Research Article

Journal of Environmental Nanotechnology

TiO_2 Nano-photocatalyst-driven CO_2 Biofixation and Lipid Accumulation in Brackish Water Cyanobacteria for Sustainable Biodiesel Production

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Received: 24.06.2025 Accepted: 26.08.2025 Published: 30.09.2025
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ABSTRACT

This study focused on the effect of CO_2 concentration on cyanobacterial cultivation using BG-11 medium to enhance biomass and lipid productivity. Different cyanobacterial species isolated from brackish water were screened and identified using molecular characterisation. From the isolated cyanobacterial species, two rapidly growing species, *Phormidium uncinatum* and *Oscillatoria salina* were selected. Their biomass and lipid productivity were optimised by varying the CO_2 concentration. These strains were subjected to tolerance tests by changing the conditions of the cyanobacterial growth medium, pH, CO_2 concentration, and temperature. Biochemical parameters were also estimated. *Oscillatoria salina* exhibited the highest biomass production, reaching 956 ± 0.04 mg/L at a 15 % CO_2 concentration. A high protein content of 25.23 ± 0.32% was found in *P. uncinatum* compared to *O. salina*, whereas *O. salina* showed higher lipid and biomass productivity than *P. uncinatum*. Furthermore, O. salina was utilised for the production of biodiesel using a nanophotocatalyst (TiO_2). It was found to enhance the biodiesel up to 98%. The biodiesel was tested for fuel properties and compared with the ASTM Standards.

Keywords: CO_2 concentration; Biomass and lipid productivity; Cyanobacteria; *Phormidium uncinatum*; *Oscillatoria salina*; TiO_2 nanoparticles.

1. INTRODUCTION

The daily demand for energy has increased due to population growth, rapid industrialisation, and urbanisation. Currently, 80 % of the world's energy needs are met by fossil fuels, resulting in the depletion of these resources and the emission of greenhouse gases into the atmosphere, which contributes to global warming. Hence, an alternative fuel is required to satisfy the global energy demand (Olabi *et al.* 2023). Recently, scientists worldwide have begun to focus on cyanobacterial biomass for various biofuel production, including biodiesel, bioethanol, biomethane, and biohydrogen (Mukherjee *et al.* 2013; Singh *et al.* 2016; Nainangu *et al.* 2020; Carpine and Sieber 2021; Kar *et al.* 2022; Żymańczyk-Duda *et al.* 2022). Large-scale biofuel production through some bioenergy options has failed; however, there are no limitations to using microorganisms for bioenergy production (Ramamurthy *et al*. 2021). Various value-added products derived from cyanobacteria have recently been utilised to produce biofuels (Verseux *et al.* 2016; Pathak *et al.* 2018; Singh *et al.* 2019; Chittora *et al*. 2020). Due to their high oil content, greater photosynthetic ability, and higher intake of solar energy, they produce higher biomass compared to other traditional bioenergy sources. Conversely, cyanobacterial species can convert carbon dioxide into value-added products (Tiwari *et al.* 2019). Growing cyanobacteria require simple ingredients and a simple genomic structure adaptable to environmental changes (Zahra *et al.* 2020). Several studies have been conducted on microalgae at elevated CO_2 concentrations, resulting in enhanced biomass and lipid productivity. The flue gases from the 4 MW coal-fired power plant were utilised for microalgal growth. A lesser concentration of CO_2 resulted in a higher growth rate in isolated microalgal species, such as *Scenedesmus dimorphus NT8c*, *Chlorella vulgaris*, and *Limnothrix planctonica*. CO_2 is applied in smaller quantities to facilitate better adaptation, thereby increasing growth and stability. Adaptation to CO_2 directly depends on the nature of the cyanobacterial strains. In Malaysia, the flue gases obtained from coal-fired power plants were utilised to cultivate microalgae, including *Nannochloropsis sp.*, *Tetraselmis sp. and Isochrysis sp.* The results showed that *Isochrysis sp.* achieved the maximum CO_2 fixation. The higher concentration of CO_2 released from the exhaust gases of thermal power plants can be used as a carbon source for microalgae growth.

The ability to apply several biochemical processes to cyanobacteria makes them extremely beneficial for generating various fuels, such as biogas, cellulosic ethanol, and biodiesel (Sitther *et al.* 2020).

Some of the benefits of cyanobacteria are their high lipid content, quick growth rate, and low land requirements. Due to their advantages, microalgae-based oils may be an attractive option to meet the need for transportation fuels from renewable energy sources (Karatay and Dönmez, 2011). A limited number of studies have been conducted on converting cyanobacterial lipids to biodiesel. Yadav *et al.* (2021) compared the biodiesel potential of *Phormidium sp.* FW01, *Phormidium sp.* FW02, *Oscillatoria sp.* FW01 and *Oscillatoria sp.* FW02. The study revealed lipid contents of 6.7 %, 8.2 %, 10.2 %, and 9.4 %, respectively. Lipid content of *Oscillatoria sp.* FW01 was higher at 2000 lx and 37–40 °C. Under ideal conditions, the highest lipid concentrations were 42.8 % for *Synechococcus sp.*, 45.0 % for *C. aponinum*, and 38.2% for *Phormidium species*. *Phormidium* species produced the largest yields of saturated compounds and C16 and C18 methyl esters, as reported by Karatay and Dönmez (2011). Sharafi *et al.* (2021) found that by optimising the composition of the growth medium, it may be possible to develop *Synechocystis sp.* MH01, particularly to increase lipid production for use as a potential raw material in biofuel generation.

According to Janiad *et al.* (2024), *Oscillatoria sp.* produces more biodiesel compared to other filamentous strains. Potential sources of third-generation biofuels include cyanobacteria and nanomaterials, which are often used for the transesterification reaction. In recent years, photocatalysts have garnered attention for their role in converting lipids into biodiesel, owing to their reusability and cost-effectiveness (Gadore *et al.* 2024) Generally, TiO_2 is a promising candidate for nanomaterial-based photocatalytic transesterification due to its lower recombination rate (He *et al.* 2020) Metabolically modified cyanobacterial strains have significantly higher potential to produce biofuels (Eungrasamee *et al.* 2019). This study involves isolating cyanobacteria strains from brackish water and utilising them to enhance biomass and lipid productivity at various CO_2 concentrations. The lipid produced by high-lipid-containing cyanobacteria was used in a nanoparticle-based photocatalytic transesterification reaction to yield fatty acid methyl ester.

