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Recovery of AutoFluorescent Pigment from Different Species of Marine Micro Algae

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Abstract

In the present work, two different species of microalgae: Spirogyra and Volvox were collected from the Palk Bay region and were selected as raw materials for the recovery of multifunctional extracts. After sampling, the micro algal samples are viewed under the fluorescent microscope for biofluorescent emission. The presence of pigments which absorb light energy and them reemit it at a longer wavelength, creating a visible fluorescent glow. To Prepare an appropriate culture medium like f/2 medium (Guillards Medium) to promoting micro algal growth at 18- 25ºC for several days to weeks under 12:12hours light/dark conditions and50–100 µmol photons m^{-2} s^{-1}.Ultrasonic assisted extraction (UAE) is a process that uses sound waves to break down particles in the algal culture using different solvents was applied under specific optimized conditions. Organic solvents such as hexane, acetone and ethanol, as well as food grade solvents, such as, soybean oil and phosphate buffer (pH=7) were used. 10-20 mL of extraction solvent are sonicated at 40 kHz for 10 mins. The total carotenoid and chlorophyll a content in the extracts was determined for all species using UV-Visspectrophotometry. (Rodrigo Martins et al 2023). Taking into consideration the nature of the solvent used and the total pigment content, acetone and ethanol can be replaced by limonene for the extraction of total chlorophyll from Spirogyra and Volvox. HPLC (High Performance Liquid Chromatography) pigment analysis for micro algae to identify and quantify the various photosynthetic pigments present in microalgae, such as chlorophyll, Xanthophyll, carotenoids, and phycobiliproteins by separating them with a diode array detector (DAD).(Guillermo Linares and Meliza Lindsay Rojas et al 2022)

Keywords: micro algae, biofluorescent, chlorophylls, xanthophyll, ultrasound-assisted extraction

Introduction

Microalgae are microscopic algae that are usually found in the marine system. They are important photosynthetic microorganisms that have the ability to convert about 10% of the energy from solar light in chemical energy via photosynthesis. Microalgae contain high protein content, with balanced amino acids pattern, carotenoids, polysaturated fatty acids, vitamins, polysaccharides, sterols, phycobilin and other biologically active compounds, more efficiently than traditional crops. Biofluorescence in micro algae is fascinating phenomenon in which these microscopic organisms absorb light at wavelength and re emit it at a longer wavelength. The property is primary due to the presence of specialized pigments such as chlorophyll, xanthophyll, phycobiliproteins, and carotenoids which play a crucial role in photosynthesis and environmental adaptation.

Microalgae including Spirogyra and Volvox exhibit biofluorescence due to their unique pigment composition. Spirogyra is a genus of filamentous green algae belonging to the division Chlorophyta which contain Chlorophyll a, chlorophyll b and carotenoids. These pigments contribute to its green colour and natural fluorescence, particularly in the red region of the spectrum when exposed to UV or blue light. It is commonly found in freshwater environments and also in marine environments. Spirogyra consists of unbranched, cylindrical filaments made up of a series of cells. Its most distinctive feature is presence of spirally arranged chloroplasts inside each cell, which contain chlorophyll for photosynthesis. Spirogyra contributes to oxygen production in aquatic ecosystems and serves as food for various microorganisms and aquatic organisms. Volvox is a genus of colonial green algae that belongs to the division Chlorophyta and it often found in marine and fresh water environments. It composed of thousands of flagellated cells embedded in a gelatinous matrix. Each cell contains chlorophyll a, chlorophyll b, carotenoids, which enable fluorescence emission. (Damisane Mahlanga, Keletso Mphahlele and Francesco De Paola *et al* 2024).

Biofluorescence in microalgae has significant applications in marine ecology, environmental monitoring, and biotechnology. Fluorescent pigments act as natural biosensors, helping researchers detect heavy metal contamination, assess water quality. By analysing the fluorescence intensity and spectra shifts to their pigments, researcher can assess algal health, detect pollution, and explore potential applications in biotechnology and biofuel production. (Luis Porras Reyes, Ivo Havlik and Beutal *et al* 2024).

Materials and Methods

Sample Collection

Micro algal samples were collected from **Palk Bay** region. Different species of micro algal samples were collected in sterile glass container containing sea water and transported to the laboratory under dark and temperature-controlled conditions (4^{o}C) to minimize photobleaching and metabolic changes.

Cultivation of Micro algae

Microalgae were cultured in appropriate liquid media **(f/2 medium)** for marine species. Sterilize the medium by autoclaving and maintain sterile working conditions to prevent contamination. To prepare 250-300 mL of medium and it inoculated with microalgae into sterile culture vessels. The culture was incubated at 20–25°Cand it was maintained 12:12 h light-dark cycle under condition of 100 µmol photons ms^{-1} light intensity.

