

Isolation, identification, growth optimization of marine algae from Arabian Sea coast of Kerala

Anjali Yadav¹, Sharma Mona², Santosh Bhukal^{1*}

¹ Department of Environmental Science and Engineering, Guru Jambheshwar University of Science & Technology, Hisar-125001, Haryana, India

² Department of Environmental Studies, School of Interdisciplinary and Applied Sciences, Central University of Haryana, Mahendergarh-123031, Haryana, India

* Corresponding author's e-mail: santoshesegju@gmail.com

ABSTRACT

Marine microalgae have been isolated from four different locations of the Arabian Sea coast at Kozhikode (Calicut), Kerala, India. A Magnus MLXi plus light microscope has been used to isolate and morphologically identify ten different microalgal species. XRD and FTIR have been used to describe four pure isolates (Ag1 to Ag4) of two Chlorophyceae and two Cynobacteria species (*Halochlorococcum marinum*, *Oocystis borgei*, *Chroococcopsis chroococcoides*, and *Desmodesmus abundans*). Proteins, carbohydrates, and lipids with a variety of functional groups have been identified in the FTIR spectrum. Traces of quartz/siliceous material at 29–30°, which are commonly identified as calcite when present as biomineral or as precipitated carbonate from the growth media, are represented by the XRD peak at 26.6°. To determine the optimal growth for future use, the culture conditions for the selected microalgae have been optimized using a variety of culture media, including Walne's medium and Bold's Basal Medium (BBM), by varying salinity concentration. The Ag1 (*Halochlorococcum marinum*) and Ag2 (*Oocystis borgei*) strains grow in BBM at 35 ppt salinity having 8.54×10^{-6} cells/ml and 10.2×10^{-6} cells/ml. Ag3 (*Chroococcopsis chroococcoides*) and Ag4 (*Desmodesmus abundans*) grew 0.83×10^{-6} cells/ml and 0.86×10^{-6} cells/ml in Walne medium, despite the salinity of 30 ppt. This suggests that the strains are extremely halotolerant, means they can thrive in conditions with higher salinity. The results reveal that these microalgal strains are a viable choice for large-scale production in salinity conditions ranging from brackish to marine. Ag4 may be a more robust and biotechnologically advantageous strain for applications requiring steady pigment production since it is enriched in both accessory pigments (Chl b) and protective carotenoids, but Ag3 is superior in primary photosynthetic pigment (Chl a) accumulation. Due to their ability to survive in a variety of climatic conditions, the microalgal strains that have been separated during the succession stage may be mass-produced outdoors for industrial uses.

Keywords: marine microalgae, culture media, salinity, chlorophyll, etc.

INTRODUCTION

Phytoplankton (derived from the Greek words phyton meaning “plant” and planktos meaning “wandering”) comprises plant-like components of plankton, microscopic in size, photosynthetic organisms that float freely and passively in water column. As a fundamental biological component of aquatic ecosystems, phytoplankton forms the primary base of food webs, supporting higher trophic levels. These are unable to actively swim and can only survive as filaments, colonies, or

free cells. Rather, their movements depend on the aquatic environment's dynamics. As an alternative, these might be mobile, like ciliates or flagellates, but their range of movement is limited and impacted by the aquatic environment around them (Riouchi et al., 2024; Gravdahl et al., 2025).

The euphotic zone, where phytoplankton is most frequently found, is the uppermost illuminated layer of water bodies and whose lower limit is depth that receives 1% of incident light. While it makes up only 1% to 2% of the world's plant biomass and is at the base of all aquatic trophic

systems, it ironically fixes between 30% and 60% of the world's inorganic carbon each year. A significant portion of atmospheric oxygen is produced by phytoplankton, which also acts as a carbon dioxide pump (Papachristou et al., 2023). As photosynthetic microorganisms, microalgae are thought to have produced oxygen first, which is necessary for the majority of living beings to respire (Naselli et al., 2023).

Naturally found in freshwater and marine environments, microalgae are microscopic photosynthetic organisms that produce a variety of valuable biomolecules, including vitamins, lipids, proteins, polysaccharides, carbohydrates, and pigments. Microalgae are photosynthetic microorganisms that fall into the eukaryotic and prokaryotic categories. These can be unicellular, multicellular, filamentous, or siphonaceous (Lithi et al., 2024). Approximately 200,000 species of microalgae are the greatest primary producers in the world (Balasubramaniam et al., 2020). Algal biomass provides unique nutrient content as well as bioactive substances that have a wide range of economic applications in diverse industries. These are mostly used in cosmetics, aquaculture, medicine, and as a food or feed supplement for living beings (Srimongkol et al., 2022). Moreover, the biomass of microalgae can be transformed into biofuel as bioethanol, biodiesel, biogas, biohydrogen, etc., using a variety of techniques, such as thermal (transesterification) or biochemical (anaerobic digestion) processes, and provide microalgae an excellent source of renewable energy (Balasubramaniam et al., 2020; Arunachalam et al., 2022). The growth and development of bioactive compounds are affected by various environmental parameters such as light intensity, temperature, pH, presence of other microorganisms like bacteria, viruses, and fungi and nutrients (P, N, and K) in cultivation medium (Amit et al., 2021; Rani et al., 2022).

Temperature, light intensity, pH, carbon dioxide, and nutrients in growth media are some of the variables that might affect microalgae cultivation. Light and temperature are two major environmental parameters affecting algal development and biomass production; the optimized temperature and light requirements differ for each algal species. A reduction in biomass output and growth rate is noted when these are grown under stressful conditions, such as insufficient nutrients in culture medium and extremely high or low light intensities and temperature (Zhu et al., 2015). Variations in environment as well as cultural conditions,

like light intensity, temperature, pH, and the nutritional composition of culture medium, have an impact on the macronutrient composition of the microalgae biomass generated, as lipids, proteins, and carbs (Metsoviti et al., 2019).

The present study aims to isolate and characterize microalgal strains from the Arabian Sea coast at Kozhikode (Calicut), Kerala, India. A detailed morphological study has been performed to document and validate the taxonomic identification of collected marine microalgae. Furthermore, the study assesses the influence of seasonal environmental variation on species composition, with particular emphasis on comparative analyses of growth period, biomass yield, and nutrient profiles of identified algal strains.

MATERIAL AND METHODOLOGY

Study area and sampling site

Algal samples have collected from the Arabian Sea coast at Kozhikode (Calicut), Kerala, India (Figure 1). Four different locations have been sampled in order to ensure a representative collection of the local algal diversity. Each site's geographic coordinates, as determined by a GPS, device are as follows: Beypore: 75.80177°E, 11.16313°N; Kappad: 75.12060°E, 11.38028°N; Kozhikode: 75.76567°E, 11.26177°N; Kadakundi: 75.82798°E, 11.1846°N. These locations have been chosen to represent a range of coastal habitats, such as rocky shorelines that are exposed, intertidal zones, and regions that may have been impacted by different human activities (Praseetha et al., 2024).