2. MATERIALS AND METHODS

2.1 Isolation and Screening of Cyanobacteria

The samples were collected from the Parangipettai coastal area of Cuddalore district, Tamil Nadu. The cyanobacterial species were grown using a BG-11 medium. This medium consisted of 1500 mg/L $NaNO_3$, 40 mg/L K_2HPO_4, 75 mg/L $MgSO_4 \cdot 7H_2O$, 36 mg/L $CaCl_2 \cdot 2H_2O$, 6 mg/L citric acid, 6 mg/L ferric ammonium citrate, 1 mg/L $EDTANa_2$, 20 mg/L Na_2CO_3, and 1 mL/L A5 trace metal solution. It also had trace metal solutions like 2.86 g/L H_3BO_3, 1.86 g/L $MnCl_2 \cdot 4H_2O$, 0.22 g/L $ZnSO_4 \cdot 7H_2O$, 0.39 g/L $Na_2MoO_4 \cdot 2H_2O$, 0.08 g/L $CuSO_4 \cdot 5H_2O$, and 0.05 g/L $Co(NO_3)_2 \cdot 6H_2O$. Initially, 1 mL of the wastewater sample was added to the test tube containing 9 mL of distilled water. From this 10 mL stock solution, 1 mL was transferred to another test tube containing 9 mL of saline (0.45 %). A multiple of 10 dilutions was used to reduce the logarithm of the sample. This method systematically reduced the unknown entities and repeated this dilution tenfold. The pure isolated colonies were selected and viewed under a compound microscope. Finally, colonies were subcultured in BG-11 medium, and then the sample was kept in a rotary shaker for a week. The culture was maintained at a room temperature of 30 ± 2°C in a 16:8 light-to-dark phase with a light intensity of 2000 lx, using a rotary shaker at 100 rpm. The obtained pure colonies were used for the morphological identification study.

2.2 Morphological Identification

The cyanobacterial strains were characterised and identified using a compound light microscope at 40X and 100X magnification. For species identification, each strain was examined to assess cell length, width, shape, sheath morphology, and attenuated apical cells. The primary taxonomic publication was used for identification comparison. During the exponential phase, cyanobacterial strains were selected, and DNA was extracted from cyanobacterial cells. Following the procedure described by Yang *et al.* (2013), the genomic DNA of the cyanobacterial strains was extracted. To the cyanobacteria cells, 500 µL of Tris-EDTA buffer, 100 µL of lysozyme, followed by 50 µL of proteinase were added. At 70 °C, the mixture was incubated for 1 hour, and then 0.5 mL of chloroform:phenol (1:5) was added. After incubation, the mixture was thoroughly mixed and then centrifuged. The supernatant obtained was collected by adding absolute ethanol, and then the total DNA was separated by precipitation. The precipitate was washed with 70 % ethanol and centrifuged for sequencing analysis.

2.2.1 DNA Amplification Using PCR and Sequencing

The 16S rRNA gene was amplified using RT-PCR with a specific pair of designed cyanobacterial primers. A complete genome database of cyanobacterial 16S rRNA gene-specific primer sequences was developed. From the NCBI GenBank, randomly selected partial gene sequences of the 16S rRNA gene were obtained. To the 1 µL 100-500 ng of template DNA suspended in water, 1.8 mM of $MgCl_2$, 10 µm of each primer, 1 IU of Taq DNA polymerase, and 0.5 mM of each deoxynucleotide triphosphate (dATP, dTTP, dGTP, and dCTP) were added. Polymerase chain reaction amplification was carried out using a total volume of 25 µL. The conditions for PCR amplification were as follows: an initial denaturation step set for 4 minutes at 96 °C, followed by 35 cycles of denaturation at 94 °C for 30 seconds. After denaturation, annealing was performed

for 30 seconds at 50 °C, followed by elongation for 90 seconds at 72 °C, and the final elongation was carried out for 8 minutes. The obtained PCR products were resolved using a 1% agarose gel and a 1 kb ladder was run simultaneously with the PCR products in the agarose gel. The size of amplified gene was determined by comparing bands with the ladder.

2.2.2 DNA Sequencing and Editing

Before the PCR products were purified, the samples were sequenced using forward and reverse primers. Then, 200 µL of chloroform was added to the amplified DNA in 200 µL of water, and the mixture was vortexed gently. The mixture was centrifuged for 3 minutes at 4 ℃ at 1000 rpm. The supernatant containing DNA was collected and transferred to a new tube. Sodium acetate (3 M, 20 µL) was added and gently mixed by inversion, followed by the addition of 200 µL of isopropanol. At 1000 rpm, the contents were centrifuged for 15 minutes. The obtained pellet was washed with 70 % ethanol, air-dried overnight, and stored in 40 µL of distilled water. The sequence was submitted for Sanger sequencing using BioEdit 7.1.9 and the sequences were analysed. After analysing the sequences, they were submitted to BLAST. All four edited sequences were deposited in GenBank to get a specific accession number.

2.2.3 Phylogenetic Tree

Similar 16S rRNA sequences were identified using the NCBI BLAST tool. The closely related species of the isolated cyanobacterial strains were analysed by performing multiple sequence alignments. The multiple alignment sequences were analysed using the MUSCLE 3.7 program. The poorly aligned positions and the deviating regions were removed using Gblocks, and significantly aligned sequences were preserved.

2.3 Determination of Growth Curve

The stock culture (10 mL) was inoculated into 3 L of medium at room temperature, with continuous light illumination and aeration achieved by bubbling air through the air line in the photobioreactor. Each day, the cell proliferation and growth of the cyanobacterial strains were monitored by measuring absorbance values at 750 nm using a UV-visible spectrophotometric technique. When each culture reached the stationary phase, the cyanobacterial residue was harvested by centrifugation and dried at 50 ℃ for 24 hours.

2.4 Analysis of Pigments

The crude extract of the two cyanobacterial species was prepared, and the phycocyanin content in the two species was determined using Silvera's method. In the dark, 10 mL of sodium phosphate buffer was taken, and 1 g of cyanobacterial powder was added. The mixture was then incubated for 72 hours at 30 °C (Silveira *et al*. 2007). After 72 hours, the extracts were centrifuged, and the absorbance was measured at 615 and 652 nm by UV-Vis spectroscopy. Phycocyanin contents present in the two cyanobacterial species were determined using the formula:

$$\text{Phycocyanin (mg. mL}^{-1}) = A_{615}-(0.474\times A_{652})/5.34 \ldots (1)$$

2.5 Analysis of Total Carotenoid

Initially, 10 mL of fully grown cyanobacterial culture was homogenised by vortexing and then centrifuged at 4 ℃ for 10 minutes at 1500 rpm. The pigment was stored in a dark environment overnight at 4 ℃, in 85 % acetone as the solvent and the carotenoid pigment was extracted. The collected supernatant was measured at 450 nm using UV-visible spectroscopy (Naziri *et al*. 2014).

