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Bioprospecting Indigenous Microalgae for Pigment Production and Stability Assessment for Textile Applications

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A B S T R A C T

The severe environmental and health hazards posed by synthetic dyes in the textile industry such as toxic effluent discharge and water pollution necessitate the exploration of sustainable, eco-friendly alternatives. Microalgae present a highly viable source of natural pigments due to their rapid growth rates, high pigment content, and minimal land and freshwater requirements compared to terrestrial crops. This study aimed to bioprospecting and evaluate indigenous freshwater microalgae from local Mumbai habitats for natural pigment production and subsequent application as textile colorants. Three distinct microalgal samples were sourced for this study: an enriched culture provided by the Microbiology Department of Khalsa College (*Tetradesmus* spp.), an isolate from a domestic fish tank (*Chlorella* spp.), and an isolate from Bhavan's College Lake (*Scenedesmus* spp.). Initial enrichment trials established that BG-11 medium optimally supported the phototrophic growth of all three indigenous isolates. Furthermore, nitrogen availability emerged as a critical driver for both biomass accumulation and pigment biosynthesis. Cultivation using a 2.25 g/L sodium nitrate concentration (representing a 50% excess from the standard baseline) maximized both the overall biomass generation (yielding 0.428 g, 0.251 g, and 0.473 g for Samples 1, 2, and 3, respectively) and the total pigment concentration across all isolates. Intracellular pigment extraction was executed utilizing an ultrasonic-assisted method (80% amplitude with nine cycles of 10-second pulses and 5-second pauses). Solvent screening confirmed that 80% aqueous acetone was significantly superior to chloroform and hexane for pigment recovery. Quantitative spectrophotometric analysis utilizing the Arnon-Lichtenthaler equations determined that the extracts were chlorophyll-dominant. The *Chlorella* spp. isolate yielded the highest overall pigment concentrations, recording Chlorophyll a at 116.0 µg/g and Chlorophyll b at 294.6 µg/g of dry biomass. For textile application, the extracted natural pigments successfully imparted a green coloration to cellulosic cotton fabrics, facilitated by 5% alum and 5% tannic acid mordanting pre-treatments. However, rigorous stability assessments exposed critical vulnerabilities. The dyed textiles demonstrated exceptionally poor wash fastness, experiencing substantial colour loss within 15 minutes of mechanical agitation in mild detergent, alongside severe photodegradation and limited light fastness under continuous sunlight exposure. Ultimately, while this study successfully establishes the feasibility of utilizing indigenous microalgae for sustainable dye production, the inherent chemical instability of the chlorophyll-based pigments underscores the critical necessity for advanced encapsulation, chemical modification, and optimized mordanting strategies prior to commercial textile integration.

Introduction

The global textile industry heavily relies on synthetic dyes derived from petrochemicals, which present severe environmental and health hazards, including toxic effluent discharge, bioaccumulation, and potential carcinogenicity (Costa et al., 2022). Consequently, there is an escalating demand for sustainable, eco-friendly colorants derived from natural resources. While traditional natural dyes sourced from terrestrial plants and insects offer biodegradability, their commercial scalability is hindered by strict seasonal availability, extensive arable land and freshwater requirements, and extended cultivation periods. Microalgae have emerged as a superior alternative for natural pigment production. These photosynthetic microorganisms offer rapid growth rates with cellular doubling times of 24 to 48 hours, year-round cultivation capabilities, and high pigment accumulation without competing for arable land (Arashiro et al., 2020). Furthermore, microalgal cultivation can be coupled with wastewater treatment and carbon dioxide sequestration, substantially enhancing the overall sustainability of the production process.

Microalgal pigments primarily serve physiological functions in light harvesting and cellular photoprotection. Chlorophylls act as the primary photosynthetic pigments, while carotenoids function as accessory pigments and potent antioxidants that protect cells from photo-oxidative damage (Keddar et al., 2025; Kerner et al., 2025; Balasubramaniam et al., 2024). Freshwater green microalgae belonging to the *Chlorophyceae* class, particularly the families *Scenedesmaceae* and *Chlorellaceae*, are highly valued for their dense intracellular accumulation of these pigments. Genera such as *Chlorella*, *Scenedesmus*, and *Tetradesmus* are robust candidates for natural dye production due to their high chlorophyll content and proven adaptability to various culture conditions (Hamidian et al., 2022; Wizi et al., 2022).

Although commercial strains are widely utilized, bioprospecting indigenous microalgae remains a critical strategy for discovering novel strains with superior pigment production capabilities. Strains isolated from local aquatic ecosystems are naturally adapted to regional climatic fluctuations, including variable nutrient availability, temperature extremes, and shifting light intensities. Cultivating locally adapted isolates can significantly reduce the energy inputs required for environmental control in large-scale systems, thereby lowering overall production costs. India's diverse freshwater ecosystems, particularly the tropical urban water bodies of Mumbai, provide an ideal, largely unexplored reservoir for bioprospecting. Furthermore, utilizing indigenous biodiversity aligns with the Nagoya Protocol's emphasis on the sustainable use and equitable sharing of microbial genetic resources.

Therefore, this study aims to systematically isolate, optimize, and classify pigment-producing indigenous microalgae from local aquatic habitats, extract their intracellular pigments, and critically evaluate their structural stability and applicability as sustainable textile colorants.

Microalgae as a Source of Natural Pigments

Microalgae are increasingly recognized as prominent candidates for the sustainable production of natural pigments, including carotenoids, phycobiliproteins, and chlorophyll derivatives. Beyond imparting vivid colours, these pigments exhibit potent antioxidant, anticancer, and anti-inflammatory properties, making them highly valuable across the nutraceutical, cosmetic, textile, and pharmaceutical industries. Unlike synthetic dyes, which pose severe environmental and health risks, microalgae-derived pigments are biodegradable, renewable, and align with global shifts toward green chemistry (Del Campo et al., 2007).

Among microalgal pigments, chlorophyll *a* and *b* are the primary photosynthetic and accessory pigments, absorbing light primarily in the blue and red spectrums. Carotenoids, such as beta-carotene, lutein, and violaxanthin, serve as accessory pigments that protect cells from photo-oxidative damage.

The industrial relevance of these metabolites is well documented; for instance, *Haematococcus pluvialis* and *Dunaliella salina* are extensively studied for their ability to hyper-accumulate astaxanthin and beta-carotene, respectively, under induced stress conditions. Phycobiliproteins from *Spirulina platensis* have even gained regulatory approval as food colorants, highlighting the commercial viability of algal pigments (Yazdani & Tavakoli, 2019).

