

Comparative Assessment of Lipid Yield and Biofuel Production in *Nannochloropsis oceanica* and *Tetraselmis astigmatica*

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ABSTRACT

This study investigates *Nannochloropsis oceanica* and *Tetraselmis astigmatica* isolated from the Threspuram region along the Thoothukudi coast to evaluate their suitability for biofuel production. Biochemical profiling revealed that the biomass and lipid content was high in *Nannochloropsis oceanica* and *Tetraselmis astigmatica* comprised with carbohydrates and proteins content, indicating a favourable lipid content for biofuel applications. Fourier-transform infrared spectroscopy confirmed the presence of characteristic functional groups in the algal oil, with peaks at 1735.27 and 1734.76 cm^{-1} in *Nannochloropsis oceanica* and *Tetraselmis astigmatica* corresponding to C=O stretching vibrations, signifying ester compounds associated with lipid-derived biodiesel. The extracted oil was subsequently transesterified into fatty acid methyl esters using an acid catalyst and the composition was characterised by gas chromatography–mass spectrometry. Major fatty acids identified included myristic and stearic are the favourable fuel properties in biodiesel present in *Nannochloropsis oceanica*. Overall, the findings suggest that *Nannochloropsis oceanica* yields more biodiesel than *Tetraselmis astigmatica*. Nevertheless, both microalgae possess strong potential for producing cost-effective and sustainable biodiesel, thereby contributing to the growth of renewable energy resource

Keywords: *Nannochloropsis oceanica*, *Tetraselmis astigmatica*, Biomass, Fatty acid methyl ester and biofuel

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1. Introduction

Renewable fuels have gained increased attention in recent years because of the global energy crisis, the depletion of fossil fuel reserves, restricted petroleum availability and stricter emission regulations. At present, the transportation sector relies heavily on fossil fuels, with diesel being the most widely used fuel. (Boutesteijn *et al.*, 2017; Atmanli, 2020). First-generation biofuels are produced from edible feedstocks such as corn, soybean, sugarcane and rapeseed, whereas second-generation biofuels are obtained from non-edible sources like jatropha, miscanthus and switchgrass. However, their production is still insufficient to meet rising energy demands and large-scale cultivation requires extensive land areas, creating competition with food crop production. This makes both first and second-generation biofuels unsustainable (Mandotra *et al.*, 2016; Zainol *et al.*, 2024). Thus, third generation biofuel feedstocks include macro and microalgae that reveal the probability of high biomass yields devoid of arable lands, no seasonal influences and owning the credits of being cultivated in any containment offshore (Chi *et al.*, 2019; Koley *et al.*, 2019). A promising alternative source for biodiesel-convertible lipids are algae. Microalgae are photosynthetic microorganisms characterized by rapid growth, high photosynthetic efficiency and the ability to accumulate substantial amounts of lipids under favourable conditions. They produce a wide range of lipids, including triacylglycerols, glycolipids, phospholipids and free fatty acids, making them a valuable resource for biofuels, cosmetics, nutraceuticals and pharmaceutical applications. (Nguyen *et al.*, 2017; Morshchinin, 2025). Microalgae-based biodiesel offers a sustainable and environmentally friendly alternative to conventional fuels, as it can be produced using renewable resources such as sunlight and wastewater, which has a lower carbon footprint compared to fossil fuels. (EL-Seesy *et al.*, 2021; Chandrasekhar *et al.*, 2022). Many previous studies have investigated microalgal lipids as a sustainable and environmentally friendly source for biodiesel (Wang *et al.*, 2016; Almarashi *et al.*, 2020). However, achieving economic feasibility requires microalgal cultivation to deliver high biomass yields along with efficient lipid production. (Touliabah *et al.*, 2020).

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Therefore, the present study evaluates the potential of selected *Nannochloropsis oceanica* and *Tetraselmis astigmatica* green microalgae as renewable energy sources, emphasising their advantages for biofuel production and their role in advancing sustainable energy strategies. By utilising the unique characteristics of microalgae, this research aims to address global energy challenges while supporting both ecological and economic sustainability.