$$\text{Carotenoids (mg/mL)} = D\times V\times F\times 10/2500 \ldots (2)$$

F – Dilution Factor; V – The volume of the extract; D– Absorbance at 450 nm

2.6 Analysis of Chlorophyll-a

Initially, 10 mL of each cyanobacterial suspension was taken, homogenised, and centrifuged at 2000 rpm for 10 minutes. Using 90 % cold methanol, Chlorophyll-a was extracted by incubating the sample in the dark overnight at 4 ℃. Chlorophyll-a, present in the supernatant of cyanobacterial strains, was measured at 663 nm using UV-visible spectroscopy (Shawer *et al.* 2023).

$$\text{Total Chlorophyll (µg/g)} = A663\times 12.7 \times \text{Volume of the extract} \ldots (3)$$

2.7 Analysis of Total Phenolic Content (TPC)

Phenolic content in the crude extract of the two cyanobacterial species was analysed using the Folin-Ciocalteu reagent method. Approximately 1 mL of methanolic extract from the two cyanobacterial strains was transferred to different test tubes, and 20 µL of Folin-Ciocalteu reagent and 5 mL of distilled water were added to each test tube. Then, both test tubes were incubated at 25 °C for 30 minutes, followed by the addition of 1 mL of Na_2CO_3 (7.5 %) and distilled water. The mixture was incubated in complete darkness for 90 minutes at room temperature. Different standard solutions of gallic acid were prepared at concentrations of 100, 125, 150, 175, and 200 µg/mL. The absorbance of the sample was measured at 760 nm. From the standard curve, the total phenolic content was measured.

2.8 Analysis Total Flavonoid Content (TFC)

The cyanobacterial strains were analysed using the colorimetric method to identify the total flavonoid

content. Initially, 2.5 mL of methanol, 100 µL of 1 M potassium acetate, and 100 µL of 10% (w/v) aluminium chloride were added to 500 µL of each sample extract in a test tube. The sample was incubated in the dark for 30 minutes, and the absorbance was measured at 415 nm. The standard curve plots the flavonoid content in milligrams of quercetin equivalent. Phenol and flavonoids were analysed using the procedure described by Jain *et al.* (2015).

2.9 Analysis of Total Protein Content

The protein content in the two cyanobacterial strains was estimated using Lowry's Method (Lowry *et al.* 1951). According to Lowry's method, 1 ml of each cyanobacterial strain was hydrolysed in 10 ml of 1 M NaOH solution for 24 hours. A supernatant was used for protein estimation. In 5 mL of reagent A, 1 mL of hydrolysate supernatant was added and mixed for 10 minutes at room temperature. Then, 1 mL of B reagent was gently mixed and incubated at room temperature for 30 minutes. The protein content of the cyanobacterial strain was estimated by measuring the absorbance using UV-visible spectroscopy at 750 nm.

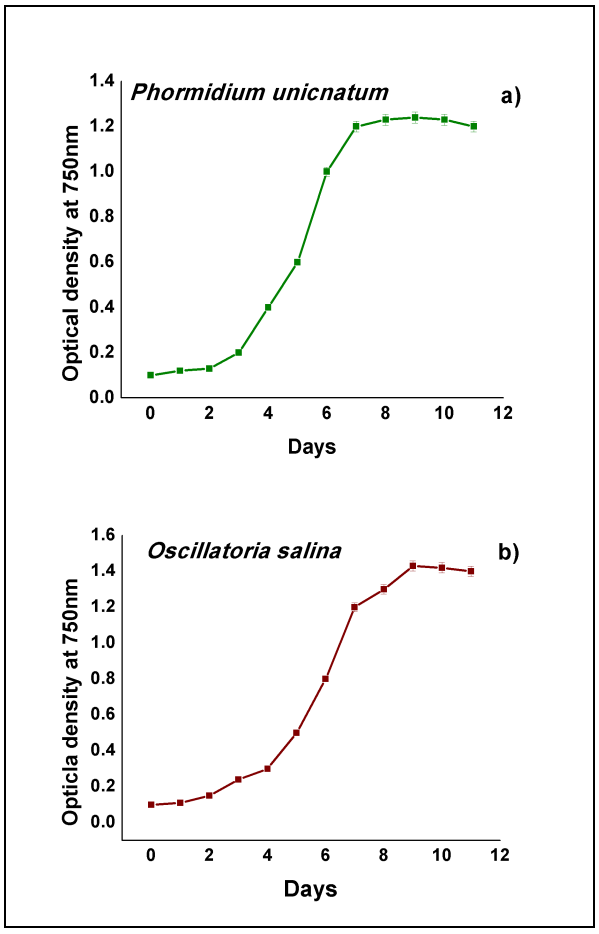


Fig. 1: Growth curve of the cyanobacterial species (a) *Phormidium uncinatum* and (b) *Oscillatoria salina*

2.10 Extraction of Exopolysaccharides

Exopolysaccharides were extracted from two cyanobacterial strains. The two cyanobacterial strains were taken, and 10 mL of warm water was added. The mixture was then shaken well, and 0.1 M EDTA was added. The solution was centrifuged, and the pellet was removed. Further purification was done by dialysis and desalting processes. The final product was stored at -20 °C, precipitated with an isopropanol solution (1:1), and then lyophilised.

2.11 Total Carbohydrates and their composition

A phenol sulphuric acid assay was carried out to determine the purified total carbohydrate content (total neutral sugars). For reference, standard glucose was used. The total carbohydrate content, containing monosaccharides, was removed and identified using HPLC analysis. Initially, 2 mL of 0.5 M trifluoracetic acid was added to the 2 mg of lyophilised EPS sample from the previous procedure, and then the mixture was combined, boiled at 100 °C for 4 hours, and treated with diethyl ether. The aqueous phase containing excess solvent was removed using a rotary evaporator.

2.12 Effect of pH

By varying pH, the growth of the two cyanobacterial strains was observed. In a 500 mL Erlenmeyer flask containing 250 mL of BG-11 medium, the two cyanobacterial strains were grown in the medium. The pH values were adjusted by adding 0.1 mol/L sulfuric acid, and the strains were grown at different pH conditions ranging from 3 to 6. Both strains, with varying pH levels, were maintained in a rotary shaker at 150 rpm for 12 days, with continuous illumination for 24 hours. From the 1st to the 12th day, the biomass concentration and lipid productivity of the cyanobacterial strain were calculated.

2.13 Effect of CO_2 Concentration

Carbon dioxide was bubbled into the photobioreactor containing the cyanobacterial growth medium at different concentrations (5 %, 10 %, and 15 %) to analyse the biomass concentration and lipid productivity. Optimal pH was maintained throughout the reaction. From the 1st to the 12th day, the biomass concentration and lipid productivity of the cyanobacterial strain were calculated.

