.5 can yield a higher lipid accumulation in most microalgae species ^[45]. Lipid content for *Chlorella* genus varied from 17 % to 45 % at different pH setting. However, the high lipid content came at the cost of lower biomass productivity ^[46]. Light intensity effect on lipid content and accumulation is contradictory in the literature, since it has been reported to be only affected to a minor extent in some studies, while lipid content increases with light intensity with an optimal irradiance for lipid accumulation in other studies ^[47].

The most promising microalgae for biofuel production should have simultaneous growth, lipid accumulation, and respond to little stress without significantly damage the growth rate. Lipid content of 30 % is quite common in most microalgae proposed for biodiesel production, while some, like *Nannochloris sp.* (56%), *Chlorella sp.* (53%), and *Neochloris oleoabundans* (65%), have higher content. However, high-oil strains grow slower; e.g., 80% oil microalgae grow 30 times slower than 30% oil ones ^[45]. In this aim, pH stress could be a robust and an effectiveness approach to increase lipid content and to improve the fatty acids quality without affecting biomass production.

3.4 Fatty acid composition and derived biodiesel quality

The most common FAMES are palmitic acid (C16:0), oleic acid (C18:1n9), linoleic acid (C18:2n6), and linolenic acid (C18:3n3) (table 1). They are mainly rich in C16:0 which are 43.25 %, 44.98 % and 28.23 % for LCS, CR and LCN, respectively. Oleic acid content known to be a main component of biodiesel was 19.86%, 17.87%, and 11.49% of total fatty acids in LCS, CR and LCN, respectively.

FAs content showed that LCS and CR were predominated by saturated and monounsaturated fatty acids, with values (SFA+MUFA) of 81.49 % and 72.06 %, respectively. LCN shows equilibrium between SFA and PUFA with values of 43.24 % and 45.27 %, respectively.

Physical cultivation parameters influence the FAs profile and hence both the quantity and quality of lipids, such as light intensity and light duration, temperature, nutrient limitation, pH, oxidative stress [47]. It was reported that low level of CO₂ favors the accumulation of saturated FAs, while high CO₂ concentrations stimulate unsaturated FAs [47]. [42] have found that local *Chlorella vulgaris* (LCN) showed a FAs composition highly rich in C16:0 and C18:2 at the end of exponential phase (12th day of cultivation, 500 mgNH₄-N/L, pH 7.8). FAs composition was found to be dominated by the C18:2 at the stationary phase, which are similar to results found in this study. [48] found under N-deprivation *Chlorella vulgaris* shifts from the production of polyunsaturated FAs (C18:2 and C18:3) to saturated or monounsaturated FAs (C18:0 and C18:1).

Only one study reported the fatty acid profile of *Coelastrella thermophila var. globulina* isolated from an Algerian hot spring [49]. Its FAs profile consisted of MUFA (51%), SFA (27%), and PUFA (22%) (35°C, 75 µmol/m² s, 16:8 light/dark cycle). In the current study, the FAs profile consisted of 60% of SFA; this could be due to the high light intensity and photoperiod. [47] reported that an increase in light intensity and light duration was shown to be related to increased SFAs and decreased MUFAs and PUFAs.

TABLE 1:

Table 2 shows the different parameters used for Biodiesel quality according to the current standards. Different FAs profile confer different properties to the derived biodiesel and accurate prediction of biodiesel properties from FAs content is challenging due to complex relationships of FAs properties, such as the chain length, the number and geometry of double bonds of the fatty acids ^[50].

The CN is an indicator of the ignition quality of a diesel fuel, which depends strongly on compounds structure ^[51]. Standards ASTM D6751 and EN 14214 for biodiesel fuel requires a minimum CN of 47 and 51, respectively. The values obtained for different microalgae species range from 57.78 for LCN, 64.51 for CR and 69.62 for LCS.

Estimated oxidative stability (OS) for the studied strains are presented in table 2. The relatively high OS for LCS is explained by its low C18:3 content compared to the other strains. Low OS for LCN and CR could be explained by the high PUFA content for each microalgae strain, in particular for LCN strain (table 1).

The European standard EN14214 defines a maximum iodine value (IV) of $120 \text{ g I}_2 \text{ 100 g}^{-1}$, which is necessary to avoid the polymerization of glycerides and deposits formation or deterioration of the lubricating oil. Obtained IV for biodiesel issued from the three microalgae strains are in accordance with the EN14214, since the highest value of 89.62 was found for LCN.