Samples collection and preservation

During low tide, samples of algae from the subtidal and intertidal zones have been collected. To maintain the integrity of the thallus, specimens have been meticulously collected by hand for bigger macroalgae and with a sterile scalpel for scraping epilithic forms. At the same time, water samples have been taken for additional physicochemical examination from every location. Every algal sample has been promptly put into sterile plastic bottles and sampling bags that has already been labeled. Samples have kept in a refrigerated cooler box with ice packs after collection in order to keep the temperature down while being transported to the lab. Algal material meant for morphological

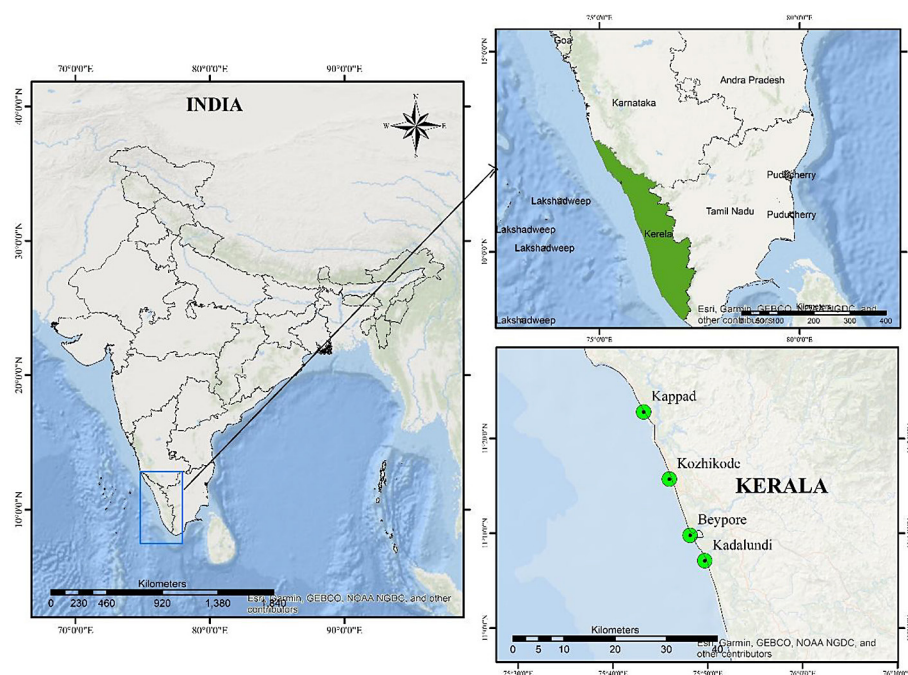


Figure 1. Study area Arabian Sea coast at Kozhikode (Calicut), Kerala, India

examination has been preserved by treating it with Lugol's iodine solution, which fixed the specimens and prevented them from degrading. After that, the samples have been kept at 4 °C in a refrigerator until they could be processed further. Standard procedures have been followed to preserve the water samples for further examination.

Sample enrichment, isolation, and identification of algal strains

Sterile Blue-Green Medium (BG-11) has been added to 150 mL of each freshwater sample in individual transparent glass bottles as soon as the samples arrived at the lab. The cultures have been kept in a controlled culture chamber with white fluorescent lighting at 26 ± 2 °C for 14 days throughout sample enrichment. The cultural enrichment phase also included a 14-hour light-dark cycle. Following initial enrichment, repeated dilutions and streak plating were used to isolate the samples. The quickest, easiest, and most economical way to isolate algae is through serial dilutions (Duong 2016). In this study, certain filamentous microalgae that did not grow well on solidified material were isolated using repeated dilutions (Figure 2).

Several serial dilutions have been performed in order to isolate non-filamentous microalgae, and then microscopic inspections have been performed using a Magnus MLXi plus. The samples have been streak-plated on appropriate

enrichment medium solidified using bacteriological agar (Hi Media, India) following several serial dilutions. Until separate unialgal colonies have been seen, streaking was done repeatedly. After that, the individual colonies have been selected and injected into several 250 mL Schott bottles that had the appropriate liquid media. A phase contrast microscope has been then used to identify the isolated unialgal species. Algal strain analysis has been also conducted using FTIR, FESEM and XRD.

Optimization of growth conditions

For the pure isolates of algal species, the growth conditions, including salinity and culture medium, have been optimized in order to investigate the impact of physical characteristics. To assess the growth requirement of pure isolate, two growth media- BBM medium and Walne medium, along with five salinity conditions 20, 25, 30, 35, and 40 ppt, were individually optimized. First, 15ml test tubes containing various growth media have been injected with 1ml of isolated strains of Ag1, Ag2, Ag3, and Ag4 (*Halochlorococcum marinum*, *Oocystis borgei*, *Chroococcopsis chroococcoides*, and *Desmodesmus abundans*) at an initial concentration of 0.5×10^6 cells/ml. Every experiment has been carried out using fluorescent light bulbs with a light level of 3000 lux at 26 ± 1 °C in the phycology lab.

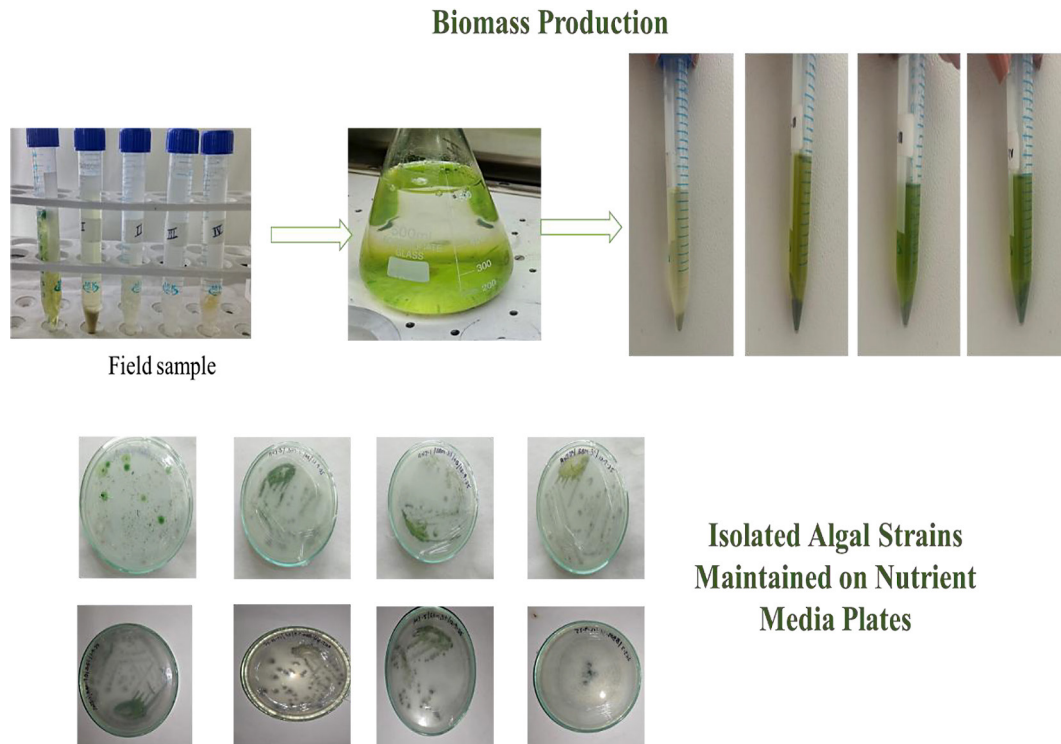


Figure 2. Visual methodology from field sample collection to isolation

Using a hemocytometer under a light microscope, biomass of microalgae from each treatment has been assessed every other day during the 15-day experiment. Each microalga's growth curve has been plotted against the quantity of cells per milliliter and the number of days. Using the usual formula, cell density of cultivated strain has been determined:

$$\begin{aligned} \text{Cell density} &= \text{Average number of cells} \times \\ &\times \text{number of squares (16)} \times 10.000 = \\ &= \text{million cells/ml} \end{aligned} \quad (1)$$

