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Physiological characterization of *Oscillatoria salina* and *Phormidium uncinatum* under various growth conditions

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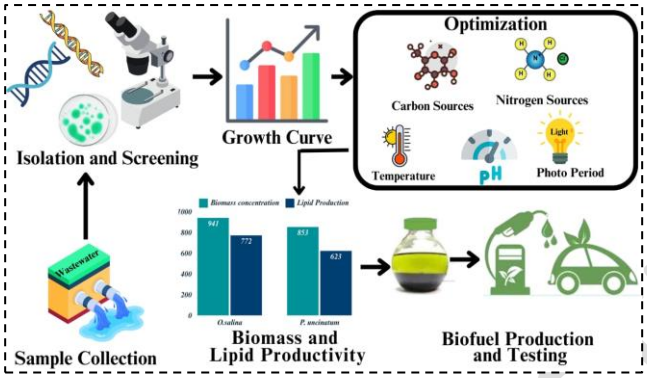
Graphical abstract



Abstract

This study focuses on isolating and cultivating cyanobacterial strains from seawater for enhanced biomass and lipid production under optimized mixotrophic conditions, with the goal of sustainable biodiesel development. Two cyanobacterial species, *Oscillatoria salina* and *Phormidium uncinatum*, were successfully isolated and cultivated in four different culture media: BG-11, BBM, WC, and ASN-III. Among these, BG-11 supported the highest biomass yield. The influence of various organic carbon (glucose, sucrose, glycerol, sodium acetate) and nitrogen (sodium nitrate, ammonium nitrate, potassium nitrate) sources on biomass production was assessed, with sucrose and sodium nitrate showing the most favourable effects. Environmental conditions such as pH, light intensity, and Photoperiod were varied to determine their impact on biomass accumulation, with optimal growth observed at pH 10, light intensity of 125 ± 2 µmol photons m^{-2} s^{-1}, and a 16:8 light-dark cycle. Biochemical analysis of the cultured strains confirmed the presence of key pigments and macromolecules. Lipid extracts were analyzed using GC-MS, revealing the presence of four major fatty acids—nonadecanoic, octadecanoic, hexadecanoic, and tetradecanoic acids—suitable for biodiesel production according to international standards. These findings underscore the potential of *Oscillatoria salina* and *Phormidium uncinatum* as promising candidates for biofuel generation under tailored culture conditions.

Keywords: Biomass, lipid production, organic carbon and nitrogen sources, brackish water

1. Introduction

Cyanobacteria are the largest, widely dispersed group of photosynthetic prokaryotes, blue-green algae, oxygenic, and ubiquitous. Various cyanobacteria species are used as biofertilizer, for biofuel production, and in other industrial applications. They are independent autotrophs that fix atmospheric nitrogen in the soil and are grown in large numbers in rice paddies (Chittora *et al.,* 2020; Prabha *et al.,* 2022). The nitrogen content in the soil and wastewater is utilized for their growth metabolism and is a valuable resource in the chemical, food, and biofuel industries (Vi *et al.,* 2020). Like other biomass resources, they can use solar energy to convert into chemical energy and exhibit remarkable molecular aspects, functional similarity, and structural similarity. Cyanobacteria can absorb atmospheric CO_2 and enhance the lipid content and triglycerides (Agarwal *et al*., 2022). Its adaptable nature allows it to change its metabolism in response to environmental factors, producing different secondary metabolites with unique bioactivity and structural characteristics (Nandagopal K *et al.,* 2021; Sánchez-Bayo *et al.,* 2020; de Morais *et al.,* 2015; Sukenik *et al.,* 2009; Venkatraman, M., *et al*, 2025; Suresh Maruthai *et al*., 2025). As a result, it can thrive in various harsh environmental conditions. Cyanobacteria are a sustainable natural source in food production, pharmaceuticals, and bioenergy industries (Ampofo and abbey, 2022; salami *et al.,* 2021). Recently, cyanobacteria have drawn increasing attention to producing biohydrogen, biomethane, and biodiesel. Artificial light sources can improve photosynthetic efficiency to enhance lipid productivity in cyanobacteria for biofuel production, while genetic engineering can further optimize the metabolic pathways involved (Nozzi *et al*., 2013). Cyanobacterial feedstocks offer higher biomass and lipid productivity than first- and second-generation biofuels; however, their large-scale use is limited by barriers such

as low photosynthetic efficiency, high cultivation and harvesting costs, and complex lipid extraction processes. Despite these challenges, their ability to sequester CO_2 significantly lowers greenhouse gas emissions and supports sustainable energy production. (Afzaal *et al.,* 2022; Udayan *et al.,* 2022; Bošnjaković and Sinaga, 2020; Andrew *et al.,* 2017; Patil *et al*. 2008; Mahmood *et al.,* 2022). Generally, utilizing the nutrients in the wastewater, cyanobacteria exhibit significant growth compared to other feedstock materials (Arias *et al.,* 2020; Jiang *et al.,* 2019; Shoener *et al*. 2018; Sood *et al.,* 2015; Patnaik and Mallick, 2021; Taofeeq *et al.,* 2021; Hossain *et al.,* 2019; Khan *et al.,* 2018; Baracho and Lombardi, 2023; Dagnino-Leone *et al.,* 2022). The biomass produced by cyanobacteria is employed for biomethane, biodiesel, bioethanol production, and bioelectricity with biodiesel production (Benedetti *et al.,* 2018; Maurya *et al.,* 2016;). According to Bhooshan *et al*., (2018), both abiotic and biotic environmental variables play a significant role in cyanobacteria growth. Abiotic factors include light, temperature, pH, carbon dioxide, nutrient concentration, and oxygen, whereas biotic factors include bacteria, fungi, and viruses (Yadav *et al.,* 2021). The present study was to isolate cyanobacterial strains in water samples and to compare and optimize the cultivation conditions for the isolated cyanobacterial strains *Oscillatoria salina* and *Phormidium uncinatum,* which can be used for biodiesel production.

