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PII: S2949-8813(26)00016-8

DOI: https://doi.org/10.1016/j.decarb.2026.100162

Reference: DECARB 100162

To appear in: *DeCarbon*

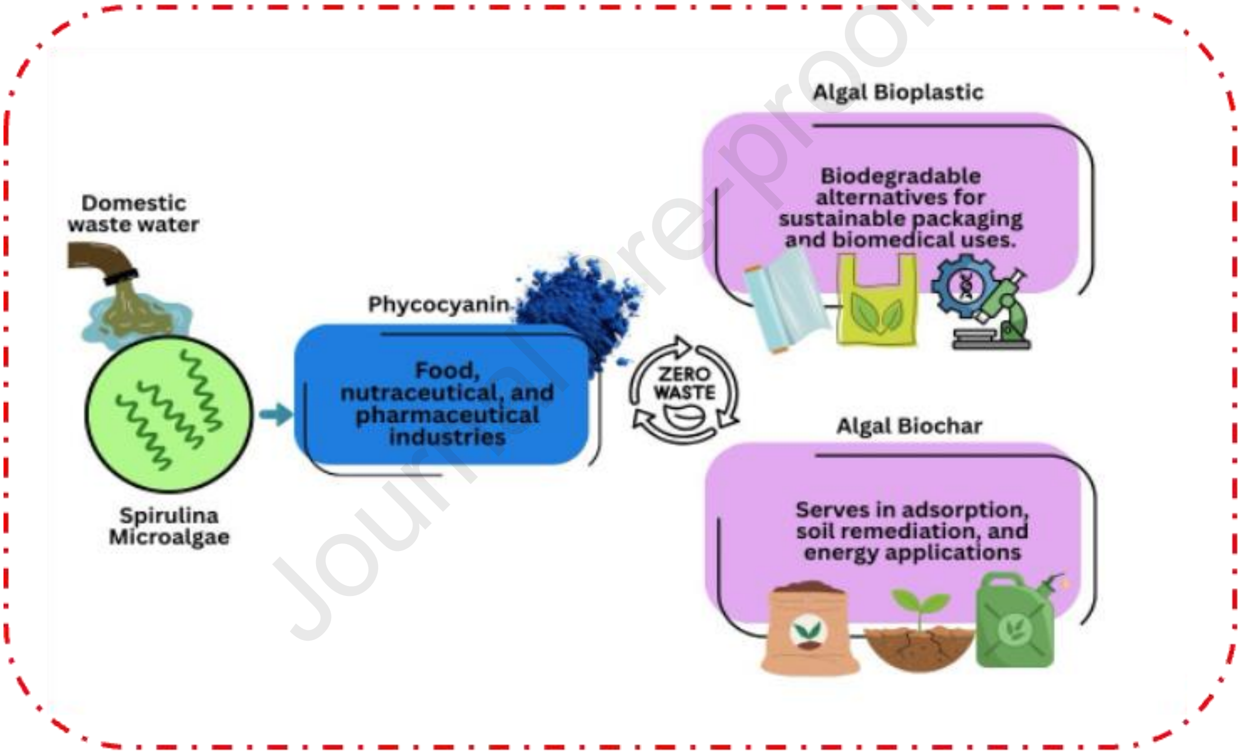
Received Date: 4 February 2026

Revised Date: 30 March 2026

Accepted Date: 1 April 2026

Please cite this article as: A. E, M. Goel, G. Kumar, C. VP, V. Priya V, C. Ngamcharussrivichai, F. G, V. Ashokkumar, Biochar Production with concomitant biopolymer accumulation of Microalgae-Based Wastewater treatment for carbon neutrality, *DeCarbon*, https://doi.org/10.1016/j.decarb.2026.100162.

Graphical Abstract



Biochar Production with concomitant biopolymer accumulation of Microalgae-Based Wastewater treatment for carbon neutrality

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Abstract

Environmental Pollution caused by plastic and wastewater discharge demands sustainable solution. To address this, microalgal species *Spirulina* effectively utilized for domestic wastewater (DWW) valorization and sustainable bioproduct development. During a 16-day cultivation period in DWW, biomass productivity increased steadily reaching a maximum of 0.83 g L^{-1} on day 12. Meanwhile, the methanol-water solvent combination produced the highest amount of pigment phycocyanin of about 22.5 mg/L from the *Spirulina* biomass, this shows that it is possible to recover valuable co-products. The residual algal biomass further utilized for the production of PHAs and biochar. FTIR analysis of *Spirulina*-derived PHA bioplastic showed characteristic peaks at 3270.1 cm^{-1} (O–H), 2979.2 cm^{-1} (C–H), and a strong ester carbonyl band at 1626 cm^{-1}. Additional peaks at 1387 cm^{-1} and 1241.6 cm^{-1} correspond to methylene, methyl, and methine groups, while bands in the 1527–1050 cm^{-1} range further confirm the polymer backbone structure ,indicating functional groups associated with PHA polymers. The PHA-bioplastic film shows a uniform thickness of 0.12±1 mm, water absorption 16%, water solubility 33.21% and balanced chemical resistance, indicating the biodegradability of the material. In addition, torrefaction of the residual biomass produces biochar yield of 31%, demonstrating effective biomass valoårization. Thermogravimetric analysis shows that the torrefied biochar contained lower volatile matter, higher fixed carbon content and improved thermal stability. This integrated approach shows the potential of DWW as an efficient medium for sustainable *Spirulina* cultivation, enabling pigment recovery and valorization of residual biomass into bioplastic and biochar. However, PHA yield optimization, the variability in wastewater composition and detailed product characterization was not yet investigated and could be addressed in future research.

Keywords: Domestic wastewater, *Spirulina*, Polyhydroxyalkanoates, Phycocyanin, Torrefaction, Biochar.

1. Introduction

Recently there has been a huge rise in the number of human-made wastes that ends up in both water and land. This is mostly due to the unregulated growth of cities and industries, which has caused serious environment problems. Among these, plastic pollution and wastewater play a pivotal role in degrading environment and the development of several health diseases. For, instance each year approximately around 460 million metric tons of plastics are produced for a wide range of uses, including in the automobiles industry, for packing, building, textile, electronics, healthcare and food sectors **[1]**.Therefore, escalated growth of plastic industries, along with inadequate plastic waste management has caused surface-level plastic deposition to potentially triple by 2060 **[2]**. Consequently, it is hazardous due to incorporation of chemicals in the production of plastics which has high chances of disrupting endocrine systems **[3].** In addition, Globally, about 359 billion m³ of wastewater were produced annually, though only 52% is treated and the wastewater remediation process also consumes high energy as more places expands water sanitation **[4]**. Consequently, wastewater retains wide range of pollutants such as organic matter, excess nutrients, toxic heavy metals, hazardous organic compounds, pesticides, when these chemicals were released untreated, they result in environmental pollution, harmful algal blooms, habitat destruction, coral reef destabilisation; biodiversity loss waterborne disease outbreaks and economic damage **[5]**. Furthermore, the excessive discharge of toxic chemicals via wastewater harm aquatic life and spread faecal-borne diseases, posing health risks to humans and sanitation workers **[6]**. Therefore, bioplastics serve as an alternative to conventional plastics and can be produced by utilizing wastewater as a resource, provides dual benefits include wastewater valorization and sustainable bioplastic production. For instance, ***EUBIO Admin*** **[7]** reveals that global bioplastic production is projected to rise, supported by emerging end-use industries, this momentum predicts that global biobased plastic production capacity will double from 2.31 million tonnes in 2025 to about 4.69 million tonnes in 2030 indicating the intensifying demand for bioplastic position in global market. Therefore, the most sustainable plastics are those derived from biomass and capable of biodegradation. These biomass-based biodegradable plastics can be categorized as polylactic acid (PLA), starch-based biodegradable plastics, cellulose-based biodegradable plastics, and polyhydroxyalkanoates (PHA) **[8]**.