To determine the differences between the groups, a one-way ANOVA is used, followed by a post hoc multiple comparison test using SPSS, version 22.0. After being separated and placed in 100% acetone, the weighted samples have been homogenized for one minute at 1000 rpm. After passing through two layers of cheesecloths, the homogenate has been centrifuged for 10 minutes at 2500 rpm. After the supernatant has been removed, the Shimadzu UV-260 spectrophotometer is used to measure the absorbances between 400 and 700 nm. Total carotene has been found to have the highest absorbance at 470 nm, lycopene at 505 nm, chlorophyll a at 663 nm, and chlorophyll b at 645 nm. The amounts of these pigments have been determined using the following formulas (Rinawati et al., 2020):

$$\text{Chlorophyll a (Chl a)} = 11.75 A_{662} - 2.350 A_{645} \quad (2)$$

$$\text{Chlorophyll b (Chl b)} = 18.61 A_{645} - 3.960 A_{662} \quad (3)$$

$$\begin{aligned} \text{Total carotene} &= 1000 A_{470} - \\ &- 2.270 \text{ Chl a} - 81.4 \text{ Chl b} / 227 \end{aligned} \quad (4)$$

$$\text{Lycopene } (\mu\text{g/ml}) = (A_{505} \times 31.2) / 1.76 \quad (5)$$

RESULTS AND DISCUSSION

A total of 10 species of marine microalgae have been identified and isolated from samples taken from various locations on the Arabian Sea coast at Kozhikode (Calicut), Kerala, India. Isolation of algal strains using BG-11 media and every week fresh plating and the serial dilution of samples are performed. The resultant samples of microalgae have been studied under a microscope after isolation for their identification. A total of four Chlorophyceae and six Cyanobacteria, identified as *Desmodesmus abundans* (Kirchner); *Cyanobium bacillare* (Butcher); *Leptolyngbya africana* (Lemmermann); *Chroococcopsis chroococcoides* (F.E.Fritsch); *Planktolyngbya minor* (Geitler & Ruttner); *Desmodesmus armatus* (Chodat); *Pleurocapsa fuliginosa*; *Oocystis borgei*; *Myxosarcina*

salina; *Halochlorococcum marinum* have been confirmed by morphological characterization. Figures 3a–3j show the microscopic pictures of a pure strain of microalgal species.

The initial phase of this study involved isolating and purifying ten algae species. A comparative growth study has been conducted in order to identify which species are most suitable for further research. According to the findings, four of the ten species had noticeably better growth and are more productive. As a result, the research's focus has been pointed to these four elite species as shown in Figure 4a–4d. The use of these particular isolates provides the foundation for all subsequent work on morphological and biochemical characterization, as well as growth condition optimization.

Several functional groups linked to macromolecules like proteins, carbohydrates, and lipids were detected in the *Halochlorococcum marinum*, *Oocystis borgei*, *Chroococcopsis chroococcoides*, and *Desmodesmus abundans* algal samples FTIR spectra (Figure 5). The O–H and N–H stretching vibrations are represented by a large absorption band seen at about 3400–3300 cm^{-1} , which suggests the presence of hydroxyl groups and amide bonds from proteins and polysaccharides. The C–H stretching vibrations of aliphatic groups, which are typical of lipid and fatty acid

chains, are represented by the peaks located between 2920 and 2850 cm^{-1} . The band at about 1540 cm^{-1} belongs to amide II (N–H bending and C–N stretching), but the strong absorption band found near 1650–1630 cm^{-1} is attributed to amide I (C=O stretching of proteins). The contribution of organic acids is confirmed by peaks in the 1400–1380 cm^{-1} range, which show the existence of carboxylate groups ($-\text{COO}^-$). Furthermore, absorption bands corresponding to C–O–C and C–O stretching vibrations, which are linked to polysaccharides and nucleic acids, are located between 1240 and 1030 cm^{-1} . The presence of phenolic compounds may be indicated by the band at 875–800 cm^{-1} , which could be the result of aromatic C–H bonds bending out of plane. The FTIR study validates the algal biomass's complex biochemical composition, which includes the co-existence of proteins, lipids, carbohydrates, and trace amounts of secondary metabolites.

The algal samples *Halochlorococcum marinum*, *Oocystis borgei*, *Chroococcopsis chroococcoides*, and *Desmodesmus abundans* (Ag1–Ag4) XRD spectra show a mostly amorphous organic matrix with trace amounts of sample-dependent crystalline contributions (Figure 6). The low-angle region (10–30° 2 θ) of all four traces has a broad, low-intensity hump, which is characteristic

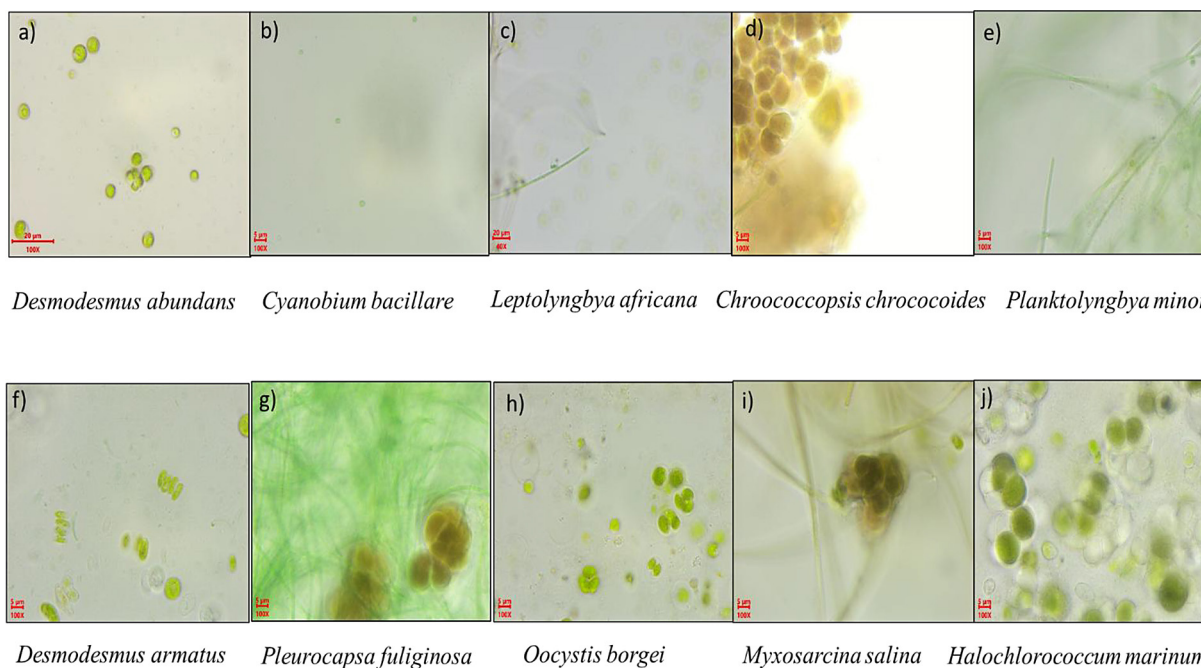


Figure 3. Microscopic image of isolated strain of microalgae's at 5µm and 100x: *Desmodesmus abundans* (Kirchner); *Cyanobium bacillare* (Butcher); *Leptolyngbya africana* (Lemmermann); *Chroococcopsis chroococcoides* (F.E.Fritsch); *Planktolyngbya minor* (Geitler & Ruttner); *Desmodesmus armatus* (Chodat); *Pleurocapsa fuliginosa*; *Oocystis borgei*; *Myxosarcina salina*; *Halochlorococcum marinum*

