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# Evaluation of *Nannochloropsis oculata* as Potential Feedstock for Sustainable Biodiesel Production

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**Abstract:**The increasing global focus on renewable and sustainable energy sources has highlighted microalgae as a promising feedstock for biodiesel production. This study evaluates the potential of *Nannochloropsis oculata*, a green alga species known for its high lipid content, in producing environmentally friendly biodiesel. The microalga was cultured in F2 medium under controlled environmental conditions to analyze its growth behavior, biomass yield, and lipid accumulation. A distinct sigmoidal growth curve was observed, with the stationary phase reached at day 25 and a baseline lipid content was recorded of 19.8%. Further experimental modifications, particularly adjusting the nitrogen-to-phosphorus (N:P) ratio and light exposure, led to notable improvements in both biomass and lipid production, achieving a maximum lipid content of 30.91% under a 1:1 N:P ratio. GC-MS profiling identified major fatty acids such as palmitic acid (C16:0), oleic acid (C18:1), and stearic acid (C18:0), which are crucial for optimal biodiesel characteristics. Using Biodiesel Analyzer software, key fuel properties were predicted, including a high cetane number (97.65) and a low cold filter plugging point (-2.72°C), meeting international biodiesel standards. These findings demonstrated that *Nannochloropsis oculata* holds strong potential as a sustainable and efficient source for biodiesel, offering a carbon-neutral alternative that addresses both ecological and economic challenges.

**keywords:** Nannochloropsis oculata, Biodiesel, Microalgae, Lipid content, Renewable energy

## 1.Introduction

There is growing interest among researchers in discovering renewable energy sources that are both economically viable and environmentally sustainable; however, this field still requires extensive investigation. Energy plays a vital role in modern society, being essential for virtually all human endeavours. The development of the steam engine and the ensuing reliance on fossil fuels have significantly advanced various sectors. Nevertheless, the overuse of these limited resources has led to serious environmental consequences, including pollution of land and water, release of harmful gases, and global climate change [1]. Considering that fossil fuel reserves could be depleted within the next 50 years [2], the search for renewable and sustainable energy options has become increasingly urgent.

Renewable energy, which is naturally replenished over short time periods, encompasses carbon-neutral forms such as solar, wind, hydro, tidal, geothermal, and biomass energy. Among these, bioenergy obtained from organic matter has attracted significant attention due to its potential to cut greenhouse gas emissions and lessen dependence on fossil fuels [3]. Biofuels derived from biological materials like plants, animals, and microorganisms are non-toxic and can decompose naturally [4]. However, first-generation biofuels—produced from food crops such as corn, sugarcane, and oil palm—have been criticized for causing ecological harm and

contributing to food scarcity [5]. These concerns have encouraged the development of second- and third-generation biofuels, which rely on non-food biomass, such as agricultural waste, microbial biomass, and urban sludge [6].

Microalgae and other microorganisms hold significant promise for biofuel production due to their high lipid content, effective photosynthetic capabilities, and resilience in harsh environments [7]. These organisms can generate various forms of biofuels, such as biodiesel, bioethanol, and biohydrogen, while also generating valuable by-products used in industry [8]. Microalgal lipids, which contain high levels of oleic, palmitic, and stearic acids, are especially well-suited for biodiesel production through the process of transesterification [9].

Certain microalgae species, such as *Chlorella vulgaris* and *Scenedesmus*, can accumulate lipids amounting to up to 43% and 18% of their dry biomass, respectively, under optimal growth conditions [10]. Transesterification transforms these lipids into methyl esters (biodiesel) and glycerol [11]. Compared to conventional diesel, algal biodiesel has higher viscosity and a greater concentration of unsaturated fatty acids, which are crucial for fuel performance [12]. Importantly, microalgae-based biofuels do not compete with food crops, thereby reducing threats to food security [13]. Their ability to grow in a wide range of water sources—including seawater and wastewater—also boosts their sustainability credentials [14].

Utilizing microalgae for biofuel production contributes to both energy independence and environmental protection by lowering $CO_2$ emissions and using non-food feedstocks [15]. Their high lipid yield, strong $CO_2$ absorption capacity, and effective photosynthetic activity make them an ideal candidate for biofuel generation [16,17]. Additionally, the application of biovalorization methods helps lower production costs and creates useful co-products, enhancing economic viability [12].

This study explores the potential of the selected *Nannochloropsis oculate* green microalga as renewable energy sources, highlighting their advantages in biofuel production and their contribution to developing sustainable energy strategies. By harnessing the unique properties of microalgae, this research aims to help tackle global energy challenges while promoting ecological and economic sustainability.

## 2. Materials and methods

### 2.1. Culture Conditions of Microalgae strain

#### 2.1.1. Microalgae Strains and Taxonomic Classification

A single green microalgae *Nannochloropsis oculata* (Korshikov), was selected in this study. The strain was obtained from the Phycology Laboratory at the Faculty of Fish Resources, Suez University, Egypt. It was collected using a plankton net with a mesh size ranging from 1 to 5 µm.

**The taxonomic classification of the microalgae species is as follows:**

*Nannochloropsis oculata*: Domain: Eukaryota, Kingdom: Chromista, Phylum: Heterokontophyta, Class: Eustigmatophyceae, Order: Eustigmatales, Family: Monodopsidaceae, Genus: *Nannochloropsis*, Species: *Nannochloropsis oculata*.