Biofluorescence Analysis

Cell Harvesting

Centrifuge the collected algae at 4000 rpm for 10 minutes to ensure complete separation of cells. Transfer the pellet to sterile tubes for cell lysis processing.

Cell lysis

Ultra sound assisted extraction (UAE) were carried out in an ultrasound. The micro algal samples were placed in a beaker with 50 ml. Place the pellet in 10-20 mL of extraction solvent and sonicate for 5-10 minutes at 40 kHz.

***Pigment Extraction*:** 10 mL of microalgal culture was centrifuged at 5000 rpm for 10 min to obtain a pellet. The pellet was resuspended in 90% acetone-methanol solution. Samples were incubated in the dark at 4°C for 24 h to ensure complete pigment extraction. The mixture was centrifuged at 8000 rpm for 15 min, and the supernatant was collected for further analysis.

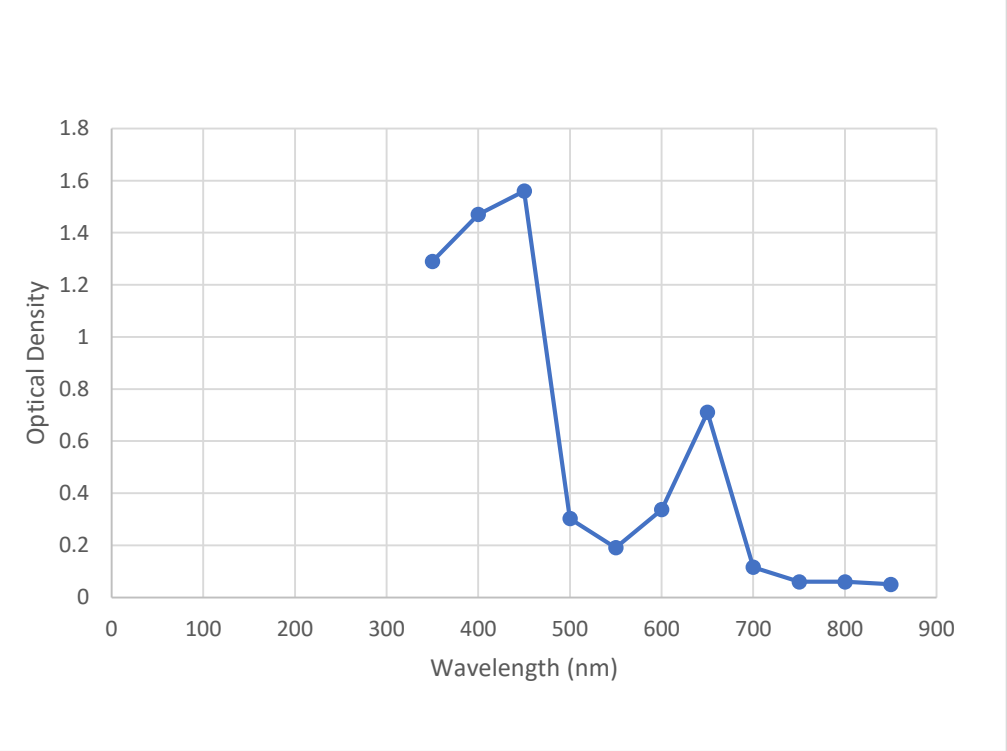
Fluorescence Spectroscopy

Fluorescence emission and excitation spectra were recorded using a fluorescence spectrophotometer. To confirm the presence of biofluorescent pigments, measure the absorption spectra of the extract using a **UV- Vis spectrophotometer**. Set the spectrophotometer to scan between 350- 750nm, covering the absorption ranges of chlorophyll (680- 700nm) and carotenoid (450-470nm). Record the absorbance.