The cold filter plugging point (CFPP) is a parameter usually used for the prediction of the flow performance of biodiesel at low temperatures. The CFPP obtained for the different microalgae oils varied from -7.61 to 29.72° C . LCS biodiesel had poor CFPP (29.8° C), which is due to its high SFAs content (59.33 %)

High heating value (HHV) is an important fuel property that characterize the fuel energy content. Biodiesels derived from the studied microalgae strains showed a good HHV of 39 MJ/kg.

Fuel with high viscosity tend to form larger droplets on injection which cause poor fuel atomization during the spray, and leads to increase exhaust smoke and emissions ^[51]. The viscosities were 3.80,

3.93 and 4.14 mm²/s, for LCN, CR and LCS, respectively. These values are in accordance with ASTM6751 and EN14214 specifications (table 2).

Density affects the mass of fuel injected into the combustion chamber and the air-fuel ratio ^[26]. The microalgae strains densities were the same with a value of 0.860 g/cm³, namely in the range of biodiesel density values, between 0.864 g/cm³ and 0.901 g/cm³ at 20°C, according to the results of ^[52].

TABLE 2

The high omega-6 FA content in LCN lipids (35.91%, w/w) is advantageous for versatile biodiesel production, enhancing process flexibility, particularly at larger scales. Regarding LCS strain biodiesel, its blending with fossil diesel or other feedstocks can alleviate poor cold flow properties. Using multiple microalgal species with complimentary FAs profiles can improve biodiesel quality.

4- Conclusion

This study identified and analyzed indigenous microalgae strains, specifically *Chlorella vulgaris* and *Coelastrella thermophila var. globulina*, from two distinct locations in Algeria. Molecular identification confirmed their identity and the microalgae exhibited notable growth characteristics. Microalgae's biochemical composition was higher than the median composition reported for microalgae for proteins, carbohydrates, lipids. Lipid content spanned from 17% to 30%, with *Coelastrella thermophila var. globulina* having the lowest and local *Chlorella vulgaris* the highest lipid content. Isolated microalgae presented a substantial potential for biodiesel production due to its adequate FAs profile and predicted biodiesel qualities are complying with ASTM6751 and EN14214 standards. Local *Chlorella vulgaris* standouts as a candidate for biodiesel production due to its high lipid accumulation with adequate FAs profile. Cold flow properties of biodiesel derived from *Coelastrella thermophila var. globulina*, may necessitate blending with other feedstocks or fossil diesel. The use of complementary FAs profiles could address biodiesel properties. Culture optimization is needed to enhance biomass and lipid yields at large scale for the local isolated microalgae strains.

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Data Availability Statement: Authors declare that all relevant data are available within the article.

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The manuscript does not contain experiments using animals.

The manuscript does not contain human studies.

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Tables section

TABLE 1: Fatty acids composition of microalgae strains

Fatty acid / Sample id	LCN	CR	LCS
C12:0	7.01 ± 0.6	9.20 ± 0.53	
C14:0	8.00 ± 0.01		2.38 ± 0.08
C14:1			
C15			
C16	28.23 ± 0.16	44.98 ± 1.3	43.25 ± 0.98
C16:1			2.30 ± 0.01
C17:1			
C18:0			10.90 ± 0.12
C18:1n9 omega 9	11.49 ± 0.14	17.87 ± 0.12	19.86 ± 0.15
C18:2 n6 trans omga6	35.91 ± 0.05	17.22 ± 0.41	12.43 ± 0.18
C18:3n3 omega 3	9.36 ± 0.04	10.72 ± 0.51	6.08 ± 0.17
C22:0			1.38 ± 0.05
C24:0			1.43 ± 0.05
C22:6 n3			
ΣSFA	43.24 ± 0.14	54.19 ± 0.09	59.33 ± 0.55

Σ MUFA	11.49 \pm 0.19	17.87 \pm 0.38	22.16 \pm 0.14
Σ PUFA	45.27 \pm 0.16	27.94 \pm 1.2	18.51 \pm 0.19

TABLE 2: Predicted properties of biodiesel from microalgal lipids compared to ASTM 6751 and EN 14214 standards.