**Table 1:** Chemical Composition (g $L^{-1}$) of F2 Medium.

| No. | Components | Amount |
|---|---|---|
| | **Macronutrients** | **g $L^{-1}$** |
| 1. | $NaNO_3$ | 75 g $L^{-1}$ |
| 2. | $NaH_2PO_4$ | 5 g $L^{-1}$ |
| 3. | **Trace metals** | |
| | $Na_2H_2EDTA$ | 4.36 g $L^{-1}$ |
| | $FeCl_3.6H_2O$ | 3.15 g $L^{-1}$ |
| | $MnCl_2 .4H_2O$ | 180 mg $L^{-1}$ |
| | $ZnSO_4$ | 22 mg $L^{-1}$ |
| | CoCl2·6H2O | 10 mg $L^{-1}$ |
| | $CuSO_4·5H_2O$ | 9.8 mg $L^{-1}$ |
| | $Na2MoO_4.2H_2O$ | 6.3 mg $L^{-1}$ |
| | $Na_2SiO_3$ | 30 g $L^{-1}$ |
| **4.** | **Vitamins** | |
| | Thiamin HCl | 200 mg $L^{-1}$ |
| | Cyanocobalamin | 10 mg $L^{-1}$ |
| | Biotin | 100 mg $L^{-1}$ |

To prepare the F2 medium, 1 ml of each stock solution (numbers 1, 2, and 4) with 0.1 mg of trace metal solution (number 3) were mixed into 1 liter of filtered natural seawater. The pH of the mixture was then adjusted to 8.0 using either 1M NaOH or 1M HCl prior to autoclaving.

### 2.1.2. Culture Media and Growth Conditions

*Nannochloropsis oculata* was cultivated in specific culture media in F2 medium (Table, 1) [18]. The cultures were maintained in 250 ml Erlenmeyer flasks at 25 ± 2°C under a 16:8-hour light-dark photoperiod with a light intensity of 1.2 klux. The purity of the cultures was confirmed through regular microscopic examination.

### 2.2. Growth Measurement and Biomass Determination

The growth of microalgae was evaluated by monitoring cell density and optical density at 640 nm. Throughout a 30-day cultivation period, daily measurements were taken using a ZEISS PRIMO STAR binocular light microscope and a microplate spectrophotometer (Infinite 200 PRO series, TECAN). For biomass quantification, algal samples were dried at 60°C until a constant weight was achieved, and the results were expressed as dry weight (mg DW) per liter of culture. The specific growth rate (μ, $d^{-1}$) was calculated using the following formula:

$$\mu = \frac{\ln(W_t/W_0)}{d}$$

where $W_0$ is the initial biomass, $W_t$ is the final biomass, and d is the duration of incubation.

### 2.3. Lipid Content Determination

The lipid content of the studied alga determined by Soxhlet solvent extraction following the method described by [19]. A chloroform-methanol solution was used to extract lipids from the dried algal biomass over a six-hour period. After extraction, the solvent was removed using a rotary evaporator, and the lipid content was calculated as a percentage of the dry biomass weight [20].

### 2.4. Gas Chromatography-Mass Spectrometry (GC-MS) Analysis

The GC/MS analysis was carried out using a Thermo Scientific Trace GC Ultra system coupled with an ISQ Single Quadrupole mass spectrometer. A TG-5MS fused silica capillary column (30 m × 0.25 mm, 0.1 μm film thickness) was employed for separation. The mass spectrometer operated in electron ionization mode at 70 eV. Helium was used as the carrier gas at a constant flow rate of 1 ml/min. The injector and MS transfer line temperatures were both set to 280°C. The oven temperature program started at 45°C, held for 2 minutes, then ramped to 150°C at 7°C/min, followed by an increase to 270°C at 5°C/min (with a 2-minute hold), and finally to 310°C at 3.5°C/min, where it was held for 10 minutes. Quantification of components was based on relative peak area measurements, and preliminary identification of compounds was performed by comparing retention times and mass spectra with entries in the NIST and WILLY libraries integrated into the GC/MS software.

### 2.5. Morphological Identification

*Nannochloropsis oculate* was primarily identified morphologically according to features described by [21, 22] using ZEISS PRIMO STAR BINOCULAR light microscope.

### 2.6. Optimization of the selected promising species

Growth trials were carried out in 250 ml Erlenmeyer flasks, each holding 100 ml of F2 medium (for *Nannochloropsis oculata*), and incubated over a 7-week period. A 20 ml inoculum from a two-week-old culture of the chosen species, with a dry biomass concentration of about 0.05 g $L^{-1}$ (dried at 60°C), was used to start the cultures. To ensure statistical reliability, all growth experiments were conducted in triplicate.

### 2.7. Effect of N/P Mass Ratio

The study investigated the impact of varying N:P mass ratios (1:4, 1:1; 1:0.5) compared to the control (4:1) on biomass, lipid, and EPS production in the selected algal species. Cultures were incubated for four weeks, after which algal biomass (g $L^{-1}$) and lipid yields ($g \cdot g^{-1}$) were measured from the cell-free medium

### 2.8. Effect of Illumination

The influence of light intensity (1.2 ± 0.2 klux, 2.6 ± 0.2 klux [control], 3.0 ± 0.2 klux, and 4.2 ± 0.2 klux) on biomass and lipid production in *Nannochloropsis oculata* was evaluated. Cool white, fluorescent tubes maintained stable temperatures, and cultures were incubated at 25 ± 1°C with a 16:8 h light: dark photoperiod. After four weeks, dry weight

biomass and lipid content production were analyzed.

### 2.9. Extraction and determination of total lipids

The total lipid content of *Nannochloropsis oculata* was determined according to the method described by [19]. The alga was cultivated in 1.5-liter plastic bottles containing 1 liter of optimized growth medium, with three replicates per species. Cultures were incubated at 25 ± 2°C under 1.2 klux light intensity with a 16:8-hour light/dark photoperiod for 14 days. Post-incubation, algal biomass was filtered, dried, and subjected to lipid extraction using the Soxhlet method with a chloroform-methanol solvent mixture for 6 hours. The extracted lipids were concentrated using a vacuum rotary evaporator, transferred to a pre-weighed beaker, and dried under mild airflow until a constant weight was achieved. Lipid content was quantified as a percentage of dry biomass (g lipid/g biomass) [20].