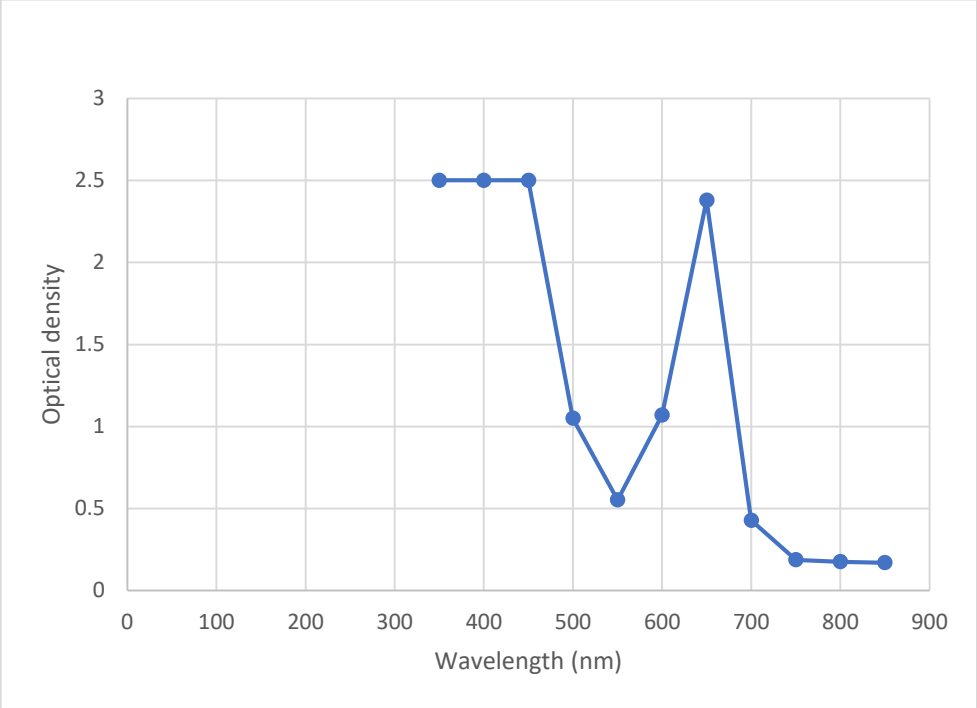
Table 1: Spectrophotometric analysis for different micro algal species

Wavelength (nm)	*Spirogyra* (OD)	*Volvox* (OD)
350	1.29	2.50
400	1.47	2.40
450	1.56	2.10
500	0.303	1.05
550	0.191	0.552
600	0.337	1.07
650	0.711	2.38
700	0.116	0.428
750	0.06	0.187
800	0.05	0.175
850	0.04	0.170

Graph 1: S*pirogyra* fluorescent emission



Graph 2: *Volvox* fluorescence emission



Fluorescence Microscopy

Fluorescence imaging was performed using a **Fluorescent Microscope.**

Result and Discussion

Fluorescence Spectroscopy Analysis

In this study of Micro algae**, Chlorophyll- a** was emitted by***Volvox* sp.** Chlorophyll-a showed a strong red fluorescence emission at **680 nm**. **Carotenoid** pigment was emitted by ***Spirogyra*** sp. Carotenoid-associated **fluorescence** was observed at **510 nm**, suggesting photoprotective mechanisms.

Microscopic Fluorescence Imaging

In this present work, fluorescent imaging demonstrated distinct fluorescence patterns among different microalgal species. *Spirogyra* exhibited prominent **Green Fluorescence** due to carotenoid accumulation, whereas *Volvox* displayed **Red Fluorescence**, likely due to chlorophyll-rich extracellular matrix components.

Sample Collection

Figure 1: Micro algae

Cell Lysis

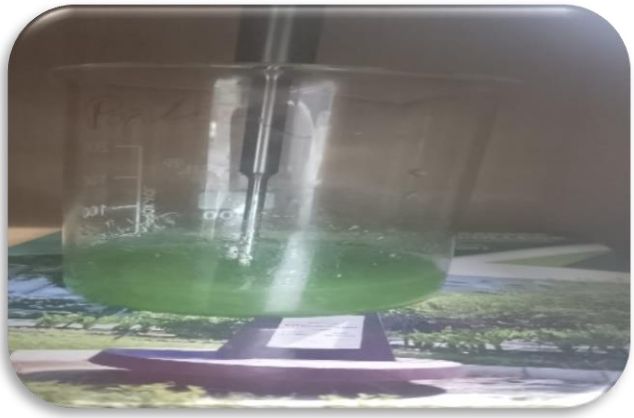


Figure 2: Ultra sonication

Fluorescence Microscopy

Figure 3: Microscopic View of *Spirogyra*

Conclusion

In this study, the autofluorescent pigment recovery from *Spirogyra* and *Volvox* collected from the Palk Bay region was investigated. Fluorescence microscopy confirmed the presence of biofluorescent pigments, highlighting distinct emission patterns corresponding to chlorophyll and carotenoid content. The application of ultrasonic-assisted extraction (UAE) with different solvents effectively extracted these pigments, with UV-Vis spectrophotometry quantifying total chlorophyll and carotenoid content. The study also explored the feasibility of using alternative solvents like limonene, presenting a more sustainable approach for pigment recovery. Additionally, HPLC analysis enabled the identification and quantification of key photosynthetic pigments, including chlorophyll, xanthophyll, carotenoids, and phycobiliproteins.

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