Among these biodegradable plastic materials, PHAs act as a significant biodegradable polymer, with various environmental advantages due to their inherent degradability and sustainability **[9].** The attributes of PHA biopolymer relies on the composition and distribution of their monomeric units. In accordance with carbon chain length, PHAs are categorized into short chain-length (scl-PHAs, 3–5 carbons), medium chain-length (mcl-PHAs, 6–14 carbons), and long chain-length PHAs (lcl-PHAs, >15 carbons). Variations in chain length influence the crystallinity and tensile strength of PHAs **[10]**. In contrast, the production cost of PHA restricts their wide utilization. For example, the production cost of PHAs ranges from about 2.2–3.7 €/kg, which is comparatively higher than the petrochemical plastics (~1 €/kg), meanwhile the industrial cost to be around 4–6 $/kg, making PHAs about five to six times more expensive. This high cost is mainly due to expensive feedstocks and complex processing required to avoid contamination **[11].** Thus, PHA production from organic sources generated from waste materials offers a significant solution for largescale production. *Spirulina* cultivated from wastewater serves as an efficient feedstock for the sustainable production of PHA.

Furthermore, *Spirulina* can grow rapidly under sunlight and can demonstrate high nutrient uptake while simultaneously generating high-value biomass enriched with carbohydrates and essential nutrients **[12]**. In addition to this, *Spirulina* synthesizes a blue pigment namely C-phycocyanin a water-soluble pigment, possess antioxidant, anticancer and pharmacological properties. Yet, it still remains under-utilized because of low yield and instability of the pigment during purification and storage **[13]**. Moreover, microalgae can produce PHAs autotrophically using CO_2 and sunlight, offering both bioplastic production and carbon sequestration while reducing costs. They can grow on non-arable land and wastewater, enhancing pollutant removal and synthesis of high-value compounds. Although PHA yields are generally low, improving production efficiency remains a key research focus, with recent studies highlighting innovative strategies **[14].** Furthermore, ***Zhila et al.,*** **[15]** study investigates on PHA production from two different microalgal species includes *Chlorella vulgaris* and *Spirulina platensis* which yields PHA polymers up to 20.0%, 16.2% respectively. Followed by PHA extraction, the algal biomass residues have attracted significant interest due to their potential application in biofuels especially biochar and other uses. Numerous studies demonstrate that the thermal conversion of algal biomass into biochar can function as a superior carbon source, capable of being converted into biofuels **[16]**. Microalgae are a significant resource for the production of renewable energy, particularly biofuel as compared to fossil fuels using them reduces CO_2 emission and produces green energy **[17]**. Furthermore, this integrated strategy facilitates establishments of microalgal biorefineries, while it is considered as financially feasible method to achieve sustainable product development **[18].** However, various studies have been done in *spirulina* cultivation using wastewater, yet the effective valorization of the *spirulina* biomass produced from wastewater were unexplored. Therefore, the present study focuses on sustainable cultivation of *Spirulina* by utilizing DWW, for biomass production. The resulting biomass is utilized for the extraction of phycocyanin, followed by this the residual biomass after phycocyanin extraction can be used for the production of bioplastic and biochar. This integrated approach combines wastewater utilization, high value pigment extraction and valorization of residual biomass into bioplastic and biochar, thereby contributing to the development of zero-waste biorefinery system. The overall schematic representation of the present work was shown in **fig 1**.

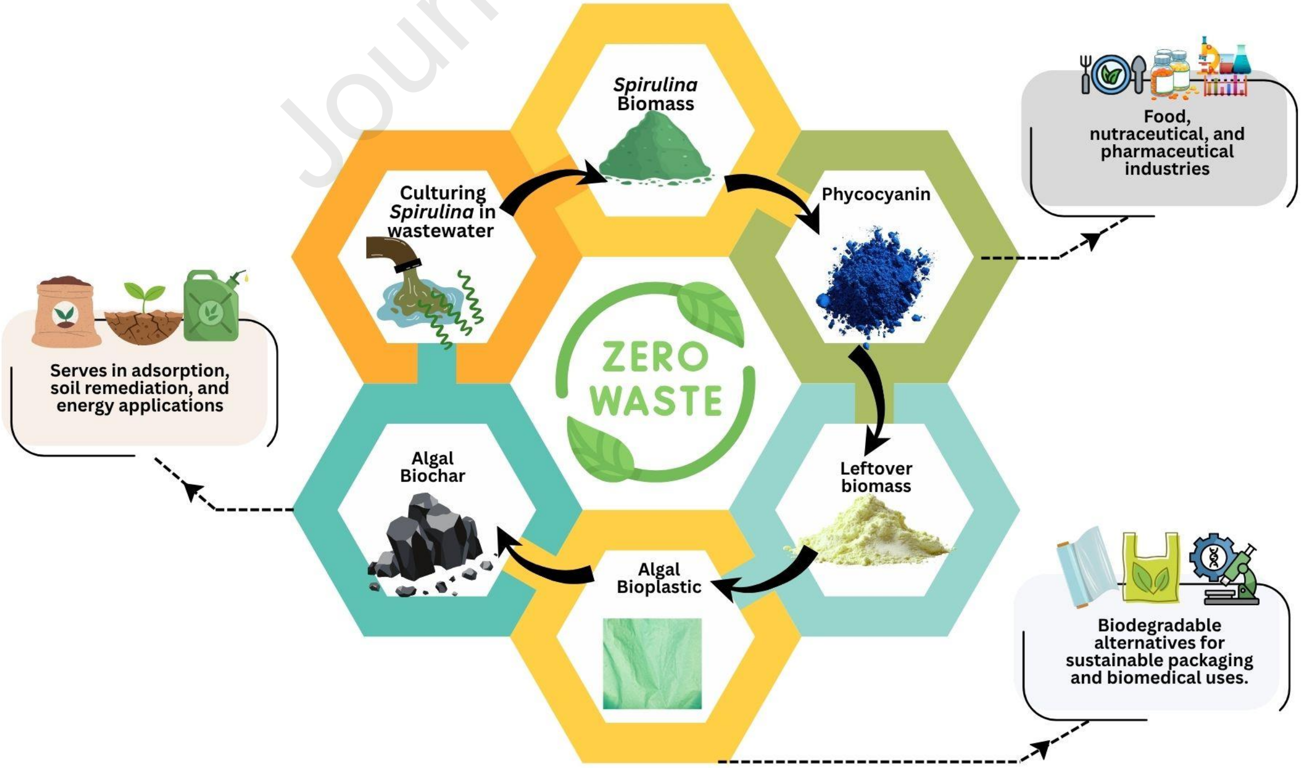


Fig.1. Overall schematic representation of valorization of *Spirulina* biomass for various high value- added products development.

2. Materials and methods

2.1. Collection of domestic waste water

The DWW was collected from Saveetha University, and it was undergone both primary and secondary treatment, then the treated DWW taken for algal cultivation. The collected wastewater sample was studied for physicochemical characterization by the Tamil Nadu Water Supply and Drainage Board (TWADB), in accordance to Indian Standards (IS) and American Public Health Association (APHA), finally the results were evaluated against Central Pollution Control Board (CPCB) standards to determine potential and compliance for algal biomass cultivation.

