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Extraction & Analysis of Algal Pigments by Thin Layer Chromotography & Spectrophotometric Analysis

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ABSTRACT: The study focuses to extract and analyse the algal pigments present in the lithophilic algae collected from various sites in the concrete walls of Department of Biotechnology, University of Madras. The samples collected were stored in sterile plastic ware and cultured in a growth medium in the laboratory for further analysis. Significant algal growth was observed in media enriched with $CaCo_3$.Microscopic observations reveals filamentous algae of the family Cyanobacteria. The cultured algal biomass was treated with solvent and the pigments were extracted. The pigment extracts were analysed using two-dimension thin layer chromatography (TLC) and confirmed using UV-Vis Spectrophotometer. The filamentous algae have significant amount of Chlorophyll a &b and Carotenoids.

KEYWORDS: Lithophilic algae, Pigments, TLC.

INTRODUCTION

Biological pigments or biochrome is a substance that is produced by the living organism which produces colour as a result of selective colour absorption. Algae are diverse, ubiquitous photosynthetic organisms, which possess photosynthetic pigments, which absorbs light and harvest the chemical energy. The algal pigments include chlorophylls, phycobiliproteins, fucoxanthins, xanthophylls, and carotenes (Rowan, K.S.1989). Pigments other than chlorophyll serve to trap the energy of light and lead it to chlorophyll as it is responsible for initiating oxygenic photosynthesis reactions. There is a diverse group of algae ranging from blue-green algae to long complex kelps. The algae can be classified based on their habitat, which includes: aerial/terrestrial, aquatic, and algae in unusual habitats such as thermal algae found in very high temperatures, algae that are attached to the stones, and rocky areas. The algae that habitat in the stones and rocky surfaces are known as the “Lithophytes”. These lithophytes are of two types: Endolithic- habitats inside the stones and rocks and Epilithic- habitats outside the stones and rocks. These lithophilic algae habitats on highly illuminated areas where there is an availability of sufficient light energy for growth. They acquire nutrients easily as they grow on fissures in rock and concretes where soil or organic substances accumulate. Oblique lithophytes solely habitats on the rocks, whereas the facultative grow on a rock and simultaneously on other substances.

“Biodeterioration” is a damage/ destruction or decomposition of a man-made material caused by the presence of fungi, algae, bacteria, mold, etc., or their by-products. The damage in deterioration is mainly due to the secretion of inorganic and organic acids which are capable of leaching the minerals in the matric by weakening the binding system (C. Ferrari et al., 2015). The process of deterioration can occur due to weathering and soiling and it can also be organic and non-organic. In case of organic deterioration, A biofilm, i.e., the development of a community of microorganisms in a polymeric matrix that is adherent to living or inert surface, the metabolic molecules produced by the organism of the biofilm contribute to the damage. These biofilms majorly contain cyanobacteria, algae, and fungi. The organic deterioration is influenced by wind, temperature, rainfall, sunlight, and the water availability in the material which acts as an essential element for microbial metabolism. The wet concrete promotes the growth of autotrophic organisms which leads to increased biofouling during the rainy seasons. The damage due to biodeterioration leads to “Bioreceptivity” – the ability of the material to colonise with the living organism. Under favourable conditions such as high amount of moisture, optimum temperature, and lighting even new concrete can be easily colonised with the organisms. In case of algae, the most commonly found in biodeterioration are the cyanobacteria and other lithophilic algae (Muscio, et. al., 2007). They habitat as Endoliths which protects them from intense temperature and heat. Cyanobacteria generally habitats in shady region which retains the humidity by reduced illumination. During the dry seasons, the biofilms of cyanobacteria appear grey in colour whereas in humid and rainy conditions it is green in colour. Algae are present in an environment that has high atmospheric humidity or the surface is damp. Phylum Chlorophyta is majorly involved in the process of colonization. Algae can habitat in various materials such as brick,

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stone, concrete, steel, paint, etc. The taxonomical diversity of algae and cyanobacteria involved in the colonisation is dependent on the climatic, environmental conditions along with the level of bio receptivity of the material (C. Ferrari et al., 2015).

MATERIALS AND METHODS

- **COLLECTION OF SAMPLES:**

The algae samples were collected from various sites in concrete walls from. (Department of Biotechnology, University of Madras, January 2018) The scrapped algae samples were collected in a sterile plastic bottle with distilled water and taken to the laboratory for further analysis.

Figure 1. Site of Sample Collection

- **IDENTIFICATION OF ALGAE:**

The collected algae samples were identified using the LABOMED VISION 2000 BINOCULAR microscopic unit.