2. Materials and Method

2.1 Sample collection:

Marine water samples were collected from Threspuram, Thoothukudi coast using a plankton net made of tightly woven silk fabric with a mesh size of 20 µm during June 2023 and February 2024. The collected samples were stored in clean polyethylene containers and carefully transported to the laboratory for further analysis

2.2 Isolation of microalgae

Microalgae were isolated using the serial dilution and streak plate techniques. Subsequent cultures were cultivated and maintained in F/2 medium (Guillard, 1975). The pure strain was grown in the algal culture room for 21 days at 23±2°C with a light-dark cycle of 16:8 hours. Regular shaking was performed to enhance growth and biomass production.

2.3 Measurement of growth (Lustigman *et al.*, 1995)

The growth of microalgae strains and microalgae consortium was measured using a UV-visible spectrophotometer at an optical density of 750 nm

2.4 Estimation of carbohydrates (Dubois *et al.*, 1956)

1 mL of the sample was mixed with 4 mL of anthrone reagent and the mixture was incubated in a boiling water bath for 15 minutes. The tubes were then cooled on ice and absorbance was measured at 620 nm. The carbohydrate content was determined from a standard calibration curve prepared using a glucose solution.

2.5 Estimation of protein (Lowry, 1951)

The washed pellet was treated with 5 mL of 10% trichloroacetic acid and incubated in a boiling water bath. After cooling, the sample was centrifuged at 5000 × g for 5 minutes. The resulting pellet was resuspended in 1 mL of 1 N sodium hydroxide and boiled for 30 minutes. A 0.1 mL aliquot was then taken and diluted to 1 mL with distilled water. Subsequently, 4 mL of alkaline reagent was added, and the mixture was incubated at 37°C for 3 minutes. Following incubation, 0.5 mL of Folin–Ciocalteu phenol reagent was added, and the mixture was allowed to stand for 30 minutes at room temperature. Absorbance was measured at 750 nm. Protein concentration was determined from a standard calibration curve prepared using bovine serum albumin and expressed as µg of protein.

2.6 Biomass estimation

After 21 days of cultivation, the microalgae were harvested by centrifugation at 5000 rpm for 10 minutes and were washed with distilled water. The resulting pellet was subsequently freeze-dried, and the dry weight of the algal biomass was determined gravimetrically.

Biomass productivity (mg L⁻¹ day⁻¹) was calculated using the following equation:

$$P = \frac{(X_2 - X_1)}{(t_2 - t_1)}$$

Where, X₁ and X₂ are the dry cell weight (DCW) (mg/L) at time t₁ and t₂, respectively.

2.7 Lipid extraction (Bligh and Dyer, 1959)

At the specified incubation period, the cells were harvested by centrifugation at 10,000 rpm for 10 min at 4 °C. The pellets were washed with distilled water and centrifuged again at 3000 rpm for 5 min at 4 °C. The resulting biomass was homogenized using a chloroform–methanol mixture (2:1, v/v). The supernatant was collected and transferred into a separating funnel, washed with distilled water and left to stand overnight. After 24 h, the lower organic layer containing crude lipids was carefully collected for further analysis.

$$\text{Lipid content} = \frac{\text{Post lipid weight} - \text{Pre lipid weight}}{\text{Total biomass weight}} \times 100$$

2.8 FT – IR

Microalgal lipids were characterized using Fourier-transform infrared spectroscopy. The dried lipid samples were analysed over a spectral range of 4000–400 cm⁻¹ at a resolution of 4 cm⁻¹. The spectra obtained were compared with reference standards to identify the functional groups present in the samples.

2.9 Transesterification (Praveenkumar *et al.*, 2012)

The lipid and methanolic sulphuric acid (2% H₂SO₄ in methanol) were refluxed for 4 h. The contents were mixed thoroughly with equal volume of distilled water in a separating funnel. The aqueous layer was extracted twice

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with ethyl acetate. The ethyl acetate extract containing the FAME was collected, dried over anhydrous sodium sulphate to remove any excess moisture, and concentrated under vacuum. The dried FAME samples of both normal and nitrogen-starved cultures were analysed by gas chromatography.

$$\text{Oil extraction yield (\%)} = \frac{\text{Weight of extracted oil}}{\text{Weight of algal biomass}} \times 100$$