2.2. Microalgae culturing using wastewater

The alga *Spirulina sp.,* cultures were sourced from the Centre for Waste Management and Renewable Energy, Chennai, Tamil Nadu. The microalgae were grown in Zarrouk medium, comprising of macro-nutrients including NaCl, $CaCl_2$, $NaNO_3$, EDTA, $FeSO_4$, K_2SO_4, $MgSo_4$, $NaHPO_4$, K_2HPO_4, and micro-nutrient such as H_3BO_3, $ZnSO_4$, $MnCl_2$, $NaMoO_4$ and $CuSo_4$ purchased from SRL (Chennai). Cultivation was conducted in 1L Erlenmeyer flasks under axenic condition with the medium adjusted to pH 10 to 11 and maintained at a temperature range from 22-24° C. The culture was subjected to a controlled light regime of 16 h of continuous white light illumination followed by 8 hours of darkness. To ensure homogeneity in nutrient availability and light exposure, manual agitation was performed every 2 h. The lab grown *Spirulina* was subsequently acclimatized using DWW for further scale up process.

2.3. Cultivation of microalgae *Spirulina* in mini open raceway

The mini open raceway pond was designed for 20 L capacity with 85cm length × 18cm breath × 13cm. The initial inoculum strength was maintained up to 20 mg/L (cell dry weight). This experimental study was carried out for a period of 16 days under natural sun light. The raceway pond was equipped with a motorized four-blade paddle wheel at 40 rpm to maintain uniform mixing and surface flow. The cultivation system was operated under open pond conditions. The culture temperature was governed by ambient environmental conditions and ranged from 22 to 30 °C. Natural sunlight served as the primary light source for the cultures, while continuous mixing was provided by a paddlewheel rotating at 40 rpm to maintain uniform circulation of the culture medium. Unialgal conditions were maintained through regular microscopic checks to ensure culture purity. Evaporation rate during daytime was frequently monitored and compensated by replenishing with treated wastewater.

2.4. Biomass productivity

Algal growth was assessed every 4 days interval. The biomass yield was determined using the gravimetric method and calculated using **equation 1** and specific growth rate μ (div. d^{-1}) was calculated using **equation 2.**

$$\text{Biomass productivity (wt\%)} = \frac{\text{N2}-\text{N1}}{\text{t2}-\text{t1}} \quad \textbf{(1)}$$

Where, N2 represent the weight of the fresh biomass, N1 denotes the dry biomass weight, and (t_2-t_1) corresponds to the total duration of the cultivation period.

$$\text{Specific algal growth rate } \mu\ (\text{div. d} - 1) = \frac{In\,X2 - In\,X1}{\text{t2}-\text{t1}} \quad \textbf{(2)}$$

Where, X_2 refers to the final biomass concentration, X_1 is the initial biomass concentration and (t_2-t_1) represents the time interval over which growth was measured.

2.5. Phycocyanin extraction and quantification from *Spirulina* using various solvents

One gram of dried *Spirulina* biomass was suspended in 50 mL of various solvents including water, methanol, chloroform, hexane and methanol-water mixture (1:1). This biomass was mechanical pretreated to induce cell disruption, after 24 h stirring was performed to increase the phycocyanin extraction. For the final disruption of the cells, the algal samples were centrifuged using a Thermo Scientific Multifuge ×3R centrifuge at 8000 rpm for about 15 mins at 4° C. After centrifugation, the supernatants containing extracted phycocyanin were collected for analysis.

Phycocyanin concentration in each extract was quantified spectrophotometrically, absorbance measurements were taken at 620 nm and 652 nm, the peak absorption wavelength of phycocyanin. The concentration of C-PC was calculated with the **equation 3**:

$$\text{C} - \text{PC}\left(\frac{\text{mg}}{\text{mL}}\right) = \frac{Abs620 - 0.474\,(Abs652)}{5.34} \quad \textbf{(3)}$$

Abs_{620} is absorbance at 620 nm, Abs_{652} at 652 nm, 0.474 is correction factor accounting for pigment overlap and 5.34 is the empirically derived extinction coefficient specific to C-PC.

The **equation 4** was applied to compute the final yield of phycocyanin, relative to biomass.

$$\text{Yield } (\frac{\text{mg}}{\text{g}}) = \frac{C-PC \times Volume}{\text{Biomass}} \quad \textbf{(4)}$$

2.6. PHA extraction from *Spirulina* biomass

The residues after extraction of phycocyanin was used here in order to extract the PHA, which was physically grinded using a mortar and pestle. Following cell lysis, the hydrolysis occurred using 4% sodium hypochlorite and kept 1 h at 37° C. Which selectively removes non-PHA components. The residual impurities were rinsed thoroughly using distilled water and acetone. Finally, the purified PHA components were solubilized using chloroform for stable yield of dry polymer mass. At last, the Fourier Transform Infrared Spectroscopy (FTIR) was employed to assess the composition and structural integrity of the recovered biopolymer.

2.7. Production of Biodegradable Plastics

The bioplastic was synthesized by blending extracted algae-based PHA, gelatine, sorbitol of 2.70% equally, additionally the plasticizing solution of glycerol 1.80% and distilled water 90.10% was employed to attain optimal flexibility, texture and uniformity. The homogenate was combined well and heated to a high temperature with uninterrupted stirring. The heating was stopped once the desired consistency required, stirring was continuously prolonged for a while. The warm mixture was transferred onto a sterilized glass petri plates and evenly distributed to achieve a uniform layer. After thorough drying, the bioplastic sheet was detached from the glass petri plate resulting in a flexible sheet for characterization. Followed by a commercially available bioplastic was concurrently prepared simultaneously processed to examine its moulding and plasticizing properties under equivalent conditions.

2.8. Physical, Chemical Characterization of *Spirulina* Bioplastic

2.8.1 Physical Characterization

2.8.1.a. Thickness

An electronic vernier Caliper (0-6 inch/ 0-150 mm) with LCD display was used to measure the thickness of the bioplastics. In order to check the thickness of the synthesized algal-derived bioplastic, commercially bioplastic and polyethylene, the films were sectioned into 1×1 cm pieces for the evaluation. The thickness was measured at the random site on each film was further recorded. Following this, the average thickness of each film was calculated and employed in additional testing evaluation.

2.8.1.b. Solubility

Samples were sectioned into 1×1 cm square for solubility assessment, during which the initial weight (W0) was recorded prior to water immersion. Following immersion in 20 mL of aqueous solution within a sterile petri dish for about 24 h, the sample were dried and their final dry weight (W1) was recorded while, solubility was determined utilizing **equation (5).**

$$Water\ solubility\ \% = \frac{W0-W1}{W0} \times 100 \quad \textbf{(5)}$$

2.8.1.c. Water absorption percentage

Each sample was sectioned into uniform 1cm^2 pieces, prior to immersion the initial weight of the samples was recorded (M0). Consequently, the sample was immersed in a 20 mL aqueous solution for roughly 24 h, after immersion the samples were carefully removed and thickness were measured (M1) using vernier Caliper. The water absorption percentage was calculated using **equation (6)**.

$$Water\ absorption\ \% = \frac{M1-M0}{M0} \times 100 \quad \textbf{(6)}$$

2.8.2 Chemical Characterization

2.8.2.a. Bioplastic chemical sensitivity utilizing different solvents

The chemical stability of the bioplastic film as well as commercially available plastic was evaluated, by immersing 1×1 cm piece of test samples in 0.1N HCl (acid), 0.1N NaOH (alkaline), NaCl (saturated salt solution) and 50% ethanol (organic solvent) for an about 24 h. Meanwhile, the morphological changes occurring during incubation was observed and documented.

2.9. Biochar production from phycocyanin extracted biomass

The residual biomass obtained from the post phycocyanin extraction process was further utilized for biochar production. Biochar production was carried out by torrefaction method, in which the biomass first dried in a hot air over at 120°C for 4 h to remove moisture present in the residues. After drying, the biomass was allowed to cool and was further underwent torrefaction at 250°C in an inert nitrogen environment (99.99 vol%) with a constant gas flow rate of 20 mL min^{-1}. Upon concluding the torrefaction procedure, the resultant torrefied biochar was pulverized to achieve a fine and uniform particle size.