Diversity and Cultivation of Freshwater Green Microalgae

Freshwater green microalgae (Chlorophyta) represent a

highly diverse group of photosynthetic microorganisms. Within this division, the families *Scenedesmaceae* and *Chlorellaceae* are extensively studied for biotechnological applications. The genus *Tetradesmus* (formerly classified within *Scenedesmus*) and *Scenedesmus* comprise colonial green algae characterized by robust biomass and pigment production. Similarly, *Chlorella* species are unicellular green algae valued for their rapid growth rates, high protein content, and adaptability to varied culture conditions (Wizi et al., 2022; Degala et al., 2025; Kerner et al., 2025).

The successful cultivation of these strains is fundamentally dependent on the culture medium. Standard media formulations such as BG-11, Bold's Basal Medium (BBM), WC, and COMBO offer distinct nutrient compositions tailored to specific microalgal physiological requirements. Nitrogen, a critical macronutrient in these media, profoundly influences biomass accumulation and pigment biosynthesis. Under nitrogen-replete conditions, microalgae exhibit rapid growth and high chlorophyll content. Conversely, nitrogen limitation triggers adaptive metabolic shifts, often reducing chlorophyll synthesis while upregulating the accumulation of storage compounds like lipids and carotenoids. Therefore, optimizing species-specific nitrogen supplementation is essential for maximizing desired production outcomes (Kilham et al., 1998).

Pigment Extraction Techniques

Efficient extraction of intracellular pigments is a critical bottleneck for downstream applications. Traditional solvent extraction utilizing organic solvents such as acetone, methanol, and ethanol remains the conventional approach. Solvent selection is dictated by the target pigment's polarity; for instance, aqueous acetone (typically 80-90%) is widely favoured for comprehensive chlorophyll extraction due to its efficacy in disrupting cell membranes and solubilizing chlorophylls while maintaining molecular stability. For quantitative analysis, the Arnon equations have been extensively validated for 80% acetone extracts *(Porra et al., 1989)*.

To improve recovery efficiencies and reduce solvent consumption, advanced extraction techniques have been developed. Ultrasonic-assisted extraction (UAE) is particularly effective, utilizing acoustic cavitation to generate localized shearing forces that disrupt robust microalgal cell walls, thereby enhancing mass transfer and reducing extraction times (Degala et al., 2025; Kerner et al., 2025; Balasubramaniam et al., 2024).

Application in Textile Dyeing

The application of microalgal pigments in textile dyeing has gained significant traction as a sustainable alternative to synthetic colorants. Cotton, a widely used natural cellulosic fiber, is the primary focus of this research. However, applying natural dyes to cotton strictly requires mordanting the application of metal salts or natural tannins to improve dye uptake and structural fixation (Linhares *et al.,* 2016; Wizi *et al.,* 2022).

Mordanting relies on the formation of coordination complexes between metal ions, mordant compounds, and dye molecules, creating chemical bridges that anchor the pigment to the fiber. For example, pretreating cotton with tannic acid followed by alum mordanting generates aluminum-tannate complexes that significantly enhance colour absorption and fastness. Despite the clear environmental benefits of algae-based textiles, significant challenges remain; natural microalgal dyes struggle to achieve wash and light fastness properties comparable to synthetic dyes. Current research emphasizes the need for innovative application methods, such as nano-suspension systems and advanced encapsulation, to construct functional, colour-fast textiles using microbial pigments *(Zhang et al., 2024; Kwan et al., 2022; Ghazal et al., 2025).*

Materials and Methods

Culture Media Preparation

To determine the most conducive nutritional environment for microalgal proliferation, five standardized microbiological media were prepared: Woods Hole Culture Medium (WC), COMBO, BG-11, Bold's Basal Medium (BBM), and Chu-10. The media were formulated using precise macro- and micronutrient stock solutions dissolved in distilled water, adjusted to their respective optimal pH ranges, and sterilized via autoclaving at 121°C for 15 minutes. Following the sterilization and cooling process, Vitamin B12 was aseptically incorporated into all media formulations.

Sample Collection and Enrichment

Microalgal samples were procured from three distinct sources to ensure a diverse screening of indigenous

strains. Sample 1 was given by the Microbiology Department of Khalsa College. Sample 2 was isolated from an indoor domestic fish tank aquarium, representing a nutrient-rich ecosystem driven by organic matter. Sample 3 was collected from the open-air lake at Bhavan's College, utilizing clean plastic bottles submerged 10 to 15 centimetres below the water surface to bypass transient floating debris.

For the initial enrichment phase, 20 ml aliquots of the raw environmental water samples were directly inoculated into sterile flasks containing 300 ml of the prepared cultivation media. To stimulate cellular proliferation, the enrichment media were initially supplemented with Vitamin B12 at a concentration of 50 mg/100 ml.

Optimization of Cultivation Conditions

Nitrogen Optimization: To maximize biomass yield and intracellular pigment production, the primary nitrogen source in BG-11, sodium nitrate ($NaNO_3$), was systematically optimized. An experimental gradient of six modified media was prepared by adding excess $NaNO_3$ relative to the standard baseline concentration, featuring 1.65 g/L, 1.95 g/L, 2.25 g/L, 2.55 g/L, 2.70 g/L, and 3.00 g/L supplemental nitrogen. Aliquots of 50 ml of each modified medium were inoculated with 1 ml of unialgal culture and incubated in duplicate under natural sunlight.

Pigment Extraction

A comparative solvent screening and sonication-assisted protocol were employed to recover intracellular photosynthetic pigments. Three distinct extraction solvents were evaluated: hexane, chloroform, and 80% aqueous acetone. Precisely 1 g of dried microalgal biomass was suspended in 20 ml of the respective solvent. To protect heat-sensitive pigment molecules, the mixtures were pre-cooled before mechanical disruption (Porra et al., 1989).

Ultrasonic-assisted extraction (UAE) was executed using a probe sonicator set to an 80 MHz frequency. Acoustic cavitation was induced utilizing a standardized pulsed sequence of 10 seconds ON and 5 seconds OFF, repeated for a total of 9 complete cycles. Following sonication, the cellular debris was partitioned via cold centrifugation at 5000 rpm for 15 minutes at a strictly maintained temperature of 5°C. The clarified pigment supernatants were subsequently collected for quantitative analysis (Balasubramaniam et al., 2024).

Spectrophotometric Analysis and Quantification

The clarified pigment extracts were quantitatively analysed Type equation here. utilizing a UV-Visible spectrophotometer, with 80% aqueous acetone serving as the baseline calibration blank. Optical absorbance (A) was measured at three specific wavelengths: 470 nm for total carotenoids, 645 nm for Chlorophyll b, and 663 nm for Chlorophyll a.