2.10 GC-MS analysis

GC-MS analysis of the transesterified algal oil was performed using a GC Clarus 500 Perkin-Elmer system comprising an AOC-20i autosampler and gas chromatograph interfaced to a mass spectrometer (GC-MS) equipped with an Elite-1 fused silica capillary column (330 mm × 0.25 mm ID × 1µm df, composed of 100% Dimethyl polysiloxane). For GC-MS detection, an electron ionization system with an ionizing energy of 70 eV was used. Helium gas (99.999%) was used as the carrier gas at a constant flow rate of 1 ml/minute, and an injection volume of 0.5µl was employed (split ratio of 10:1). The injector temperature was set to 250°C, and the ion-source temperature to 280°C.

3. Result and Discussion

3.1 Identification and Growth rate of microalgae

The microalgae were isolated and morphologically identified as *Nannochloropsis oceanica* and *Tetraselmis astigmatica*. The growth of *Nannochloropsis oceanica* and *Tetraselmis astigmatica* were examined at 750 nm from 0th day to 15th day of cultivation (Figure 1). Both the microalgae showed highest growth rate on 15th day of cultivation. Among the two microalgae, *Nannochloropsis oceanica* was found to be high in optical density of 0.869 on 15th day and *Tetraselmis astigmatica* was found to be lower in optical density of 0.618. A recent study reported that *Chlorella vulgaris* exhibited higher growth on the 12th day, which was influenced by the growth conditions and the culture medium used (Wong *et al.*, 2017).

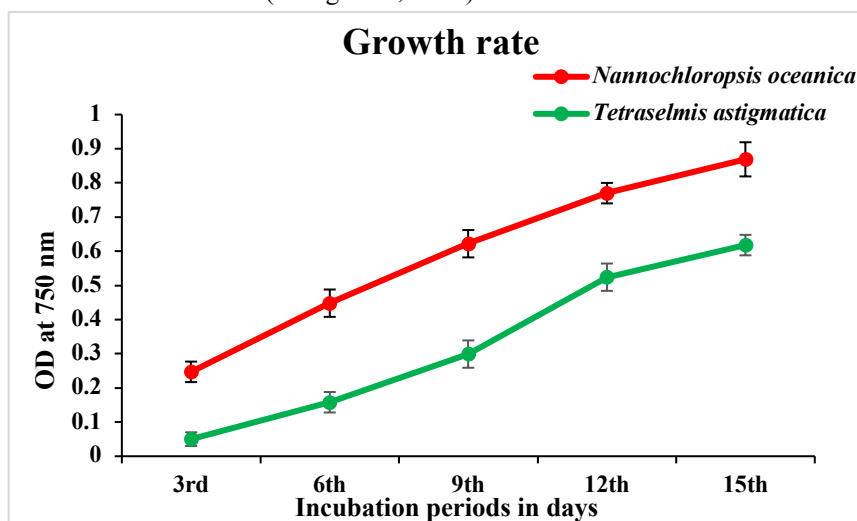


Figure 1: Growth rate of *Nannochloropsis oceanica* and *Tetraselmis astigmatica*

3.3. Biochemical content

The carbohydrate content of 0.51 ± 0.211 (mg/mL) was found to be high in *Tetraselmis astigmatica* and 0.366 ± 0.092 (mg/mL) found to be low in *Nannochloropsis oceanica*. Similarly, protein content of 0.66 ± 0.015 (mg/mL) was found to be high in *Tetraselmis astigmatica* and 0.419 ± 0.028 (mg/mL) found to be low in *Nannochloropsis oceanica* (Table 1). However, the strain under investigation observed low protein content. Microalgae with high protein content generally do not accumulate substantial amounts of lipids and are therefore mainly utilized in food or cosmetic applications. Conversely, low carbohydrate and protein content indicates that the both *Nannochloropsis oceanica* and *Tetraselmis astigmatica* is more suitable for biofuel production. Biomass production was high in *Nannochloropsis oceanica* (0.56 ± 0.05 mg/L) than *Tetraselmis astigmatica* (0.492 ± 0.033 mg/L). Osman *et al.*, (2023) examined *Oocystis pusilla* under KC medium it showed highest biomass of 1.73 g/L while comparing with Kühl medium