2.14 Effect of Temperature

The isolated cyanobacterial strains were grown in a BG-11 culture medium, with the temperature range varying from 20 °C to 40 °C. The optimal pH value was maintained throughout the experiment. From the 1st to the

12th day, the biomass concentration and lipid productivity of the cyanobacterial strain were calculated.

2.15 Estimation of Biomass Productivity

The biomass productivity of the isolated cyanobacterial strains was calculated up to 12 days, and the biomass productivity was estimated using a UV-Vis spectrophotometer at 750 nm. The cyanobacterial cells were harvested from the culture medium, washed with ammonium bicarbonate, and then centrifuged and filtered through Whatman filter paper. The cyanobacterial biomass was dried at 80 ℃ for 1 hour to remove the moisture content present in the sample. Biomass productivity was calculated using the standard formula.

$$\text{Biomass Productivity (g/L. d)} = \frac{W2-W1}{t} \quad \ldots (4)$$

W_1 – Initial weight of the biomass; W_2 – Last day biomass concentration; T - reaction time (day)

2.16 Determination of Lipid Content

The grown cyanobacterial culture was centrifuged at 5000 rpm at 4 ℃ for 10 min. The pellet obtained was washed with distilled water to remove impurities present in the medium and then freeze-dried at -50 ℃. The Bligh and Dyer (1959) method measured the lipid content present in the cyanobacterial strain. According to the Bligh-Dyer method, 10 mL (v/v-2.5:5:2) of chloroform and methanol was added to 100 mg of freeze-dried microalgae for lipid extraction. The mixture was kept for 24 hours at RT and sonicated for 10 minutes. Again, 7.5 mL of chloroform and water were added to the mixture and centrifuged for 10 minutes at 5000 rpm. The centrifuge tube was pre-weighed, the organic phase was collected, and the excess solvent was removed from the distillation process. The following equation was used to measure the lipid content:

$$\text{Lipid content (g/L.d)} = \frac{1}{t}\frac{(W_2-W_1)(g)}{volume\ (L)} \quad \ldots (5)$$

where W_1 is the empty weight of the centrifuge tube (g), W_2 is the weight of the centrifuge tube after freeze drying (g), and t is the duration of the experimental run (day).

Biomass productivity was calculated using the following formula (Hamedi *et al.*2016)

$$Lipid\ productivity\ \left(\frac{g}{L}.d\right) = \frac{(Lipid\ content\ of\ cells(g/g) * Dry\ cell\ weight\ (g/L))}{Cultivation\ Period(d)} \quad \ldots (6)$$

2.17 Determination of Fatty Acid Composition

Gas chromatography with mass spectrometry (GC-MS) was used to analyse the fatty acid composition of the lipids present in the cyanobacterial strains (Kebelmann *et al.* 2013). Briefly, 3 mL of a saturated KOH-CH_3OH solution was added to 20-50 mg of freeze-dried cyanobacterial lipid-containing samples, and the samples were heated for up to 1 hour in a hot water bath at 80℃. Then, 5 mL of hexane was added to the sample. After vortexing, the fatty acid present in the upper layer was analysed using GC-MS. A flame ionisation detector with a (50 m × 250 µm × 0.25 µm) DB-5MS chromatographic column was used in the GC-MS analysis. The temperature in the injection was 300 ℃, and the initial column temperature was 150 ℃. It was increased using a temperature gradient by increasing the temperature by 10 ℃ per minute, up to 300 ℃, with a 5-minute time interval. The experiments were repeated, and finally, based on the GC chromatogram and mass spectrometer analysis, the fatty acids present in the lipids of the isolated cyanobacterial strains were identified.

2.18 Synthesis of Photocatalytic TiO_2 Nanomaterials

TiO_2 nanoparticles were synthesised using the co-precipitation method. Initially, an alkoxide was formed by slowly adding 0.01 mol of titanium (IV) isopropoxide to 20 mL of deionised water dropwise. The obtained product was subjected to constant magnetic stirring for an hour. A white precipitate formed after one hour, and it was collected. The residue was washed repeatedly with ethanol to attain a pH of 7.0, and then the precipitate was washed with deionised water. The compound obtained was then dried for hours at 80 ℃ and subsequently calcined at 500 ℃ in a muffle furnace for 6 hours (Saini *et al.* 2018).

2.19 Photocatalytic Transesterification of Cyanobacterial Lipid into Biodiesel

A photocatalytic reactor consists of a cylindrical vessel with a mercury UV lamp added, which is then filled with reactants, including lipid isolated from a high-lipid-containing cyanobacterial strain and methanol (molar ratio 1:12). TiO_2 at 4 wt% is subsequently added to the reaction vessel. The whole reaction solution was stirred under a magnetic stirrer for 120 minutes. Every 30 minutes, 2 mL of the sample was drawn and analysed in GC-MS.

3. RESULTS AND DISCUSSION

From the collected brackish water samples, different cyanobacterial strains were isolated. Microbial growth was seen after one week of inoculation. Various fungi and bacteria were present in the petri dish. Predominantly found cyanobacterial colonies were selected and individually cultured using the spread plate method. After 12 days, the colonies were taken using dilution to reduce the contamination and isolate the pure culture. The last dilution was used for the further mass culturing of cyanobacteria. Four different colonies were obtained as pure cultures and grown under suitable