The absolute volumetric concentrations (µg/ml) of the pigments were calculated using the standard Arnon-Lichtenthaler empirical equations:

$$Chl\_a = 12.7[A\{663\}] - 2.69[A\{645\}]$$

$$Chl\_b = 22.9[A\{645\}] - 4.68[A\{663\}]$$

$$Carotenoids = \frac{1000[A\{470\}] - 1.82[Chl\_a] - 85.02[Chl\_b]\}}{198}$$

The resultant volumetric concentrations were then normalized to the initial dry biomass weight to determine the absolute pigment yield, formally reported as micrograms of pigment per gram of dry biomass (µg/g).

Textile Dyeing and Stability Assessment

To evaluate practical applicability, standard cotton fabric was cut into uniform 2x2 swatches. Surface modification was performed using freshly prepared 5% tannic acid and 5% alum aqueous solutions. The fabric swatches were submerged in these mordanting solutions for a strictly timed 10-minute pretreatment phase, after which excess solution was removed and fabrics were partially air-dried. The dyeing process was conducted iteratively by immersing the swatches directly into the extracted pigment solutions in distinct 5-minute intervals, repeated 4 to 5 times until satisfactory visual colour intensity was achieved (Zhang et al., 2024; Linhares et al., 2016).

Stability assessments were executed under acute physical stress conditions

Light Fastness: Photostability was assessed through controlled exposure to direct, natural outdoor sunlight. Swatches were monitored in duplicate for a total of 3 hours, with photographic documentation captured at 0, 1, 2, and 3-hour intervals to comparatively analyse progressive pigment photodegradation.

Wash Fastness: The mechanical and chemical resilience of the dye-fiber bond was evaluated by immersing the dyed swatches in an aqueous wash bath formulated with a mild detergent.

The fabric samples were subjected to continuous mechanical agitation, with washing trials conducted and documented in precise 5-minute intervals (0, 5, 10, and 15 minutes). After each interval, fabrics were thoroughly rinsed with distilled water, fully air-dried at room temperature, and photographically analysed to document total colour loss.

Results and Discussion

Taxonomic Identification and Morphological Characterization

Following the initial enrichment phase and subsequent transfer to natural sunlight, robust phototrophic growth was observed exclusively within the BG-11 medium.

The selective environmental pressures, combined with the specific physiochemical profile of the BG-11 broth, facilitated the successful isolation of unialgal cultures.

Taxonomic classification to the family and genus levels was established through detailed morphological evaluation under bright-field light microscopy at 45x magnification.

The identification criteria focused on cellular geometry, chloroplast morphology, and colonial architecture.

Isolate 1: Tetradesmus spp.

Isolate 1, an enriched culture explicitly provided by the Microbiology Department of Khalsa College, was taxonomically assigned to the family *Scenedesmaceae* and identified as *Tetradesmus spp*.

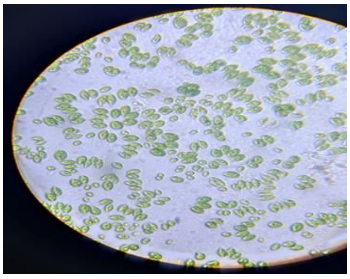


Figure 1:- Morphological identification of microalgal sample1 by hanging drop microscopy. The micrograph (45×, hanging drop) shows green, non-motile cells that are oval to ellipsoidal with smooth, well-defined cell walls. Cells possess prominent parietal chloroplasts and occur both singly and in characteristic coenobial groupings. Distinct bow-like (slightly curved) morphology was observed, with cells arranged in groups of 2, 4, 6, and 8, which is typical of members of the family Scenedesmaceae. The coenobia appear loosely organized with occasional elongated cells showing tapered ends. No flagella or active motility were observed. Based on the bow-shaped cellular morphology and colonial arrangement pattern, the isolate was tentatively identified as Tetradesmus spp.

Morphologically, the isolate presented predominantly as solitary vegetative cells, exhibiting a distinct fusiform (spindle-shaped) geometry that tapered sharply into acute, pointed poles. The intracellular space was largely occupied by a prominent parietal chloroplast, indicative of active photosynthetic metabolism and robust cellular health.

Isolate 2: Chlorella spp.

Isolate 2, procured from a nutrient-rich domestic aquarium ecosystem, was classified within the family *Chlorellaceae* as *Chlorella spp.*

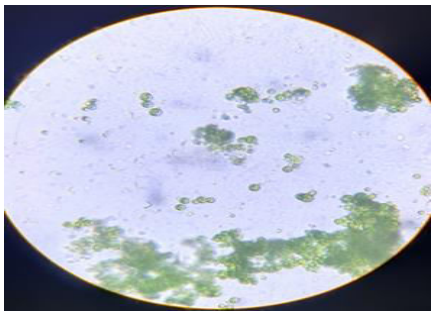


Figure 2:- Morphological identification of microalgal sample2 by hanging drop microscopy. The micrograph obtained by hanging drop microscopy (45×) shows small, green, non-motile cells that are predominantly circular to subspherical in shape. Cells possess smooth, well-defined cell walls and exhibit a distinct cup-shaped chloroplast occupying a large portion of the cell interior. The cells occur mostly singly or in loose aggregations without coenobial organization. These morphological features are characteristic of members of the family Chlorellaceae, supporting the tentative identification of the isolate as belonging to the genus *Chlorella spp.*

The culture was characterized by dense populations of non-motile, unicellular microalgae exhibiting a strict spherical to sub-spherical morphology.

A primary diagnostic feature confirming this classification was the presence of a single, prominent, cup-shaped chloroplast occupying a large proportion of the cellular volume, which serves as the hallmark morphological trait of the Chlorella genus.

Isolate 3: *Scenedesmus* spp.

Isolate 3, sourced from the open-water environment of Bhavan's College Lake, was identified as *Scenedesmus spp*., also belonging to the family Scenedesmaceae.