Table 1: Biochemical content of *Nannochloropsis oceanica* and *Tetraselmis astigmatica*

	<i>Nannochloropsis oceanica</i>	<i>Tetraselmis astigmatica</i>
Carbohydrates(µg/mL)	0.366 ± 0.092	0.41 ± 0.211

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Protein($\mu\text{g/mL}$)	0.419 ± 0.028	0.56 ± 0.015
Biomass (mg/mL)	0.56 ± 0.05	0.492 ± 0.033
Lipid (g/L)	18.2 ± 0.3	16.6 ± 0.8
Biodiesel (%)	71.43 ± 0.02	60.85 ± 0.4

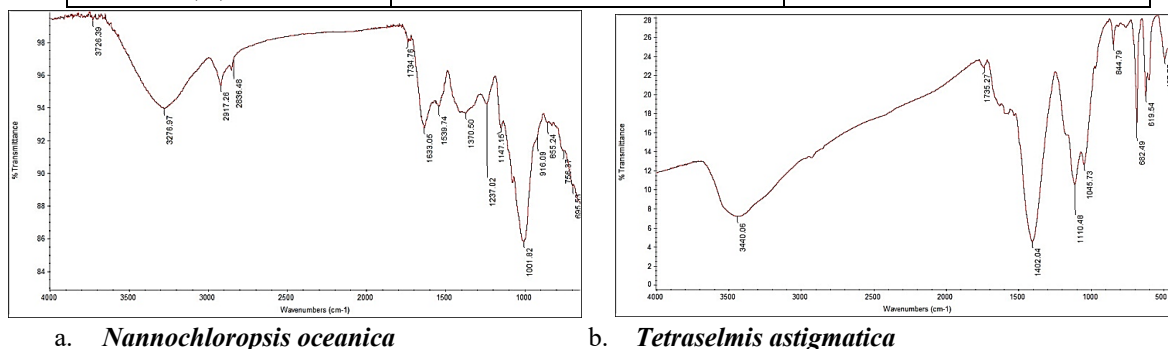


Fig 2: FT-IR spectrum showing the major functional groups present in the lipid

3.3. Lipid content and FTIR analysis

Lipids are bio-molecules soluble inorganic solvents but insoluble in water. Based on the chemical structure and polarity of the molecular head group, they can be classified as polar and non-polar. Polar lipids are used by the microalgae to form cell membranes and include phospholipids and glycolipids, while nonpolar lipids (or neutral lipids) are used as energy source and comprise acyl-glycerols (mono, di, and tri) and free fatty acids (Satia *et al.*, 2019). Lipid content was high in *Nannochloropsis oceanica* (18.2 ± 0.3 g/L) than *Tetraselmis astigmatica* (16.6 ± 0.8 g/L) similarly, Biodiesel was high in *Nannochloropsis oceanica* with 71.43% than *Tetraselmis astigmatica* with 60.85%. FTIR analysis was performed to determine the functional groups present in the microalgal oil. *Nannochloropsis oceanica* and *Tetraselmis astigmatica* lipid content were examined to analyse their biomolecular changes and lipid accumulation through FTIR spectrum. The findings are presented in **Tables 2a and Figures 2a**. In this investigation, *Nannochloropsis oceanica* exhibited 9 compounds ranges from 3440.06 cm^{-1} to 487.77 cm^{-1} . An increase in peak at 3440.06 cm^{-1} is associated with O-H stretching vibrations indicates the increase of moisture content. The peak at 1735.27 cm^{-1} is related to C=O stretching indicated the ester compound which was present for the algal based biodiesel. The peak at 1402.04 cm^{-1} represents sulfonyl chloride. The peak at 1110.48 cm^{-1} and 1045.73 cm^{-1} is related to C-O stretching and CO-O-CO stretching indicates aliphatic ether and anhydride. The peak at 844.79 cm^{-1} and 682.49 indicates C-Cl and C-Br stretching in halocompounds. The peak at 487.77 indicates M-O stretching in Metal-oxygen.