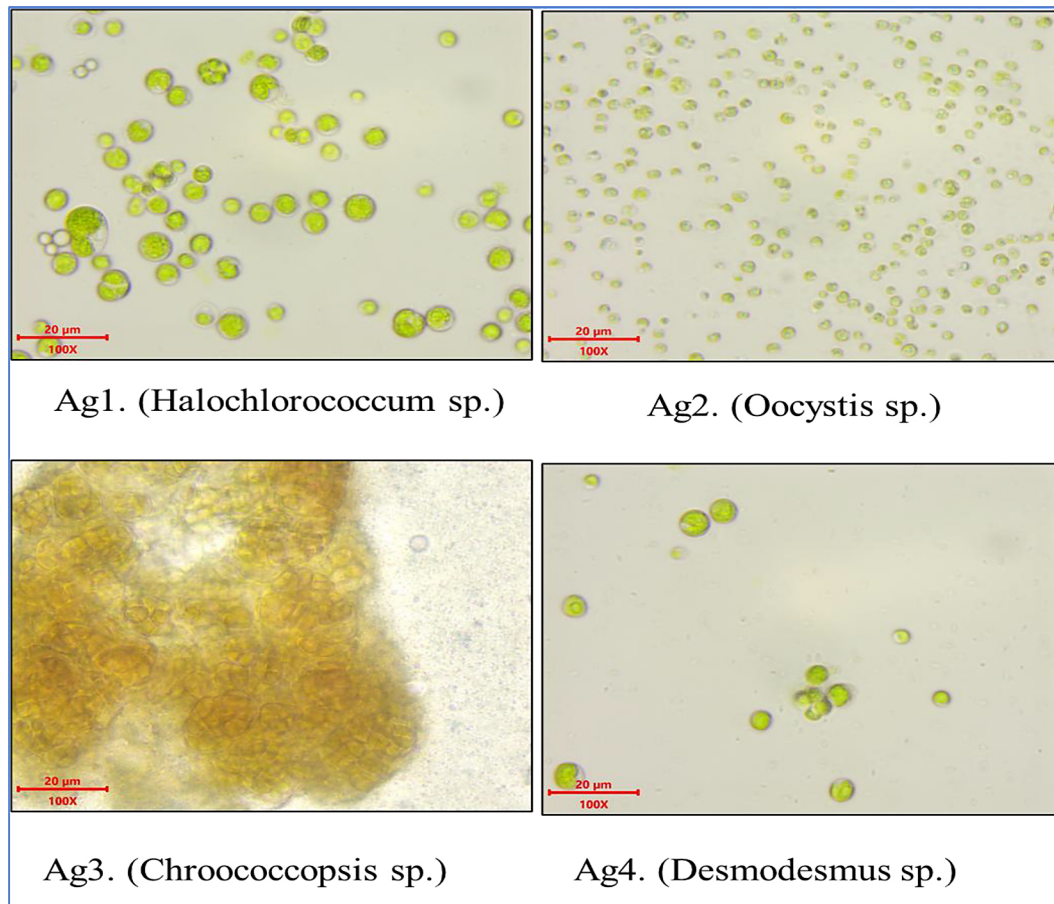


Figure 4. Microscopic image of best four identified strain of microalgae

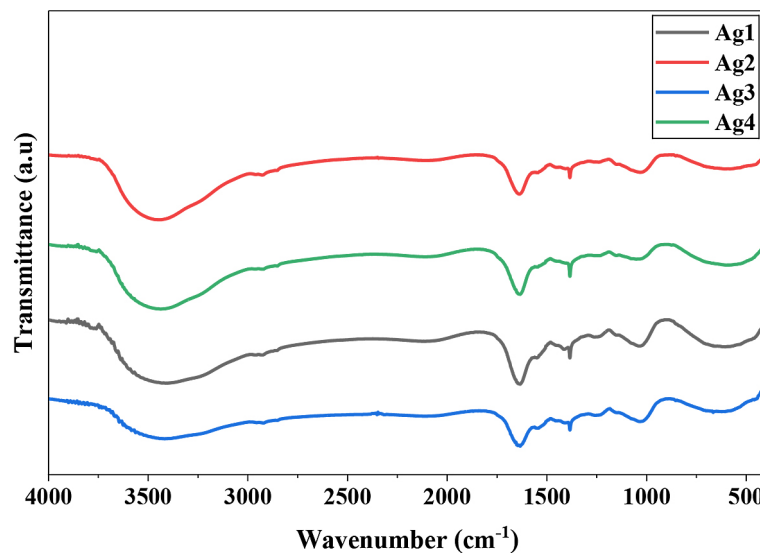


Figure 5. FTIR spectra of Ag1-Ag4 microalgal strains

of non-crystalline organic matter (proteins, polysaccharides, lipids, and other cellular ingredients) and suggests that the majority of the biomass is amorphous (Waqif et al., 2024; Abbas et al., 2025). A few weaker but repeatable, sharper

reflections that differ in strength between samples are superimposed on the amorphous background. These are especially noticeable at 22°, 26.6°, and 29–30°. The following are tentative assignments: a peak at 26.6° ($d = 3.35 \text{ \AA}$) consistent with traces

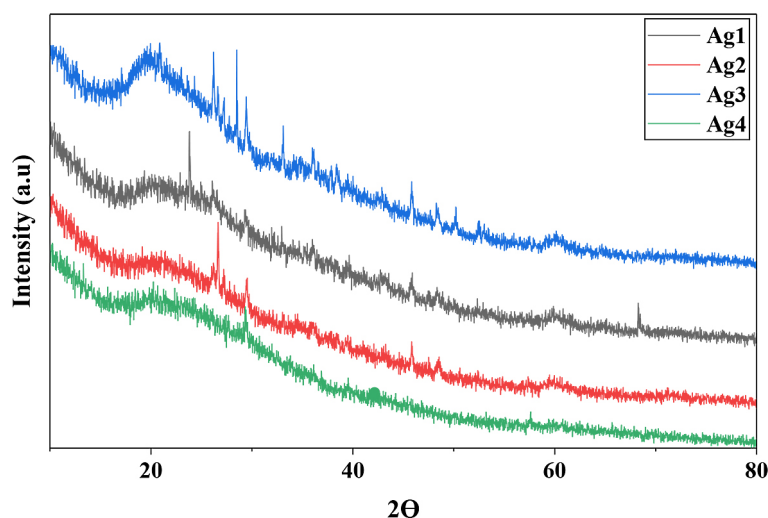


Figure 6. XRD pattern of microalgal strains (Ag1-Ag4)

of quartz/siliceous material (possible if diatom frustules or silica contamination are present); a reflection near 22° ($d \approx 4.04 \text{ \AA}$) that can be linked to partially ordered cellulose/biopolymer domains or organized polysaccharide regions in the cell wall; and a signal around $29\text{--}30^\circ$ ($d \approx 3.04 \text{ \AA}$) that is frequently attributed to calcite (CaCO_3) when present as biomineral or as precipitated carbonate from the growth medium. Secondary mineral phases or small crystalline salts originating from media residues or sample drying/processing are indicated by additional, weaker reflections at higher angles (Yu et al., 2025).