2.9.1. Proximate analysis

Thermogravimetric analysis of the *Spirulina* biochar was conducted to evaluate its thermal behaviour and composition. Thermogravimetric analysis (TGA) assesses the transition of mass with increasing temperature under a controlled atmosphere. From TG and DTG curves, parameters such as volatile matter, ash content, and fixed carbon can be evaluated.

3.Results and discussion

3.1. Biomass productivity of *Spirulina* in wastewater

In order to determine if the DWW was suitable for reuse, the current study evaluated its physicochemical characteristics and compared them to the permitted limits set by IS 10500:2012. Turbidity (1.2 NTU) and total dissolved solids (2808 mg/L) are examples of physical characteristics. Chemical characterization pH (7.3), total alkalinity as $CaCO_3$ 356 mg/L, total hardness as $CaCO_3$ 570 mg/L, other necessary nutrients as calcium 108 mg/L, magnesium 73 mg/L, sodium 588 mg/L, potassium 35 mg/L, iron 0.10 mg/L, free ammonia 3.90 mg/L, nitrite 1.37 mg/L, fluoride 0.40 mg/L, sulphate 268 mg/L, and phosphate 0.29 mg/L. The microalgae species may potentially show increased biomass productivity under these circumstances. The laboratory-grown *Spirulina* culture was initially evaluated under laboratory-scale conditions using different concentrations of DWW acclimatization ratios (25%, 50%, 75%, and 100%), while 100% Zarrouk's growth medium served as the control. Based on laboratory observations, the cultivation system was scaled up to a larger unit to assess its practical performance. Therefore, the large-scale cultivation of DWW shows that over a 16 days span of cultivation period in which *Spirulina* exhibited a significant rise in the biomass concentration. Initially, the growth was very low at starting period of cultivation with 0.002 (g L^{-1} d^{-1}) on day 1. By the day 4, the biomass concentration increased to 0.12 (g L^{-1} d^{-1}) with a sharp increase of 0.65 (g L^{-1} d^{-1}) on the day 8. The highest yield was recorded on day 12 with 0.83(g L^{-1} d^{-1}), after which a slight reduction to 0.78 (g L^{-1} d^{-1}), was observed on day 16 marking the transition of stationary phase of growth **(fig 2)**. This steady growth suggests proficient utilization of the nutrients in the wastewater highlighting as an efficient and medium of sustainable *Spirulina* production. Similarly, the study of ***Chaiklahan et al.,*** **[19]** reported a productivity of *Arthrospira platensis* using photobioreactor under semi-continuous conditions of about 0.62 (g L^{-1} d^{-1}), although the photobioreactor maintained consistent productivity of biomass yield, the open cultivation system in the present study achieved a higher peak of biomass concentration promoting minimal energy usage and sustainability through wastewater recycling. This comparison states the significant utilization of open cultivation system for expandable biomass production notably under economically or resource challenged setups. In a related to lab-scale cultivation the study by ***Rodas-Zuluaga, Mora-Godínez and Pacheco.,*** **[20]** the biomass productivity was improved by addition of 25% CO_2 for culture enrichment, achieving concentration of 0.18±0.01 (g L^{-1} d^{-1}) *Desmodesmus abundans* and 0.21±0.01 (g L^{-1} d^{-1}) *Chlorella vulgaris,* respectively. Although this biomass values reported above were lower than the current study, were the controlled CO_2 enrichment in lab-scale setup promotes a promising cultivation strategy for boosting biomass production which collectively demonstrate adaptable nature of algal cultivation systems. While the consistency and precision are the hallmarks of the closed system which ensures the stable yield in the biomass, whereas the open system using wastewater offers significant yield in the biomass production. The microalgae are autotrophic, photosynthetic microorganisms that have attracted considerable interest because of their rapid growth rate, high photosynthetic efficiency and ability to produce a wide range of bioactive metabolites **[21]**. In this context of wastewater treatment and bioenergy generation, they offer significant potential since the organic pollutants presents in wastewater can act as carbon sources, while inorganic compounds provide essential nutrients to support **[22]**. The current study demonstrated effective biomass accumulation of *Spirulina* cultivated in domestic wastewater, reaching a peak concentration of 0.83 (g L^{-1} d^{-1}) on the day 12. This result reflects strong nutrient utilization and adaptability to open system conditions. Comparatively, **Hassan et al., [23]** investigated the growth of *Tetradesmus obliquus* in three different wastewater streams raw wastewater (RW), recycled wastewater (RE) and final effluent (EF) and reported the highest biomass yield of 1.59 (g L^{-1} d^{-1}) on the day 16th in a blended medium of 75% RW and 25% RE. While the biomass concentration reported in **Hassan et al., [23]** was twice as high as in the present study which was optimized using different types of wastewaters with longer cultivation period. The disparity in biomass productivity is due to variations in species selection, wastewater characteristics and most specifically cultivation strategies. Therefore, both the studies affirm the potential of utilizing wastewater as a nutrient source for the algae growth, while *Tetradesmus obliquus* favoured optimized condition whereas, in the current study *Spirulina* proved to be an effective in open systems which highlights its sustainable and practical potential. The microalgal culture showed a maximum biomass concentration of 0.83 g L^{-1} on day 12. The calculated specific growth rate (μ) was 0.50 day^{-1}, with a doubling time of 1.38 days and 0.73 cell divisions per day. The overall biomass productivity during the exponential phase was 0.069 g L^{-1} day^{-1} **(Table 1)**. This consistent growth indicates that the wastewater is being utilized effectively in terms of its nutrient content, showcasing the wastewater as a medium that is both efficient and sustainable for the production of *Spirulina*. The performance of the current investigation was compared with standard published article for both biomass production and nitrogen removal efficiency. For instance, a membrane photobioreactor (MPBR) system that was utilized in order to cultivate the microalgae *Chlorella vulgaris* which had a volumetric biomass productivity of 42.6 mg L^{-1} d^{-1} and also showed strong nutrient removal rates, with 86.1% for total nitrogen (TN) and 82.7% for total phosphorus (TP) **[24]**. Following this, **Jialing Tang et al., [25]** conducted a study that utilized an anaerobic membrane effluent and municipal wastewater. The results of this study demonstrated that 40% of the anaerobic membrane effluent had a biomass productivity of 52.9 mg L^{-1} d^{-1} under optimal conditions. Furthermore, the microalgal systems that were based on wastewater had high nutrient removal efficiencies of approximately 97% for ammonia and 90% for phosphate. This study demonstrates the efficacy of wastewater-based microalgal systems for simultaneous mass production and the removal of pollutants. **Álvarez-González et al.'s [26]** used *Synechocystis sp. Phormidium sp* and *Scenedesmus sp* in Secondary urban wastewater in which the *Scenedesmus sp.* biomass productivities were found to be 48 and 73 mg L^{-1} d^{-1}, respectively. Followed by effective nutrient removal with a reduction of 73% nitrogen and 59% phosphorus, highlighting the importance of adjusting growing conditions to achieve the best possible balance between biomass production and nutrient removal performance.