conditions in four distinct culture media. Maximal growth was observed in the mixotrophic condition; therefore, this condition was used to cultivate cyanobacterial strains in BG-11 medium. The obtained colonies were identified based on their shape, colour, size, filament structure, and sheath, which are key taxonomic features. *Phormidium sp.* has a thin thallus, appears green in color, contains a sheath and cross-wall, and is filamentous. The size of *Phormidium sp.* was approximately 2.5 µm in length and 1-2 µm in width. Another filamentous species found was *Oscillatoria sp.*, which is commonly found in water sources. They are cylindrical and straight, measuring 60 µm in length and 2 µm in width, and appear pale green, exhibiting oscillating movement. Two cyanobacterial strains were isolated and morphologically identified as *Phormidium* and *Oscillatoria sp.* Then, the DNA was isolated from rapidly growing cyanobacterial species and amplified by PCR. A primer was designed to target specific sequences. The newly designed primers were (i) Forward primer: CCTAGCCGTAAACGATGGATAC; Reverse Primer: CAGTATGCTGACCTGGGATTAC; (ii) Forward primer: GGGAGCGAATGGGATTAGATAC; Reverse Primer: ATTCACCGACCTGGCGATTAC. The molecular characterisation was done using 16S RNA, a phylogenetic tree was constructed, and the molecular characterisation studies identified the cyanobacterial species as (*Phormidium uncinatum* VR6-OQ543112) and (*Oscillatoria salina* JRV-OQ781225). The cyanobacterial strains *Phormidium uncinatum* and *Oscillatoria salina* were selected and grown in BG-11 medium. The culture was taken every day, and absorbance was measured for each sample at an optical density of 750 nm. Fig. 1 shows the growth curve of the cyanobacterial species, monitored up to the 12th day, when it reached the stationary phase, and then declined until the 18th day. Similarly, the cyanobacterial species were analysed after harvest to determine the levels of chlorophyll, carotenoids, and phycocyanin. Saini *et al.* (2018) reported three primary pigments present in the light-harvesting complex of cyanobacteria. These pigments are bioactive compounds that can be potentially used in various industrial applications (Molina *et al.* 2023). The cyanobacterial strains fluoresce at different wavelengths and exhibit a more significant antioxidant potential (Ni *et al.* 2018). The quantity of pigments mainly depends on light intensity and photoperiod (Niangoran *et al.* 2021). *Phormidium sp.* was found to be a potential candidate for phycocyanin production, with reported levels of up to 2.39 mg/mL of phycocyanin (Shanmugam *et al.* 2017; Aoki *et al.* 2021; Shaikhoun *et al.* 2022). In both harvested cyanobacterial species, *Phormidium uncinatum* and *Oscillatoria salina*, the phycocyanin content was determined and enhanced using sodium nitrate as a nitrogen source. Fig. 2 shows the phycocyanin content for both the optimum condition and the control. Under optimum conditions, phycocyanin production increased compared to the control in both cyanobacterial species.

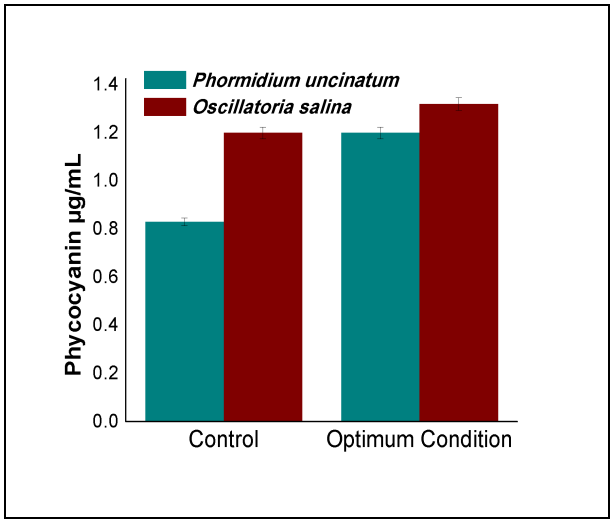


Fig. 2: Phycocyanin content *Phormidium uncinatum* and *Oscillatoria salina*

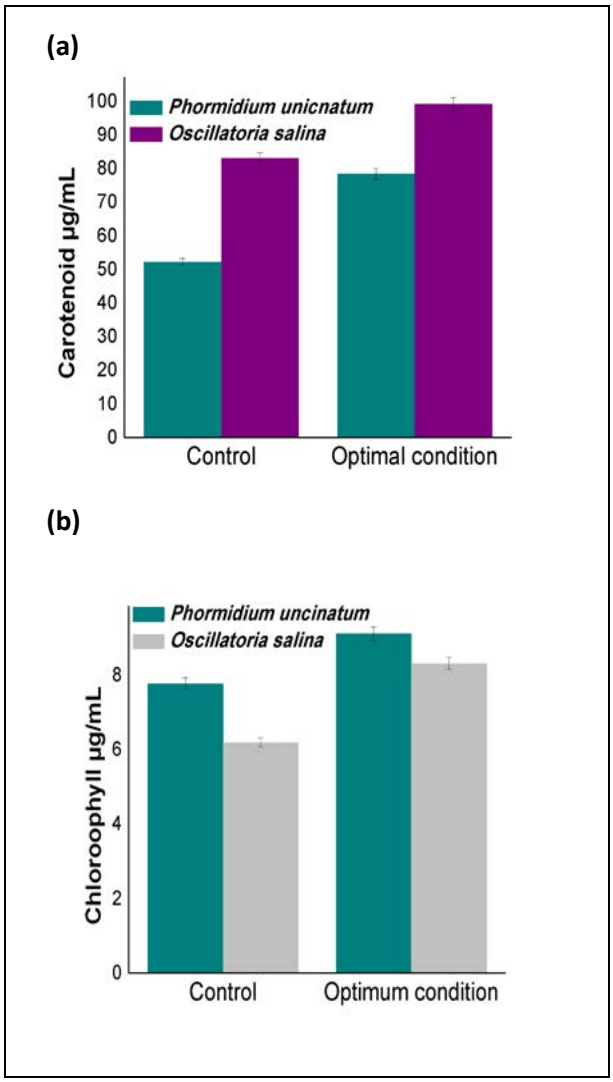


Fig. 3: Carotenoid and chlorophyll content of *Phormidium uncinatum*. *Oscillatoria salina* under control and optimum conditions

Phormidium sp. generally produces 4 µg/ mL of carotenoid, which can be enhanced up to 76.2 µg/mL by adding extra nutrient supplements (Santhosh *et al.* 2011). *Phormidium* contains carotenoid components, including β-carotene, lutein, violaxanthin, and neoxanthin, which have high antioxidant activity and promote free radical scavenging, thereby helping to protect against chronic diseases (Pagels *et al.* 2021). Both *Oscillatoria sp.* and *Athrospira sp.* contain myxoxanthophyll and oscillaxanthine. However, their structures were not established, previous studies (Hertzberg and Liaaen, 1966; Young and Lowe, 2018; Maoka, 2020) reported that carotenoids in cyanobacteria interact with reactive oxygen species, scavenge free radicals, and contribute to protection against various diseases. that the carotenoids present in cyanobacteria interact with reactive oxygen species, exhibit antioxidant activity, and promote free radical scavenging, thereby helping to protect against various diseases. The amount of chlorophyll-a present in *P. unicnatum* under regular conditions was found to be 7.783 µg/mL, as shown in Fig. 3b. The maximum chlorophyll content increased to 9.125 µg/mL under optimal conditions. In *O. salina*, the maximum chlorophyll content obtained in optimal conditions was 8.32 µg/mL, compared to *P. unicnatum*, which produces the most significant chlorophyll content (Hotos and Antoniadis, 2022). Recently, a chlorophyll content of 9.3 µg/mL was reported in marine-based *Phormidium sp.* by Sultana *et al.* (2009). The phytochemical extraction was done using methanol, as it has high polarity (Scholz and Liebezeit, 2006; Truong *et al.* 2019). The phenolic content found in *Oscillatoria salina* was 20.09 mg/g, and *Phormidium uncinatum* consists of 4.21 mg/g of phenolic content. High phenolic content was present in *Oscillatoria salina* compared with *Phormidium uncinatum* (Andriopoulos *et al.* 2022; Singh *et al.* 2017).