This study examined the optimal growth of isolated strains at different salinity levels (20.0, 25.0, 30.0, 35.0, and 40.0 ppt) using two types of culture media, BBM and Walne's medium. At 35.0 ppt salinity, Ag1 (*Halochlorococcum marinum*) strain grew more readily in BBM ($8.54 \times 10^{-6} \text{ cell/ml}$) than in Walne's medium ($8.13 \times 10^{-6} \text{ cell/ml}$). From the first day of inoculation to the thirteenth day of the growth phase, the cells growth was observed to increase progressively. Later, on the fifteenth day, the cells transitioned into the lysis phase. On thirteenth day of culture in BBM, followed by Walne's medium, the greatest growth was noted. Salinity and culture media had a substantial impact ($p < 0.05$) starting on the third day, according to statistical analysis. In BBM medium, Ag1 grew the fastest at 35 ppt salinity, suggesting that it prefers moderately salinized environments that are nutrient-rich. On the thirteenth day of culture, the growth was observed at BBM (7.5 to $8.54 \times 10^{-6} \text{ cell/ml}$) and Walne's medium (7.5 to $8.13 \times 10^{-6} \text{ cell/ml}$). In terms of salinity, growth was found to be lowest at lower

salinity 20 ppt and 25 ppt, greatest at 30 and 35 ppt in all growth media, and gradually declining at 40 ppt, as illustrated in Figure 7 (Hashem et al., 2023; Hotos et al., 2023).

At 35 ppt and 30 ppt salinity, Ag2 (*Oocystis borei*) recorded a high density of $9.9 \times 10^{-6} \text{ cell/ml}$ in Walne's medium and $10.2 \times 10^{-6} \text{ cell/ml}$ in BBM. On the eleventh day of culture in both media, the highest cell density was noted. Ag2 increased steadily until the eleventh day, at which point it began to slightly decrease on days 13 and 15. After the third day of culture, the effects of medium and salinity were determined to be significant ($p < 0.05$). As shown in Figure 8, maximum cell density was noted in BBM medium at 30 ppt salinity, indicating that Ag2 prefers comparatively lower saline conditions and benefits from the nutritional content of BBM (Hasnain et al., 2023).

The Ag3 (*Chroococcopsis chroococcoides*) cells gradually improved in both growth media, although at a salinity of 30 ppt, growth was greatest in the Walne medium ($0.83 \times 10^{-6} \text{ cell/ml}$) and BBM ($0.79 \times 10^{-6} \text{ cell/ml}$). In both media, cell growth entered stationary phase on the ninth day of culture. On the eleventh, thirteenth, and fifteenth day of culture, cell transitioned into lysis phase. At 30 ppt, both media displayed their maximal growth. Figure 9 depicted that there was no discernible difference ($p < 0.05$) in Ag3 sp. growth between the various growth media and salinities. Ag3 sp. grows more effectively in both media at salinity levels of 30 and 35 ppt, while growth was slower in other conditions. This implies that the strain is highly halotolerant, enabling it to flourish in high salinity environments (Li et al., 2023; Sharma et al.,

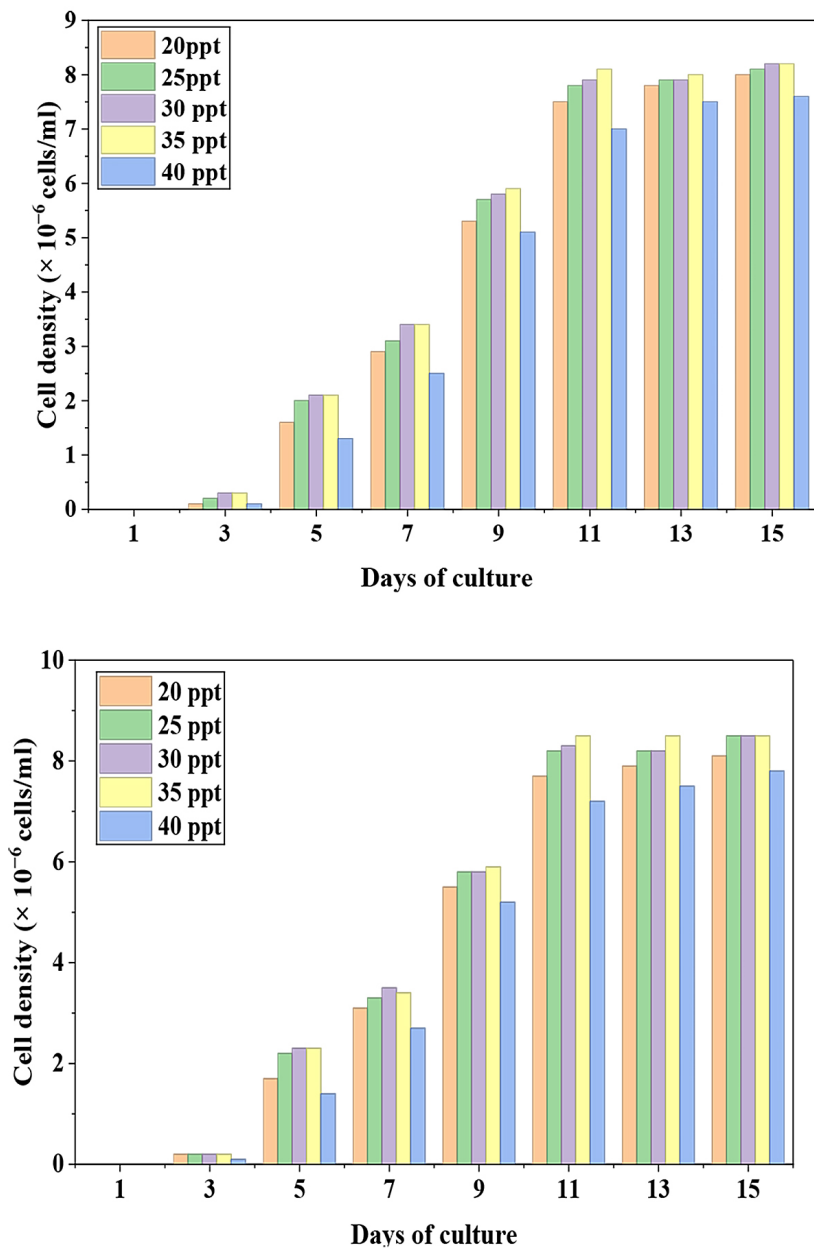


Figure 7. Salinity effect on Ag1 (*Halochlorococcum marinum*) cell growth using Walne media and BBM media

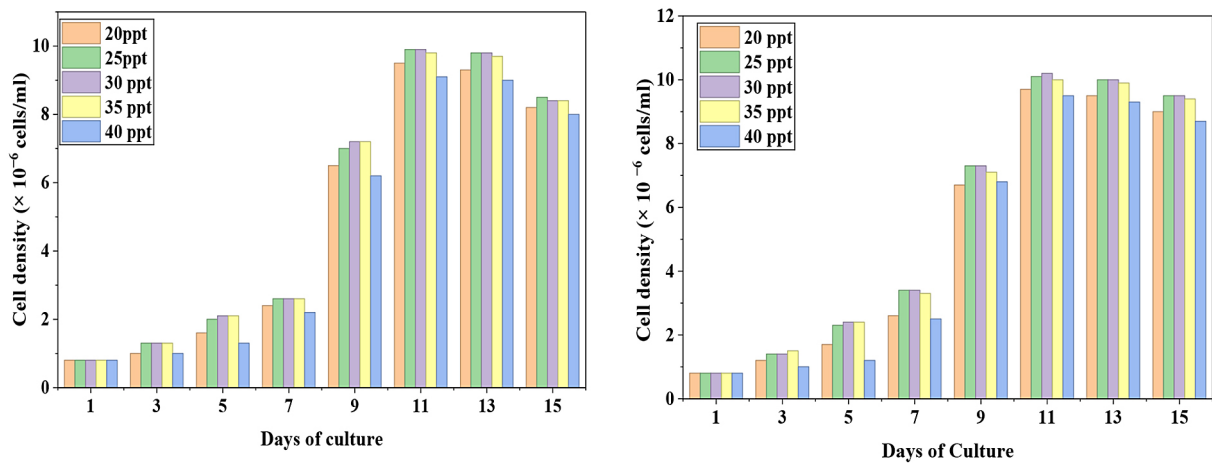


Figure 8. Salinity effect on Ag2 (*Oocystis boregi*) cell growth using Walne media and BBM media