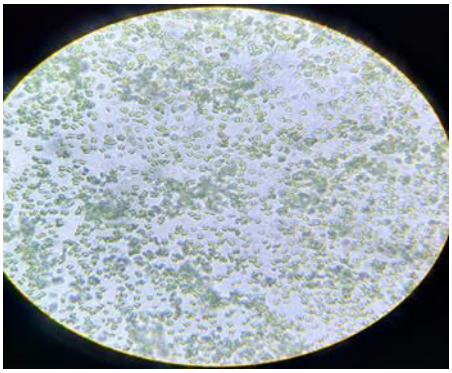


Figure 3:- Morphological identification of microalgal sample3 by hanging drop microscopy. The micrograph obtained by hanging drop microscopy (45×) shows green, non-motile cells arranged in characteristic coenobial formations. Cells are oval to slightly elongated with smooth cell walls and distinct chloroplast pigmentation. The isolate exhibits typical raft-like colony morphology, with cells aligned in organized groups of 2, 4, 6, and 8, a diagnostic feature of members of the family Scenedesmaceae. The regular linear arrangement and compact coenobia support the tentative identification of the organism as belonging to the genus *Scenedesmus* spp.

In contrast to the solitary cells of Isolate 1, this strain exhibited a distinctly colonial architecture, forming flat, linear coenobia typically comprising four to eight cells. The individual cells within the coenobium were oblong-ellipsoid to cylindrical, arranged laterally in a single parallel row. Furthermore, the terminal cells of the coenobial arrangements frequently displayed distinct structural appendages or polar spines, representing characteristic morphological adaptations for buoyancy and predation defines typical of the *Scenedesmus* genus in freshwater ecosystems.

Optimization of Biomass and Pigment Production by Nitrogen Supplementation

Nitrogen availability is intimately linked to both cellular division and pigment synthesizing capacity, as nitrogen atoms form the core structure of the porphyrin ring in chlorophyll molecules. In the standard commercial BG-11 medium, sodium nitrate (NaNO3) serves as the primary nitrogen source. To determine the optimal nitrogen availability, a supplementation strategy was employed utilizing modified NaNO3 concentrations: 1.65 g/L, 1.95 g/L, 2.25 g/L, 2.55 g/L, 2.70 g/L, and 3.00 g/L.

The concentration of available nitrogen exerted a profound and direct influence on overall biomass accumulation. As detailed in Table 1, a distinct dose-dependent response was observed: biomass generation increased proportionally and steadily as the NaNO3 concentration increased from 1.65 g/L to 2.25 g/L. The absolute maximum biomass yield across all isolates was recorded precisely at the 2.25 g/L concentration. Specifically, Sample 1 (*Tetradesmus* spp.) reached a peak biomass of 0.428 g, Sample 2 (*Chlorella* spp.) reached 0.251 g, and Sample 3 (*Scenedesmus* spp.) achieved the highest overall biomass of 0.473 g.

Pigment Production Trends

The quantitative data for pigment production perfectly mirrored the trends observed in biomass accumulation. Pigment concentrations, measured both volumetrically (µg/ml) and per unit of dry weight (µg/g), peaked at the 2.25 g/L NaNO3 concentration. At this optimum level, Sample 1 yielded maximum pigment concentrations of 0.821 µg/ml and 9.59 µg/g.

Sample 2 yielded 0.783 µg/ml and a notably high relative concentration of 15.59 µg/g, indicating highly efficient intracellular pigment synthesis despite a lower overall biomass weight. Sample 3 yielded 0.638 µg/ml and 6.74 µg/g.

Interestingly, increasing the nitrogen concentration beyond the optimal 2.25 g/L threshold resulted in diminishing returns. At 2.55 g/L, 2.70 g/L, and 3.00 g/L concentrations, biomass yields began to plateau or slightly decline. Concurrently, pigment synthesis

pathways appeared to be down-regulated at these higher concentrations.

This continuous decline confirms the inhibitory effect of hyper-concentrated NaNO3 on the metabolic efficiency of these indigenous strains, suggesting that concentrations above 2.25 g/L may induce mild substrate inhibition or osmotic stress, thereby stunting further cellular proliferation.

Table.1 Effect of Varying Nitrogen Concentrations on Microalgae Biomass and Pigment Production

Concentration of Nitrogen (g/L)	Khalsa College (S1) Biomass (g/L)	Khalsa College (S1) Pigment Yield (µg/ml)	Khalsa College (S1) Pigment Yield (µg/g)	Fish Tank (S2) Biomass (g/L)	Fish Tank (S2) Pigment Yield (µg/ml)	Fish Tank (S2) Pigment Yield (µg/g)	Bhavans College Lake (S3) Biomass (g/L)	Bhavans College Lake (S3) Pigment Yield (µg/ml)	Bhavans College Lake (S3) Pigment Yield (µg/g)
1.65	0.348	0.526	7.55	0.104	0.319	15.33	0.381	0.483	6.33
1.95	0.351	0.513	7.30	0.086	0.216	12.55	0.342	0.361	5.27
2.25	0.428	0.821	9.59	0.251	0.783	15.59	0.473	0.638	6.74
2.55	0.416	0.781	9.38	0.219	0.674	15.38	0.433	0.491	5.66
2.70	0.408	0.743	9.10	0.221	0.406	9.18	0.416	0.422	5.07
3.00	0.412	0.779	9.45	0.206	0.386	9.36	0.384	0.398	5.18

Table.2 Effect of Solvent Type on Total Pigment Extraction Efficiency

Microalgae Sample	80% Acetone Yield (µg/ml)	Chloroform Yield (µg/ml)	Hexane Yield (µg/ml)
Sample 1 (*Tetradesmus* spp.)	136.6	NA	NA
Sample 2 (*Chlorella* spp.)	410.6	NA	NA
Sample 3 (*Scenedesmus* spp.)	143.6	NA	NA

Pigment Extraction and Quantification

To determine the most efficacious solvent for pigment recovery, three different extraction systems were evaluated: hexane, chloroform, and 80% aqueous acetone.

During the physical mixing phase, the biomass proved highly immiscible in hexane (sinking completely) and immiscible in chloroform (floating on the surface). In contrast, the 80% aqueous acetone solution mixed uniformly with the biomass, facilitating proper suspension and solvent penetration.

Quantitative analysis of the total pigment yield confirmed these physical observations. As detailed in Table 2, 80% acetone significantly outperformed the other solvents across all three samples.

The superior performance of 80% acetone is attributed to its intermediate polarity, which effectively solubilizes both polar chlorophyll molecules and moderately polar carotenoids, while the 20% aqueous component hydrates and disrupts the cell membrane to enhance pigment release.

Spectrophotometric analysis of the 80% acetone extracts, derived from biomass cultivated under the optimized 2.25 g/L NaNO3 conditions, revealed that Chlorophyll a was the dominant pigment fraction across all samples.

Furthermore, all three isolates exhibited a highly consistent Chlorophyll a/b ratio ranging from 0.39 to 0.69, which is a standard physiological indicator for green microalgae cultivated under moderate lighting.