Parameters	**value**
Specific growth rate (μ)	0.50 day^{-1}
Doubling time (td)	1.38 days
Divisions per day (k)	0.73 day^{-1}

Table 1. **Growth Kinetics of *Spirulina* sp. Cultivated in Domestic Wastewater**

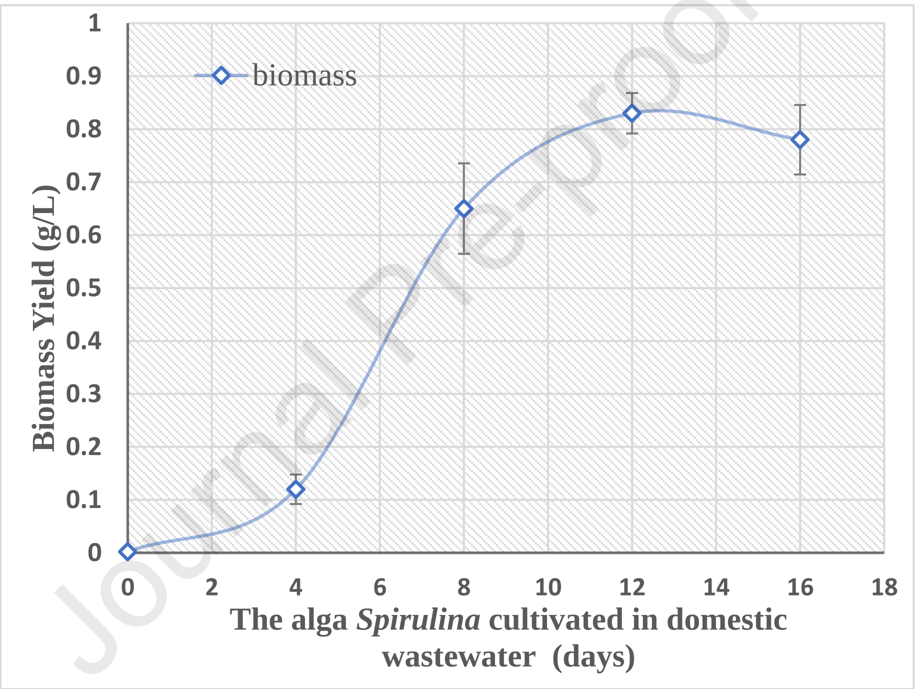


Fig.2. Biomass productivity of *Spirulina* cultivated in domestic wastewater, measured at 4 days interval over a 16 days period.

3.2. Extraction and Yield of Phycocyanin from *Spirulina* sp.

Among the tested solvents, extraction with methanol-water combination yielded the most substantial phycocyanin with 22.5 mg/L. This was followed by water, showed a notable concentration of about 20 mg/L. Comparatively, lower yields were observed with methanol at 17.2 mg/L and chloroform at 11.6 mg/L. Finally, the lowest extraction of phycocyanin was observed with hexane at approximately 4 mg/L **(fig 3)**. Therefore, the result indicate that the methanol-water combination emerged as the most potent solvent for the extracting phycocyanin from *Spirulina,* with water being the next option for the effective extraction of phycocyanin. This is due to compatibility and polarity nature of solvent, which significantly influence the efficiency rate of extraction. In contrast, hexane a non-polar solvent is being less effective for phycocyanin extraction. Compared to other solvent, the methanol-water mixture yielded a higher pigment extraction rate, highlighting the critical role of solvents polarity influence in improving phycocyanin solubility and extraction rate **[27]**. This is due to phycocyanin's water solubility and highly polar nature, which allows it to dissolves most effectively in the solvents of intermediate polarity, such as methanol combined with water preserving its structural integrity **[28]**, where solvent integration enhances the binding interaction with hydrophilic sites on phycocyanin, supporting efficient permeability and solubility functionality within *Spirulina* cell matrix **[29]**. The solvent polarity is directly or strongly correlated with the dielectric constants (ε), demonstrating that greater values are more proficient in stabilizing molecules which are polar. Unlike nonpolar solvents, methanol which possesses a polarization coefficient, displays a significant hydrogen-bonding were this attribute to easier elimination of non-pigmented compounds. Similarly, the yield of extraction are also shows that the boiling points of the solvents affects the procedure, this is because the methanol (67°C) is less volatile than the water (100°C), which permits less pigment degradation and advances the mass transfer mechanisms **[30]**. These results suggest that the molecular affinity, polarity, and boiling point of solvents can directly or indirectly influence the purity and yield of *Spirulina* phycocyanin. Moreover, earlier studies utilize various methods to extract phycocyanin, **Salazar-González et al., [31]** study reports a phycocyanin yield of 37.0 ± 1.9 mg/g, but it involves ultrasound-assisted extraction and membrane filtration, which increase process complexity, operational cost, and may limit scalability despite the moderate yield. In addition, **Lin et al., [32]** study reveals under optimized conditions and solvent combination of glycerol and glucose maximum yields 67.86 mg/g, although higher phycocyanin yields have been reported this method involves complex extraction techniques and requires higher cost. In contrast, present study involves simpler methanol-based extraction approach coupled within a biorefinery framework, using residual biomass for bioproduct development.

Beyond solvent selection, process optimization strategies can further enhance production efficiency and economic feasibility. For example, **Kumar et al., [33]** showed that use of specially designed membranes in upstream and downstream, moreover they are low-cost and eco-friendly separation, purification and concentration enrichment of algal by products Collectively, these findings highlight that integrating solvent selection with process optimization can maximize both yield and quality. In this context, the methanol–water mixture stands out as the simpler extraction solvent, as it ensures superior cell permeabilization, preserves pigment integrity. Furthermore, polar solvents like methanol, ethanol, aquadest were used for polar pigment extraction, the employment of these solvents will extract polar compounds in dry cells of *Spirulina plantensis* **[30]**. The removal of polar components from *spirulina* biomass by phycocyanin extraction process enhance the suitability of residual biomass for further valorization into bioplastic and biochar.

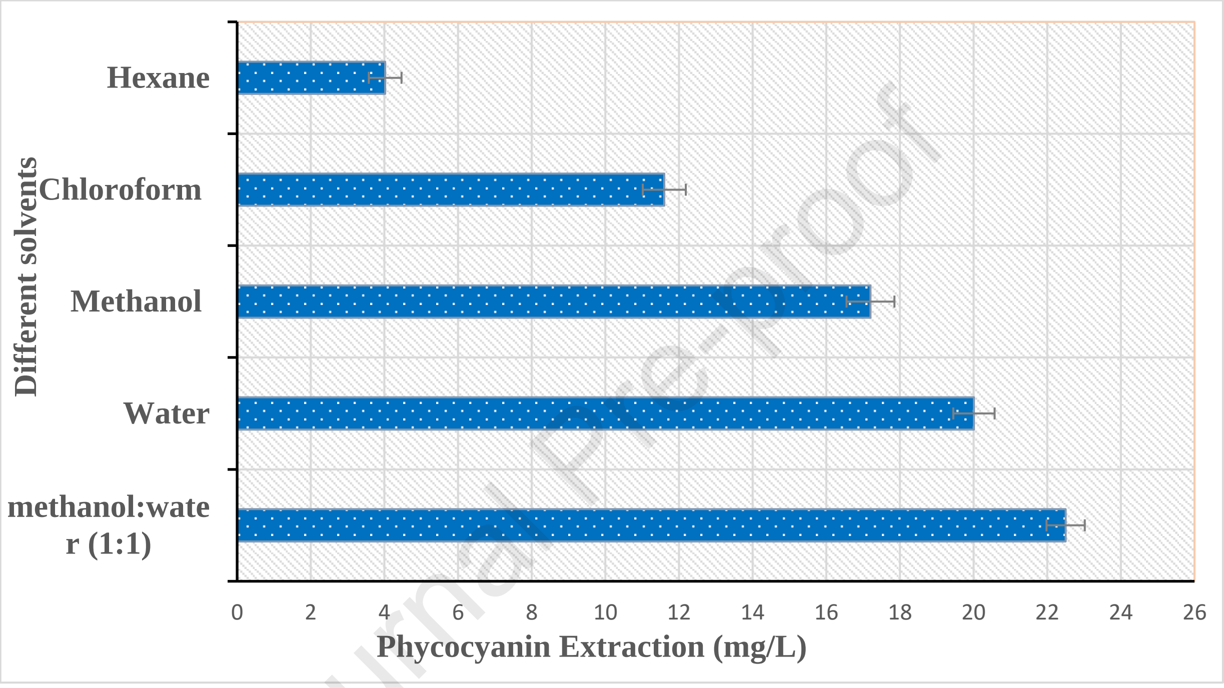
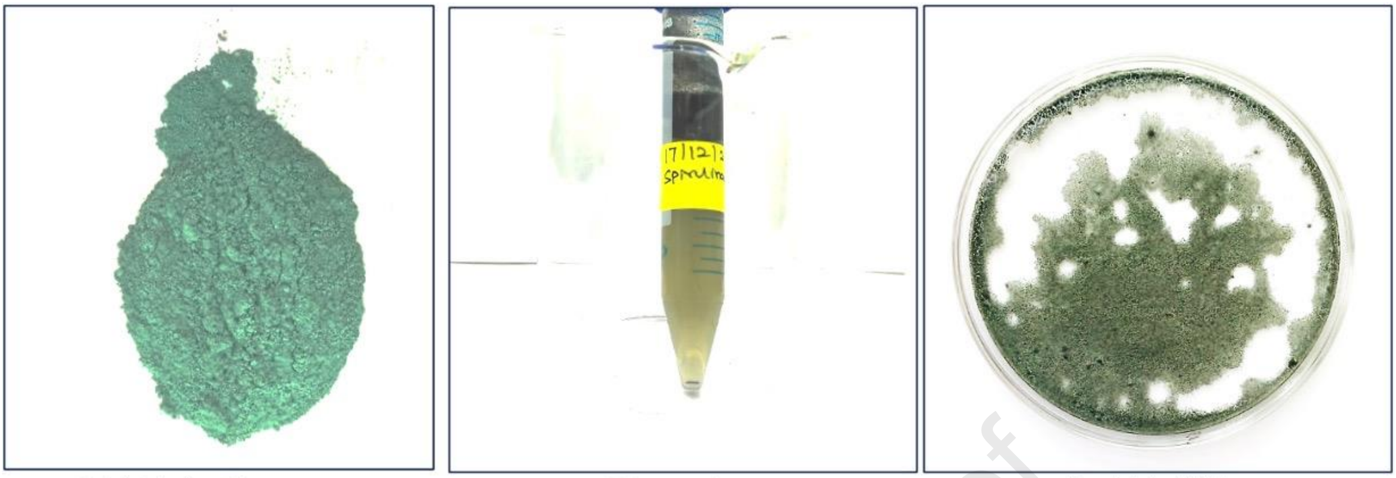