Property	LCN	CR	LCS	ASTM D6751	EN14214
SN	172.53	162.96	155.90	-	-
IV (gI_2 100 g^{-1})	89.62	67.97	51.97	-	120
CN	57.78	64.51	69.62	47 min	51 min
Oxidative stability; h	7.60	13.88	16.69	3 min	8 min
HHV	39.39	39.38	39.04	-	-
Kinematic viscosity (40 $^{\circ}$ C; mm^2s^{-1})	3.80	3.93	4.14	1.9–6.0	3.5–5.0
LCSF	2.82	4.89	14.70	-	-
CFPP ($^{\circ}$ C)	-7.61	-2.34	29.72	-	-
Density at 20 $^{\circ}$ C (g/cm^3)	0.88	0.87	0.86	-	-

Figures section

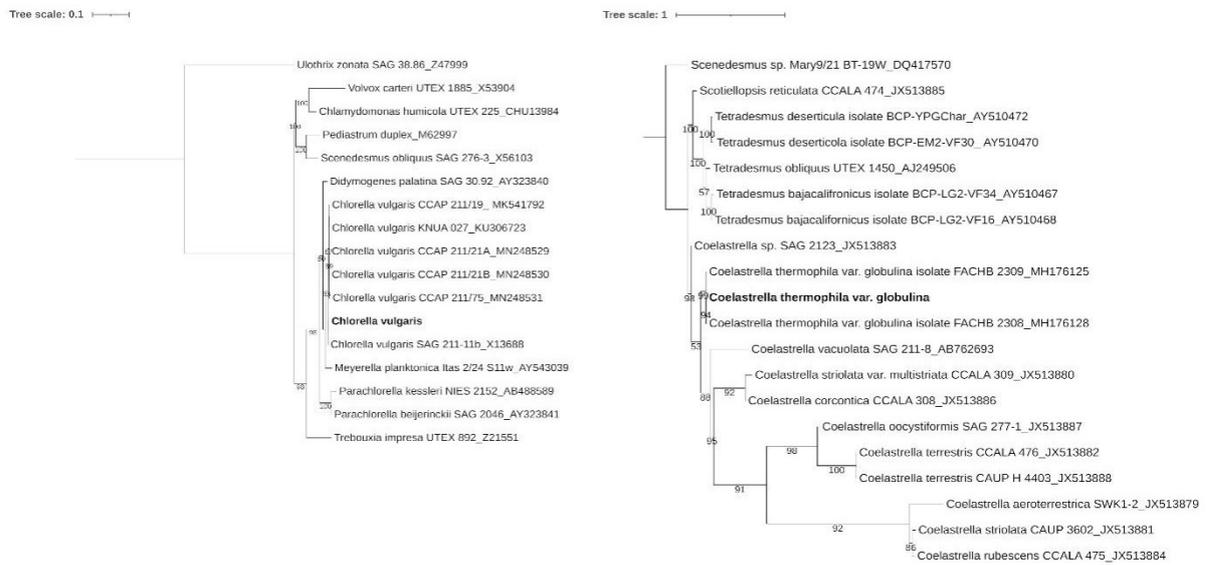


Figure 1: Phylogenetic analysis based on the 18S rDNA and ITS region amplification. Comparison was based on most similar sequences retrieved from NCBI nucleotide database