Total carotenoid content was proportionally lower, representing approximately 10% to 12% of the total measured chlorophyll content.

Table.3 Pigment Characterization and Quantification of Microalgal Isolates

Sample	Chlorophyll a (µg/ml)	Chlorophyll a (µg/g)	Chlorophyll b (µg/ml)	Chlorophyll b (µg/g)	Carotenoid (µg/ml)	Carotenoid (µg/g)	Chlorophyll a/b Ratio
Sample 1 (*Tetradesmus* spp.)	2.28	45.6	3.32	66.4	1.23	24.6	0.69
Sample 2 (*Chlorella* spp.)	5.80	116.0	14.73	294.6	NA	NA	0.39
Sample 3 (*Scenedesmus* spp.)	2.38	47.6	3.5	70.0	1.30	26.0	0.68

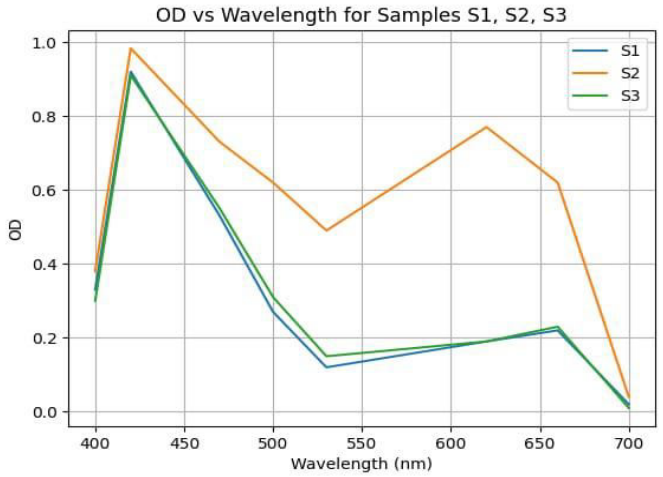


Figure.4 UV-Visible Spectrophotometric absorbance profile of pigment extracts from microalgal isolates.

The graph shows the optical density (OD) of pigment extracts from isolates S1, S2, and S3 measured across the visible wavelength range (400-700 nm). All samples exhibited a prominent absorption peak in the blue region (~420-430 nm), characteristic of chlorophyll and carotenoid pigments. A secondary absorbance shoulder was observed in the red region (~650–660 nm), corresponding to chlorophyll-associated absorption. Among the isolates, S2 consistently showed higher absorbance values across the spectrum, indicating comparatively greater pigment concentration, while S1 and S3 displayed similar but lower intensity profiles. These spectral patterns confirm successful pigment extraction and the presence of photosynthetic pigments in the isolates.

Textile Dyeing and Fastness Assessment

Initial dyeing experiments demonstrated that the extracted microalgal pigments could successfully impart a green coloration to cotton fabric. The visual intensity varied, with Sample 2 (*Chlorella spp*.) producing the most intense green shade. To facilitate dye uptake, a pretreatment process utilizing tannic acid followed by alum mordanting visibly enhanced colour absorption into the fibers. However, subsequent fastness testing revealed significant limitations regarding the structural stability of the applied pigments.

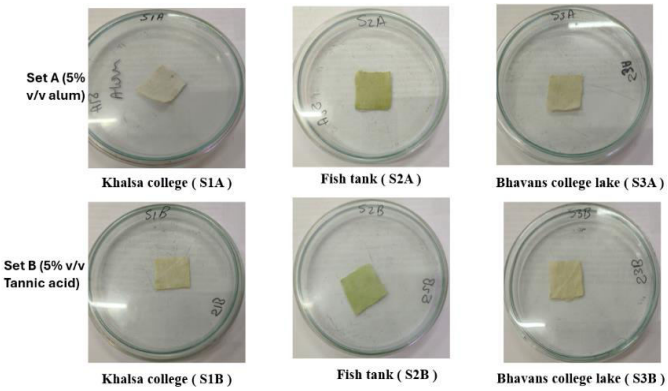
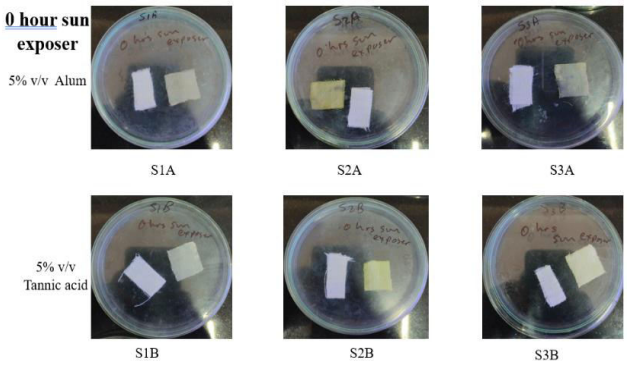


Figure.5 Effect of mordant type on cotton dyeing using microalgal pigments.

The figure compares dye uptake on cotton fabric using two mordants: Set A (5% w/v alum) for samples S1A, S2A, and S3A, and Set B (5% w/v tannic acid) for samples S1B, S2B, and S3B. Visual assessment of colour development indicates differential dye fixation among the isolates. Sample S2 exhibited the most intense bright green coloration with both alum and tannic acid mordants, demonstrating superior dye affinity. Sample S3 showed moderate light green coloration, indicating intermediate dye uptake. In contrast, sample S1 displayed a comparatively dull green shade, suggesting lower pigment fixation on the cotton substrate. Overall, the results highlight S2 as the most efficient dye-producing isolate under both mordanting conditions.

Sunlight Stability (Light Fastness): To evaluate the practical viability of the microalgal pigments under environmental stress, the photostability of the dyed cellulosic (cotton) fabrics was rigorously assessed. The mordanted, dyed swatches were subjected to continuous, direct exposure to natural sunlight to simulate standard wear conditions. Visual colorimetric degradation was monitored and documented at precise chronological intervals of 0, 1, 2, and 3 hours. The empirical observations revealed a precipitous decline in colour intensity across the extracts from all three microalgal isolates. Initially, following the tannic acid and alum mordanting process, the fabrics exhibited vibrant, saturated green hues. However, upon exposure to ambient ultraviolet (UV) and visible solar radiation, the extracted chlorophyll-dominant pigments demonstrated acute photochemical vulnerability Within the first hour of sunlight exposure, a noticeable onset of fading and loss of pigment vibrancy was recorded. By the critical 2-hour threshold, the structural integrity of the chromophores was severely compromised; the previously distinct green coloration had visibly dulled and begun to bleach. By the conclusion of the 3-hour trial, the original green pigment had largely degraded, leaving behind only faint, residual yellowish-brown tones on the fabric surface. This rapid rate of photodegradation indicates that the porphyrin ring structure of the extracted chlorophyll molecules is highly susceptible to photo-oxidation when removed from the protective microenvironment of the algal chloroplast and exposed to atmospheric oxygen and UV light. Consequently, the microalgae-derived pigments yielded exceptionally low stability grades on the light fastness scale, underscoring that raw, unencapsulated microalgal extracts currently lack the inherent structural resilience required to withstand standard environmental light exposure in commercial textile applications.