Oscillatoria salina contains a high flavonoid content, with a concentration of 11.09 mg/dry weight, compared to *Phormidium uncinatum*. Polyphenols are the largest group of flavonoids and potent antioxidants due to their ability to donate hydrogen. The flavonoid's primary mechanism is scavenging the free radical (El-Aty *et al*. 2014). The phytochemicals present in cyanobacteria, such as phenols, flavonoids, steroids, and alkaloids, enhance the DPPH free radical scavenging activity of the cyanobacterial strain. *Oscillatoria salina* exhibited 89.2 % free radical scavenging activity compared to *Phormidium uncinatum* (Chatterjee *et al.* 2014; Ismaiel *et al.* 2014; Renugadevi *et al.* 2018; Yasin *et al.* 2019; Srinivasan *et al*. 2014). Similarly, the protein content in *Phormidium uncinatum* was high, at 25.23 %, compared to 18.93 % in *Oscillatoria salina*. *Phormidium uncinatum* contains 139 mg/g of exopolysaccharides, and *Oscillatoria salina* contains 334.8 mg/g. Both species consist of polysaccharides composed of glucose, fructose, fucose, ribose, xylose, mannose, rhamnose, galactose, galacturonic acid, galactosamine. Exopolysaccharides from the *Phormidium* species have anti-inflammatory properties (Nicolaus *et al.* 1999; De-Philippis *et al.* 2001; Bhatnagar and Bhatnagar, 2019; Zampieri *et al.* 2020; Elkomy and Ismail, 2021; Moia *et al.* 2023). Cyanobacteria are extremophiles because they thrive in extreme conditions, including ionising radiation, high pH levels, high temperatures, and water-deprived environments. (Pade and Hagemann, 2014; Singh *et al.* 2018; Shahbazi *et al.* 2021; Yadav *et al.* 2022).

3.1 pH Resistance

Phormidium uncinatum and *Oscillatoria salina* were grown at various pH conditions. In alkaline pH conditions, the biomass productivity of the cyanobacterial strains increased compared to neutral pH conditions. Consequently, at lower and higher alkaline pH levels, biomass concentration was also reduced, affecting the growth of the cyanobacterial strain. At pH 8, *Oscillatoria salina* exhibited the highest dry weight of 897 ± 0.03 mg/L, as shown in Fig. 4. Both cyanobacteria showed the highest biomass production at a pH of 8. It is reported that blue-green algae do not grow in low pH environments due to the highly acidic conditions (Brock, 1973). Cyanobacteria can grow quickly at neutral or alkaline pH levels, which favors their growth (Fang *et al.* 2018).

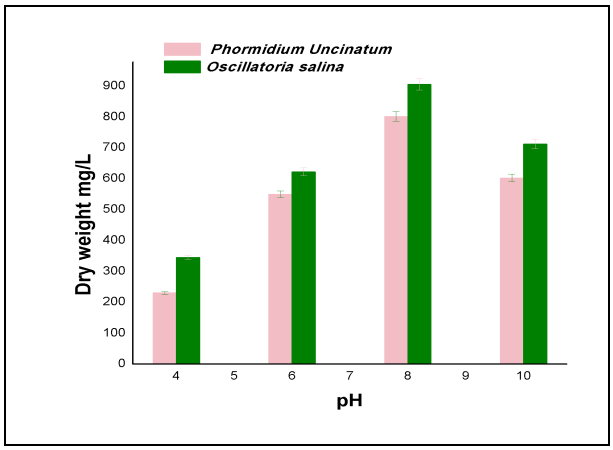


Fig. 4: Effect of pH on biomass concentration of *Phormidium uncinatum* and *Oscillatoria salina*

3.2 Influence of CO_2 Concentration

The growth of *Phormidium uncinatum* and *Oscillatoria salina* was enhanced by supplying CO_2 at different concentrations (5 %, 10 %, and 15 %). Increasing the CO_2 concentration enhances the biomass production rate observed in both species. Both species showed the highest biomass concentration at 15 % CO_2 concentration. This *Oscillatoria salina* exhibited the highest biomass production, reaching 956 ± 0.04 mg/L at a 15 % CO_2 concentration. The increase in biomass concentration relative to CO_2 concentration is illustrated in Fig. 5

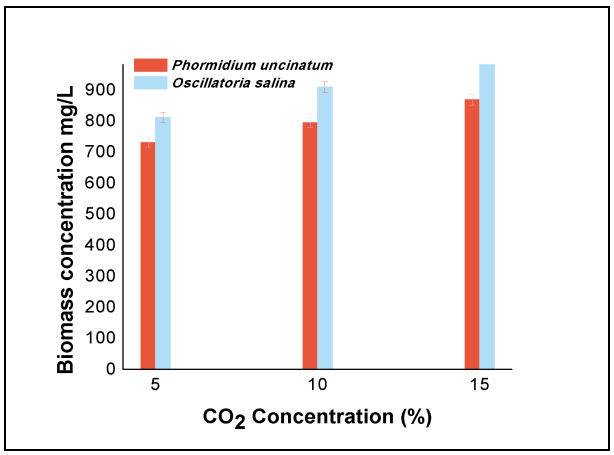


Fig. 5: Effect of CO_2 concentration on biomass concentration *Phormidium uncinatum* and *Oscillatoria salina*

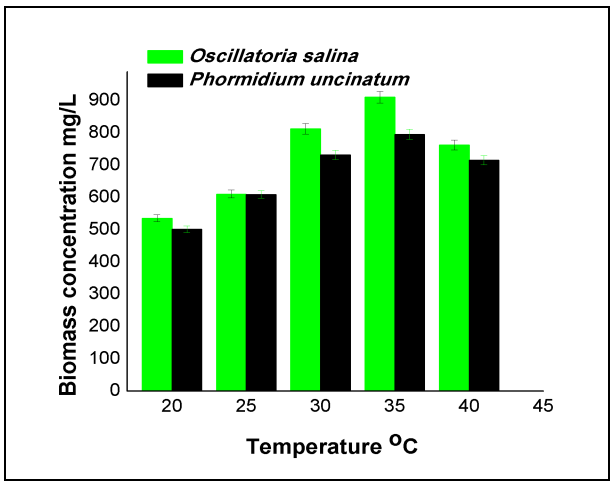


Fig. 6: Effect of temperature on biomass production of *Phormidium uncinatum* and *Oscillatoria salina*

3.3 Influence of Temperature

Phormidium uncinatum and *Oscillatoria salina* were grown under optimal media conditions with different temperature ranges from 20 °C to 45 °C. There is an increase in biomass concentration with increasing temperature, and a decrease in biomass at lower temperatures. Both species exhibited the highest biomass concentration at an optimum temperature of 35 °C. *Oscillatoria salina* exhibited the highest biomass production, reaching 896 ± 0.04 mg/L at 35 °C which is shown in Fig. 6 Native cyanobacteria species are present in lakes and their biomass production increases with rising temperatures (Thomas and Litchman, 2016). Cyanobacteria can also tolerate low temperatures; photosynthesis occurs at lower temperatures (Nalley *et al.* 2018).