### 2.10. Preparation of the fatty acid methyl esters

The method [23] was used to saponify and methylate the dried lipid extract, transforming it into fatty acid methyl esters (FAMEs), which are the main components of biodiesel. In the first step, 250 mg of oil was mixed with 5.0 ml of 0.50 $mol.L^{-1}$ sodium hydroxide in methanol and heated under reflux for 5 minutes. Then, 15.0 ml of an esterification mixture—prepared by dissolving 2.0 g of ammonium chloride in 60.0 ml of methanol and adding 3.0 ml of concentrated sulfuric acid—was added, and the solution was refluxed for an additional 3 minutes.

The resulting mixture was transferred to a separatory funnel containing 25.0 ml of petroleum ether and 50.0 ml of deionized water. After thorough mixing, the lower aqueous layer was drained off. The organic layer was then washed twice with 25.0 ml portions of deionized water to remove any remaining moisture. Once separated, the organic phase was collected, and the solvent was removed using a rotary evaporator. The purified methyl esters were subsequently dissolved and prepared for analysis by gas chromatography.

### 2.11. Quantification of Fatty Acids by Gas chromatography–mass spectrometry (GC-MS) analysis

The GC/MS analysis was carried out using a Thermo Scientific Trace GC Ultra system coupled with an ISQ Single Quadrupole mass spectrometer. A TG-5MS fused silica capillary column (30 m × 0.251 mm, 0.1 µm film thickness) was used for separation. Detection was performed using electron ionization at 70 eV, with helium serving as the carrier gas at a constant flow rate of 1 ml/min. Both the injector and the MS transfer line were kept at 280°C. The oven temperature program started at 150°C, where it was held for 4 minutes, then increased to 280°C at a rate of 5°C per minute, followed by an additional 4-minute hold. Compounds were quantified based on their relative peak area percentages. Preliminary identification of the detected substances was conducted by comparing their retention times and mass spectra against entries in the NIST and WILLY libraries within the GC/MS software

### 2.12. Analysis of Biodiesel Properties Using Biodiesel Analyzer Software

After determining the fatty acid profile of the produced biodiesels through gas chromatography (GC-MS), the data was input into the Biodiesel Analyzer software, a user-friendly tool designed to predict the properties of prospective biodiesel [25]. This software relies on a robust model previously developed and validated by [24].

## 3. Results and Discussion

### Morphological identification of the selected green algal species

The isolated green algal species, was identified based on its morphological characteristics as *Nannochloropsis oculata*, following the traits outlined by [21] using ZEISS PRIMO STAR BINOCULAR light microscope. The morphological appearance of the green algal species was characterized by its spherical shape and small size, typically ranging from 2:3 µ in diameter. The cell wall is composed of a glycoprotein layer, providing structural support and protection. Internally, the cell houses a single, cup-shaped chloroplast that occupies a significant portion of the cell volume as shown in figure. 1.

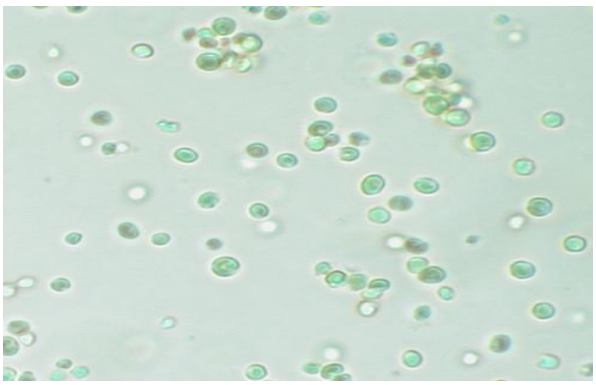

**Figure 1**: Photo of *Nannochloropsis oculate*

**Growth Curve of *Nannochloropsis oculata***

**Spectrophotometric procedure**

Cell quantification was performed using a Sedgewick Rafter chamber. *Nannochloropsis oculata* was grown in 250 ml Erlenmeyer flasks containing 100 ml of F2 medium. An initial cell concentration, determined via the Sedgewick Rafter, was introduced into each flask and maintained for 30 days at 25 ± 2°C under 1.2 klux illumination with a 16:8-hour light-dark cycle. Optical density (O.D.) at 640 nm was monitored daily using a microplate spectrophotometer (Infinite 200 PRO series, TECAN). The growth curve of the *Nannochloropsis oculata* was illustrated in figure 2 illustrate distinct patterns of population dynamics over a 30-day period. Each curve represents the change in optical density (a proxy for biomass or cell concentration) over time.

*Nannochloropsis oculata*: displays a robust sigmoidal growth curve with a clear lag phase followed by a sharp exponential increase. The stationary phase is reached around day 25, with the highest peak optical density among the five species. The subsequent decline is gradual, indicating a stable transition into the death phase as shown in figure 2.

**Biomass technique**

Following incubation, the sample was passed through filter paper to isolate the algal biomass from the culture medium, and the mass of the collected algae was subsequently measured. *Nannochloropsis oculata* achieved ~0.12 g $L^{-1}$, suggesting efficient resource utilization during the exponential phase as shown in figure 3.

*Nannochloropsis oculata* displayed highly variable growth metrics, with substantial standard deviations, particularly after 2 weeks ($\mu = 0.208 \pm 0.332$ g.$d^{-1}$) as illustrated in table 2, suggesting inconsistent performance possibly due to environmental or biological factors.