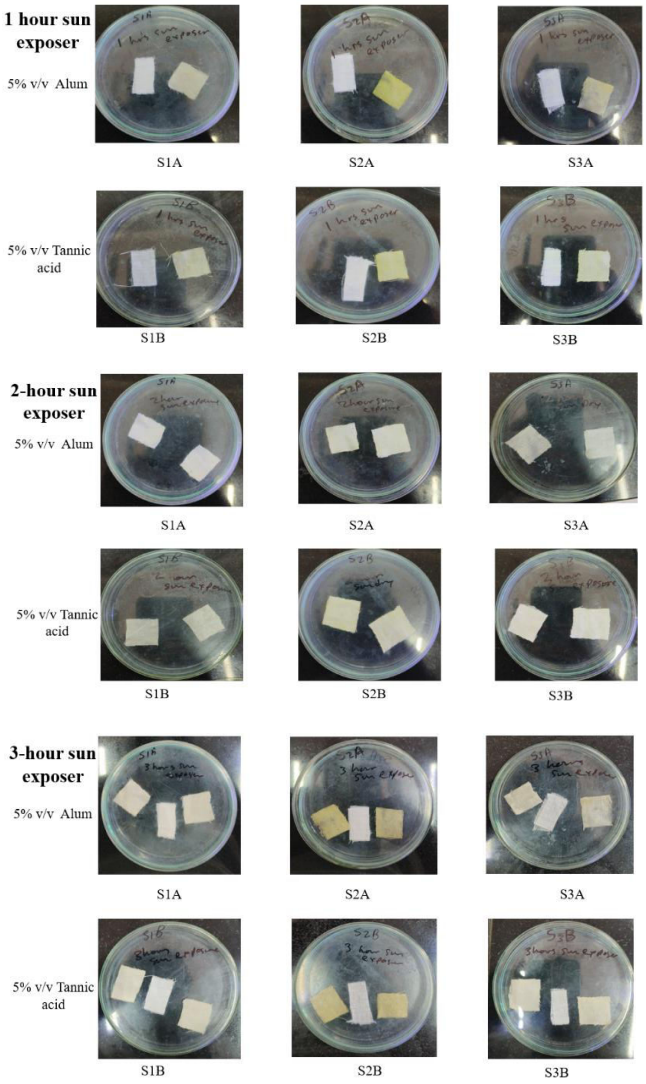


Figure.6 Effect of sunlight exposure on colour stability of algal-dyed cotton fabric

The figure shows cotton samples dyed using algal pigments and mordanted with 5% (w/v) alum (S1A–S3A) and 5% (w/v) tannic acid (S1B–S3B), followed by sunlight exposure monitored at hourly intervals up to 3 hours. Up to 1 hour of exposure, the dyed fabrics retained colour intensity comparable to the 0-hour control, indicating good short-term photostability. After 2 hours, noticeable fading of the green dye was observed, suggesting the onset of photodegradation. By 3 hours, the pigment exhibited clear oxidative changes, with the fabric showing a greenish-brown discoloration, indicative of chlorophyll oxidation and reduced colour stability under prolonged sunlight exposure.

Washing Stability (Wash Fastness)

To systematically evaluate the mechanical and chemical resilience of the dye-fiber bond, a standardized wash fastness assay was conducted on the dyed cotton fabrics. The cellulosic swatches, which were previously pretreated with a dual mordanting system utilizing 5% tannic acid and 5% alum, were fully immersed in an aqueous wash bath formulated with a standard mild detergent. To accurately simulate rigorous physical laundering conditions, the submerged fabric samples were subjected to continuous mechanical agitation throughout the testing period. Visual colorimetric evaluations and photographic documentation were performed at strict chronological intervals of 0, 5, 10, and 15 minutes to track pigment retention. The empirical results demonstrated a rapid and severe loss of pigment retention across the textile samples treated with extracts from all three microalgal isolates.

Initially, the fabrics displayed a vibrant green coloration; however, the combined application of continuous mechanical stress and the chemical surfactant action of the detergent quickly compromised the adherence of the natural dye. By the 10-minute mark of continuous agitation, significant visual fading and dye leaching into the surrounding wash liquor were recorded. By the conclusion of the 15-minute trial, the structural integrity of the dye-fiber complex had fundamentally failed, and the green coloration was almost completely washed out from the cotton substrate.

While all samples exhibited critically low wash fastness overall, comparative visual analysis indicated that the pigment extracted from Sample 2 (*Chlorella spp.*) maintained a slightly higher degree of pigment retention compared to the extracts from Sample 1 (*Tetradesmus spp.*) and Sample 3 (*Scenedesmus spp.*).

Ultimately, this exceptionally poor wash fastness conclusively suggests that despite the tannic acid and alum mordanting pretreatments, the chemical bonding established between the microalgal pigment molecules and the cellulosic cotton fibers remains exceedingly weak. Consequently, while these extracted microalgal pigments demonstrate initial viability as natural colorants, the empirical data confirms they are not currently stable enough for practical, commercial textile applications without significant further optimization of the mordanting and pigment fixation processes.

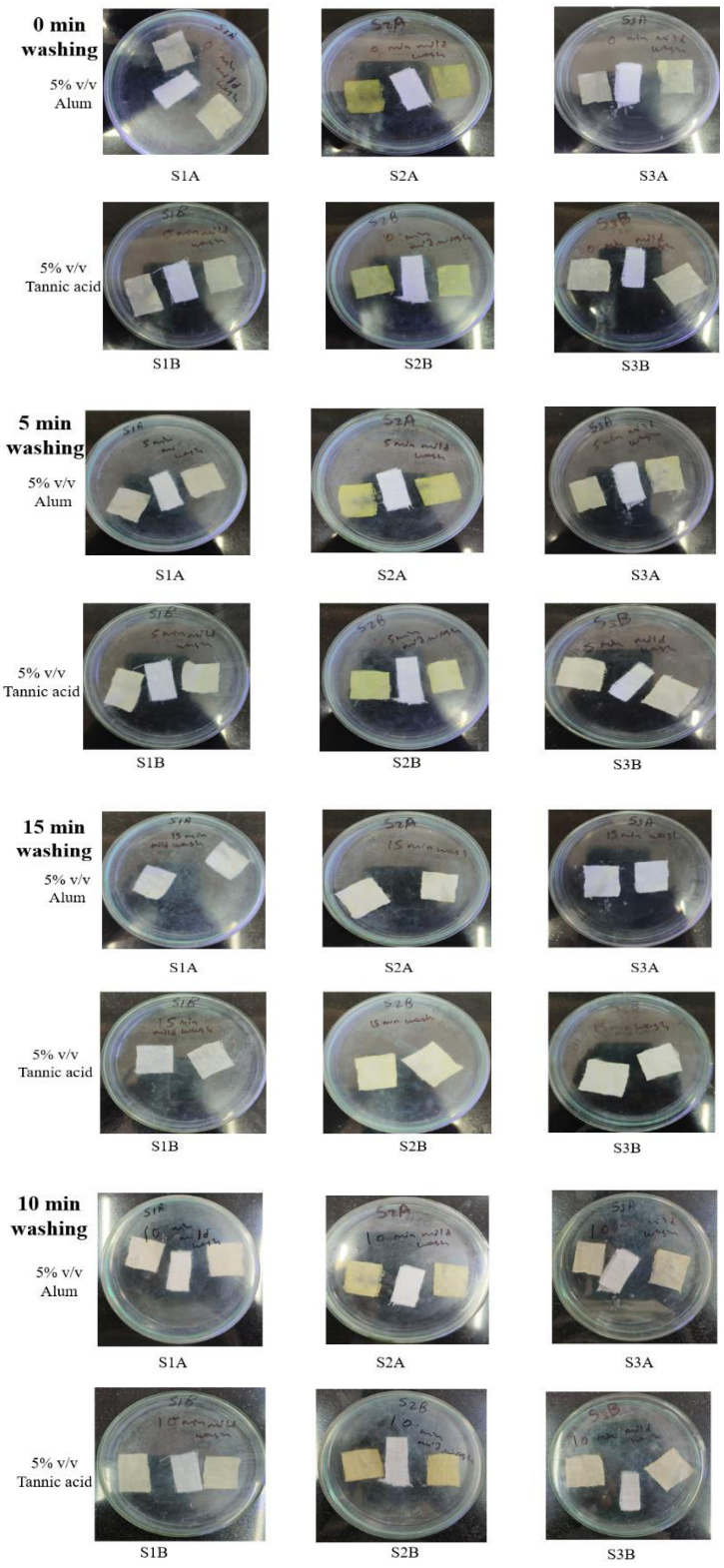


Figure.7 Washing fastness of algal-dyed cotton fabric at different washing intervals

The figure illustrates the washing stability of cotton fabrics dyed with algal pigments and mordanted with 5% (w/v) alum (S1A–S3A) and 5% (w/v) tannic acid (S1B–S3B). Washing was performed up to 15 minutes, with observations recorded at 0, 5, 10, and 15 minutes. After 5 minutes of washing, the dyed fabrics appeared slightly brighter compared to the 0-minute samples, likely due to the removal of excess mordant or loosely bound pigments from the fabric surface. At 10 minutes, gradual fading of the colour became noticeable. By 15 minutes, significant colour loss was observed in some samples; S1 showed marked fading, S3 was almost completely faded, while S2 retained a light green coloration, indicating comparatively better washing stability of the pigment from sample S2.

Media Suitability and Micronutrient Dynamics

The exclusive success of the BG-11 enrichment medium for the isolated indigenous strains (*Tetradesmus spp*., *Chlorella spp*., and *Scenedesmus spp*.) is primarily attributed to its specific macronutrient and physiochemical profile. BG-11 was formulated with a high baseline nitrogen concentration (1.5 g/L $NaNO_3$, equivalent to approximately 247 mg/L of nitrogen) and an alkaline pH ranging from 7.5 to 8.0. This alkalinity significantly enhances the availability of dissolved inorganic carbon in the form of bicarbonate (HCO_3^-, which is highly favourable for green algae belonging to the *Scenedesmaceae* and *Chlorellaceae* families. Furthermore, the inclusion of EDTA as a chelating agent ensures that critical trace metals such as iron, manganese, zinc, and copper remain bioavailable without precipitating as insoluble hydroxides or carbonates in the alkaline environment (Gabrielyan et al., 2026).

Nitrogen Dynamics and Metabolic Limitations

Nitrogen availability is a fundamental driver of microalgal growth and pigment biosynthesis, as nitrogen atoms form the core structure of the tetrapyrrole ring in chlorophyll molecules. The optimization trials demonstrated that increasing the $NaNO_3$ concentration to 2.25 g/L successfully relieved baseline nutrient limitations, allowing cells to allocate nitrogen effectively toward protein synthesis and chlorophyll accumulation, resulting in a 37% increase in biomass compared to the standard BG-11 formulation.

However, exceeding this 2.25 g/L optimal threshold proved physiologically detrimental. The continuous decline in biomass and pigment yield at concentrations of 2.55 g/L, 2.70 g/L, and 3.00 g/L suggests that hyper-concentrated $NaNO_3$ induced severe osmotic stress, reducing cellular water availability and impairing metabolic processes. Additionally, excessive nitrate uptake is accompanied by the release of hydroxyl ions (OH^-), leading to extreme pH fluctuations that can limit carbon dioxide availability and inhibit photosynthesis. At these elevated concentrations, nitrogen assimilation likely consumed excessive carbon skeletons and energy, leaving insufficient metabolic resources for other critical cellular processes, thereby stunting proliferation (Wizi et al., 2022; Hamidian et al., 2022; Degala et al., 2025).

Solvent Polarity and Pigment Yield Discrepancies

The superior extraction efficiency of 80% aqueous acetone is governed by the polarity characteristics of both the solvent and the target pigments. Acetone acts as a polar aprotic solvent with intermediate polarity (dielectric constant \epsilon=20.7), making it highly effective at solubilizing moderately polar chlorophyll molecules (due to their coordinated magnesium ion and phytol tail) as well as lipophilic carotenoids. The 20% water component is critical; it hydrates and disrupts the microalgal cell membranes through osmotic stress while preventing the aggregation of polar pigments. This biphasic capability allowed it to significantly outperform highly non-polar solvents like hexane, which only solubilized the most lipophilic fractions. Furthermore, ultrasonic-assisted extraction (UAE) amplified this efficiency by utilizing acoustic cavitation to generate localized high-pressure zones that mechanically sheared the robust cell walls, facilitating rapid pigment release.

Despite achieving successful extraction, the absolute pigment yields obtained from these indigenous isolates were substantially lower than literature values for optimized commercial strains. For example, the indigenous *Chlorella spp*. produced 410.6 µg/g of total chlorophyll, whereas optimized *Chlorella* strains have been reported to yield up to 60,000 µg/g representing an approximately 146-fold discrepancy. Similarly, the *Tetradesmus spp*. yielded 112.0 µg/g compared to literature values of 10,080 µg/. This massive variance highlights the physiological limitations of cultivating wild-type strains under standard laboratory conditions. The cultivation protocol utilized a batch mode with relatively low light intensity (50 µmol photons m^{-2} s^{-1})

and lacked atmospheric carbon dioxide (CO_2) supplementation, which is often a limiting factor for photosynthetic activity. Crucially, high-value pigments, particularly carotenoids, accumulate to commercial levels primarily under targeted abiotic stress conditions such as severe nitrogen starvation or high light intensity which were deliberately omitted in this baseline growth study *(Porra et al., 1989; Balasubramaniam et al., 2024; Kerner et al., 2025)*.

Chemical Limitations in Textile Fastness and Structural Stability

The most significant barrier to utilizing microalgal pigments in commercial textiles is their inherent chemical and photochemical instability. The critically poor wash fastness observed during the trials indicates that the pigment-fiber interactions are structurally weak. Cotton is a cellulosic fiber composed of glucose polymers with hydroxyl functional groups. Chlorophyll molecules lack reactive functional groups capable of forming robust covalent bonds with cellulose; instead, they rely on weak van der Waals forces and hydrogen bonding, which are easily disrupted and hydrolyzed by aqueous detergent solutions and micelle formation. While tannic acid and alum were utilized as mordants to create coordinating bridging complexes between the dye and the fiber, these aluminum-tannate bridges proved insufficiently stable against mechanical agitation in surfactant environments.

Equally problematic is the severe failure in light fastness, which is driven by the inherent photochemical reactivity of the chlorophyll molecule. Chlorophylls are evolutionary designed to absorb high levels of light energy. In a living microalgal cell, this energy is safely funnelled into the photosynthetic electron transport chain, protected by an organized chloroplast structure and neutralizing antioxidant carotenoids. However, once extracted and applied to an air-exposed fabric, the unsupported porphyrin ring is highly vulnerable. Ambient UV light and abundant molecular oxygen trigger rapid photo-oxidation, generating destructive singlet oxygen and reactive oxygen species (ROS) that aggressively attack and cleave the tetrapyrrole ring structure. This structural degradation directly results in the rapid bleaching, dulling, and formation of colourless breakdown products observed on the dyed textiles. Overcoming these foundational chemical vulnerabilities will require advanced stabilization strategies, including polymeric encapsulation, cross-linking agents, or synthetic chemical modification of the pigment structures (*Linhares et al., 2016; Wizi et al., 2022; Zhang et al., 2024; Kwan et al., 2022; Ghazal et al., 2025*).

In conclusion, this study successfully demonstrated the viability of bioprospecting indigenous freshwater microalgae from local Mumbai habitats as a sustainable, renewable source of natural pigments for textile applications. Three distinct green microalgal species were isolated and identified: *Tetradesmus spp*., obtained as an enriched culture from the Microbiology Department of Khalsa College; *Chlorella spp*., isolated from a domestic fish tank aquarium; and *Scenedesmus spp*., isolated from Bhavan's College Lake.

The study established that BG-11 is a highly effective, versatile culture medium for these indigenous Chlorophyceae strains. Nitrogen availability was identified as a critical driver for both biomass and pigment biosynthesis; cultivating the strains with a 2.25 g/L sodium nitrate ($NaNO_3$) concentration yielded the absolute optimal results. Under these specific nutritional parameters, maximum dry biomass yields of 0.428 g, 0.251 g, and 0.473 g were achieved for *Tetradesmus spp., Chlorella spp.*, and *Scenedesmus spp*., respectively.

For downstream processing, ultrasonic-assisted extraction utilizing 80% aqueous acetone proved to be a highly efficient method for intracellular pigment recovery, significantly outperforming highly non-polar solvents like chloroform and hexane. Quantitative spectrophotometric analysis confirmed that the extracts were chlorophyll-dominant, with the *Chlorella spp*. isolate exhibiting the highest overall pigment content, yielding 116.0 µg/g of Chlorophyll a and 294.6 µg/g of Chlorophyll b (totalling 410.6 µg/g of dry biomass). While these extractions were successful, comparative analysis revealed that the absolute pigment concentrations derived from these wild-type indigenous isolates were substantially lower ranging from 30- to 260-fold than those reported for domesticated commercial strains. This discrepancy underscores the necessity for targeted physiological stress induction (such as nitrogen starvation or high light intensity), the implementation of carbon dioxide (CO_2) supplementation, and the transition from batch to semi-continuous cultivation modes to maximize secondary metabolite accumulation.

Crucially, while the extracted microalgal pigments successfully imparted a visual green coloration to cotton fabrics utilizing alum and tannic acid mordants, rigorous stability assessments exposed severe limitations. The dyed cellulosic textiles demonstrated critically poor wash fastness, experiencing substantial colour loss within 15 minutes of mechanical washing, alongside severe photodegradation and limited light fastness under continuous ambient sunlight exposure. These inherent chemical vulnerabilities represent the primary barrier to the commercial application of chlorophyll-based microalgal pigments in the textile industry.

Ultimately, while microalgae present an environmentally sustainable alternative to synthetic, petrochemical-derived dyes offering rapid growth rates, minimal freshwater dependency, and a drastically reduced ecological footprint their raw pigments lack the structural stability required for practical textile integration. Future research and development must prioritize advanced stabilization strategies, including polymeric encapsulation, synthetic chemical modification of the pigment structures, the application of UV absorbers, and the optimization of robust nano-suspension mordanting systems, to fully realize the commercial potential of microalgal pigments.

Author Contributions

Aarif Riyazuddin Ansari: Investigation, formal analysis, writing—original draft. Lavina Pinto: Validation, methodology, writing—reviewing. S. V. Raut:—Formal analysis, writing—review and editing.

Data Availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethical Approval Not applicable.

Consent to Participate Not applicable.

Consent to Publish Not applicable.

Conflict of Interest The authors declare no competing interests.

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