Fig.3. Comparative Phycocyanin Recovery from *Spirulina* Using Various Solvent Systems

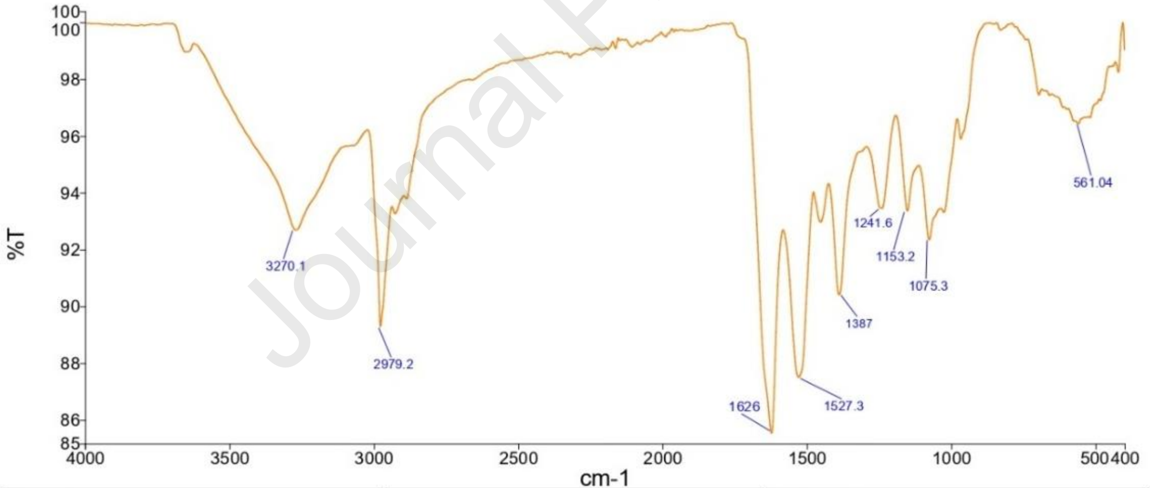
3.3. Extraction of Algal PHA and its utilization as a biopolymer

To study the synthesis of inert PHA utilizing microorganisms in the lyophilized form of biomass, the Fourier Transform Infrared (FTIR) spectroscopy is an instrument that has been demonstrated to provide accurate analysis of the produced biopolymeric components **[34]**. This also provides comprehensive data on other macro and micro compounds, such as proteins, carbohydrates, lipids and nucleic acids, making it an appropriate method for the quick detection of PHA-producing microorganisms **[35].** This analytical technique necessitates a minimal amount of sample volume, indicating its efficient and potential, while also facilitating the detection and characterization of absorption bands, which are primarily those related to ester and carbonyl stretches **[36].** However, its application in PHA quantification is still remained constrained. In majority of the studies this analytical FTIR is considered as a semi-quantitative method, which is capable of providing a relative approximation instead of exact measurements. Therefore, the current study utilized FTIR analysis to confirm the polymeric characteristic of the recovered algal-derived PHA **(Fig 4)**, were the FTIR spectra of the isolated *Spirulina* PHA bioplastic exhibited significant absorption bands at 3270.1 cm^{-1} (O-H), 2979.2 cm^{-1} (C-H), a pronounced ester carbonyl stretch at 1626 cm^{-1}, alongside with additional peaks 1387 cm^{-1} and 1241.6 cm^{-1} which are associated with -CH2, -CH3 and -CH (methylene, methyl and methine) groups **(Fig 5)**. Additionally, bands were found in between 1527 and 1050 cm^{-1} back up the structure of the polymer backbone. These bands undoubtedly show that there are polymeric functional groups that are typical PHAs. Similarly, the research conducted by **Thepsuthammarat et al., [37]** utilizing FTIR on the polymeric component isolated from the microorganisms *Coelastrella sp.,* KKU-P1 and *Acutodesmus sp.,* KKU-P2 revealed a dominant peak value of 1453, 1379 and 1279 cm-^{1} confirming the presence of standards PHB and PHBV, where the observed peaks align with the distinctive fingerprint region of polymeric component PHB and PHBV, therefore affirming the existence of these polymers in the microalgal sources. So, the present study's FTIR spectra of *Spirulina*-derived PHA substantially consistent with those of PHB from KKU-P1 and KKU-P2, especially in the alkyl and carbonyl areas showing a significant resemblance in chemical structure. The carbonyl (C=O) vibration of stretching in *Spirulina* PHA was identified at a slightly reduced wavelength (1626 cm-^{1}) compared to the standard value of around 1720 cm-^{1} found in conventional PHB/PHBV. These changes are typically attributed to disparities in the polymeric chain context, crystalline structure or copolymer composition. These finding align with the current research identifying *Spirulina* as a PHB-producing microalgae, with multiple studies demonstrating PHB accumulation and spectrum features, including ester, carbonyl and alkyl peaks similar to those observed in normal PHB. Therefore, the study's FTIR spectral features confirm that *Spirulina*-derived PHA possesses the fundamental properties of PHB, hence affirming its promise as a viable source of biopolymers for sustainable bioplastic fabrication.



Dried Spirulina Biomass PHA extraction Precipitated PHA

Fig.4. Stages of PHA bioplastic production from *Spirulina*.



Sample Name	Description	Quality Checks
Spirulina PHA	Sample 189 By PEService Date Tuesday, December 17 2024	The Quality Checks give rise to a Weak Bands warning for the sample.