3.4 Biomass and Lipid Productivity of the Isolated Cyanobacterial Strains

The factors that influence the growth and lipid content of the cyanobacteria were examined and optimised using a fully grown culture. Fig. 7 shows that the lipid content of the cyanobacteria increases with respect to cultivation time. Optimised pH (8), CO_2 concentration (15 %), and temperature (35 °C) cultures were grown in BG-11 medium for up to 10 days. The lipid content was examined in both cyanobacterial strains, and the lipids were extracted; the yield was then quantified. The lipid content was higher in *Oscillatoria sp.* than in *Phormidium sp.* Both species contain mono-unsaturated fatty acids suitable for biodiesel production. *Phormidium uncinatum* exhibited 832.45 mg/L of biomass production, while *Oscillatoria salina* showed 958.93 mg/L and also the lipid productivity shown under optimised condition. The highest lipid content was found in *Oscillatoria salina*. High-quality biodiesel yields were reported in *Oscillatoria sp.* (Cao *et al.* 2014; Singh *et al.* 2018).

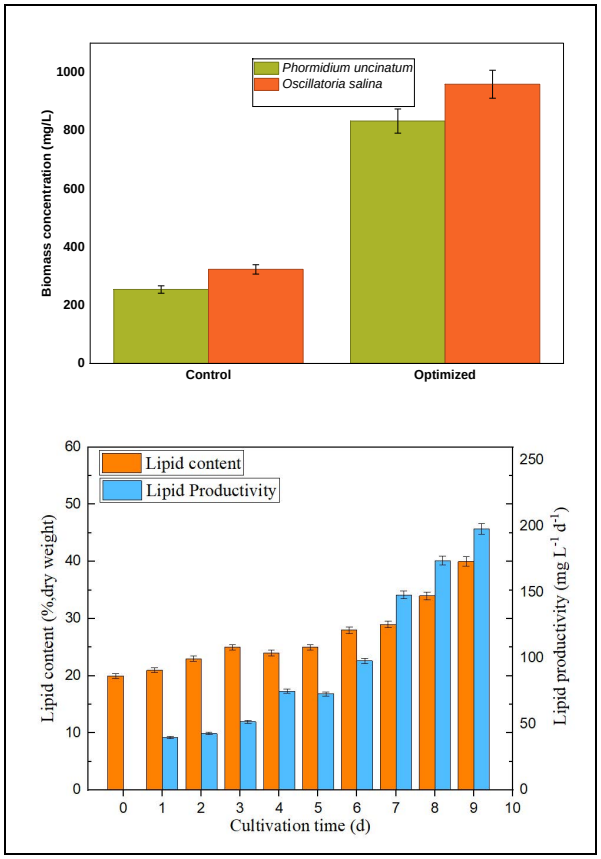


Fig. 7: Illustrates the growth increase of lipid content to lipid productivity of *Oscillatoria salina*

3.5 Fatty Acid Composition

Oscillatoria salina cyanobacterial strains contain fatty acids like palmitic acid (C16:0) (0.82–78.84 %), palmitoleic acid (1.44–13.82 %), (C16:1) (7.43–38.37% concentration) and myristic acid (C14:0) (1.13–46.76 %). The fatty acid profile serves as the basis for determining the biodiesel quality criteria. The

cyanobacterial strain *Oscillatoria salina*, grown on BG-11 media, exhibited the highest values and produced higher-quality biodiesel. The primary fatty acids present in the cyanobacterial strains are suitable for making biodiesel. Most cyanobacteria and oleaginous microalgae synthesise lipids of 4–28 carbons, including polyunsaturated, mono-unsaturated, and saturated fatty acids. For the generation of biodiesel, strains with high levels of saturated and monounsaturated fatty acids are more suitable (Karageorgou *et al.* 2022)

3.6 Characterisation of Nano TiO_2 Photocatalysis

The peaks observed in the XRD of nano TiO_2 were 25.4 °, 27.5 °, 37.9 °, and 36.3 °, which correspond to the TiO_2 phase, including both anatase and rutile crystalline phases. The pattern was in good agreement with the standard pattern (JCPDS Card No. 96-900-7433). Enhanced thermal stability and photocatalytic activity have been reported when both anatase and rutile phases are present in the sample (Yu and Yang, 2010). The average crystal size of the synthesised nanocomposite was found to be 26.2 nm, calculated using the Scherrer equation. The XRD pattern shows distinctive diffraction (2θ) peaks at 25.4 ° and 27.5 ° corresponding to the polymorphic form. Narrow peaks obtained in the XRD pattern show the nanomaterial with high crystallinity, enhanced interfacial strains, and lattice distortion, and the average crystallite size for the nanoparticles was calculated using the following equation:

$$D = \frac{k\lambda}{B\cos\theta} \quad \ldots (7)$$

where B is the full-width half maximum, k is the Scherrer constant, D is the crystallite size, and λ is the wavelength of the X-ray. The band gap range of the titanium dioxide nanomaterial was calculated using the Kubelka-Munk function and Tauc's plot. The sample was examined using the Kubelka-Munk function F(R), as specified by Eq. (7). A pure TiO_2 exhibits a 3.0 eV intrinsic band gap. TiO_2 activates in the UV region due to its intrinsic band gap of 3.2 eV (Feng *et al.* 2016) SEM-EDAX shows the structure and elemental composition of the synthesised nanoparticle. Characterisation of the nanomaterial is shown in Fig. 8.