Table 2a. FT-IR spectrum of *Nannochloropsis oceanica*

Frequency range	Group	Compound class
3440.06	N-H stretching	Primary amine
1735.27	C=O stretching	Esters
1402.04	S=O stretching	Sulfate
1110.48	C-O stretching	Secondary alcohol
1045.73	C-O stretching	Alkyl aryl ether
844.79	C-Cl stretching	Halo compound
682.49	C-H bending	Monosubstituted benzene derivative
619.54	C-Br stretching	Halo compound
487.77	M-O stretching	Metal oxygen

Table 2b. FT-IR spectrum of *Tetraselmis astigmatica*

Frequency range	Group	Compound class
3276.97	O-H stretching	Carboxylic acid
2917.26	C-H stretching	Alkane
2836.48	O-H stretching	Alcohol
1734.76	C=O stretching	Esters
1633.05	C=C stretching	Alkene

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1539.74	N-O stretching	Nitro compound
1370.50	S=O stretching	Sulphonamide
1237.02	C-O stretching	Alkyl aryl ether
1147.15	C-O stretching	Aliphatic ether
1001.82	C=F stretching	Fluoro compound
916.09	=C-H out-of-plane bending	Vinyl group
855.24	C-Cl stretching	Halocompound
756.37	C-Cl stretching	Alkyl halide
695.53	C=C bending	Alkene

Tetraselmis astigmatica exhibited 15 compounds ranges from 3276.97 cm⁻¹ to 695.53 cm⁻¹ (**Table 2b and Figure 2b**). An increase in peak at 3726.39 cm⁻¹ doesn't have any functional group. The peak at 3276.97 and 2836.48 cm⁻¹ are associated with O-H stretching vibrations indicates the increase of carboxylic acid and alcohol group. The peak at 2917.26 cm⁻¹ is a C-H stretching indicates the presence of alkane group. The peak at 1734.76 cm⁻¹ is related to C=O stretching vibration confirms the presence of ester functional groups in the algal-derived biodiesel. The peak at 1633.05 and 695.33 cm⁻¹ represents alkene group corresponding to amide I and II bands in proteins, respectively (Ji *et al.*, 2020). The peak at 1539.74, 1370, 11001.82 cm⁻¹ and 916.09 cm⁻¹ is related to N-O, S=O, C=F stretching and =C-H out-of-plane bending stretching indicates nitro compound, sulfonamide, fluoro compound and vinyl group. The peak at 1237.02 cm⁻¹ and 1147.15 indicates C-O stretching in alkyl aryl ether and aliphatic ether. The peak at 855.24 and 756.37 indicates C-Cl stretching in halocompound and alkyl halide. In FTIR, the absorption bands at 1700–1750cm⁻¹ characteristics of C=O groups in lipid esters and the absorption bands at 2,800–3,000cm⁻¹ characteristics of CH₂ and CH₃ groups in lipid acyl chains (Caporgno *et al.*, 2016). These findings indicate that *Nannochloropsis oceanica* and *Tetraselmis astigmatica* possesses considerable lipid levels, underscoring its suitability for enhancing biomass composition in biofuel production.

3.4. FAME analysis

The composition of fatty acid methyl esters plays a critical role in determining the fuel properties of biodiesel. The microalgal lipids contained fatty acids with carbon chain lengths ranging from 15 to 18 carbons and comprised both saturated and unsaturated types. Polyunsaturated fatty acids typically enhance the cold-flow characteristics of biodiesel, whereas saturated fatty acids contribute to better oxidative stability and improved ignition and combustion performance (Atmanli, 2020). The fatty acid profiles of *Nannochloropsis oceanica* and *Tetraselmis astigmatica* were analysed using GC-MS and the corresponding retention times, lipid number, molecular formula and molecular weight are presented in **Tables 3** and **Figures 3a and 3b**. *Nannochloropsis oceanica* and *Tetraselmis astigmatica* exhibited 26 and 23 types of fatty acids, including saturated fatty acid of Cyclopentanol, 2-methyl-, 2(3H)-Furanone, 5-ethoxydihydro-, 2-Ethoxyethyl 3-methylbutanoate, 3-(Hexyloxy)-1,2-propanediol, Phosphonous dibromide, cyclohexyl-, 2-Ethyl-1-hexanol, methyl ether, Dodecane, Tridecane, Pentadecane, Methyl 14-methyl-eicosanoate, Dibutyl phthalate, Methyl stearate, Cycloundecane, 1,1,2-trimethyl-, 1-Pentacosanol essential fatty acids that are favourable for efficient biodiesel production.

Table 3: Fatty acid profile of *Nannochloropsis oceanica* and *Tetraselmis astigmatica*

Name	Molecular formula	Molecular weight	R.Time	
			<i>N. oceanica</i>	<i>T. astigmatica</i>
Cyclopentanol, 2-methyl-	C ₆ H ₁₂ O	100	3.621	3.689
2(3H)-Furanone, 5-ethoxydihydro-	C ₆ H ₁₀ O ₃	130	5.297	-
2-Methoxyethyl 3-methylbutanoate	C ₈ H ₁₆ O ₃	160		5.269
2-Ethoxyethyl 3-methylbutanoate	C ₉ H ₁₈ O ₃	174	5.784	5.765
Pentanoic acid, 2-propenyl ester	C ₈ H ₁₄ O ₂	142	6.323	6.315
3-(Hexyloxy)-1,2-propanediol	C ₉ H ₂₀ O ₃	176	6.591	-
Phosphonous dibromide, cyclohexyl-	C ₆ H ₁₁ Br ₂ P	272	6.963	-
2-Pentene, 2,4-dimethyl-	C ₇ H ₁₄	98	-	6.949
1-Decene	C ₁₀ H ₂₀	140	7.481	7.48
2-Propyl-1-pentanol, methyl ether	C ₉ H ₂₀ O	144	-	8.45

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2-Ethyl-1-hexanol, methyl ether	C ₉ H ₂₀ O	144	8.44	-
1-Dodecene	C ₁₂ H ₂₄	168	11.736	11.736
Dodecane	C ₁₂ H ₂₆	170	11.885	11.885
Tridecane	C ₁₃ H ₂₈	184	13.55	13.551
1-Tetradecene	C ₁₄ H ₂₈	196	14.955	14.955
Pentadecane	C ₁₅ H ₃₂	212	16.453	-
Phenol, 3,5-bis(1,1-dimethylethyl)-	C ₁₄ H ₂₂ O	206	16.61	16.613
1-Nonadecene	C ₁₉ H ₃₈	266	17.682	17.683
Hexadecane	C ₁₆ H ₃₄	226	-	17.76
1,4-Naphthoquinone, 6-acetyl-2,5-dihydroxy-	C ₁₂ H ₈ O ₅	232	18.076	-
Methyl tetradecanoate	C ₁₅ H ₃₀ O ₂	242	19.272	-
1-Nonadecene	C ₁₉ H ₃₈	266	20.102	20.101
Phenol, 2,4,6-tri-tert-butyl-	C ₁₈ H ₃₀ O	262	-	20.649
9-Hexadecenoic acid, methyl ester, (Z)-	C ₁₇ H ₃₂ O ₂	268	21.32	-
Pentadecanoic acid, 14-methyl-, methyl ester	C ₁₇ H ₃₄ O ₂	270	-	21.539
Methyl 14-methyl-eicosanoate	C ₂₂ H ₄₄ O ₂	340	21.552	-
Benzenepropanoic acid, 3,5-bis(1,1-dimethylethyl)-4-hydroxy-, methyl ester	C ₁₈ H ₂₈ O ₃	292	-	21.631
Dibutyl phthalate	C ₁₆ H ₂₂ O ₄	278	21.906	-
1-Nonadecene	C ₁₉ H ₃₈	266	22.286	22.284
Methyl octyl phthalate	C ₁₇ H ₂₄ O ₄	292	-	22.624
6-Octadecenoic acid, methyl ester, (Z)-	C ₁₉ H ₃₆ O ₂	296	23.349	23.348
Methyl stearate	C ₁₉ H ₃₈ O ₂	298	23.595	-
2-Ethylhexyl methyl isophthalate	C ₁₇ H ₂₄ O ₄	292	-	23.638
Cycloundecane, 1,1,2-trimethyl-	C ₁₄ H ₂₈	196	23.987	-
1-Pentacosanol	C ₂₅ H ₅₂ O	368	24.274	24.275

Unsaturated fatty acid of Pentanoic acid, 2-propenyl ester, 1-Decene, 1-Dodecene, 1-Tetradecene, Phenol, 3,5-bis(1,1-dimethylethyl)-, 1-Nonadecene, 1,4-Naphthoquinone, 6-acetyl-2,5-dihydroxy-, 9-Hexadecenoic acid, methyl ester, (Z)-, 6-Octadecenoic acid, methyl ester, (Z). Similarly, *Tetraselmis* sp. contained major fatty acids in its lipid extracts, including the methyl esters of palmitic acid (C16:0), palmitoleic acid (C16:1), hexadecadienoic acid (C16:2), hexadecatrienoic acid (C16:3), hexadecatetraenoic acid (C16:4), stearic acid (C18:0), oleic acid (C18:1), linoleic acid (C18:2), linolenic acid (C18:3), octadecatetraenoic acid (C18:4), and eicosapentaenoic acid (C20:5) (Shin *et al.* 2018).

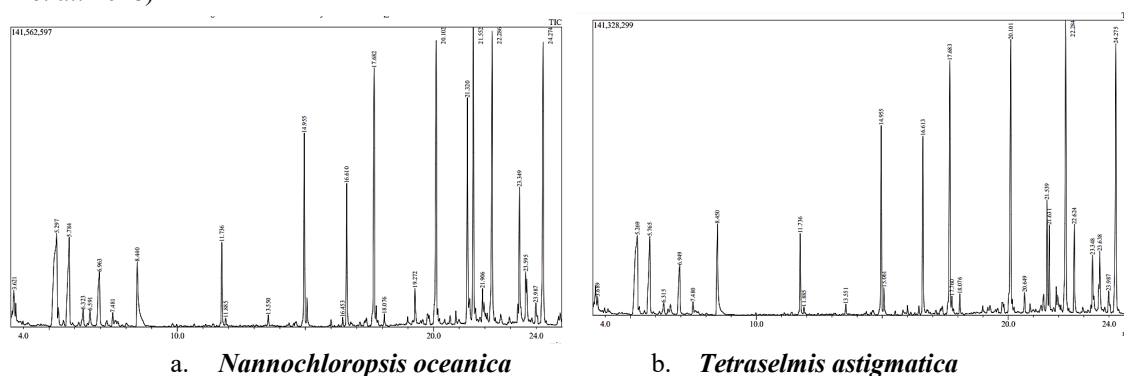


Figure 3: Chromatogram of fatty acid methyl esters

Major fatty acids identified included myristic as methyl tetradecanoate and stearic as methyl stearate, which contribute to favourable fuel properties in biodiesel and only present in *Nannochloropsis oceanica*. Overall, the

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findings confirm that both *Nannochloropsis oceanica* and *Tetraselmis astigmatica* are suitable candidates for biofuel production due to their high biomass productivity, lipid accumulation and appropriate fatty acid composition.

Conclusion

Nannochloropsis oceanica and *Tetraselmis astigmatica* were investigated for their biomass, lipid content and fatty acid composition. Biomass, lipid content and fatty acid methyl ester were found to be high in *Nannochloropsis oceanica*. FT-IR confirmed the presence of characteristic functional groups in the algal oil, with peaks at 1735.27 and 1734.76 cm^{-1} in *Nannochloropsis oceanica* and *Tetraselmis astigmatica* corresponding to C=O stretching vibrations, signifying ester compounds associated with lipid-derived biodiesel. The GC-MS analysis of fatty acid methyl ester showed the presence of methyl tetradecanoate and methyl stearate these fatty acids are ideal for biodiesel. The obtained results showed that *Nannochloropsis oceanica* is more suitable than *Tetraselmis astigmatica*. Thus, this result will help us to determine the right candidate for biodiesel production

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CRediT authorship contribution statement

Monisha: Writing – review & editing, Writing – original draft, Methodology, Formal analysis. Arockia Jenecius Alphonse: Writing – review & editing, Resources. Vellammal: Writing – review & editing, Resources.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.