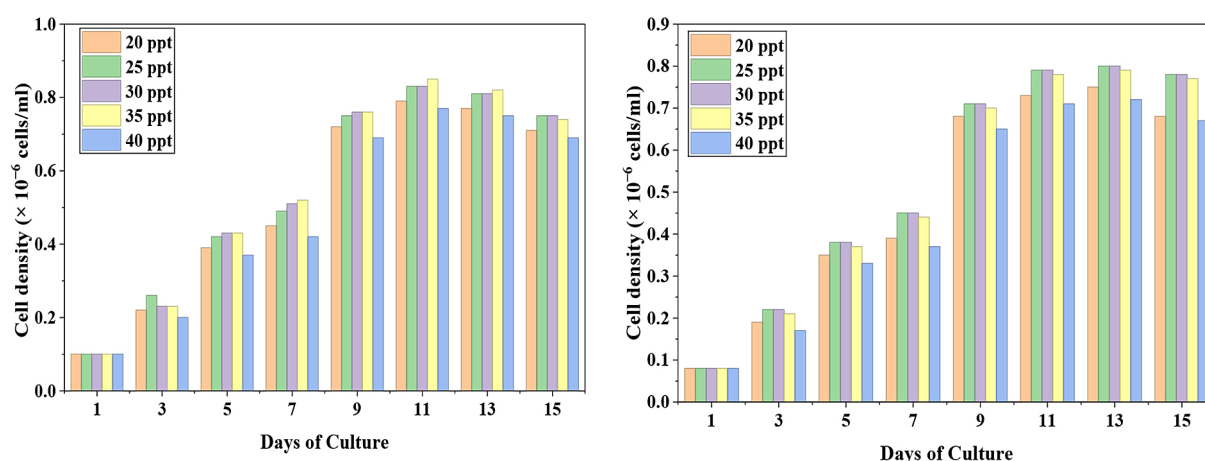


Figure 9. Salinity effect on Ag3 (*Chroococcopsis chroococcoides*) cell growth using Walne media and BBM media

2024). The results reveal that Ag3 is a promising option for large-scale production in brackish to marine salinity environments (Gharaei et al., 2022; Derafshi et al., 2024).

Figure 10 demonstrates, Ag4 (*Desmodesmus abundans*) grows in response to variations in salinity and growth media. At 25–35 ppt salinity, high in Ag4 cell density (0.86×10^{-6} cell/ml) was observed in Walne's medium, which was followed by BBM (0.83×10^{-6} cell/ml). After day 3, a two-way ANOVA depicted substantial variance ($p < 0.05$) in both culture medium and salinity. Ag4's extraordinary resilience to an extreme saline environment was confirmed by its optimal development at 35 ppt salinity in Walne media. These results show that Ag4 is quite halophilic and works best in saline, nutrient-rich conditions. However, lysis phase of cell growth was suggested by a marked decrease in cell density after the eleventh day. At 30 and 35 ppt, highest cell density was noted in both growth media (Saaad et al., 2023; Alzahmi et al., 2024).

Chlorophyll content

Depending on the type of microalgae growth, several techniques are used for harvesting them. In present study, microalgae were harvested using a centrifuge. The Centrifuge is used for the centrifuge procedure, which involves adding culture to a 50 mL Corning bottle and using this process, microalgae cultures are harvested. For the analysis of chlorophyll, 100 mL of each volume of culture of microalgae has been isolated, and the remaining volume has been used for carotenoid and lycopene analysis. The technique of extracting

chlorophyll has been carried out utilizing Becker's method (1982). Using an appropriate solvent to separate the substance from the mixture is known as extraction. To obtain biomass, each sample is placed into a bottle for subsequent 50 mL Corning centrifuge and filtered. Acetone is the solvent used in the chlorophyll extraction procedure. Acetone (CH_3COCH_3) has a dielectric constant of 21, making it a semi-polar solvent. Chlorophyll is a non-polar pigment that requires extraction using organic solvents with a specific polarity (polarity index 5.2), such as acetone. Compared to chlorophyll b, chlorophyll a is more polar. As a result, acetone is employed as a solvent to extract chlorophyll (Rinawati et al., 2020).

The absorbance of each microalgae sample was then measured at 645 and 663 nm. The greatest wavelength at which chlorophyll can absorb light is this one. Next, the highest absorption spectra of chlorophyll with a wavelength ranging between 300 and 700 nm are characterized. The results have been depicted as a spectrum of chlorophyll absorption. The following Table 1 provides a description of microalgae's chlorophyll content for the studied algal species.

Chlorophyll and carotenoid accumulation pattern varies significantly during the culture period, as per the pigment analysis of the four algal species (Ag1–Ag4). Ag3 had the highest concentration of chlorophyll a (Chl a) among them on fifteenth day, representing that this species maintained an active electron transport chain and a more effective photosynthetic apparatus under the conditions of the culture (Khan et al., 2025). On the other hand, while the Chl a level of the other three species (Ag1, Ag2, and Ag4) are similar,

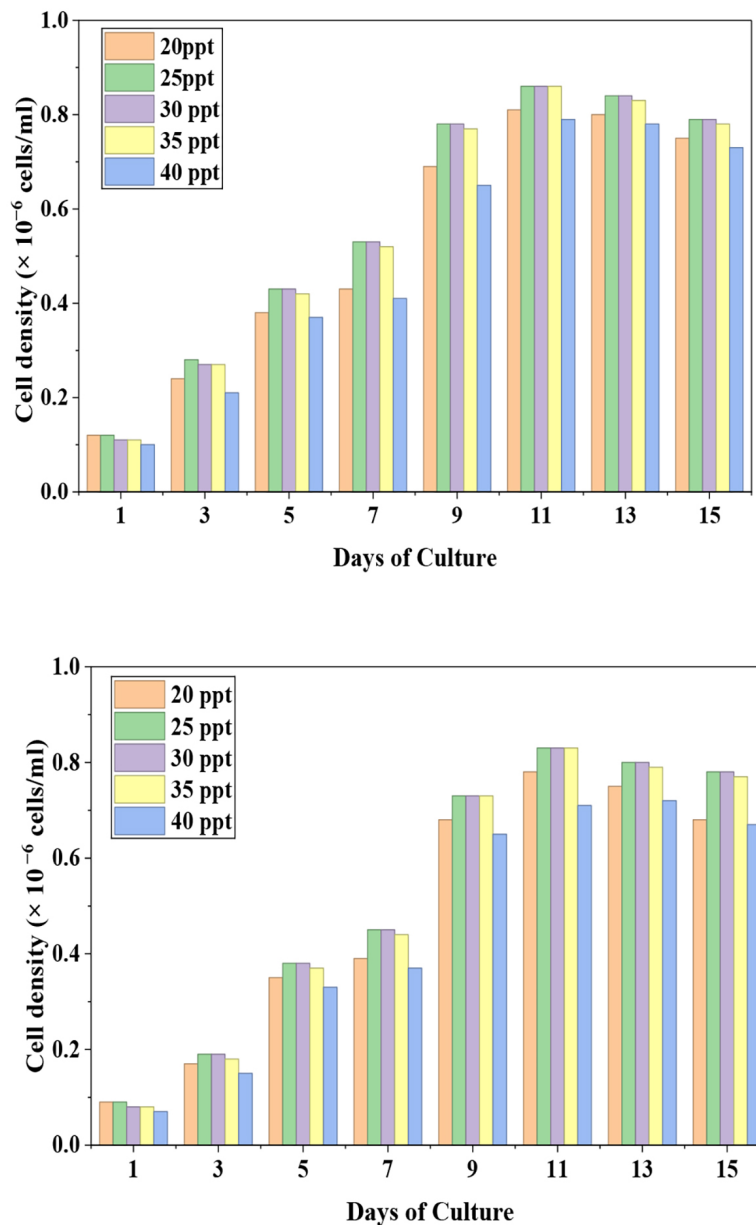


Figure 10. Salinity effect on Ag4 (*Desmodesmus abundans*) cell growth using Walne media and BBM media

Ag4's values are slightly higher, suggesting that this strain maintained efficient light harvesting activity in comparison to Ag1 and Ag2 algal strains.