The variability in *Nannochloropsis oculata's* growth rate observed in this study is comparable to that described by [10], who noted its sensitivity to fluctuations in culture parameters such as light intensity and nutrient composition

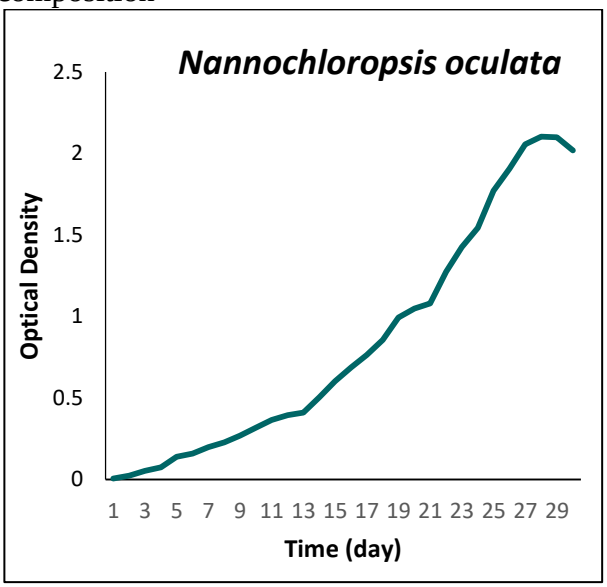


**Figure 2:** Growth curve of the green algae Nannochloropsis oculate grown on F2 medium under standard growth conditions.

**Growth rate calculation:**

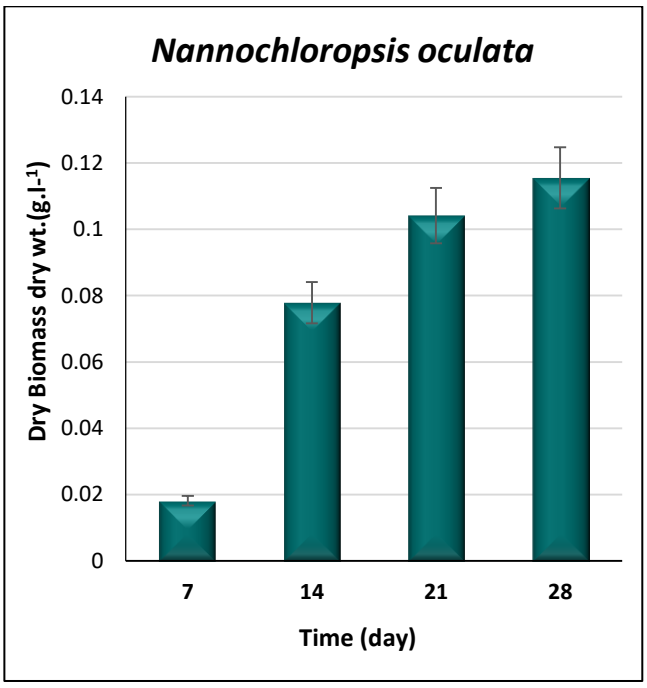


**Figure 3:** specific growth rate of the green algae *Nannochloropsis oculata* after 4 weeks of incubation.

**Table 2:** Measurements of biomass yield, specific growth rate (μ), daily doubling rate ($Dd^{-1}$), and generation time (G) were obtained for Nannochloropsis oculate.

| *Nannochloropsis oculata* | μ | $Dd^{-1}$ | G |
|---|---|---|---|
| **2 weeks** | 0.208 ±0.332 | 0.300 ±0.479 | 3.331 ±2.458 |
| **3 weeks** | 0.125 ±0.166 | 0.180 ±0.240 | 5.554 ±3.491 |
| **4 weeks** | 0.088 ±0.114 | 0.127 ±0.165 | 7.866 1 4 |

## Lipid Production of *Nannochloropsis oculata*

The analysis of lipid production in *Nannochloropsis oculate* displayed lipid yields of 0.198 $g.g^{-1}$ with corresponding lipid contents of 19.8% as shown in table 3. This finding aligns with [26], who reported similarly high lipid content in *Nannochloropsis oculate.*, indicating its efficient conversion of nutrients into storage lipids and confirming its suitability for scalable biodiesel production.

**Table 3:** Average lipid production ($g.g^{-1}$) after growth period of 28 days

| Strain | lipid production ($g.g^{-1}$) |
|---|---|
| *Nannochloropsis oculata* | 0.198±0.017 |

## Analysis of Lipid Composition Using Gas Chromatography and Mass Spectrometry (GC/MS)

The fatty acids and fatty acids esters investigated by GC-MS composition of *Nannochloropsis oculata* and their Retention time (RT), compound names, area % of, molecular formula, common name of each compound, and carbons double bonds.

## Analysis of Lipid Composition of Using Gas Chromatography and Mass Spectrometry (GC/MS) *Nannochloropsis oculata*

Different compounds($C_{14}$-$C_{37}$) were identified from the lipid extract of *Nannochloropsis oculate* (Table, 4). The Gas Chromatography and Mass Spectroscopy (GC/MS) analysis of the lipid composition of *Nannochloropsis oculata* reveals a diverse profile comprising 25 distinct compounds, each characterized by unique functional groups that contribute to its potential applications in biofuel production and other biotechnological uses as shown in figure 4. Among the identified compounds, fatty acids and their derivatives dominate the profile, including key components such as 9-Octadecenoic acid ($C_{18}H_{34}O_2$, MW 282) and pentadecanoic acid ($C_{15}H_{30}O_2$, MW 242), which are critical for biodiesel synthesis due to their transesterification efficiency. These compounds feature carboxyl functional groups (-COOH), essential for forming ester linkages during biofuel production.

Alcohols, such as tridecanol ($C_{13}H_{28}O$, MW 200), were also detected, contributing to hydroxyl (-OH) functional groups that influence fuel properties. Ketones and aldehydes, like germacetin ($C_{26}H_{34}O_{11}$, MW 522), demonstrate the presence of carbonyl groups (C=O), which enhance oxidative stability. Esters, including hexadecanoic acid, 2,3-dihydroxypropyl ester ($C_{19}H_{38}O_4$, MW 330), highlight ester functional groups crucial for biofuel applications.

Aromatic compounds, such as dasycarpidan-1-methanol, acetate (ester) ($C_{20}H_{26}N_2O_2$, MW 326), add complexity to the lipid profile, impacting combustion characteristics. Nitrogen-containing compounds, like cathine ($C_9H_{13}NO$, MW 151), introduce amide and nitro functionalities, potentially affecting fuel stability. Terpenoids, such as phytol ($C_{20}H_{40}O$, MW 296), represent structures with multiple hydroxyl and ketone groups, often associated with antioxidant properties. Steroids, such as cholestan-3-one, cyclic 1,2-ethanediyl acetal ($C_{29}H_{50}O_2$, MW 430), present steroidal structures with various functional groups, including hydroxyl and ester functionalities. Long-chain hydrocarbons, such as heptadecane, 9-hexyl- ($C_{23}H_{48}$, MW 324), provide aliphatic chains influencing fuel viscosity and cold-flow properties. Brominated compounds, like 5-(dibromomethyl)-1,3-bis (tribromomethyl) benzene ($C_9H_4Br_8$, MW 744), indicate halogenated functional groups. Miscellaneous hydrocarbons, such as docosane ($C_{22}H_{46}$, MW 310), further contribute to the lipid profile's complexity.

**Table 4:** Analysis of the Lipid Profile in *Nannochloropsis oculata* Using Gas Chromatography-Mass Spectrometry (GC/MS)

| No | Chemical molecule | Chemical Formula | Retention Time (Min) | peak Area % | Functional group |
|---|---|---|---|---|---|
| 1 | 1H-2,8a-Methanocyclo penta[a]cyclopropa[e]c yclodecen-11-one, 1a,2,5,5a,6,9,10,10a-oc tahydro-5,5a,6-trihydro xy-1,4-bis(hydroxymeth yl)-1,7,9-trimethyl | $C_{20}H_{28}O_6$ | 27 .26 | 0.41 | Alcohol Ketone |
| 2 | 2,2-DIDEUTERO OCTADECANAL | $C_{18}H_{34}D_2O$ | 24 .18 | 0.54 | Aldehyde |
| 3 | 1-Heptatriacotanol | $C_{37}H_{76}O$ | 25 .21 | 0.40 | Alcohol |
| 4 | Docosane (CAS) | $C_{22}H_{46}$ | 25.52 | 0.54 | Hydrocarbon |
| 5 | Heneicosane, 11-phenyl- (CAS) | $C_{27}H_{48}$ | 26 .60 | 0.54 | Hydrocarbon |
| 6 | Methyl octadec-6,9-dien-12-ynoate | $C_{19}H_{30}O_2$ | 26 .98 | 0.74 | Ester Hydrocarbon |
| 7 | 1H-2,8a-Methanocyclo penta[a]cyclopropa[e]c yclodecen-11-one, 1a,2,5,5a,6,9,10,10a-oc tahydro-5,5a,6-trihydro xy-1,4-bis(hydroxymeth yl)-1,7,9-trimethyl | $C_{20}H_{28}O_6$ | 27 .26 | 0.41 | Alcohol Ketone |
| 8 | Pentadecylbenzene | $C_{21}H_{36}$ | 27 .74 | 0.64 | Hydrocarbon |
| 9 | 5-Heptadecene, 1-bromo- (CAS) | $C_{17}H_{33}Br$ | 28 .04 | 0.98 | Hydrocarbon |
| 10 | Malonic acid, 2-heptyl tetradecyl ester | $C_{24}H_{46}O_4$ | 29 .05 | 3.16 | Ester |
| 11 | E,E,Z-1,3,12-Nonadec atriene-5,14-diol | $C_{19}H_{34}O_2$ | 30 .51 | 0.76 | Alcohol |
| 12 | Zederone | $C_{15}H_{18}O_3$ | 29 .78 | 2.06 | Ketone |
| 13 | Benzene, (1-butyloctyl)- (CAS) | $C_{18}H_{30}$ | 29 .91 | 2.22 | benzene ring |
| 14 | Benzene, (1-propylnonyl)- (CAS) | $C_{18}H_{30}$ | 30 .24 | 1.33 | benzene ring |
| 15 | 2,6,10-Dodecatrienoic acid, 3,7,11-trimethyl-, methyl ester (CAS) | $C_{16}H_{26}O_2$ | 29 .16 | 1.33 | Ester |
| 16 | Benzene, (1-ethyldecyl)- (CAS) | $C_{18}H_{30}$ | 30 .84 | 0.93 | benzene ring |
| 17 | Benzene, (1-methylundecyl)- (CAS) | $C_{18}H_{30}$ | 31 .87 | 0.79 | benzene ring |
| 18 | Benzene, (1-pentyloctyl)- | $C_{19}H_{32}$ | 32 .30 | 1.89 | benzene ring |
| 19 | Benzene, (1-butylhexadecyl)- (CAS) | $C_{26}H_{46}$ | 32 .45 | 1.53 | benzene ring |
| 20 | 11-Octadecenal (spectrum disagrees) (CAS) | $C_{18}H_{34}O$ | 32 .67 | 0.50 | Aldehyde |
| 21 | 17-Octadecynoic acid | $C_{18}H_{32}O_2$ | 33 .03 | 0.42 | Carboxylic Acid |
| 22 | Ethanol, 2-(9-octadecenyloxy)-, (Z)- (CAS) | $C_{20}H_{40}O_2$ | 33 .20 | 0.37 | Alcohol |
| 23 | 13-Heptadecyn-1-ol | $C_{17}H_{32}O$ | 33 .41 | 0.61 | Alcohol |
| 24 | 3,15-Octadecadien1-ol acetate | $C_{20}H_{36}O_2$ | 33 .57 | 0.81 | Alcohol Ester |
| 25 | Dodecane, 5,8-diethyl- | $C_{16}H_{34}$ | 33 .83 | 0.74 | Hydrocarbon |
| 26 | Benzene, 1,4-didecyl- (CAS) | $C_{26}H_{46}$ | 34 .20 | 0.52 | benzene ring |
| 27 | tert-Butyl 5,5-dimethoxy-2-(2-ox ocyclopentyl)cyclopenta [b]dihydrofuran-3-carbo xylate | $C_{19}H_{28}O_6$ | 37 .94 | 0.85 | Ester Ether |
| 28 | Hexadecanoic acid (CAS) | $C_{16}H_{32}O_2$ | 36 .00 | 11.68 | Carboxylic Acid |
| 29 | Ethylene brassylate | $C_{15}H_{26}O_4$ | 36 .59 | 0.76 | Ester |
| 30 | Cyclopropaneoctanoic acid | $C_{21}H_{38}O_2$ | 37 .84 | 1.86 | Carboxylic Acid |
| 31 | Pentadecanoic acid, 14-methyl-, methyl ester | $C_{17}H_{34}O_2$ | 34 .50 | 3.46 | Ester |
| 32 | cis-Vaccenic acid (CAS) | $C_{18}H_{34}O_2$ | 39 .52 | 16.74 | Carboxylic Acid |
| 33 | cis-13-Octadecenoic acid | $C_{18}H_{34}O_2$ | 39 .59 | 6.32 | Carboxylic Acid |
| 34 | Octadecanoic acid | $C_{18}H_{36}O_2$ | 39 .80 | 5.03 | Carboxylic Acid |
| 35 | Tributyl acetylcitrate | $C_{20}H_{34}O_8$ | 40 .69 | 1.12 | Ester Carboxylic Acid |
| 36 | 9-Octadecenoic acid (Z)- (CAS) | $C_{18}H_{34}O_2$ | 40 .78 | 0.48 | Carboxylic Acid |
| 37 | cis-Vaccenic acid | $C_{18}H_{34}O_2$ | 43 .41 | 0.51 | Carboxylic Acid |
| 38 | 9-Octadecenoic acid (Z)-, | $C_{21}H_{40}O_4$ | 43 .73 | 1.32 | Carboxylic Acid |
| 39 | Cyclohexane, 1,1'-dodecylidenebis[4-m ethyl | $C_{26}H_{50}$ | 44 .01 | 0.36 | Hydrocarbon |
| 40 | Octadecanal, 2-bromo- | $C_{18}H_{35}BrO$ | 44 .12 | 0.56 | Aldehyde |
| 41 | 1-Tricosanol (CAS) | $C_{23}H_{48}O$ | 44 .79 | 0.37 | Alcohol |
| 42 | 1,2-Benzenedicarboxylic acid, bis(2-ethylhexyl) ester (CAS) | $C_{24}H_{38}O_4$ | 45 .40 | 16.86 | Ester |
| 43 | 16-Octadecenoic acid, methyl ester (CAS) | $C_{19}H_{36}O_2$ | 46 .91 | 0.66 | Ester |
| 44 | DI-2-BENZOTHIAZO LE DISULFANE | $C_{14}H_8N_2S_4$ | 47 .03 | 0.60 | |
| 45 | 9-Octadecenoic acid (Z)-, | $C_{21}H_{40}O_4$ | 47 .42 | 0.61 | Carboxylic Acid |
| 46 | 2,5-Furandione, dihydro-3-octadecyl | $C_{22}H_{40}O_3$ | 47 .55 | 1.21 | Ketone |
| 47 | Pregn-5-en-20-one, 11-(acetyloxy)-3,14-di hydroxy-12-(2-hydroxy -3-methyl-1-oxobutoxy | $C_{28}H_{42}O_8$ | 48 .08 | 0.63 | Ketone Alcohol |

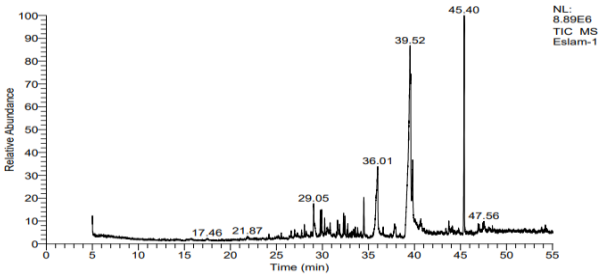


**Figure 4:** The relative abundance and retention time of fatty acids in the green microalga *Nannochloropsis oculata* were determined using GC-MS analysis.

## Optimization of *Nannochloropsis oculata*

### Effect of N: P mass ratio

A glance on table 5 clarify the effect of varying N:P mass ratios on biomass yield and lipid content of *Nannochloropsis oculata*. Under the control condition (N:P = 4:1), the algal biomass reached its highest value, approximately 0.12 g $L^{-1}$, indicating optimal growth under standard nutrient conditions. However, altering the N:P ratio to 1:4 resulted in a notable decrease in biomass yield (~0.08 g $L^{-1}$), suggesting that reduced nitrogen availability may limit overall growth. The 1:1 N:P ratio maintained relatively high biomass levels (~0.11 g $L^{-1}$), while the 1:0.5 ratio

showed intermediate biomass production (~0.09 g $L^{-1}$). In contrast, the lipid content exhibited an inverse relationship with biomass. The control condition (4:1) yielded a lipid content of approximately 20%, which was lower compared to other treatments. Notably, the 1:1 N:P ratio led to the highest lipid content (~31%), demonstrating that this nutrient imbalance strongly promotes lipid accumulation at the expense of biomass growth. The 1:4 ratio showed a slight reduction in lipid content (~19%), while the 1:0.5 ratio maintained a moderate lipid content (~20%), indicating that excessive phosphorus availability does not significantly enhance lipid synthesis but maintains reasonable lipid yields. These results closely parallel those reported by [27], who found that moderate nitrogen limitation can trigger lipid accumulation while preserving cellular viability. At the opposite extreme, the 1:4 N:P condition, which imposed phosphorus limitation alongside severe nitrogen stress, led to reductions in both biomass and lipid production.

**Table 5:** Effect of nitrogen phosphorus (N:P) ratios on dry biomass and lipid content in Nannochloropsis oculata

| **Strains** | **Nannochloropsis oculata** | |
|---|---|---|
| **N/P** | Dry biomass yield(g $L^{-1}$) | lipid content 100% |
| 4:1 (control) | 0.1186±0.0178 | 20.14 |
| 1:4 | 0.0813±0.0048 | 18.53 |
| 1:1 | 0.1084±0.0142 | 30.91 |
| 1:0.5 | 0.0895±0.0064 | 19.89 |

## Effect of light intensity

As well, dry biomass production of *Nannochloropsis oculate*, exhibited optimal growth at the control light intensity (2.6 klux), achieving a peak dry weight of approximately (0.1g). Biomass production decreased slightly but remained relatively high at 3.0 klux and 4.2 klux, indicating that moderate increases in light intensity beyond the control level did not significantly impair growth. At the lowest light intensity (1.2 klux), biomass production was notably lower, suggesting that insufficient light hindered photosynthetic activity and overall growth.

Regarding lipid content, *Nannochloropsis oculata* showed the highest percentage of lipids (approximately 33%) at the control light intensity (2.6 klux) as shown in table 6. Similar to biomass trends, lipid content remained relatively stable at higher light intensities (3.0 klux and 4.2 klux), with slight decreases observed compared to the control condition. At the lowest light intensity (1.2 klux), lipid content was lower, around 29%, indicating that inadequate light reduced the accumulation of lipids. The findings show distinct, species-specific responses, broadly consistent with earlier research such as that by [28].

**Table 6:** Effect of light intensity (klux) on dry biomass and lipid content

| **Strains** | *Nannochloropsis oculata* | |
|---|---|---|
| **Light intensity**(klux) | Dry biomass (g $L^{-1}$) | lipid content% |
| 1.2 | 0.0534±0.0027 | 29.2697 |
| 2.6 (control) | 0.1026±0.0157 | 32.6974 |
| 3 | 0.0863±0.0059 | 31.2879 |
| 4.2 | 0.0692±0.0034 | 30.5741 |

## Fatty acid methyl esters products from *Nannochloropsis oculata* microalgal lipids using transesterification

The results showed a significant amount of fatty acids after transesterification process from *Nannochloropsis oculate* as shown in figure 5. The fatty acids and fatty acids esters composition of *Nannochloropsis oculate* was determined using GC-MS analysis.

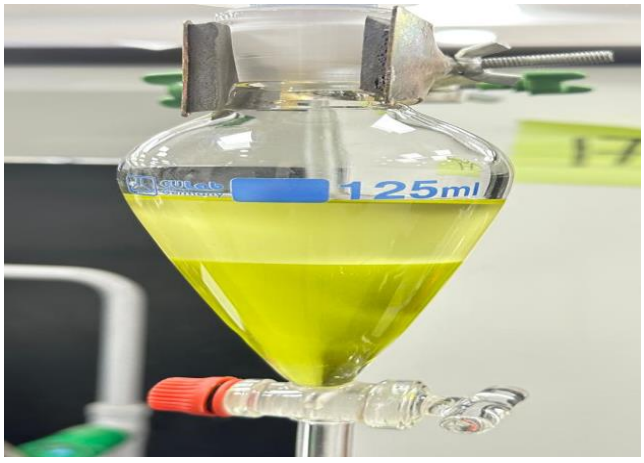


**Figure 5:** Biodiesel and glycerol formed after transesterification from selected promising green algal species *Nannochloropsis oculata.*

## Quantification of Fatty Acids by Gas Chromatography–Mass Spectrometry (GC-MS) analysis

The GC-MS analysis of the chloroform-methanol extracts of *Nannochloropsis oculata* revealed a complex mixture of fatty acids and

their methyl esters as shown in table 7. Among the identified compounds, hexadecanoic acid (Palmitic acid) was detected in two forms: as a free acid ($C_{16}H_{32}O_2$) with a retention time of 36.00 minutes and a peak area of 11.68%, and as its methyl ester derivative ($C_{17}H_{34}O_2$) at a retention time of 34.50 minutes with a peak area of 3.46%. Both forms showed no double bonds in their carbon chains (C16:0). Oleic acid, an unsaturated fatty acid, was present as both a free acid (9-Octadecenoic acid, $C_{18}H_{34}O_2$) and its methyl ester (9-Octadecenoic acid methyl ester, $C_{19}H_{36}O_2$), demonstrating one double bond in the carbon chain (C18:1). The free acid form of oleic acid eluted at 39.59 minutes with a peak area of 6.32%, while its methyl ester counterpart appeared slightly earlier at 39.52 minutes with a significantly higher peak area of 16.74%. Stearic acid (Octadecanoic acid, $C_{18}H_{36}O_2$), a saturated long-chain fatty acid, was also identified with a retention time of 39.80 minutes and a peak area of 5.03% (C18:0). Additionally, cis-Vaccenic acid ($C_{18}H_{34}O_2$), another monounsaturated fatty acid, was detected at 43.41 minutes but constituted only 0.51% of the total peak area. The presence of these fatty acids indicates that *Nannochloropsis oculata* is a rich source of both saturated and unsaturated fatty acids, which could have significant implications for biodiesel production.

**Table 7:** Fatty acids and fatty acids esters of Nannochloropsis oculata

| | Chemical Formula | RT | peak Area % | Chemical group | Carbon double bond |
|---|---|---|---|---|---|
| ***Nannochloropsis oculata*** | | | | | |
| Hexadecanoic acid, methyl ester | $C_{17}H_{34}O_2$ | 34.50 | 3.46 | Palmitic acid | C16:0 |
| Hexadecanoic acid | $C_{16}H_{32}O_2$ | 36.00 | 11.68 | Palmitic acid | C16:0 |
| 9-Octadecenoic acid, methyl ester | $C_{19}H_{36}O_2$ | 39.52 | 16.74 | Oleic acid | C18:1 |
| 9-Octadecenoic acid | $C_{18}H_{34}O_2$ | 39.59 | 6.32 | Oleic acid | C18:1 |
| Octadecanoic acid | $C_{18}H_{36}O_2$ | 39.80 | 5.03 | Stearic acid | C18:0 |
| cis-Vaccenic acid | $C_{18}H_{34}O_2$ | 43.41 | 0.51 | 11-Octadecenoic acid | C18:1 |
| Pentadecanoic acid, 14-methyl-, methyl ester | $C_{17}H_{34}O_2$ | 34.50 | 3.46 | Methyl 14-methylpentadecanoate | C16:0 (branched) |

**Calculation of algal biodiesel properties from biodiesel analyzer software**

The results obtained from the biodiesel analyzer software are presented in figure 6 and table 8. These findings highlight various characteristic parameters that contribute to the distinct properties of each type of biodiesel. The key parameters include: Saturated Fatty Acids (SFA), Monounsaturated Fatty Acids (MUFA), Polyunsaturated Fatty Acids (PUFA), Degree of Unsaturation (DU), Saponification Value (SV), Iodine Value (IV), Cetane Number (CN), Long-Chain Saturated Factor (LCSF), Cold Filter Plugging Point (CFPP), Cloud Point (CP), Allylic Position Equivalents (APE), Bis-Allylic Position Equivalents (BAPE), Oxidation Stability (OS), Higher Heating Value (HHV), Kinematic Viscosity (μ), and Density (ρ).

The predicted characteristics of biodiesel produced from *Nannochloropsis oculata* highlight its potential as a renewable fuel source as shown in figure 6, though certain properties may require further optimization for practical applications. The high cetane number of 97.65 indicates excellent ignition quality, which is favorable for diesel engines, as higher cetane numbers correlate with reduced ignition delay and smoother combustion. However, the oxidation stability of 0 hours is a significant limitation, as biodiesel with low oxidation stability is prone to degradation during storage, potentially leading to engine deposits and fuel system issues. The absence of polyunsaturated fatty acids and bis-allylic position equivalents suggests inherent resistance to oxidative degradation, yet the oxidation stability results contradict this, implying the presence of other destabilizing factors.

The cold filter plugging point of -2.72°C and cloud point of 4.79°C reflect moderate cold-flow properties, which could restrict biodiesel use in colder climates without additives or blending. The kinematic viscosity of 0.66 $mm^2/s$ and density of 0.41 $g/cm^3$ are notably lower than typical biodiesel standards, which may affect lubrication and injection performance in engines. The high degree of unsaturation and allylic position equivalents values further emphasize the need for stabilization strategies to enhance oxidation resistance as presented in table 8.

While the higher heating value of 18.64 MJ/kg aligns with conventional biodiesel, the combined properties suggest that *Nannochloropsis oculata*-derived biodiesel may require additives or blending with petroleum diesel to meet industry standards. Recent studies emphasize the role of antioxidants and chemical modifications in improving oxidation stability and cold-flow properties. Further research is needed to address these challenges and optimize the fuel's performance in real-world applications. These findings are consistent with the conclusions of [29], who emphasized the necessity of aligning algal feedstock selection with regional climatic conditions.

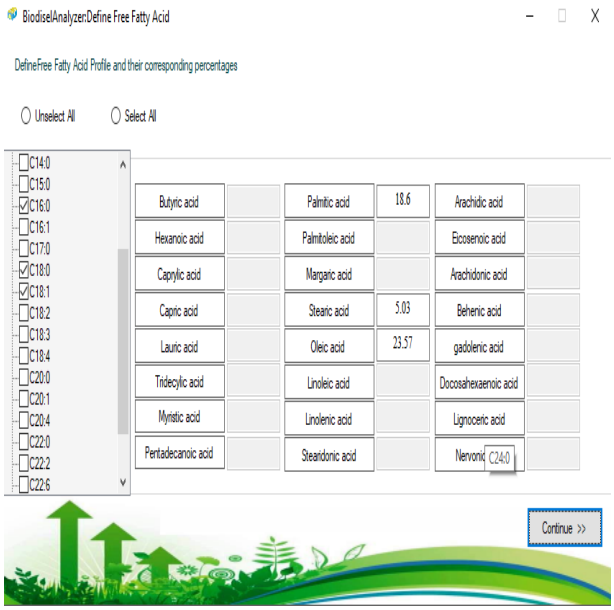


**Figure 6:** Predicted characters of produced biodiesel from selected microalgal Nannochloropsis oculate

**Table 8:** Predicted characters of produced biodiesel from *Nannochloropsis oculate.*

| Parameters | *Nannochloropsis oculata* |
|---|---|
| Saturated Fatty Acids (SFA) | 23.63 |
| Cold Filter Plugging Point (CFPP) (°C) | -2.72 |
| Polyunsaturated Fatty Acids (PUFA) | 0.00 |
| Degree of Unsaturation (DU) | 23.57 |
| Monounsaturated Fatty Acids (MUFA | 23.57 |
| Iodine Value (IV) | 21.20 |
| Cetan Number (CN) | 97.65 |
| Long-Chain Saturated Factor (LCSF) | 4.38 |
| Saponification Value (SV) | 97.25 |
| Cloud point (CP) (°C) | 4.79 |
| Allylic Position Equivalents (APE) | 23.57 |
| Bis -Allylic Position Equivalents (BAPE) | 0.00 |
| Oxidation Stability (OS) (hours) | 0 |
| Higher Heating Value (HHV) | 18.64 |
| Kinematic Viscosity (u) (mm^2/s) | 0.66 |
| Density (p) (g/cm3) | 0.41 |

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