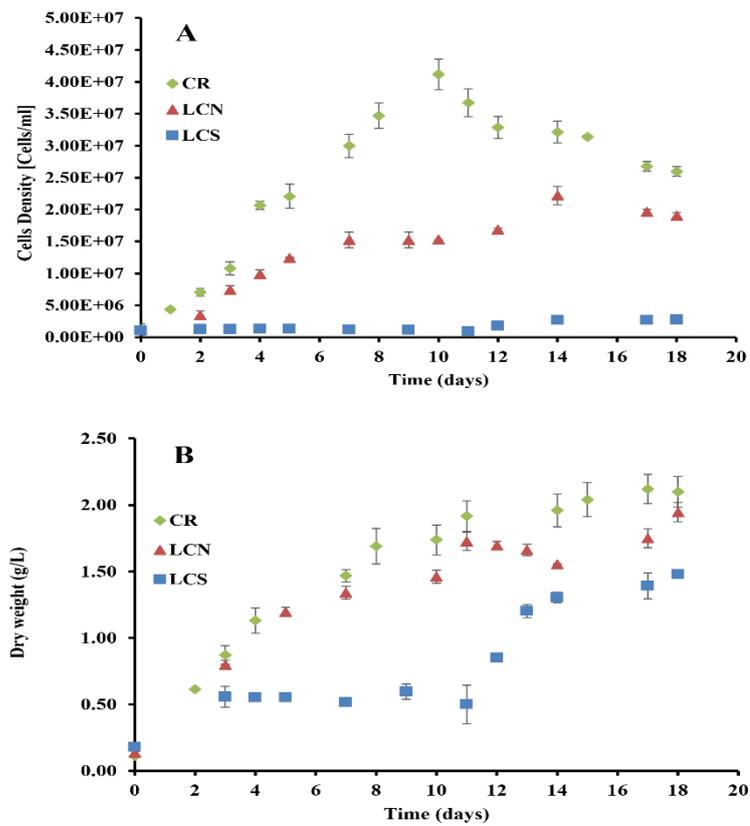


Figure 2: Growth characterization of isolated microalgae strains

(7000 Lux, 10^6 cells/ml, 25°C, pH_i 7.5)

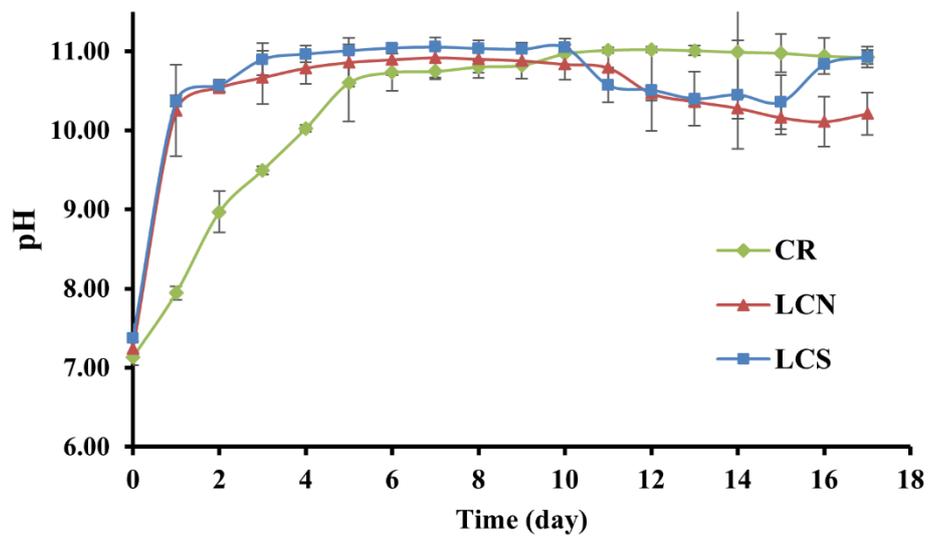


Figure 3: pH variation during CR, LCN, and LCS microalgae cultivation

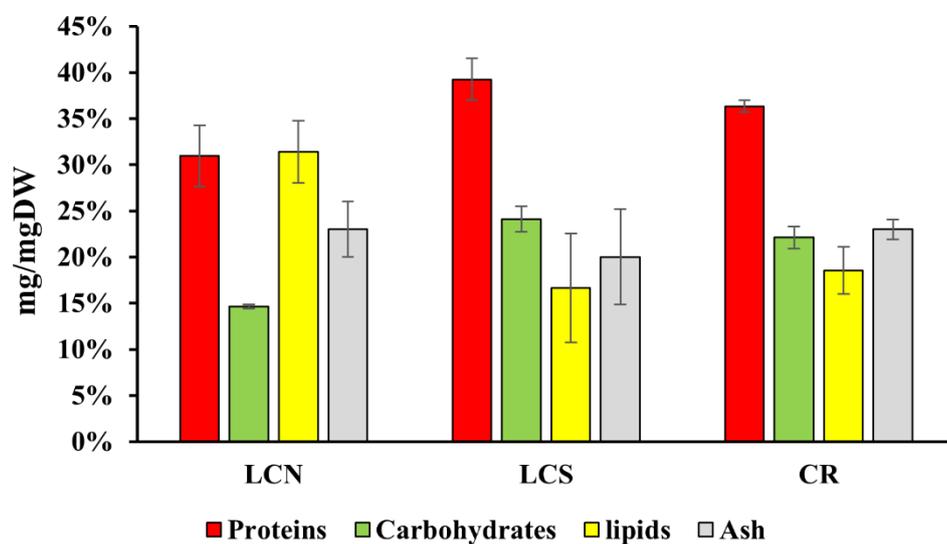


Figure 4: Biochemical composition of the microalgae strains LCN, LCS and CR

(7000 Lux, 10^6 cells/ml, 25°C, pH 7.5).