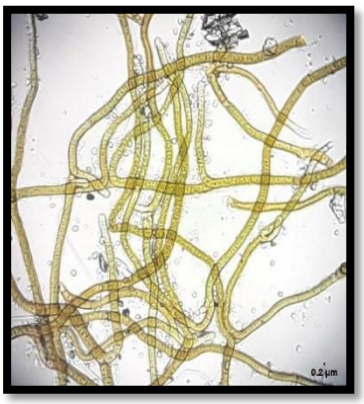

Figure 2 a

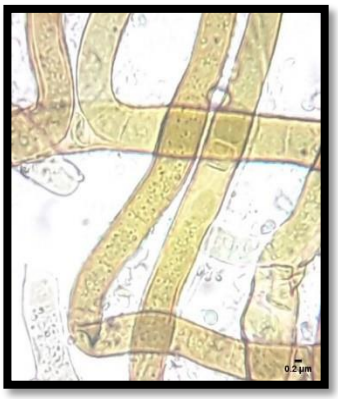

Figure 2 b

Figure 2. a Filamentous algae under 40 x, Figure 2 b Filamentous algae under 100 x

- **CULTURE OF ALGAE:**

The collected algal samples were cultured in soil water growth medium, soil water medium with $CaCO_3$, soil water medium with NH_4MgPO_4, soil water medium with organic peat and soil for the most acidophilic algae, modified Bold's Basal medium, for 4 weeks at 37 ℃ and the cultures were used for pigment extraction.

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Figure 3. Culture of Algae

- **PIGMENT EXTRACTION:**

The cultured algal samples were air dried and treated with 100% Acetone. 5ml of the algal biomass was grinded with 2 ml of 100% Acetone in a Dounce glass homogenizer (Wiltshire, K. H 2000).

- **ANALYSIS OF PIGMENT BY TLC:**

The extracted pigment was analysed using Thin layer Chromatography. Thin layer chromatography was performed in pre-coated silica gel sheet (Silica 60 F_{254}). The TLC sheet of length 7 cm and width of 2 cm was taken. The deposit line for loading the sample mixture was marked at the bottom of the TLC sheet. The different algal extracts were loaded at different spots in the deposition line in the TLC sheet. Once the sample gets dried, the TLC plates were placed in the mobile phase. The mobile phase contains the mixture of methanol, distilled water and ammonia in 9: 1: 0.0012µl ratio. Once the mobile phase reaches the solvent front. The Rf value was calculated and the TLC sheets were observed in UV- transilluminator.

Rf_{value}= Distance moved by the analyte / Distance moved by the solvent

Figure 4. a **Figure 4. b**

Figure 4. a - TLC Sheet under UV, Figure 4 b- TLC Sheet under Visible light

In Figure 4 a & b, Pigment 1- Chlorophyll a

Pigment 2- Chlorophyll b

Pigment 3- Carotenoids

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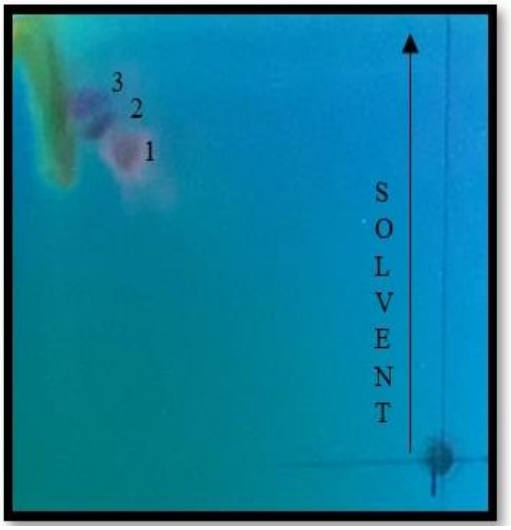

Figure 5. A

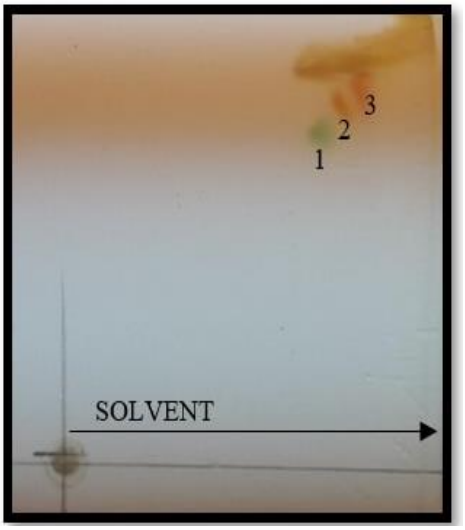

Figure 5. B

Figure 5. A- TLC sheet in UV, Figure 5 B- TLC sheet in visible light

Figure 5 A& B Pigment 1- Chlorophyll a

Pigment 2- Chlorophyll b

Pigment 3- Carotenoids

Rf $_{value}$ CALCULATION:

Table 1- Rf $_{values}$ of the pigments

Compound		**Rf value**
A	3.6/5.5	0.65
B	3.9/5.5	0.72
C	4.6/5.5	0.83
D	5.3/5.5	0.91

- **ANALYSIS OF PIGMENT BY UV-VIS SPECTROPHOTOMETER:**

The extracted algal pigments were further analysed using Shimadzu UV-Vis Spectrophotometer 1800 series. The UV-VIS Spectrophotometric analysis was done to determine the maximum absorbance of the different algal extracts at a specific wavelength. Each extract was measured for maximum absorbance at a wavelength ranging from 100 nm – 1900 nm. The maximum absorbance shown by the pigments was recorded.

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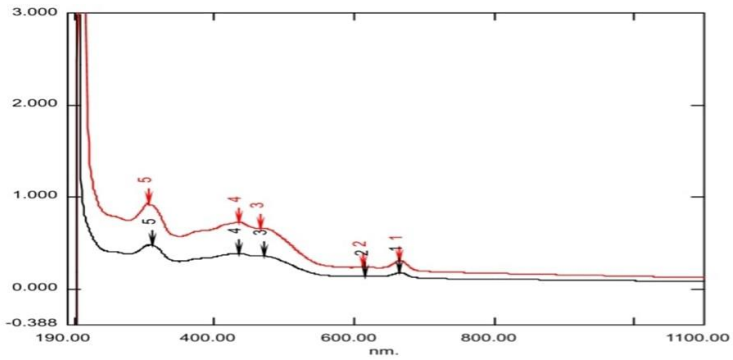

Figure 6. Absorption Spectrum of the Algal Pigments

Table 2- Maximum Absorbance of the Pigments

S.NO	SAMPLE	WAVE LENGTH	ABSORBANCE
A	1µl/ml	320nm, 423nm, 620nm, and 690nm	0.442, 0.338, 0.552, 0.553 and 0.774
B	2µl/ml	320nm, 423nm, 454nm, 620nm, and 690nm	0,559, 0.447, 0.886, 0,992 and 0.998

RESULTS & DISCUSSION

Among the five media tested to optimize the algal growth, soil water media showed significant higher growth rate compared to the other media tested. The well grown algae viewed under microscope (Figure II), filamentous algae being observed as dominant species. Based on the microscopic characteristics compared with the web resources, the filamentous algae have been identified as cyanobacteria. Further the pigments were extracted from desiccated algal samples using the extraction solvent: 100% Acetone (J. Azmir et.al., 2013). The pigments separated being identified as Chlorophyll a (pigment-1) Chlorophyll b (pigment-2) Carotenoids (pigment-3) through Thin layer Chromatography (Figure IV a & b) (Jeffrey, S. W. 1974). Further spectral analysis of the pigments was carried out and based on the absorption spectrum (Fabrowska. J et.al.,2017), it is identified that Chlorophyll a & b fall between 300nm- 400nm (Pointer 5 in the spectrum) (Figure V) and the carotenoids falls between the 400nm- 600nm (Pointer 3,2,1 in the spectrum) (Figure V).

- **Chlorophyll a & b:**

Chlorophyll a and b are the form of the photosynthetic pigment chlorophyll, found in the thylakoid membranes of the chloroplast. Chlorophyll a is known as the primary pigment and chlorophyll b as the accessory pigment. The light from the violet-blue and orange-red areas of the electromagnetic spectrum was absorbed by Chlorophyll a (Rowan, K. S. 1989). It transfers two excited electrons to the electron transport chain and transfers the energy to the reaction center in the light-absorbing antenna system. Chlorophyll b absorbs blue light and the pigment aids in expanding the absorption spectrum in the organism. The presence of chlorophyll b in the cell allows the organism to absorb and convert a higher amount of light energy into chemical energy. The structural difference between Chlorophyll a & b is the atom in the side chain in the third carbon, chlorophyll a has methyl group whereas b has aldehyde group.

- **Carotenoids:**

Carotenoids are pigments with tetraterpenoids. There are many different carotenoids found in the algae, namely α- carotene, β-carotene, alloxanthin, antheraxanthin, astaxanthin, diatoxanthin, etc. carotenoids play two major roles in algae, one absorbing light energy for use in photosynthesis another role as a photoprotective agent (Rowan, K. S. 1989). The excited state of chlorophyll has to be quenched immediately, if not it may react with the molecular oxygen and forms singlet oxygen which reacts and damages other cellular components. Carotenoids do their photoprotective action by quenching the energy of excited chlorophyll.

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CONCLUSION

The pigments from lithophilic algae have a wider biotechnological application in industries such as pharma, food production and processing. The pigments have antibacterial, antioxidant and nutritional properties. The pigments can also be used as potential natural dye replacing the synthetic dyes. The scaled-up production of these algae can increase their application and in the production of pigments, vitamins and proteins.

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