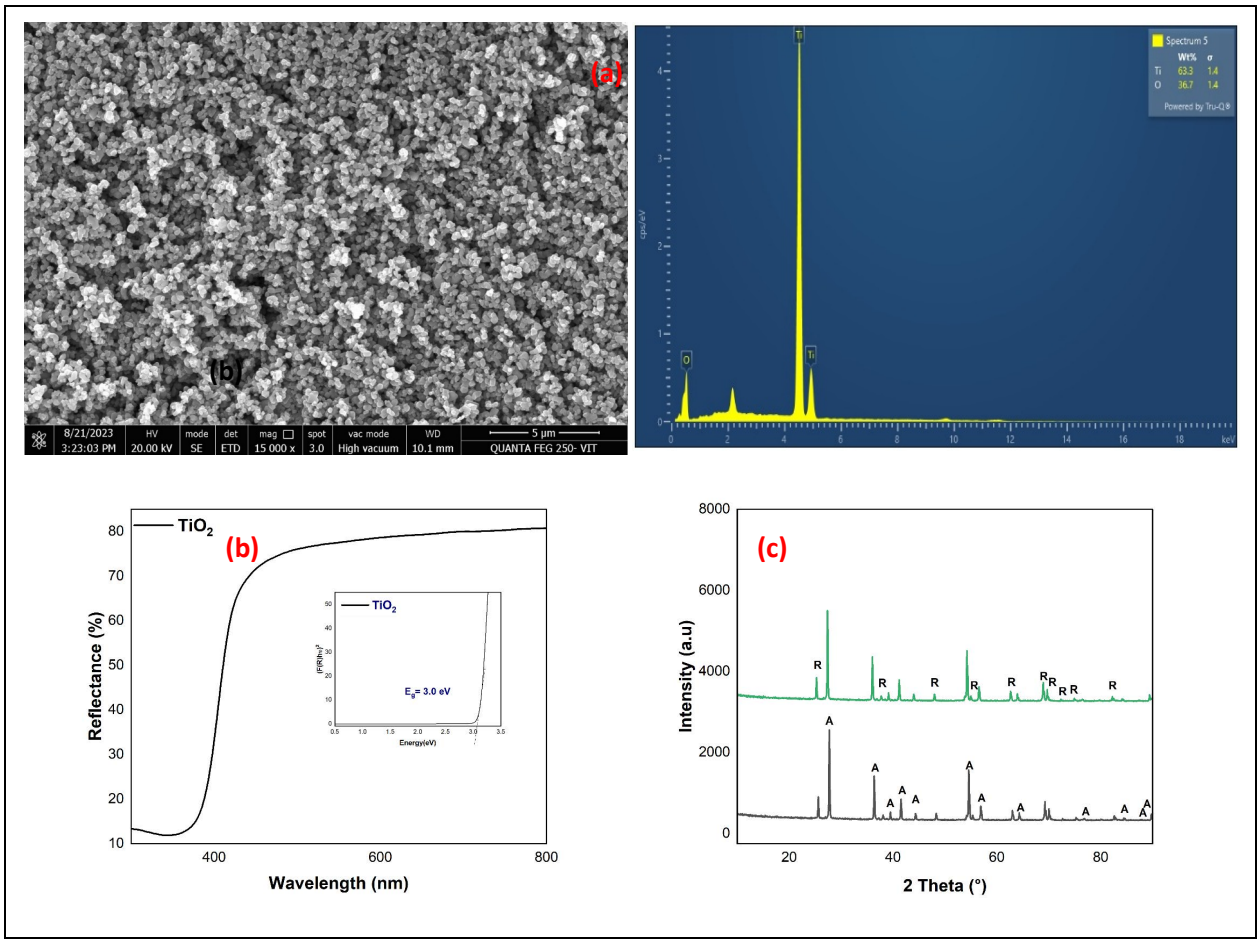


Fig. 8: (a) SEM-EDX of TiO_2 nanoparticles, (b) DRS spectrum of TiO_2 nanoparticles and (c) XRD spectrum of TiO_2 nanoparticles. Black Spectrum: Anatase phase of TiO_2, Green Spectrum: Rutile phase of TiO_2

3.7 Photocatalytic Transesterification of Cyanobacterial Lipid

The cyanobacterial lipid obtained from *Oscillatoria salina* was converted into fatty acid methyl ester using the synthesised TiO_2 nanoparticles. The photo-esterification of fatty acids and methanol was carried out in a photocatalytic batch reactor. The photocatalyst, 4 wt % TiO_2, was added to the reaction vessel under UV-light illumination. Then, methanol and cyanobacterial lipids were added to the reaction vessel to produce fatty acid methyl ester (FAME). The conversion of lipids to fatty acid methyl ester was found to be 98%. The estimated values of the mass spectra, derived from the molecular ion peaks and base peaks of the FAMEs using the GC-MS spectral data which is shown in the Fig. 9.

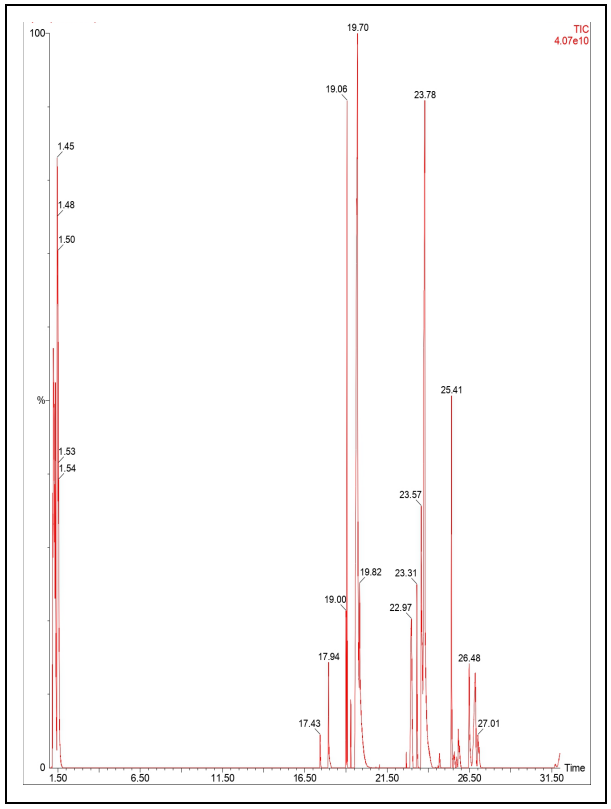


Fig. 9: Characterisation of Fatty acid methyl ester using GC-MS

4. CONCLUSION

In conclusion, this study successfully isolated and identified two indigenous cyanobacterial strains from brackish water, namely, *Phormidium uncinatum* and *Oscillatoria salina*. Increased biomass concentration and lipid content were achieved for both cyanobacterial strains at pH 8, a temperature of 350 °C, and by supplying 15 % CO_2 into the culture medium. Both cyanobacterial strains demonstrated significant antioxidant activity, containing bioactive compounds such as saponins, glycosides, steroids, and alkaloids. The biochemical composition of *Phormidium uncinatum* exhibited a higher protein content. *Oscillatoria salina* exhibited a higher lipid content and was utilised for photocatalytic transesterification using TiO_2 nanoparticles under UV radiation. The conversion of lipids to fatty acid methyl ester was found to be 98% and the obtained biodiesel was tested to identify the fuel properties and compared with ASTM standards.

FUNDING

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

CONFLICTS OF INTEREST

The authors declare that there is no conflict of interest.

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