The highest concentration of Chl b, an accessory pigment that improves light absorption in blue portion of the spectrum and transfers energy to chlorophyll a, has been found in Ag4 on the fifteenth day. This implies that Ag4 might have a more varied antenna pigment system, which would provide more flexibility in a range of light levels. A potential increase in light harvesting complex II (LHCII) density, which enhances photosynthetic efficiency by increasing the absorption spectrum, is also suggested by the comparatively higher Chl b levels in Ag4.

Species-specific differences have been further highlighted by the analysis of carotenoid pigments (total carotene and lycopene). Carotenoids play two roles in algae: they participate in light capture and act as photoprotective agents against reactive oxygen species produced under high light or stress conditions (Kumari et al., 2021). Ag4 has the highest concentrations of both carotene and lycopene among the isolates under study, followed by Ag3, Ag2, and Ag1. This gradient suggests that Ag4 has a better non-enzymatic antioxidant defense, which may increase its tolerance to oxidative stress and extend its metabolic potential. The simultaneous measurement of chlorophylls and carotenoids not only highlights

Table 1. Chlorophylls, carotenoid and lycopene content in different algal species

Chl A					
Algal sp.	Day5	Day10	Day15	Day20	Day25
<i>Halochlorococcum marinum</i>	0.836	1.823	2.868	2.791	2.534
<i>Oocystis borgei</i>	8.191	13.525	20.548	19.928	19.013
<i>Chroococcopsis chroococcoides</i>	16.883	25.982	38.824	38.314	35.084
<i>Desmodesmus abundans</i>	16.283	26.109	35.424	36.617	33.642
Chl B					
Algal sp.	Day5	Day10	Day15	Day20	Day25
<i>Halochlorococcum marinum</i>	0.218	0.538	0.911	0.891	0.831
<i>Oocystis borgei</i>	3.025	4.817	7.295	7.023	6.335
<i>Chroococcopsis chroococcoides</i>	2.121	4.186	8.014	5.909	5.482
<i>Desmodesmus abundans</i>	2.077	3.944	4.931	5.931	5.483
Cartenoid					
Algal sp.	Day5	Day10	Day15	Day20	Day25
<i>Halochlorococcum marinum</i>	0.416	0.669	0.966	0.961	0.841
<i>Oocystis borgei</i>	2.451	3.424	4.887	4.794	4.367
<i>Chroococcopsis chroococcoides</i>	3.584	4.847	6.209	6.661	6.053
<i>Desmodesmus abundans</i>	4.0974	5.373	5.555	7.282	8.362
Lycopene					
Algal sp.	Day5	Day10	Day15	Day20	Day25
<i>Halochlorococcum marinum</i>	0.985	1.882	2.735	2.572	2.338
<i>Oocystis borgei</i>	8.113	12.430	18.686	18.213	16.514
<i>Chroococcopsis chroococcoides</i>	8.429	12.854	20.919	18.696	17.061
<i>Desmodesmus abundans</i>	9.3972	14.931	21.467	20.845	18.828

interspecific differences in photosynthetic pigment composition, but also highlights each species ecological strategy in striking a balance between energy capture and photoprotection (Jiang et al., 2024; Shi et al., 2025).

CONCLUSIONS

In this study, native microalgae have been isolated from the Arabian Sea Coast at Kozhikode (Calicut), Kerala, India. Ten different microalgae strains have been characterized and show initially higher levels of growth in BG11 media. Among ten, only four different algae strains produced significantly higher biomass and were optimized by varying salinity and culture media concentration. This research contributes valuable insights into the potential of native marine water microalgae for sustainable cosmeceutical production as well as renewable energy production. It is recommended that for improving prediction and accuracy, the incorporation of machine learning yielded practical insights for environment parameter

optimization. Future studies on the combined effects of genetic and environmental factors, improved photobioreactor designs, and resource-efficient growing methods are made possible by the findings. A sustainable bioeconomy and the advancement of algae biotechnology are made possible by this revolutionary strategy.

REFERENCES

1. Abbas, M., Ni, L., Du, C. (2025). Kinetic modeling and salinity tolerance in *Chlorella vulgaris* and *Desmodesmus communis* (Chlorophyta): insights into differential growth responses. *Aquatic Ecology*, 59(1), 145–158. <https://doi.org/10.1007/s10452-024-10153-y>
2. Alzahmi, A. S., Daakour, S., Nelson, D., Al-Khairi, D., Twizere, J. C., Salehi-Ashtiani, K. (2024). Enhancing algal production strategies: Strain selection, AI-informed cultivation, and mutagenesis. *Frontiers in Sustainable Food Systems*, 8, 1331251. <https://doi.org/10.3389/fsufs.2024.1331251>
3. Amit, Dahiya, D., Ghosh, U. K., Nigam, P. S.,

- Jaiswal, A. K. (2021). Food industries wastewater recycling for biodiesel production through microalgal remediation. *Sustainability*, 13(15), 8267. <https://doi.org/10.3390/su13158267>
4. Arunachalam Sivagurulingam, A. P., Sivanandi, P., Pandian, S. (2022). Isolation, mass cultivation, and biodiesel production potential of marine microalgae identified from Bay of Bengal. *Environmental Science and Pollution Research*, 29(5), 6646–6655. <https://doi.org/10.1007/s11356-021-16163-9>
 5. Balasubramaniam, V., Gunasegavan, R. D. N., Mustar, S., Lee, J. C., Mohd Noh, M. F. (2021). Isolation of industrial important bioactive compounds from microalgae. *Molecules*, 26(4), 943. <https://doi.org/10.3390/molecules26040943>
 6. Derafshi, M., Hassani, A., Amanifar, S., Babaakbari, M., Hematimatin, N., Asgari Lajayer, B., Price, G. W. (2024). Optimization of Sargassum bovinum extraction techniques for germination of wheat, canola, and corn under different salinity stress. *Agronomy*, 14(11), 2646. <https://doi.org/10.3390/agronomy14112646>
 7. Gharaei, S., Ohadi, M., Hassanshahian, M., Porsheikhali, S., Forootanfar, H. (2022). Isolation, optimization, and structural characterization of glycolipid biosurfactant produced by marine isolate Shewanella alga e B12 and evaluation of its antimicrobial and anti-biofilm activity. *Applied Biochemistry and Biotechnology*, 194(4), 1755–1774. <https://doi.org/10.1007/s12010-021-03782-8>
 8. Gravidahl, M., Arlov, Ø., Aasen, I. M., Aachmann, F. L. (2025). Fermentation of cultivated brown algae Saccharina latissima and Alaria esculenta and effects on alginate quality. *Algal Research*, 104241. <https://doi.org/10.1016/j.algal.2025.104241>
 9. Hashem, A. H., Abu-Elreesh, G., El-Sheikh, H. H., Suleiman, W. B. (2023). Isolation, identification, and statistical optimization of a psychrotolerant *Mucor racemosus* for sustainable lipid production. *Biomass conversion and biorefinery*, 13(4), 3415–3426. <https://doi.org/10.1007/s13399-022-02390-8>
 10. Hasnain, M., Munir, N., Abideen, Z., Macdonald, H., Hamid, M., Abbas, Z., Radicetti, E. (2023). Prospects for biodiesel production from emerging algal resource: Process optimization and characterization of biodiesel properties. *Agriculture*, 13(2), 407. <https://doi.org/10.3390/agriculture13020407>
 11. Hotos, G., Avramidou, D., Mastropetros, S. G., Tsigkou, K., Kouvara, K., Makridis, P., Kornaros, M. (2023). Isolation, identification, and chemical composition analysis of nine microalgal and cyanobacterial species isolated in lagoons of Western Greece. *Algal Research*, 69, 102935. <https://doi.org/10.1016/j.algal.2022.102935>
 12. Ji, F., Hao, R., Liu, Y., Li, G., Zhou, Y., Dong, R. (2013). Isolation of a novel microalgae strain *Desmodesmus* sp. And optimization of environmental factors for its biomass production. *Bioresource Technology*, 148, 249–254. <https://doi.org/10.1016/j.biortech.2013.08.110>
 13. Jiang, Y., Ye, J., Hu, Y., Zhang, J., Li, W., Zhou, X., Hu, Y. (2024). Extraction and synthesis of typical carotenoids: lycopene, β -carotene, and astaxanthin. *Molecules*, 29(19), 4549. <https://doi.org/10.3390/molecules29194549>
 14. Khan, A., Ullah, S., Ali, R., Rehman, M., Moshawih, S., Goh, K. W., Gew, L. T. (2025). Machine learning optimization of environmental factors influencing biomass and nutritional composition in local algal species. *Royal Society Open Science*, 12(4), 241336. <https://doi.org/10.1098/rsos.241336>
 15. Kumari, S., Kumar, V., Kothari, R., Kumar, P. (2022). Experimental and optimization studies on phycoremediation of dairy wastewater and biomass production efficiency of *Chlorella vulgaris* isolated from Ganga River, Haridwar, India. *Environmental Science and Pollution Research*, 29(49), 74643–74654. <https://doi.org/10.1007/s11356-022-21069-1>
 16. Li, W., Zhang, Y., Ren, H., Wang, Z., OuYang, Y., Wang, S., Qi, G. (2023). Identification of potassium transport proteins in algae and determination of their role under salt and saline-alkaline stress. *Algal Research*, 69, 102923. <https://doi.org/10.1016/j.algal.2022.102923>
 17. Lithi, U. J., Laird, D. W., Ghassemifar, R., Wilton, S. D., Moheimani, N. R. (2024). Microalgae as a source of bioavailable heme. *Algal Research*, 77, 103363. <https://doi.org/10.1016/j.algal.2023.103363>
 18. Metsoviti, M. N., Papapolymerou, G., Karapanagiotidis, I. T., Katsoulas, N. (2019). Comparison of Growth Rate and Nutrient Content of Five Microalgae Species Cultivated in Greenhouses. *Plants*, 8(8), 279. <https://doi.org/10.3390/plants8080279>
 19. Naselli-Flores, L., Padisák, J. (2023). Ecosystem services provided by marine and freshwater phytoplankton. *Hydrobiologia*, 850(12), 2691–2706. <https://doi.org/10.1007/s10750-022-04795-y>
 20. Papachristou, I., Zhang, S., Gorte, O., Ochsenreither, K., Wüstner, R., Nazarova, N., Silve, A. (2023). Direct transesterification of microalgae after pulsed electric field treatment. *Journal of Chemical Technology & Biotechnology*, 98(3), 642–650. <https://doi.org/10.1002/jctb.7293>
 21. Praseetha, A. P., Ammini, C. J., Bibina, P. B., Sree-kumar, V. B., Tessy, P. P. (2024). Taxonomic Studies on Algal Flora in Thanikkudam River Thrissur, Kerala. *International Journal on Algae*, 26(2). <https://doi.org/10.1615/InterJAlgae.v26.i2.30>
 22. Rani, V., Deepika, S., Abarna, K., Uma, A. (2022). Isolation, identification, and optimization of growth conditions for the marine microalgae isolated from

- the Gulf of Mannar, South-east coast of India. *Regional Studies in Marine Science*, 51, 102189. <https://doi.org/10.1016/j.rsma.2022.102189>
23. Rinawati, M., Sari, L. A., Pursetyo, K. T. (2020). Chlorophyll and carotenoids analysis spectrophotometer using method on microalgae. In *IOP conference series: earth and environmental science* 441(1) 012056. IOP Publishing. <https://doi.org/10.1088/1755-1315/441/1/012056>
 24. Riouchi, O., Kholssi, R., Skalli, A., Akodad, M., Moumen, A., Baghour, M. (2024). Effect of physicochemical parameters on the growth of two microalgae strains, the *Chlorella vulgaris* and *Skeletonema costatum* isolated from different sites in the nador lagoon. *Biocatalysis and Agricultural Biotechnology*, 57, 103088. <https://doi.org/10.1016/j.bcab.2024.103088>
 25. Saad, M. H., Sidkey, N. M., El-Fakharany, E. M. (2023). Identification and statistical optimization of a novel alginate polymer extracted from newly isolated *Synechocystis algin* MNE ON864447 with antibacterial activity. *Microbial Cell Factories*, 22(1), 229. <https://doi.org/10.1186/s12934-023-02240-w>
 26. Sharma, A., Sharma, P., Mona, S. (2024). Appraisal and identification of algal bloom region, prevention and management approaches. In *Algae as a Natural Solution for Challenges in Water-Food-Energy Nexus: Toward Carbon Neutrality* 79–90. Singapore: Springer Nature Singapore. https://doi.org/10.1007/978-981-97-2371-3_4
 27. Shi, X., Wang, D., Li, L., Wang, Y., Ning, R., Yu, S., Gao, N. (2025). Algal classification and Chlorophyll-a concentration determination using convolutional neural networks and three-dimensional fluorescence data matrices. *Environmental Research*, 266, 120500. <https://doi.org/10.1016/j.envres.2024.120500>
 28. Srimongkol, P., Sangtanoo, P., Songserm, P., Watsuntorn, W., Karnchanatat, A. (2022). Microalgae-based wastewater treatment for developing economic and environmental sustainability: Current status and future prospects. *Frontiers in Bioengineering and Biotechnology*, 10, 904046. <https://doi.org/10.3389/fbioe.2022.904046>
 29. Waqif, H., Munir, N., Farrukh, M. A., Hasnain, M., Sohail, M., Abideen, Z. (2024). Algal macromolecular mediated synthesis of nanoparticles for their application against citrus canker for food security. *International Journal of Biological Macromolecules*, 263, 130259. <https://doi.org/10.1016/j.ijbiomac.2024.130259>
 30. Yu, X., Li, S., Liang, J., Liu, S., Wang, Y., Yang, J., Qin, S. (2025). Isolation and identification of native microalgae from the Yellow River Delta: A dual-focus study on the tolerance assessment of microalgae and their growth enhancement on *Sesbania cannabina* under saline-alkali stress. *Algal Research*, 88, 104033. <https://doi.org/10.1016/j.algal.2025.104033>
 31. Zhu, S., Wang, Y., Shang, C., Wang, Z., Xu, J., Yuan, Z. (2015). Characterization of lipid and fatty acids composition of *Chlorella zofingiensis* in response to nitrogen starvation. *J. Biosci. Bioeng*, 120, 205–209. <https://doi.org/10.1016/j.jbiosc.2014.12.018>