Fig.5. FTIR study of PHA isolated from *Spirulina*

3.4. Fabrication of algal bioplastic

The bioplastic film fabrication using microalgae-based components, retained a semi-transparency characteristic also exhibited a uniform and smooth surface **(Fig 6)**. The film was measured to be an average of 0.12±1 mm thickness confirming the bioplastic's reliability of the synthesis method, the measured values of the control and the synthesised films are elaborated in the **table 2**. The separation of bioplastic from the petri dish required 5 days which is also influenced by the external temperature and surrounding environmental condition. Remarkably, the film showed a good stability, which could be a sustainable material for various applications and favourable for decomposition. This effective synthesis of bioplastic film from the *Spirulina* biomass, suggests that microalgae can be efficiently utilized as a feedstock for the sustainable development of biopolymer. It is also worth noting that this *Spirulina*'s is a low maintenance and rapidly proliferating biomass for the production of sustainable polymer. This method not only based on biomass utilization which also captures carbon, making the resulting algae-based bioplastic as a significant carbon-negative or carbon-neutral alternative **[38]**. Therefore, the present study using residual algal biomass for bioplastic production requires, basic processing and drying technique making it a sustainable approach.

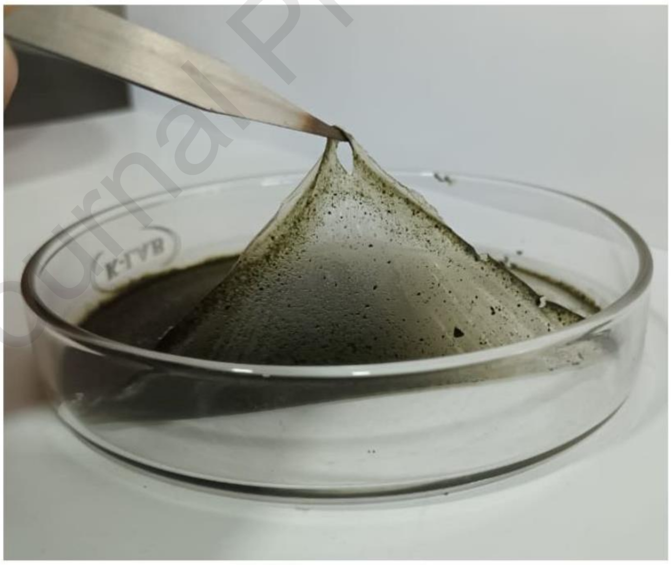


Fig.6. Synthesis of Bioplastic from *Spirulina* biomass residues

Table 2. Comparative thickness of the films

S:no	**Plastic Type**	**Initial Thickness (mm)**
1	*Spirulina* bioplastic	0.12±1

2	Commercial bioplastic	0.15±1
3	Commercially available plastic bag (polyethylene)	0.05±1

3.5. Physicochemical characterization of Spirulina-Based Bioplastics

3.5.1. Physical properties of bioplastic

3.5.1. a. Water absorption

The water absorption characteristics of the films were assessed by measuring their thickness prior and following immersion in water. The algae-derived bioplastic using *Spirulina* showed a slight increase in the thickness from around 0.12 mm to 0.14 mm, with reflecting swelling rate of 16% is listed in **Table 3**. This minimal increase in the thickness indicates its characteristics of partial hydrophilic nature, this is probably due to residual functional groups such as, carboxyl and hydroxyl with the algal cell matrix. Followed by, the commercial bioplastic exhibited a markedly elevated rate of swelling approximately 86.67%, which significantly indicate the enhanced water affinity and dropped dimensional stability in moist environment. The polyethylene film, on the other hand didn't alter in thickness indicating that it is extremely hydrophobic and non-biodegradable. Similar observations have been reported in starch-based bioplastics; for instance, **Rohindra et al., [39]**. reported a water absorption of 87% for cassava starch–based bioplastic incorporated with clove oil, highlighting that the presence of hydroxyl groups in starch strongly enhances water uptake. Therefore, the comparatively lower swelling observed in the *Spirulina* bioplastic indicates improved dimensional stability relative to other biodegradable bioplastics while still maintaining biodegradable characteristics.

Table 3. Comparative water absorption properties of algal bioplastic

S:no	Plastic Film type	Before water immersion (mm)	After water immersion (mm)	Percentage (%)
1	*Spirulina* bioplastic	0.12±1	0.14±1	16
2	Commercial bioplastic	0.15±1	0.28±1	86.67

3	Commercially available plastic bag (polyethylene)	0.05±1	0.05±1	0

3.5.1. b. Water solubility

The current study determines the solubility of the plastic films in the water by comparing initial and final weight before and after water immersion. In which the *Spirulina*-based bioplastic shows a minimal weight gain from 0.541 g to 0.0721 g equivalent to 33.21% of water absorption **(Table 4)**. This rise can be linked due to presence of hydrophilic function groups within the algal cell, which interact with the water molecules through hydrogen bonding. While, the commercial bioplastic exhibited 86.67% of moisture indicating minimal water resistance ability and dominance of polar biopolymers such as PLA and starch. Finally, as expected the commercially available polyethylene film remained constant in weight which confirms its robust hydrophobic nature and inhibition of moisture entry are the two major characteristics. In contrast, **Gurunathan et al., [40]** reported that starch based-bioplastic exhibited the water absorption of (35.69%), which can be attributed to the combined effect of sorbitol as the plasticizer and the presence of filler. Compared with these findings, the *Spirulina*-derived bioplastic in the present study demonstrates lower water absorption and improved moisture resistance, suggesting better dimensional stability and making it a more suitable biodegradable alternative for practical applications.

Table 4. Comparative solubility properties of algal bioplastic

S:no	Plastic Film type	Initial Weight (g)	Final Weight (g)	Percentage (%)
1	*Spirulina* bioplastic	0.0541±1	0.0721±1	33.21
2	Commercial bioplastic	0.15±1	0.28±1	86.67

3	Commercially available plastic bag (polyethylene)	0.05±1	0.05±1	0

3.5.2. Chemical properties of bioplastic

In this study, the films were exposed to variety of chemicals such as acids, base, salts and organic solvents in order to examine their durability, degradability and stability. This helps in evaluate the newly synthesised films and their behaviours during storage, usage across materials and degradation phase. The present study state that the *Spirulina* based film was completely disintegrated in the acid medium whereas, partially disrupted in alkaline and saturated solution while, the commercial films were shown soften appearance in 50% ethanol solution are listed in the **Table 5 and fig 7** shows the changes observed during 24 h incubation period. Therefore, this finding suggest that matrix of algal components contains hydrolysable and biodegradable groups such as hydroxyl, amides and esters which is prone to hydrolysis and biodegrade under extreme chemical conditions. on the other side, the commercial films showed signs of weak structural stability appeared to be wrinkled in 50% of ethanol medium and fully dissolved to all other aqueous solvents. In comparison with the study of **Singh et al.**, **[41]**, reported that the potato and sago starch-based bioplastic films were tested in different chemical solvents and showed solvent absorption and softening in most solutions, particularly in alkaline conditions. Complete dissolution occurred in the strong base NaOH, indicating that the bioplastic matrix is highly susceptible to alkaline degradation. Finally, the polyethylene remained no change after immersion in the chemical solutions, indicating its hydrophobic and chemically resistance nature across all chemical treatments. The present findings demonstrates that the *Spirulina*-based bioplastic film exhibits a balanced behaviour, maintaining sufficient structural stability during use while remaining chemically degradable under extreme conditions. This property highlights its potential as an environmentally sustainable alternative to conventional plastics.