2. Methodology

2.1. Material and methods

All chemicals and reagents used in this study were of analytical grade and procured from Hi-Media and SD Fine Chemicals, India. The culture media used for cyanobacterial growth included BG-11 (Blue-Green medium, Hi-Media, Cat. No. M1541). For marine species, 10 g/L sodium chloride and 1 g/L Vitamin B12 was added externally according to the technical data (HiMedia Laboratories, *Technical Data Sheet: BG-11 Medium (M1541)*, BBM (Bold's Basal Solution, Hi-Media, Cat. No. PL031.), ASN-III (Algal Salt Nutrient medium, Hi-Media, Cat. No. M1942), and WC medium was used for culturing the cyanobacterial strains according to the formulation provided by the UTEX Culture Collection of Algae (UTEX, 2025).

2.2. Isolation of cyanobacterial strain from wastewater

The brackish water rich in bluish-green mucilaginous substances were collected in a sample tube from the region of Parangipettai, Cuddalore District, Tamil Nadu, and the sample was stored meticulously in the sterile bottle at 25 ± 2 °C to prevent the culture from declining phase and to maintain the culture for further study. A traditional technique, which consists of a micropipette, was used for isolation (or) by diluting the sample serially and continuing to cultivate in agar plate (or). The well-grown colonies were selected and viewed under a compound microscope. Simultaneously, 1 mL of brackish water is serially diluted in the range of 10^{-9} to 10^{-1} as per the standard procedure. Using the dilution factor the samples were inoculated. Four different autotrophic media were chosen for the cultivation of cyanobacterial strain viz., BBM, BG-11, WC, and ASN III (Mittal *et al.,* 2022; Campos *et al.,*2013; Badr *et al.,*2021). All four media were adjusted to pH 7 using 0.1M NaOH and 0.1M HCl, and media were autoclaved under 120 °C to check, whether there was no contamination in the media, it was kept overnight then the matured cyanobacterial culture was selected and inoculated. Under different autotrophic media, cyanobacterial culture was grown in 2 L Erlenmeyer flasks. The cyanobacterial culture was kept under white fluorescent light with an intensity equal to 125 ± 2 µmol m^{-2} s^{-1} and 25 ± 2 °C temperature was maintained at 16:8 light-dark cycles. The cultivation condition of the cyanobacterial strain was based on the preliminary study.

The growth medium pH was analyzed every day, and after five days of incubation, the medium was utilized to show blue-green mucilaginous growth. The grown pure colonies were viewed under a compound microscope, cultured in broth, and kept at room temperature in the rotary shaker at 250 rpm with bright light exposure. Finally, the obtained pure culture of the isolated cyanobacterial strains was analyzed using the molecular identification method, the pure cultures of the isolated cyanobacterial strains were identified using molecular techniques based on 16S rRNA gene sequencing. Genomic DNA was extracted using the CTAB method, followed by amplification of the 16S rRNA gene using universal primers 27F (5′-AGAGTTTGATCMTGGCTCAG-3′) and 1492R (5′-TACGGYTACCTTGTTACGACTT-3′). PCR amplification was carried out with an initial denaturation at 95°C for 5 minutes, followed by 35 cycles of denaturation (95 °C, 30 s), annealing (55 °C, 30 s), and extension (72 °C, 90 s), with a final extension at 72 °C for 7 minutes. The PCR products were purified and sequenced. The resulting sequences were analyzed using the NCBI BLAST tool to determine phylogenetic affiliation, and further phylogenetic analysis was conducted using MEGA11 software. This method provides accurate taxonomic resolution for cyanobacterial identification (Neilan *et al*., 1997; Janda & Abbott, 2007). The pure cultures are used for mass culturing and further for the extraction of lipids using the Bligh Dyer method (Bligh and Dyer, 1959), for biodiesel production.

2.3. Measurement of cell growth

The growth of the cyanobacteria in the culture medium was estimated using a UV-Vis spectrophotometer at OD 750 nm. To obtain the dry weight of biomass, the cells were taken from the culture medium and washed with ammonium bicarbonate. The medium was centrifuged, and a Whatman filter paper was used to filter the supernatant. The biomass drying was carried out at 80 °C for 1 hour to remove moisture from the sample. The standard formula used to calculate biomass productivity.

$$\text{Biomass productivity } \left(g/L.d\right) = \frac{\left(W_2 - W_1\right)}{t} \qquad (1)$$

W1 – Initial weight of the biomass; W2 – Last day biomass concentration; T - reaction time (day).

2.4. Effect of external carbon sources

The two cyanobacterial cultures were grown in mixotrophic cultivation conditions. Various sources of carbon, e.g. glucose, sucrose, glycerol, and sodium acetate were added to the mixotrophic culture condition. The mixotrophic culture was incubated at 25 ± 2 °C with continuous illumination of light 125 ± 2 µmol photons $m^{-2}s^{-1}$. 0.5 g/L of different carbon sources were added to increase the biomass yield and the cell density was determined using UV-Visible spectrophotometry at OD 750 nm. Measurements of biomass concentration were conducted every day. The dry weight of the grown cyanobacterial culture was measured at the end of the experiment.

2.5. Influence of nitrogen concentration

Based on the above results, a BG-11 medium was used to grow the cyanobacterial cultures. In the mixotrophic cultivation condition, the BG-11 medium was supplied with various nitrogen sources. The nitrogen sources, such are ammonium nitrate, potassium nitrate, and sodium nitrate are added in the fixed concentration of (0.5 g/L) with continuous illumination of light of 125±2µmol photons $m^{-2}s^{-1}$ with a constant temperature of 25 ± 2 °C.

2.6. Effect of pH

The two cyanobacterial cultures were grown in mixotrophic cultivation conditions. The culture was sustained in different pH ranges of 4-10. The pH was maintained using 1N HCl and 1 N NaOH. The mixotrophic culture was incubated at 25 ± 2 °C with continuous illumination of light 125 ± 2 µmol photons $m^{-2}s^{-1}$. The measurements of the bio-mass concentration were conducted every day. The dry weight of the grown cyanobacterial culture was measured at the end of the experiment.

2.7. Effect of light intensity

Light intensity affects the cyanobacteria's environmental conditions, disturbing their growth metabolism. Different ranges of light intensity 50 to 150 and 125 ± 2 µmol photons $m^{-2}s^{-1}$ were applied to the BG-11 culture medium with a constant temperature of 25 ± 2 °C was maintained throughout the study. The light was illuminated by the cool white fluorescent lamp which can be adjusted to different intensities.

2.8. Effect of photoperiod

Photoperiod was studied in cyanobacteria at different time intervals of 6, 12, 18, and 24 hours under the mixotrophic condition in BG-11 medium with nitrogen and sucrose added as external sources. The growth of the cyanobacterial species *Oscillatoria salina* and *Phormidium uncinatum* was analyzed through optical density at 750 nm, whereas the calculation of dry biomass lasted for 11 days.

2.9. Biochemical composition and lipid extraction from the cyanobacterial biomass via the bligh and dyer method

Silvera's method determined the amount of phycocyanin present in the crude extract of the two cyanobacterial species. After adding 1g of cyanobacterial powder to 10 mL of sodium phosphate buffer, the combination was incubated for 72 hours at 30 °C in a dark environment [Silvera *et al*. (2007)]. The extracts were centrifuged after 72 hours, and UV-Vis Spectroscopy was used to measure the absorbance at 615 and 652 nm. The following formula was used to determine the amounts of phycocyanin in the two cyanobacterial species:

$$Phycocyanin\left(mg.mL\,\hat{}\,\left(-1\right)\right) = A615 - \left(0.474 \times A652\right)/5.34 \quad (3)$$

10 mL of a 14-day-old culture were collected, and vortexed for 30 seconds at medium speed (~2000 rpm) to ensure uniform suspension of the biomass, and centrifuged for ten minutes at 1500 rpm at 4 °C. The carotenoid pigment was extracted after the pigment was set for an entire night at 4 °C in a dark setting using 85% acetone as a solvent. UV-visible spectroscopy measured the supernatant at 450 nm after the overnight content was centrifuged for 10 minutes at 4000 rpm at 4 °C [Naziri *et al*. (2014)].

$$Carotenoids\left(mg/ml\right) = D \times V \times F \times 10/2500$$

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

After homogenizing 10 mL of each cyanobacterial suspension, it was centrifuged for 10 minutes at 2000 revolutions per minute. Chlorophyll-a was extracted using 90% cold methanol and left at 4 °C in the dark for the entire night. UV-visible spectroscopy determined the amount of chlorophyll-an in the cyanobacterial strains' supernatant at 663 nm [Shawer *et al*. (2023)].

$$Total\,Chlorophyll\left(\mu g/g\right) = A663 \times 12.7 \times Volume\,of\,the\,extract)$$

Under optimized culture conditions, the isolated cyanobacterial strains were grown with sucrose and sodium nitrate as carbon and nitrogen sources, respectively, with the light intensity of 125 ± 2µmol photons $m^{-2}s^{-1}$ with a constant temperature of 25 ± 2 °C at a photoperiod of 16:8 light and dark cycles. The obtained dry biomass was dried at 40 °C in an oven overnight. The Bligh and Dyer method was applied to extract the lipid from the cyanobacterial biomass (Bligh and Dyer, 1959). The protein, pigments, and carbohydrate content were analyzed for the isolated cyanobacterial strains (Lowry *et al.,* 1951, (Jensen,1978; Bennett *et al.,* 1978; MacColl *et al.,* 1941). Finally, the lipids obtained via extraction from the cyanobacterial biomass were characterized through GC-MS Spectral analysis.

3. Results and discussion

3.1. Cyanobacterial strain and culture conditions

The isolated cyanobacterial strains *Oscillatoria salina* and *Phormidium uncinatum* are examined under a compound microscope. The morphology of the two cyanobacterial species is shown in **Figure 1**. Further, the pure culture of the isolated cyanobacterial strain was analyzed using a

molecular identification method, and fragments of DNA were purified and sequenced and submitted to GenBank. Based on the Genbank, the cyanobacterial strains were identified as *Phormidium uncinatum* (Accession No: OQ781225) and *Oscillatoria salina* (Accession No: OQ543112). The size of *Phormidium uncinatum* was found to be around approx. 2.0 µm filamentous cells. *Oscillatoria salina* contains elongated cells with the size of approx. 0.8 µm. Both the isolated cyanobacteria form mucilaginous cells and are motile when visualized under a microscope. Under different media compositions both the cyanobacterial strains showed significant biomass yield in the BG-11 medium (Colusse *et al*., 2019; Jubilee *et al*., 2017), and the next highest is the BBM medium because both the cyanobacteria were found in saline water sources and WC provided glycine amino acid which increases the biomass productivity than ASN III medium. BG-11 provides vitamins, trace elements, and micronutrients for the appropriate growth of filamentous unicellular cyanobacteria (Pandey *et al*., 2023). The high ratio of N: P is the primary characteristic of BG-11 that can be used for growing cyanobacteria (George *et al*., 2014). BG-11 is used for increasing the two cyanobacterial strains showed the greatest concentration of 1.75 ± 0.03 g/L, BBM showed 1.623 ± 0.03g/L, WC showed 1.58 ± 0.03g/L and ASN III showed the least biomass concentration of 1.43 ± 0.02g/L.

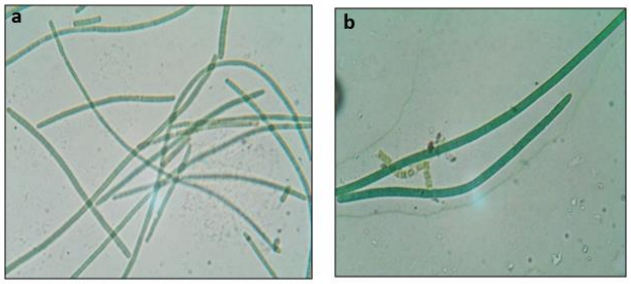


Figure 1. Morphological identification of Cyanobacteria a. *Phormidium uncinatum* b. *Oscillatoria salina*

3.2. Effect of organic carbon sources

Under mixotrophic growth conditions, both cyanobacterial strains were provided external organic carbon sources. Cyanobacteria need to boost their growth metabolism. To improve its lipid and biomass concentration (Goswami *et al*., 2022; Liu *et al*., 2021; Bashir *et al*., 2019; Jiao *et al*., 2018). various carbon sources like sodium acetate, sucrose, glucose, and glycerol, were utilized as external sources of carbon that ultimately alternate the biochemical composition of the cyanobacteria. Appropriate carbon sources enhance biomass and lipid productivity (Plohn *et al*., 2022). *Chlorococcum sp.,* and *Chlorella vulgaris* increased growth by adding glycerol as a carbon source (Gim *et al*., 2016). The biomass concentrations of *Nannochloropsis occulata*, *I. galbana*, and *Dunaliella sp.,* were increased glucose addition as the external organic carbon source, and the enhanced biomass and lipid productivity (Kong, 2013). In the present study, two cyanobacterial strains *Oscillatoria salina* and *Phormidium uncinatum* were studied under different organic carbon sources which are added externally to the culture media to enhance the biomass and lipid productivity. Different sources of carbon, like Glycerol, Glucose, Sucrose, and Sodium acetate were added at a fixed concentration of 0.5g/L. Among the various carbon sources, sucrose yielded better biomass concentration yields up to 1.93 ± 0.03g/L in *O. salina* and 1.84 ± 0.03g/L in P. uncinatum. At the beginning of the experiment, the cyanobacterial growth remained unaffected, but after 5 days it increased exponentially with green mucilaginous cells. Adding sucrose as an external organic carbon source reduces the protein and pigment synthesis in cyanobacteria, instead, it undergoes lipids and carbohydrates formation which is a precursor of biodiesel production (Bashir *et al*., 2019). In another study, it was reported that the disaccharides as an organic carbon source in *Chlorella pyrenoidosa* did not affect the microalgal biochemical composition, monosaccharides contributed to the increase of biomass concentration (Zhang *et al*., 2014). It concludes that from the present study the organic carbon source especially sucrose increases the biomass concentration in both the cyanobacterial strain (**Figure 2).**

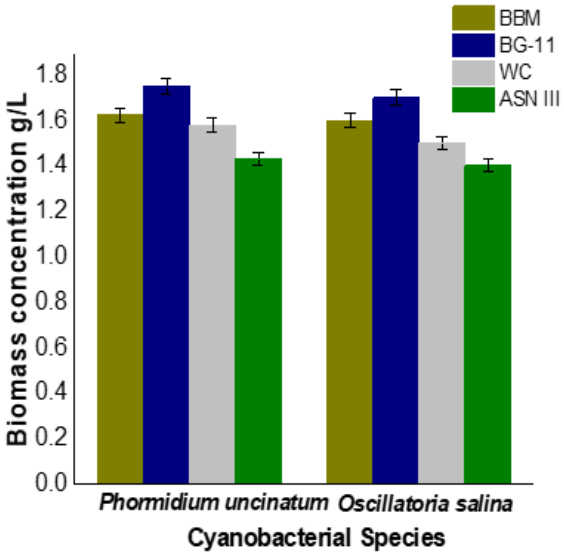


Figure 2. Biomass Concentration of *Oscillatoria salina* and *Phormidium uncinatum* under different medium conditions

3.3. Influence of nitrogen concentration

Cyanobacterial strains *Phormidium uncinatum* and *Oscillatoria salina* were cultivated in mixotrophic conditions in an optimized BG-11 media with various nitrogen sources added to the medium such as potassium nitrate, sodium nitrate, and Ammonium nitrate. All nitrogen sources were supplied in fixed concentration of 0.5 g/L. Under mixotrophic conditions, two cyanobacterial strains showed increased biomass concentration concerning sodium nitrate as a nitrogen source. *P. uncinatum* showed a biomass concentration of 1.89 g/L and 2 g/L for *O. salina*. The dry biomass weight measured was found to be 853mg/L and 941mg/L in *P. uncinatum* and *O. salina*. Compared to all nitrogen sources, sodium nitrate shows greater efficiency. *Chlorella vulgaris* and *S. dimorphus* showed a higher biomass concentration of 3.12 g/L with sodium nitrate as a nitrogen source but in

the same study, there was no change in the concentration of Tetraselmis chui (Zhu *et al*., 2019). In three different species, S quaricauda, S dimorphus, and Chlorella sp., increased biomass concentration by adding potassium nitrate source rather than sodium nitrate (Glacio *et al*., 2020). Cell growth, protein content, and lipid production were reduced by decreasing the nitrogen source (Bošnjaković and Sinaga, 2020; Udayan *et al*., 2022; Patil *et al*., 2008; Mahmood *et al*., 2022; Arias *et al*., 2020). Ammonium as a nitrogen source lowers the biomass concentration in some species due to its toxicity and reduces cell growth even in small concentration growth was reduced than the concentration produced in N-deficient media (Salbitani *et al*., 2021; Lachmann *et al*., 2019; Zhu *et al*., 2019; Xiaoting *et al*., 2018; Tam and wang 1996). This study concludes that even at a lower concentration, sodium nitrate increases the microalgae's cell growth and biochemical composition (**Figure 3a, 3b and 3c)**.

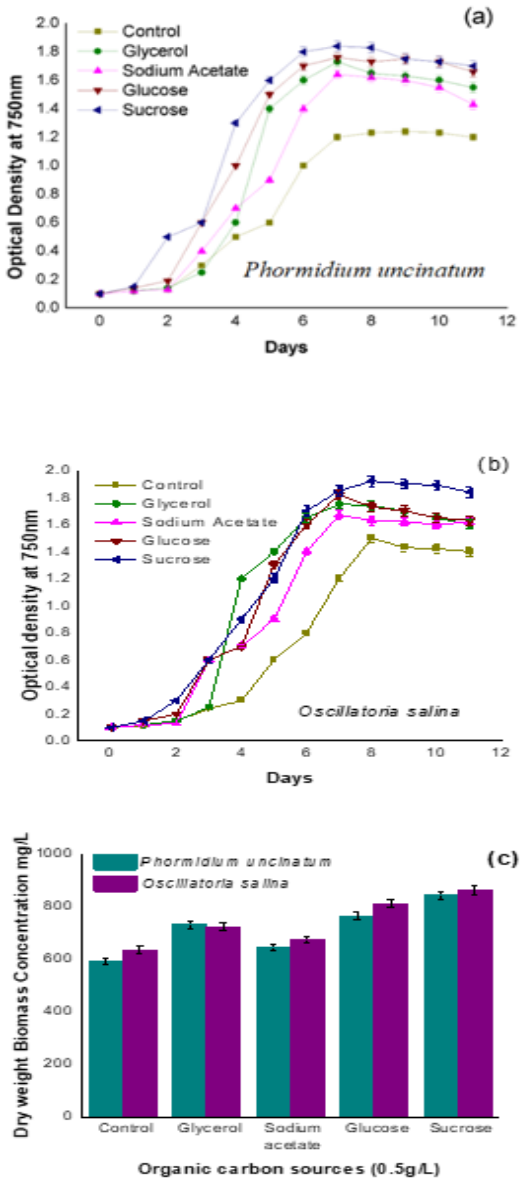


Figure 3. a) and b) Effect of organic carbon source on the growth of *Oscillatoria salina* and *Phormidium uncinatum* c) Biomass concentration of *Oscillatoria salina* and *Phormidium uncinatum* under different organic carbon sources

3.4. Effect of pH

Two cyanobacterial strains were cultivated in optimal culture conditions with different pH conditions. Biomass concentration was increased from neutral pH to alkaline pH. pH below 6 tremendously reduced the growth of the cyanobacterial strains. Both the cyanobacterial species showed better growth rates in alkaline pH. Below pH 7 photosynthetic efficiency of *Phormidium sp.,* was reduced due to lower pH stress (Farahin *et al*., 2021). Cyanobacterial blooms increase with a pH range of 6-7 (Ruangsomboon *et al*., 2013) and cell lysis leads to the release of metabolite due to low pH stress was reported in *Anabaena circinalis* and *Cylindrospermopsis raciborskii* (Charles *et al*., 2015) (**Figure 4a, 4b and 4c, Figure 5a, 5b and 5c**).

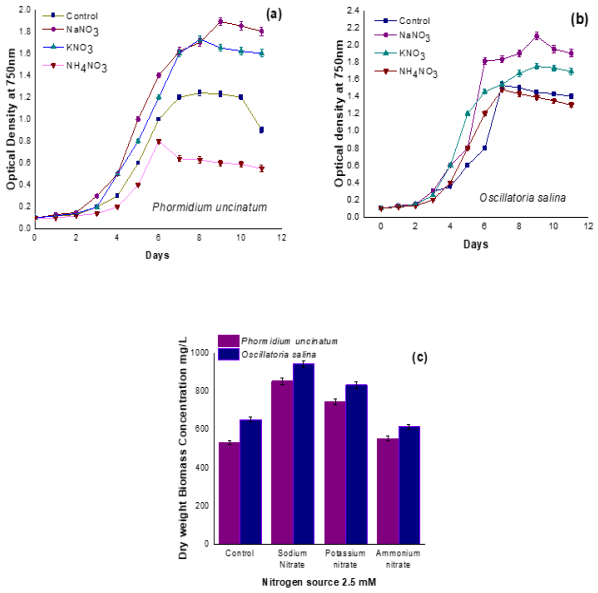


Figure 4. a) and b) Effect of external nitrogen source on the growth of *Phormidium uncinatum* and *Oscillatoria salina* c) Biomass concentration *Phormidium uncinatum* and *Oscillatoria salina* under different nitrogen sources

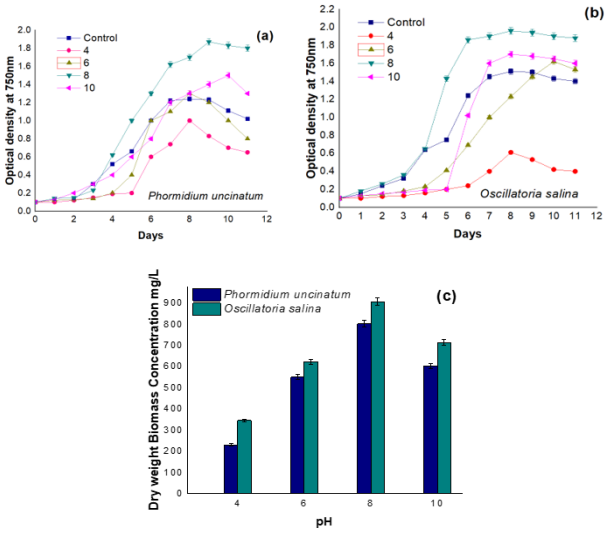


Figure 5. a) and b) show the effect of pH on the growth of *Phormidium uncinatum* and *Oscillatoria salina.* c) Biomass concentration of *Phormidium uncinatum* and *Oscillatoria salina* under different pH

3.5. Effect of light intensity

Cyanobacterial species *Phormidium uncinatum* and *Oscillatoria salina* were grown in mixotrophic conditions at different light intensities to enhance growth, which included light intensities such as 50, 75, 100, 125, as well as 150 µmol photons $m^{-2}s^{-1}$. The highest biomass concentration was obtained at 125 ± 2 µmol photons $m^{-2}s^{-1}$. The dry cell weight of *O. salina* is 911 mg/L, and *P. uncinatum* increases up to 796 mg/L. At 50 µmol photons $m^{-2}s^{-1}$, the observed concentration amounted to 1.24 g/L. This light intensity was similar to the intensity of sunlight, so the light intensity range started from 50 µmol photons $m^{-2}s^{-1}$. Increasing the light intensity by more than 125 ± 2 µmol photons $m^{-2}s^{-1}$ leads to photoinhibition of cell growth in both the cyanobacterial strain. Up to a definite point, the growth of the microalgae rapidly increases along with increasing light (Qian *et al*., 2014). In Chlorella vulgaris, it is reported that biomass concentration increased with light intensity from 25-100%, and lipid concentration increased along with light intensity as well (Maltsev *et al*., 2021; Metsoviti *et al*., 2019). In *C. Vulgaris* and *S. obliquus* there is no significant biomass concentration increase when the light intensity is increased (González-Camejo *et al*., 2019). In Scenedesmus abundans, lipid and biomass concentration were increased when light intensity was increased up to 100 µmol photons $m^{-2}s^{-1}$ (Nzayisenga *et al*., 2020) (**Figure 6a, 6b and 6c**).

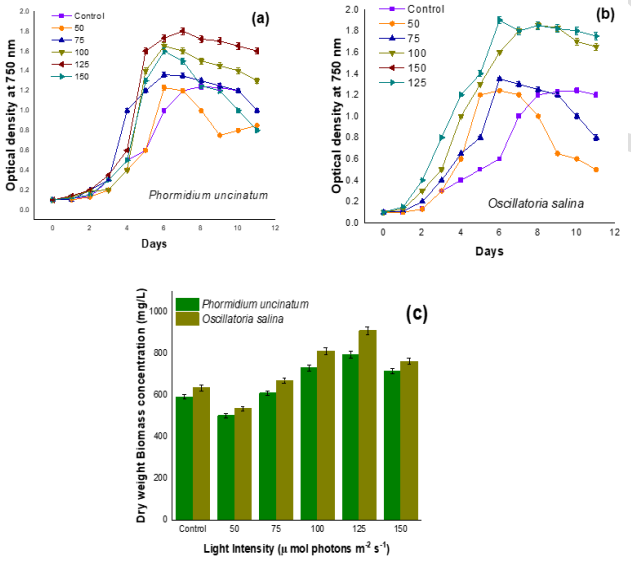


Figure 6. Images a) and b) show the effect of Light intensity in the growth of Phormidium uncinatum and Oscillatoria salina c) Biomass concentration of Phormidium uncinatum and Oscillatoria salina under different light intensities

3.6. Effect of photoperiod

The cyanobacteria utilize both external carbon and nitrogen sources to stimulate the growth. Using various photoperiods both cyanobacteria were cultivated in an optimized BG-11 medium. The light and dark Photoperiod increase the biomass concentration and enhance cyanobacteria growth up to 1.93 g/L for *O. salina* and for *P. uncinatum* producing a biomass concentration of 1.89 g/L. Increasing the Photoperiod will produce heat from the light that affects the growth of cyanobacteria. Cyanobacterial growth will be reduced even in an optimized condition with a longer dark photoperiod. So, the cyanobacterial culture should be provided with optimal light and dark cycles, which increases the biomass yield (Mandotra *et al*., 2015). In *Parachlorella kessleri*, 20:4 Photoperiod showed better efficiency in biomass concentration. (Jean-Baptiste *et al*., 2022).

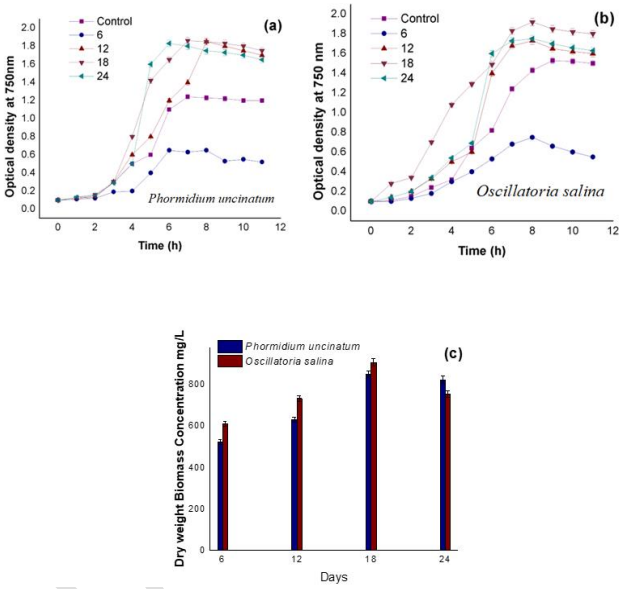


Figure 7. a) and b) present the influence of Photoperiod in the growth of *Phormidium uncinatum* and *Oscillatoria salina* c) Biomass concentration of *Phormidium uncinatum* and *Oscillatoria salina* under different Photoperiod

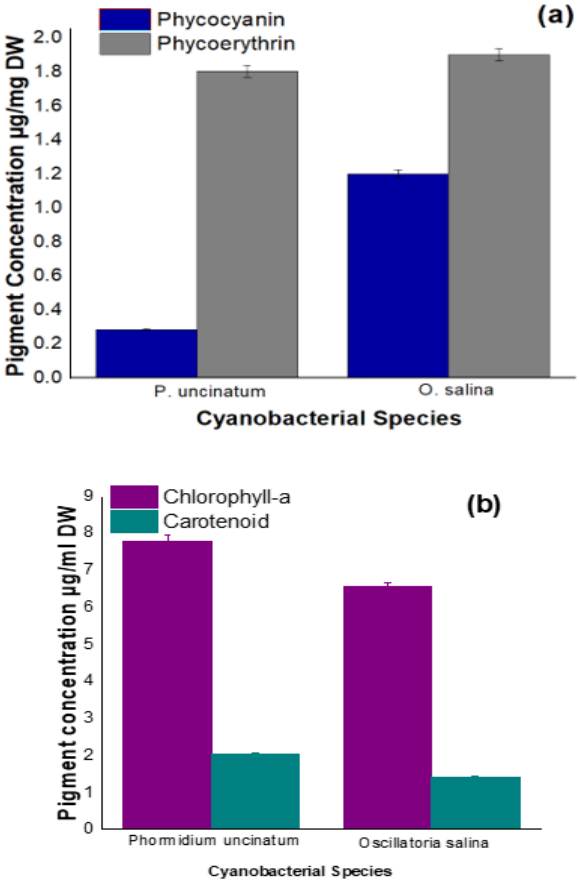


Figure 8. a) and b) shows the pigment concentration of *Phormidium uncinatum* and *Oscillatoria salina*

3.7. Analysis of biochemical composition

In this present study different biochemical compositions were analyzed under optimized conditions were mentioned in **Figure 7a and 7b**. Fatty acids present in both the algae were identified. The carbon chains found in the two isolated strains were in the range of C14 to C18. Some unidentified fatty acids were found in both the algal strains. The total protein percentage merely depends on the external source of nitrogen, by adding an external organic carbon source in the N-deficient medium reduces the protein and lipid production. Both the strains showed 4 to 15% of protein content. The carbohydrates present in both the cyanobacterial strains were found to be exopolysaccharides. The cyanobacteria-produced exopolysaccharides are a vital compound that has the potential to be used as an antiviral, antioxidant, antifungal, antibacterial, and anticancer and it is also used as an immunomodulatory agent (Laroche, 2022). Pigments like chlorophyll-a, Phycocyanin, Phycoerythrin, and carotenoid were present in both the microalgal species. Pigment formation was majorly dependent on the light intensity and Photoperiod. (Pagels *et al*., 2021) (**Figure 8a and 8b)**.

In this study, lipid content was examined in both the cyanobacterial strains. Lipid present in the cyanobacterial strains were extracted and yield was quantified. The lipid concentration was higher in *Oscillatoria salina* than in *Phormidium uncinatum*. The lipids obtained from *O. salina* and *P. uncinatum* were analyzed using ISO and EN standard procedures (**Table 2**). The density and viscosity of the lipid obtained from *O. salina* and *P. uncinatum* found in the range of 865-867 and 38-40 were within the ISO and EN standards. The acid value and iodine value of the extracted lipid of *O. salina* and *P. uncinatum* were found to be in the range of 0.33-0.35 and 45-47 respectively. The oxidation stability of the lipid is very important as an evaluation of the storage time of biodiesel. The oxidation stability of *O. salina* and *P. uncinatum* was found to be 10-11 hours at 110 °C, hours which is within the permissible limit according to EN standards. Both species contain saturated as well as unsaturated fatty acids that can be utilized in the production of biodiesel. *Phormidium uncinatum* showed 1.8 ± 0.03 g/L of lipid production and in *Oscillatoria salina* 1.92 ± 0.03 g/L. For the isolated cyanobacterial strains, in *O. salina* total of four fatty acids were identified using GC-MS spectral analysis. Based on the molecular ion peak of the GC-MS spectra as shown in **Figure 9**, the fatty acids Nonadecanoic acid, octadecanoic acid, hexadecanoic acid, and tetradeconic acid which are suitable for biodiesel production. Similarly, in *P. uncinatum,* three major prominent fatty acids were identified as Octadecanoic acid, tetradeconic acid, and hexadecanoic acid, which are also more suitable for biodiesel production as shown in **Figure 10** and **Table 1**. The fatty acid present in both species is both saturated and unsaturated, which is suitable for the production of biodiesel. High-quality biodiesel yield was reported in Oscillatoria species (Geetanjali *et al*., 2021).

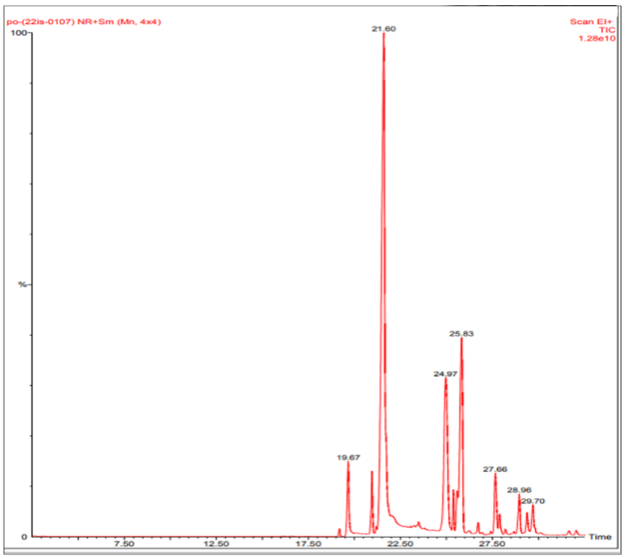


Figure 9. GC-MS Spectra of Lipid of *Oscillatoria salina*

Table 1. GC-MS spectra of the lipid obtained from Phormidium uncinatum and Oscillatoria salina

S.No	*Oscillatoria salina* RT (min)	*Phormidium uncinatum* RT (min)	Fatty Acids	Lipid Numbers	Mass (m/z)
1.	19.60	-	Nonadecanoic acid	(C19:0)	298.50
2.	20.94	19.55	Octadecanoic acid	(C18:0)	284.48
3.	21.46	23.66	Hexadecanoic acid		
4.	(C16:0)	256.43			
5.	24.92	22.89	Tetradeconic acid		
6.	(C14:0)	228.37			

Table 2. Physicochemical Properties of Lipids obtained from Phormidium uncinatum and Oscillatoria salina

S. No	Physicochemical properties of lipids	Lipids from cyanobacteria		ISO and EN standards
		Oscillatoria salina	Phormidium uncinatum	
1.	pH	8.9	8.5	-
2.	Density (kg/m^3)	867	865	ISO3675
3.	Viscosity at 40°C in (mm^2/s)	38	40	ISO3104
4.	Acid value (mg·KOH/g)	0.35	0.33	EN 14104
5.	Iodine value (mg·KOH/g)	45	47	EN 14111
6.	Oxidation stability, 110 °C, Hours	10	11	EN 14112

Figure 10. GC-MS Spectra of Lipid of *Phormidium uncinatum*

4. Conclusion

This study successfully identified, isolated, and cultivated two cyanobacterial strains, *Oscillatoria salina* and *Phormidium uncinatum*, under mixotrophic conditions for enhanced biomass and lipid production. Among the different culture media tested, BG-11 demonstrated superior performance, supporting the highest biomass concentration of 1.75 ± 0.08 g/L. Further optimization by supplementing external carbon and nitrogen sources revealed that sucrose was the most effective organic carbon source, yielding biomass levels of 1.86 ± 0.08 g/L and 1.92 ± 0.07 g/L for O. salina and P. uncinatum, respectively. Sodium nitrate was the most suitable nitrogen source for maximizing biomass productivity. Environmental parameters, including light intensity and Photoperiod, also significantly influenced growth. Optimal conditions were observed at a light intensity of 125 ± 2 µmol photons m^{-2} s^{-1} and a photoperiod of 16:8 (light: dark cycle), under which both strains achieved their peak biomass yields. Biochemical analysis of the cultivated cyanobacterial biomass revealed the presence of key pigments such as phycocyanin, phycoerythrin, chlorophyll-a, and carotenoids. Lipid extraction followed by GC-MS analysis confirmed the presence of several fatty acids — notably nonadecanoic acid, octadecanoic acid, hexadecanoic acid, and tetradecanoic acid — all of which are compatible with international biodiesel standards (ISO and EN), indicating their potential use in sustainable biofuel production.

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