Table 5. Chemical Resistance analysis of bioplastics

S:no	Solvents	*Spirulina* bioplastic	Commercial bioplastic	Commercially available plastic bag (polyethylene)
1	0.1 M NaOH	70% of film disintegrated was observed	Fully dissolved	No change
2	0.1 M HCl	90% of disintegrated was observed	Fully dissolved	No change
3	0.1 Ethanol (50%)	Soften & Partial disintegrated	Wrinkled	No change
4	NaCl solution (saturated)	Partial disintegrated	Fully dissolved	No change

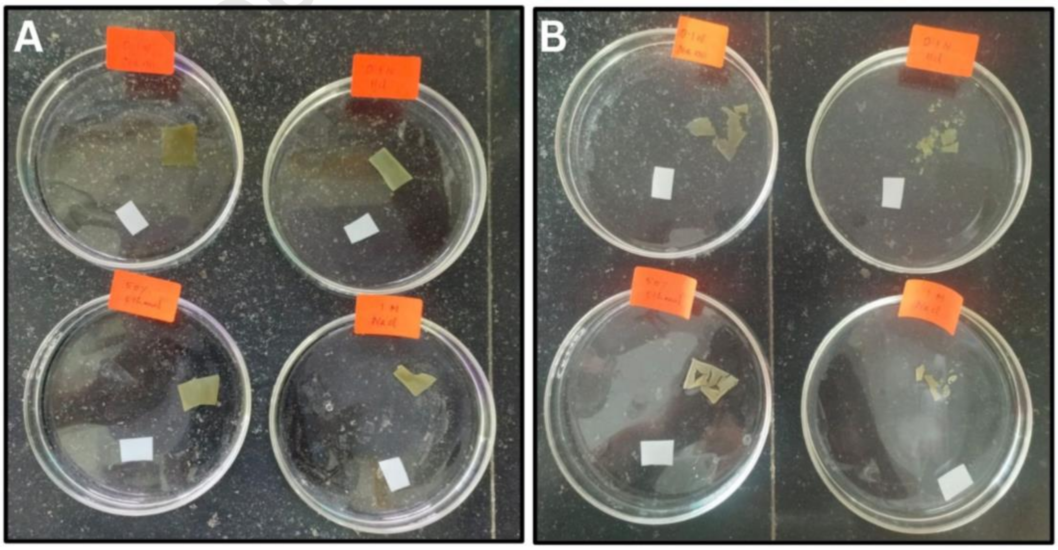


Fig.7. (A) Films before chemical immersion (B) After 24-h of chemical immersion showing structural disintegration.

3.6. Algal biochar production and characterization

Torrefaction is a thermochemical method utilized to transform biomass into biochar through regulated heating. In this study, dry torrefaction was carried out at 250 °C under an inert atmosphere, which involved in prevention of combustion and ash reduction. In prior to torrefaction, the *Spirulina* residual biomass was pretreated to eliminate impurities and obtain pure feedstock suitable for biochar production. During the early stages of heating, the moisture content in the biomass was removed. As the temperature increased, thermal degradation of organic components occurs, leading to the release of volatile compounds. This process helps in reduction of the non-carbon components in the algal biomass and simultaneously increased the carbon fraction. In which after the torrefaction procedure, about 31% of algal biochar was generated, exhibiting enhanced thermal stability, increased carbon and hydrophobicity, fine porous structure and finally with reduced bulk density. These characteristics demonstrates that torrefaction effectively stabilizes the carbon structure, enhancing the functional value of biochar. Prior studies have investigated the high-temperature pyrolysis technique for the synthesis of algal biochar. ***Kuai et al.,*** **[42]** detailed a one-step pyrolysis method that synthesised biochar with a substantial surface area, enhanced defect sites and carbonyl (C=O) functional groups, indicating efficient carbonization. In addition to this, ***Sun et al.,*** **[43]** employed high-temperature pyrolysis incorporated with KOH activation to produce highly porous algal biochar with a large specific surface area. In comparison with these intensified methods, torrefaction provides sustainable and energy-efficient conversion algal biomass into functional biochar. This is because optimal torrefaction occurs at approximately 250° C indicated by improvements in physical properties like grindability and energy density, followed chemical thermal conversion characteristics, also torrefied biomass exhibits increased carbon content, reduced H/C and O/C ratios, elevated mass energy density and chemical compositions. The torrefaction process liberates substantial amount of Cl, S and K, mitigating or eliminating ash-related issues such as slagging, agglomeration and corrosion during thermal conversion **[44]**.

The thermal behaviour of the biochar produced from *Spirulina* biomass is further assessed using thermogravimetric analysis. In this study the heating rate is fixed at f 10°C min^{-1} in an N_2 atmosphere over a temperature range of approximately 1000°C results show the thermogravimetry analysis of *Spirulina* biomass in three stages; moisture removal stage, thermal decomposition stage and carbonisation stage. Each stage involves in demonstration of elevated temperature patterns; the first stage involves in weight loss of 12.8% at 117 °C involves in the moisture removal on the biochar surfaces which can be termed as drying phase. Followed by this the weight loss of 21% occurs at 287°C corresponds to removal of volatile compounds. In the final stage, between 400 °C to 520°C, represented a weight loss of 30.6% contributing to the degradation of complex components, after char formation occurs and ash residue was produced. The results obtained from this study is consistent with earlier studies, **Khan et al., [45]** conducted thermal degradation studies on algal biochar, identifying two stages: the initial stage, characterized by a 5% weight loss below 200°C, and the final stage, around 400°C, showing a 37% weight loss due to lipid and protein degradation. In general, torrefaction is a more environmentally friendly and energy-efficient way to make functional algal biochar than high-temperature pyrolysis. Moreover, **Mondal et al., [46]** study reveals that the TGA curves of 5 different macroalgal biochar showed two-step thermal degradation, with initial weight loss due to removal of moisture content up to ~600 °C and further weight loss between 600–780 °C from combustion of carbon-rich solids, differing from raw biomass due to the pyrolytic degradation of proteins, carbohydrates, and lipids. Similar thermal degradation patterns have been reported for algal biochar in previous studies, indicating good thermal property of *Spirulina*-derived biochar.

4. Conclusion

This study has proven that the maximum biomass concentration of 0.83 (g L^{-1} d^{-1}), demonstrating that *Spirulina* could be effectively grown in an open raceway pond utilizing nutrient-rich domestic wastewater. The study's comparisons with other microalgal species and growth strategies provide more evidence of the versatility of *Spirulina* algal biomass concentration and also suggests environmentally friendly and economically viable option. After harvesting, the pigments with additional value can be recovered by extracting the inbuilt pigment phycocyanin using a solvent solution that combines methanol and water. This process yielded the highest extraction of phycocyanin. Finally, the phycocyanin-extracted residual biomass was successfully utilized as a PHA, which promotes the zero-waste approach. The FTIR analysis demonstrated that the extracted PHA has exhibited spectral features that are very similar to those of the standard PHB and PHBV polymers. This result indicates the successful production of bioplastic, while fabrication of bioplastic film demonstrates significant biodegradability. The formation of carbon-rich, thermally stable biochar was validated by TGA. Finally, this study offers a sustainable pathway to produce phycocyanin and bioplastic, biofuel from *Spirulina* grown in domestic wastewater.

However, some limitations remain, including, PHA yield optimization, wastewater variability, detailed product analyses, scale-up challenges, harvesting costs and energy balance which could be addressed in future studies.

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Highlights

- *Spirulina* biomass in domestic water rose gradually for 16 days, peaking at 0.83 g/L on day 12.
- Phycocyanin concentration methanol-water combination solvent was highest 22.5 mg/L.
- The residues comprised PHAs with spectra similar to PHB and PHBV biopolymers, according to FTIR.
- *Spirulina* showed a consistent thickness of 0.12±1 mm, 16% water absorption and 32% solubility.
- The residues turned into sustainable biochar for various industrial application

Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐ The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: