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Climate change mitigation through industrial *Spirulina* culture: a multifaceted approach for carbon sequestration and recovery of value added pigment phycocyanin

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Abstract

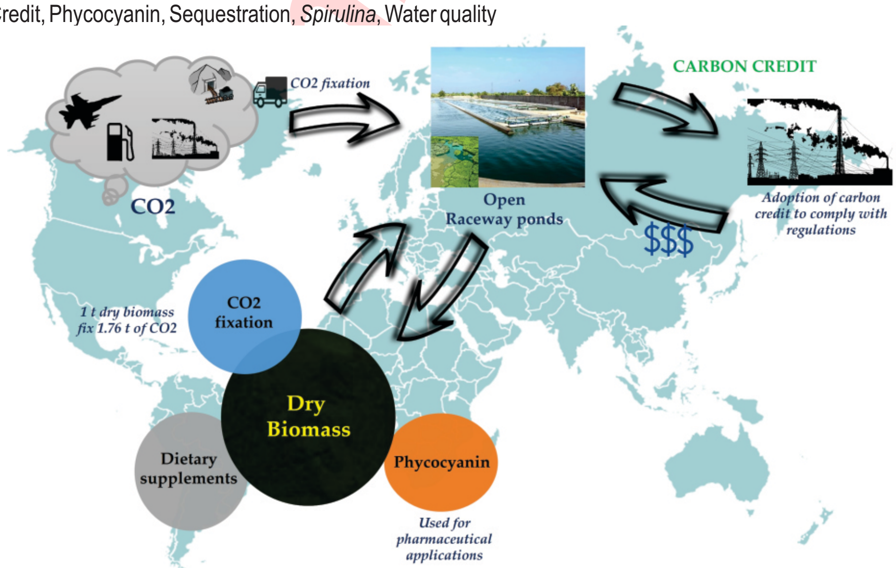
Aim: The study aimed to evaluate the carbon sequestration potential and phycocyanin recovery from industrial *Spirulina* biomass, to asses its feasibility as a sustainable biorefinery model for climate mitigation and value addition.

Methodology: Monthly *Spirulina* biomass production and water-quality parameters were recorded for one year. Carbon content in the dry biomass was analyzed using a CHNS analyzer to estimate carbon sequestration. Phycocyanin yield and purity were determined monthly using the repeated freeze-thaw method to evaluate the economic feasibility of pigment extraction.

Results: The *Spirulina* biomass showed carbon content ranging from 39.96% to 46.17%, resulting in an estimated annual CO_2 sequestration of 176.04 tonnes. Biomass productivity exhibited moderate positive correlations with electrical conductivity and carbonate levels. The total annual phycocyanin yield was 2.606 tonnes, with monthly variation influenced by seasonal environmental factors.

Interpretation: This study highlights the dual benefit of phycocyanin recovery and carbon sequestration in enhancing both environmental sustainability and economic returns from *Spirulina* cultivation. The biorefinery approach offers up to 41% additional revenue through pigment extraction and carbon credit monetization, supporting scalable, low-emission production models. These findings demonstrate practical utility for climate change mitigation and sustainable livelihood promotion in small- to medium-scale *Spirulina* enterprises.

Key words: Carbon Credit, Phycocyanin, Sequestration, *Spirulina*, Water quality



Triveni Enterprises (Educational Services Set-up)

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Introduction

Spirulina is a nutrient-rich cyanobacteriafrom the Oscillatoriaceae family, primarily represented by the genera *Arthrospira* and *Limnospira*. The main species include *Arthrospira platensis, A. fusiformis* and *A. maxima*. These filamentous, free-floating cyanobacteria possess cylindrical, multicellular trichomes. They are naturally found in tropical and subtropical lakes with high pH levels and high concentrations of carbonate and bicarbonate (Habib and Hasan, 2008). Nutritionally, they are highly regarded as a superfood, a functional food, and a dietary supplement as they are rich in high-quality protein (60–70% of its dry weight), essential vitamins like B-complex, minerals such as iron and calcium, and bioactive compounds including the antioxidant phycocyanin (Grosshagauer *et al.,* 2020; Janda-Milczarek *et al.,* 2023; Soni *et al.,* 2017). Globally, annual *Spirulina* production has surpassed 70,000 MT, with the United States as the leading producer (FAO, 2020). Its market is valued at more than USD 600 million and is poised to grow at a CAGR of 9–10% (Amin *et al.,* 2024). For instance,India is among the prominent producers, with annual output of approximately 2,000–2,500 MT(EDIDSPL, 2024), accounting 3–4% of global production. Despite the moderate production volume, India ranks second in the global *Spirulina* export market, and is expected to increase by 15–20% annually (EDIDSPL, 2024).

This trend underscores the growing economic significance of *Spirulina* in developing countries. Globally, the majority of *Spirulina* production is directed towards the nutraceutical industry, followed by applications in the food and beverage sector and personal care products. Interestingly, phycocyanin, a principal bioactive compound in *Spirulina,* exhibits strong antioxidant and anti-inflammatory properties. Previous reports suggest its critical role in scavenging free radicals and preventing oxidative stress, which are linked to multiple chronic diseases. In addition, phycocyanin demonstrates immunomodulatory effects by enhancing the antibody production, thereby strengthening the immune defense. Moreover, it is associated with improved lipid profiles, reduced blood pressure, and better glycemic control through enhanced insulin sensitivity. Together, therich nutrient composition and therapeutic potential of *Spirulina* position it as a promising natural supplement for promotion of human health. To sustain its economic and health benefits of *Spirulina*, it is essential to enhance *Spirulina* production, amid hurdles such as water quality and environmental regulation, contamination risks, high production costs, and the need for specialized infrastructure and expertise for large-scale, quality-controlled cultivation.

Mass production of *Spirulina* has gained popularity as it provides a more sustainable supply of nutrition. Depending on the purpose of cultivation, several cultivation techniques, such as open ponds, tubular photobioreactors, and glass panels, are utilized. The cost and composition of cultivation media and the growth rate are challenges to commercially sustainable production. In addition, species, photoperiod, temperature, pH, rate of cell removal, turbulence, and nutritional composition affect *Spirulina* production. Generally, *Spirulina* is cultured in bioreactors and open ponds of different sizes using Zarrouk's (Kosinski *et al.,* 2023), Rao's, OFERR, and CFTIR media (Thevarajah *et al.,* 2022). The technology and type of tanks used for culture drive the yield and CO_2 capture. However, the most influential environmental factors include light, pH, temperature, nutritional profiles, and dissolved oxygen, and biological factors such as predation, viruses and competition.

Furthermore, *Spirulina* is highly effective in CO_2 fixation, where photosynthesis acts as the basis for *Spirulina* to fix and store CO_2. Previous investigations indicate that photosynthetic efficiency is generally higher compared to other plants. Algae are responsible for 50% of the global photosynthesis process (Singh and Singh, 2014) and can synthesize biomolecules like carbohydrates, proteins, and lipids. Previous findings revealed that the average CO_2 fixation rate for *Spirulina* is 37.9%, which is higher than that ofgreen algae (de Morais and Costa, 2007). Additionally, it is believed that the CO_2 sequestration rate of *Spirulina* in freshwater is 0.152 g l^{-1} day. *Spirulina* production can be increased by adding compounds such as monoethanolamine (MEA). MEA specifically increased output by 31.4% and enhanced CO_2 fixation (Rosa, Moraes *et al.,* 2015). 1 kg of Spirulina can absorb up to 1,83 kg of CO_2 while it absorbs approximately 0.36 and 1.78 g of CO_2 g^{-1} of biomass with an overall mean of 0.78 g (Setiawan *et al.,* 2014).

Collectively, considering the commercial, nutritional, and environmental benefits, *Spirulina* production becomes economically and environmentally sustainable in the long term. Specifically, its potential to reduce greenhouse gas (GHG) emissions can earn carbon credits, which can be encashed to scale up or invest in improving relevant technologies. However, *Spirulina* production is excessively dependent on water quality management, as it thrives under specific physico-chemical conditions. Notably, pH, temperature, and the presence of essential nutrients like nitrogen, phosphorus and trace minerals play a critical role in sustaining the production throughout the year. Maintaining these parameters can be challenging due to environmental fluctuations, the buildup of metabolic waste, and the risk of contamination (Berden Zrimec *et al.,* 2024; Maulana *et al.,* 2023). Besides, diversification of products is crucial for expanding revenue streams and reducing market risk. In view of the above, this investigation aims to evaluate the impact of revenue diversification, particularly through phycocyanin production and carbon credit generation, on enhancing the profitability and ensuring the long-term sustainability of *Spirulina* cultivation.

The additional revenues from diversified products and potential carbon credit earnings can be reinvested to upgrade infrastructure associated with water treatment systems to implement quality control measures. These kinds of regular reforms and upgrades increase resilience against climate-related and operational challenges, ultimately making *Spirulina* cultivation more sustainable and profitable in the long term.

Additionally, the study will provide insights into how supplementary revenue can support the upgrading of the production facility, thereby enhancing profitability and ensuring the long-term viability of the production unit.

Materials and Methods

Sample collection: Water samples were randomly collected monthly over one year from 15 open raceway ponds, selected from a total of 40 ponds, each approximately 2000 m^2 in area, at N.B. Laboratories Pvt. Ltd., Nagpur, India, one of the country's leading *Spirulina* producers and exporters. Nagpur's tropical climate characterizedby high sunlight and warm temperatures andsupport year-round *Spirulina* cultivation. From each pond, 1000 ml of water was collected from a depth of 20–25 cm using sterile polyethylene bottles and transported to laboratory for analysis. All the samples were collected during the first week of each month to maintain uniformity. All the physico-chemical properties of water were estimated in accordance with the standard methods of APHA (2017).

Estimation of water quality parameters: Sixteen water quality parameters were estimated to assess their correlation with *Spirulina* production. Temperature and light intensity were measured with a thermometer and a lux meter, respectively, at different intervals on the sampling day (10:00 am, 2:00 pm, and 6:00 pm). Additionally, the pH was measured with a pH meter, turbidity with a nephelometer (Turbid meter AQ4500, Taiwan), transparency using a Secchi disc, salinity with a refractometer, electrical conductivity with a microprocessor EC meter, and dissolved oxygen (DO) using a DO probe. Other chemical parameters such as Nitrate (NO_3^-) Phosphate (PO_4^{3-}), Sulfate (SO_4^{2-}), Iron (Fe), Carbonate (CO_3^{2-}), Bicarbonate (HCO_3^-), Chloride (Cl^-) and Magnesium (Mg^{2+}) ions were determined following the analytical methods of APHA (2017).

Estimation of carbon: The carbon (%) content in the *Spirulina* biomass was analyzed using a Carbon, Hydrogen, Nitrogen, and Sulfur (CHNS) analyzer (Elementar, VarioMICRO) at the Central Institute of Fisheries Education (CIFE), Mumbai, India. The analysis was conducted under the following operational conditions: helium served as a carrier gas, and oxygen was used as a combustion gas A thermal conductivity detector (TCD) was employed, with the combustion and reduction tubes maintained at 1150°C and 850°C, respectively, while the detector itself operated at 59–60°C (Kumari *et al.*, 2023). Helium was delivered at a flow rate of 200 ± 10 ml min^{-1}, while oxygen was supplied at 10 ± 2 ml min^{-1} in the absence of combustion and 30 ± 2 ml min^{-1} during combustion initiation. The pressure during the analysis was maintained at 1200 ± 50 mbar (Kumari *et al.,* 2023).

Quantification of wet and dry biomass: The wet and dry biomass of *Spirulina* was estimated as reported earlier (Kumari *et al.,* 2023). Briefly, the wet biomass was obtained by filtering a defined culture volume through a 40 µm nylon net, washing with deionized water, and measuring the weight after dewatering by applying pressure. Therefore, the dewatered biomass was dried for 4 hr at 105 °C to determine the corresponding dry weight of the wet biomass. The final weight was measured on an analytical balance with a precision of ±1 g.

Quantification of carbon sequestration: Carbon sequestered was quantified as described previously (Kumari *et al.,* 2023). Briefly, carbon sequestration was estimated based on its total carbon content in dry biomass. The carbon conversion factor (CF) was derived as the ratio of carbon content (%) to 100, *i.e.,* CF = Carbon (%) / 100. The quantity of total CO_2 sequestered was calculated by multiplying the carbon content by 3.67 (ratio of molecular weight of carbon dioxide and carbon). Furthermore, the rate of carbon sequestration was calculated by multiplying the dry weight with a conversion factor.

Estimation of phycocyanin: Phycocyanin was estimated by following the repeated freeze-thaw (RFT) method (Kumari *et al.,* 2023). Briefly, biomass was suspended in 50 mM phosphate buffer (pH 6.8) in 1:30 (w/v) ratio and subjected to freeze-thaw cycles at -20 °C and 24 °C. Next, the cell debris was removed by centrifuging at 6,000 g for 20 min at 4°C, and the supernatant was used for analysis. Phycocyanin concentration was estimated spectrophotometrically at specific wavelengths (620 and 652 nm) on a double-beam spectrophotometer (Motras Scientific Pvt Ltd, India). The recorded absorbance values were later compared with the standard reference values at 235, 257, 313, and 350 nm for calibration purposes. PC was estimated by using the following formula:

$$PC\ (mg\ ml^{-1}) = \frac{(A_{620}-(0.474\ x\ A_{652})}{5.34}$$

Additionally, the grade of phycocyanin was determined by the A_{620}/A_{280} ratio, with values ≥0.7 indicating colorant-grade quality.

Statistical analyses: All the data were analyzed using SPSS. v16 (IBM, SPSS Inc., USA). Multiple comparisons were made by One-way ANOVA, followed by the Duncan Post hoc test. Additionally, correlation analysis between the physico-chemical water quality parameters and *Spirulina* productivity was performed with Pearson's correlation coefficient test. All the experiments were performed in triplicate, and the results were expressed as mean ± S.D. Statistical significance was set at 5% ($p<0.05$).

Results and Discussion

The monthly biomass production of *Spirulina* from May 2021 to April 2022 showed marked variation. Wet biomass ranged from 42,928 kg in July 2021 to a peak of 120,293 kg in November 2021, while dry biomass ranged from 5,091 to 13,347 kg in the same respective months. Together, the *Spirulina* production unit produced an average of 74488 kg of wet biomass each month. Accordingly, the estimated dry biomass was around 8758 kg per month, indicating an 88% biomass loss during conversion to dry biomass. Biomass yields were lowest during the monsoon period

(June to August) and highest during the post-monsoon and winter months (October to December), likely reflecting favorable environmental conditions, such as optimal temperatures and light intensities (Fig. 1). These results underscore the influence of seasonal variation on *Spirulina* productivity in outdoor cultivation systems.

The monthly variations in key environmental parameters influencing *Spirulina* production were assessed from May 2021 to April 2022. pH values remained relatively stable throughout the year, ranging from approximately 9.1 to 9.6, indicating favorable alkaline conditions for the growth of *Spirulina*. Light intensity (lux) showed noticeable fluctuations, with peaks observed during winter and early spring months (November to March), aligning with enhanced biomass production. The temperature exhibited a broader range (~ 18°C to 33°C), with higher values recorded during the summer months. Dissolved oxygen levels varied from around 7.5 to 10.0 mg l^{-1}, with generally higher concentrations during cooler months, likely due to increased solubility and reduced respiration rates. These trends suggest that optimal *Spirulina* growth is associated with moderate to high light intensity, alkaline pH, and stable DO levels (Fig. 2).

Moreover, NO_3 and PO_4 were assessed as they are essential nutrients in *Spirulina* ponds, supporting protein synthesis, cellular growth, and overall biomass productivity. As shown in Fig. 3, observations indicate stable monthly concentrations of PO_4 (0.5–0.6 mg ml^{-1}) and No_3 (470–490 mg l^{-1}) across the sampling period. Additionally, Seasonal monitoring indicated moderate fluctuations in turbidity (110–180 NTU), conductivity (2.1–2.7 m Scm^{-1}), sulphate (340–410 mg l^{-1}), and carbonate (350–1100 mg l^{-1}) levels in raceway ponds. Higher turbidity during monsoon suggested runoff influence, while stable conductivity and sulphate supported consistent algal growth. Periodic peaks in carbonate levels enhanced buffering and carbon fixation, contributing to optimal conditions for *Spirulina* cultivation (Fig. 4). The concentrations of Fe (0.35–0.55 mg l^{-1}), HCO_3^- (110–160 mg l^{-1}), Cl (105–120 mg l^{-1}), and Mg^2(85–135 mg l^{-1}) in the raceway ponds exhibited minor seasonal fluctuations, but largely remained within optimal ranges for *Spirulina* cultivation. (Fig. 5). These stable physico-chemical conditions support consistent growth, enhanced carbon fixation, and physiological functioning essential for sustainable biomass production. Collectively, minimal variability in most parameters suggest effective nutrient management in the *Spirulina* production pond.

In this investigation, the estimated carbon content of the dry biomass ranged from 39.96 to 46.17%. Furthermore, the monthly CO_2 sequestration observations revealed seasonal variations, indicating changes in biomass productivity and carbon assimilation efficiency. The highest CO_2sequestration occurred in November 2021, reaching 22.048 tonnes, coinciding with peak dry biomass production. October and December also showed high sequestration values of 17.817 and 16.388 tonnes, respectively, indicating favorable climatic conditions during these months. In contrast, the lowest CO_2 sequestration was recorded in July 2021 (8.103.25 tonnes). Moderate sequestration was observed during transitional months such as May, February and March, averaging around 13–14 tonne. The annual total CO_2 sequestered by the *Spirulina* production system was 176.04 tonnes, with a monthly mean of 14.37 tonne (Table 1).

Monthly estimated phycocyanin production from *Spirulina* dry biomass exhibited notable fluctuations, influenced by both environmental and physiological factors. The highest total phycocyanin yield was estimated in November 2021 (0.329 tonne), followed by April 2022 (0.308 tonne) and March 2022 (0.266 tonne), corresponding with periods of high biomass

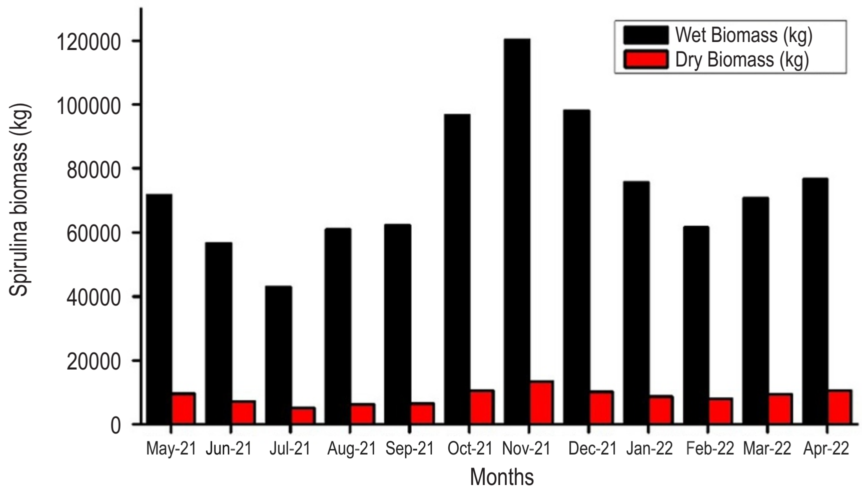

Fig. 1: Monthly data on *Spirulina* mass production in raceway ponds. The highest production was observed in November, while the lowest was reported in June and July. These findings indicate the influence of temperature on growth and production. Data obtained from N.B. Laboratories Pvt. Ltd., Nagpur, India.

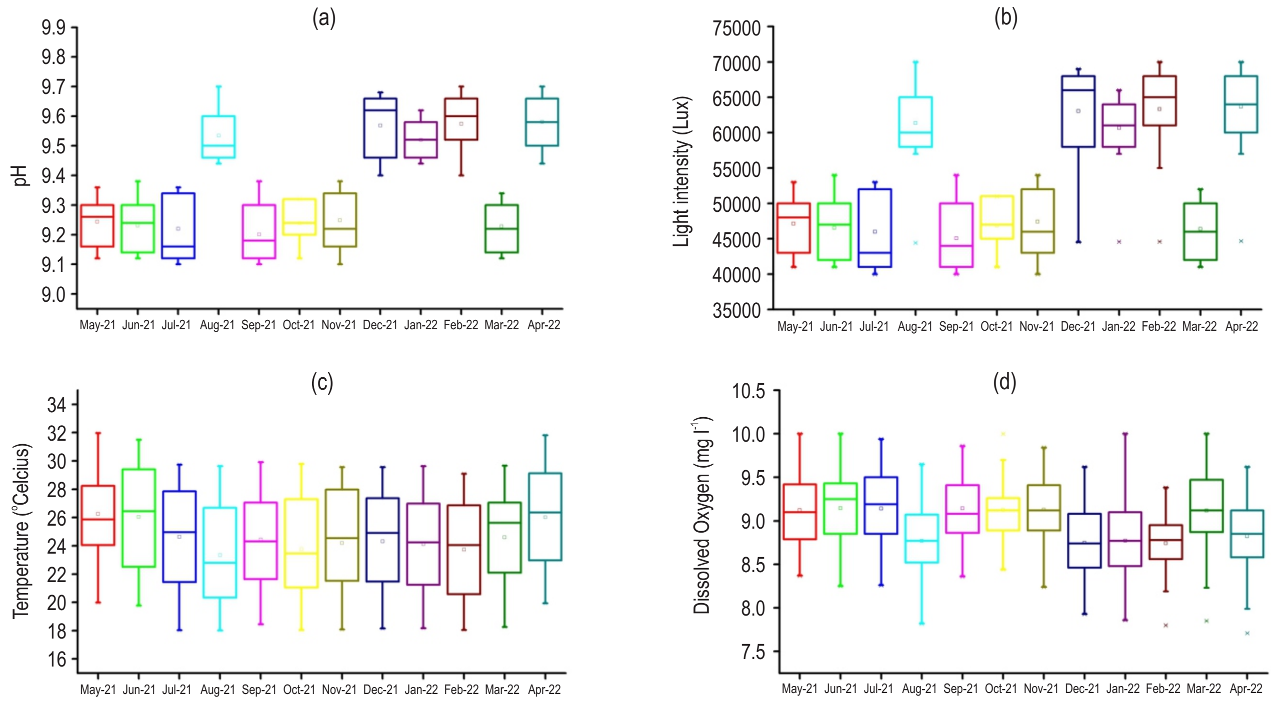

Fig. 2: Box plots illustrating the monthly variations in selected physicochemical parameters of water in *Spirulina* raceway ponds from May 2021 to April 2022. The plots depict fluctuations in (A) pH, (B) light intensity (Lux), (C) water temperature (°C), and (D) dissolved oxygen (mg l^{-1}). All numbers reported are derived from the average of three replicates. Each box represents the interquartile range, with the horizontal line indicating the median, whiskers showing variability outside the upper and lower quartiles, and points denoting outliers. These data provide insights into seasonal trends and environmental fluctuations influencing pond conditions throughout the study period.

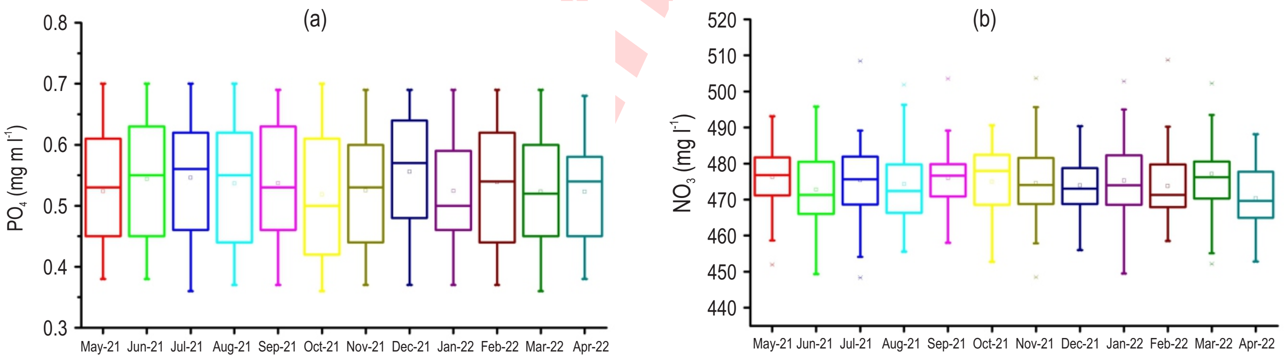

Fig. 3: Box plots showing the monthly variations in nutrient concentrations of *Spirulina* raceway ponds from May 2021 to April 2022. (A) Phosphate (PO_4, mg m l^{-1}) and (B) Nitrate (NO_3, mg l^{-1}). All numbers reported are derived from the average of three replicates. Each box indicates the interquartile range (IQR), the horizontal line represents the median, whiskers extend to the minimum and maximum values, and dots denote outliers. These data highlight seasonal fluctuations in macronutrient availability, which may influence *Spirulina* productivity and pond water quality dynamics.

production and favorable extraction conditions. In addition, purity levels varied from 0.90 to 1.45, with the highest value (1.45) recorded in August 2021. This peak occurred during a low-yield month, where reduced biomass likely minimized the accumulation of non-pigment cellular components (e.g., proteins, polysaccharides, and debris), thereby enhancing pigment purity and indicating superior pigment quality. Conversely, the lower yields and C-phycocyanin (CPC) concentrations were observed in December 2021, reflecting suboptimal growth. These observations suggest an inverse relationship between biomass yield and pigment purity, highlighting a trade-off wherein higher yields may increase cellular debris and secondary metabolites that reduce

Table 1: The annual production of dry biomass and theestimated quantity of carbon and CO_2 sequestered in a commercial production facility

Months	Dry Biomass (DB, Tonne)	Carbon sequestered (C) = D.W x CF (Tonne)	CO_2 sequestered = C X 3.67 (Tonne)
May-21	9.554	3.982	14.614
Jun-21	7.145	3.036	11.144
Jul-21	5.091	2.207	8.103
Aug-21	6.185	2.651	9.731
Sep-21	6.422	2.957	10.853
Oct-21	10.515	4.854	17.817
Nov-21	13.347	6.007	22.048
Dec-21	10.136	4.465	16.388
Jan-22	8.716	3.890	14.279
Feb-22	8.027	3.363	12.345
Mar-22	9.368	3.743	13.738
Apr-22	10.586	4.354	15.981
Total Tonne	105.092	45.509	176.04

Note: Data are based on official monthly production records from a commercial *Spirulina* facility, N.B. Laboratories Pvt. Ltd., Nagpur, India, with each value representing a single monthly measurement without independent replicates. Derived parameters (carbon sequestered and CO_2 sequestered) were estimated from these records and calculated in triplicate for consistency.

Table 2: Monthly estimated phycocyanin production from dry *Spirulina*

Months	DW Spirulina (Tonne)	Purity	% Yield	Estimated phycocyanin production (Tonne)
May-21	9.554	0.94±0.006	24.12±0.196	0.230
Jun-21	7.145	1.00±0.091	27.69±2.098	0.198
Jul-21	5.091	0.99±0.047	24.47±1.316	0.125
Aug-21	6.185	1.45±0.045	23.05±1.342	0.143
Sep-21	6.422	1.02±0.025	18.84±6.349	0.121
Oct-21	10.515	1.17±0.065	24.90±2.117	0.262
Nov-21	13.347	1.05±0.011	24.62±0.593	0.329
Dec-21	10.136	0.9±0.005	18.72±1.472	0.190
Jan-22	8.716	0.90±0.048	23.83±0.116	0.208
Feb-22	8.027	1.30±0.057	28.31±1.577	0.227
Mar-22	9.368	1.18±0.036	28.42±0.938	0.266
Apr-22	10.586	1.38±0.276	29.08±0.250	0.308
Total/ Avg	105.092	1.11±0.033	24.67±0.270	2.606

Note: Data are based on official monthly production records from a commercial *Spirulina* facility, N.B. Laboratories Pvt. Ltd., Nagpur, India, with each value representing a single monthly measurement without independent replicates. Derived parameters (carbon sequestered and CO_2 sequestered) were estimated from these records and calculated in triplicate for consistency

purity despite greater pigment availability. The total annual phycocyanin production was estimated to be 2.606 tonnes (Table 2). Seasonal variations, which are challenging to manage in open raceway ponds, can lead to reduction in biomass productivity and protein content, as reported in previous studies (Kumar *et al.*, 2011; Vonshak and Richmond, 1988). Additionally, these studies have demonstrated that precise temperature control can enhance productivity by approximately two-fold, thereby supporting the development of larger-scale cultivation facilities (Kurpan *et al.,* 2024). Furthermore, light attenuation during the monsoon months, caused by increased turbidity and persistent cloud cover, has been shown to affect pigment synthesis and reduce biomass yield significantly (Kumar *et al.,* 2011). In this study, the observations were consistent with these findings, as both biomass production and pigment synthesis were significantly reduced during the monsoon season (Jun-Sept).

The correlation analysis between water quality parameters and *Spirulina* biomass production revealed weak to moderate associations, but no significant correlations were found ($p > 0.05$). Among the variables, conductivity ($r = 0.51$, $p = 0.08$) and carbonate (CO_3) concentration ($r = 0.52$, $p = 0.07$) showed the strongest positive correlations, suggesting a potential influence of ionic strength and buffering capacity on biomass growth. Iron (Fe) and turbidity showed moderate positive correlations ($r = 0.35$ and $r = 0.34$, respectively), possibly reflecting their role in nutrient availability and light scattering. In contrast, nitrate (r = -0.05) and phosphate (r = -0.32) exhibited weak negative correlations, indicating that excessive nutrient concentrations might not directly enhance biomass accumulation. Temperature and DO levels suggested a limited impact.

However, among the various physico-chemical factors analyzed, electrical conductivity (EC) and CO_3^{2-} concentrations are of particular interest, as they demonstrated moderate positive correlations with biomass productivity. Despite variations, conductivity values ranged from 18 to 24 mS cm^{-1}, reflecting the ionic strength and salinity, both of which are known to affect the physiology and metabolic output *Spirulina* (Batac *et al.,* 2020). Interestingly, elevated carbonate levels during cooler months coincided with improved phycocyanin purity and yield despite relative moderate temperatures and light intensities. However, the correlation between biomass productivity, conductivity and carbonate level correlations were not statistically significant, which suggeststhat fine-tuning conductivity and carbonate levels could be strategically beneficial for improving biomass yield. Specifically, conductivity reflects the total ionic strength of the culture medium. It is critical for maintaining osmotic balance, cell membrane stability, and nutrient transport mechanisms across the algal cell membrane (El-Sayed and Almutairi, 2024). As a result, conductivity can be used as an indirect measure of nutrient availability in the system. Moreover, moderate elevation in conductivity has been linked to improved *Spirulina* (Ndjouondo *et al.,* 2017; Zhang *et al.,* 2015), which was notably observed during October to December in this study. While higher electrical conductivity typically reflects improved ionic availability and photosynthetic activity, the year-round variations observed in this study were not statistically significant, and can thus be considered negligible. Additionally, carbonate serves as a source of inorganic carbon and acts as a carbon reservoir, which is critical for oxygenic photosynthesis (Batac *et al.,* 2020). It is essential for maintaining pH stability despite intense photosynthetic CO_2 consumption.

In this study, the higher carbonate levels were observed during periods of high biomass production, suggesting a potential role in stabilizing pH and supporting optimal growth conditions. This is consistent with the higher correlation observed in the study. Together, the observed higher correlations of biomass production with electrical conductivity and carbonate indicate that this combination may play a critical role in influencing *Spirulina* productivity under controlled cultivation conditions. The study endorses that by virtue of a remarkably higher carbon sequestration potential, the *Spirulina* biomass production at different scales in different regions of the world offers an effective strategy for climate change mitigation. The biomass production coupled to carbon capture and fixation in the biomass, and the downstream processing of the biomass for high-value pigment, is a novel approach that can be integrated with the existing carbon removal framework. The biomass production-carbon sequestration-biorefinery concept helps to improve profitability and environmental sustainability (Thevarajah *et al.,* 2022; Vernes *et al.,* 2019). Additionally, monetizing the carbon offset potential aligns with global sustainability objectives (Maciel-Seidman *et al.,* 2024; Pande, 2024). Thus, the outcome observations of this study provide scientific insights for production and inform about the significance of investment planning, policy support, and market diversification, which are essential for the vertical development of the production facility.

The annual turnover of the selected *Spirulina* production unit ranged between ₹1 crore to ₹10 crore (approximately USD 0.12 to 1.2 million) for the fiscal year ending March 2023, reflecting a substantial 145.84% growth compared to the previous year. Based on one year of observation, the selected *Spirulina* production unit produced approximately 105.09 MT of food-grade dry *Spirulina* with no additional co-products at this stage. Assuming an average market price of ₹700 per kg for dried *Spirulina* harvested directly from cultivation ponds, it iscommonly used as an intermediate product for further processing. The estimated annual revenue generated amounts to ₹7,35,64,400 (approximately USD 0.88 million). This estimate aligns closely with publicly available turnover figures, supporting the reliability of production and market data. Interestingly, incorporating phycocyanin extraction under a biorefinery concept has the to enhance revenue from *Spirulina* production significantly. Based on the annual dry biomass output, the estimated yield of industrial-grade phycocyanin was approximately 2.606 tonnes.

At an average market price of ₹11,000 per kg, this translates to an estimated additional revenue of ₹2,86,66,000 (approximately USD 0.33 million). This represents a 38.96% increase over the previously calculated revenue of ₹73,564,400 from dry *Spirulina* biomass alone, highlighting the substantial economic advantage of integrated bioproduct recovery in commercial *Spirulina* cultivation systems. Thus, adopting the biorefinery concept in *Spirulina* production represents a transformative shift toward enhanced resource efficiency, sustainability, and economic viability, particularly for small- to medium-scale production units. In this approach, the unit is involved in the integrated processing of biomass to extract multiple high-value products, such as phycocyanin (Caporgno and Mathys, 2018). As illustrated above, the extraction of industrial-grade phycocyanin alone can increase total revenue by nearly 39%. This highlights the critical role of product diversification in improving profit margins. Additionally, the bulk *Spirulina* powder has a relatively stable market price, while value-added compounds like phycocyanin, widely used in nutraceuticals, cosmetics, and natural food colorants, offer access to premium markets with higher returns (Williams *et al.,* 2025). For small- to medium-sized units, which often face financial and operational constraints, diversification into high-value bioproducts provides resilience against market volatility and improves scalability.

Furthermore, by documenting and registering carbon sequestration activities, *Spirulina* producers can monetize their biological CO_2 capture into carbon credits. These credits can then

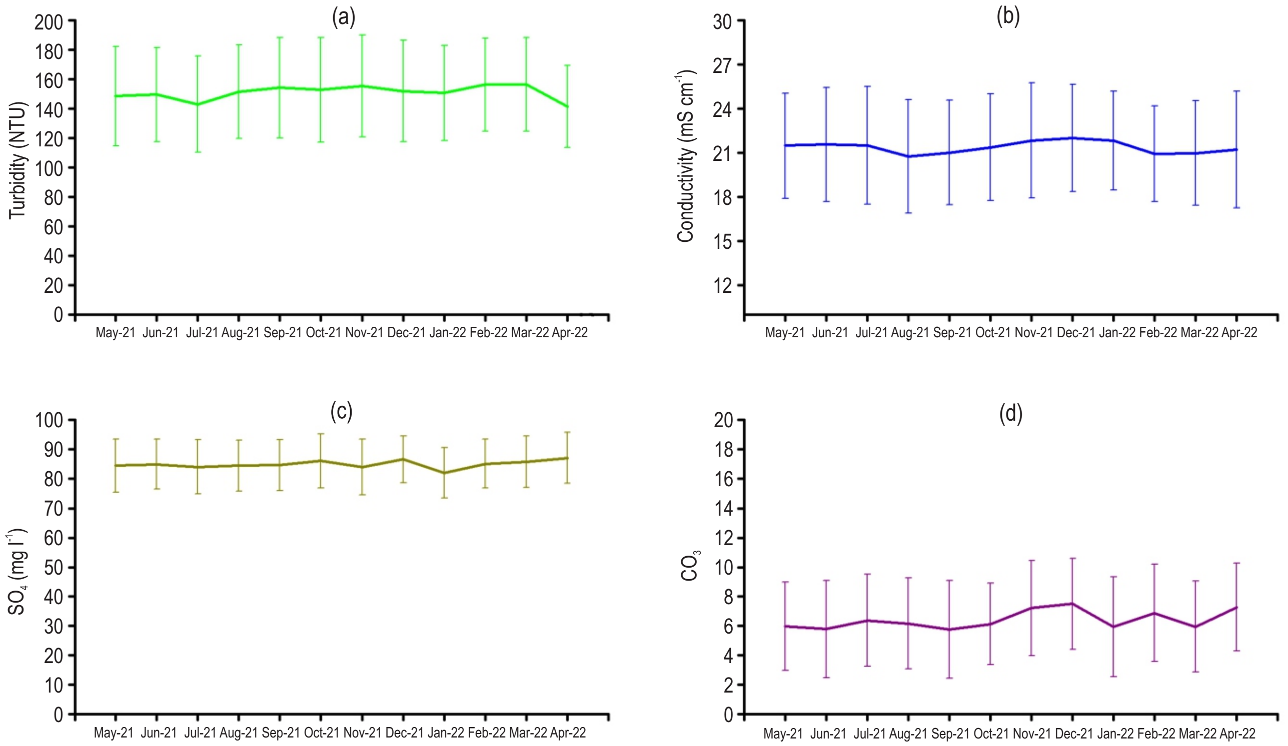

Fig. 4: Monthly variations in selected physicochemical parameters of water in *Spirulina* raceway ponds from May 2021 to April 2022. The line plots with error bars represent mean values ± S.D. for (A) turbidity (NTU), (B) conductivity (mS cm^{-1}), (C) sulfate (SO_4^{2-}, mg l^{-1}), and (D) carbonate (CO_3^{2-}, mg l^{-1}). All numbers reported are derived from the average of three replicates. These measurements illustrate seasonal fluctuations in water quality, reflecting natural variability in ionic composition and transparency, which are critical factors influencing the growth and productivity of *Spirulina* in open-pond cultivation systems.

be sold to industries seeking to offset their GHG emissions, such as those in the petrochemical and aviation sectors. For eligibility, projects must undergo registration and third-party verification under-recognized carbon credit certification standards. Upon verification, each tonne of CO_2 sequestered is equivalent to one carbon credit. As of May 29, 2025, in the compliance carbon market, each credit was valued at €72.10 (Carbon Credit, Live price, European market). Based on the annual CO_2 sequestration of ~176 tonnes at the selected *Spirulina* production facility, a total of 176 carbon credits can be issued, yielding a potential value of €12,672 (equivalent to ₹12,29,418). This contributes an additional 1.67% to the overall revenue of ₹7,35,64,400 generated from *Spirulina* dry biomass, demonstrating the added economic value of integrating carbon credit mechanisms into algal production systems. Collectively, the production unit can add ~41% additional revenue, which can be utilized to scale up with a sustainable increase in profits. The estimated ~41% increase in revenue from product diversification and carbon credit monetization can be strategically reinvested to modernize cultivation infrastructure, enhance harvesting and drying technologies, and strengthen quality assurance systems to meet export standards. Also, funds could support the installation of renewable energy systems, such as solar-powered paddle wheels and aerators, reducing dependency on fossil fuels and operational costs. Together, these upgrades not only elevate the ecological footprint of the production facility but also align it with global standards for sustainable aquaculture and green biotechnology. Future strategies could focus on optimization through life cycle assessments, automation, and value chain integration with carbon trading platforms, positioning *Spirulina* as a high-impact component in low-carbon food and health sectors.

From a broader perspective, the growing global demand for natural products present an opportunity for India to solidify its role as a major exporter of *Spirulina* and its value-added byproducts. Investments in advanced infrastructure can facilitate compliance with stringent international quality standards, enabling access to high-value markets in regions such as Europe and North America. This development not only enhances export potential but also promotes rural employment and supports the advancement of a sustainable, green economy. To further validate the present case study, a more in-depth investigation into the scalability and broader potential of approach is required. Additionally, certain ambiguities surrounding the carbon credit mechanism warrant clarification (Chen and Xie, 2023; Kreibich and Hermwille, 2021; Trouwloon

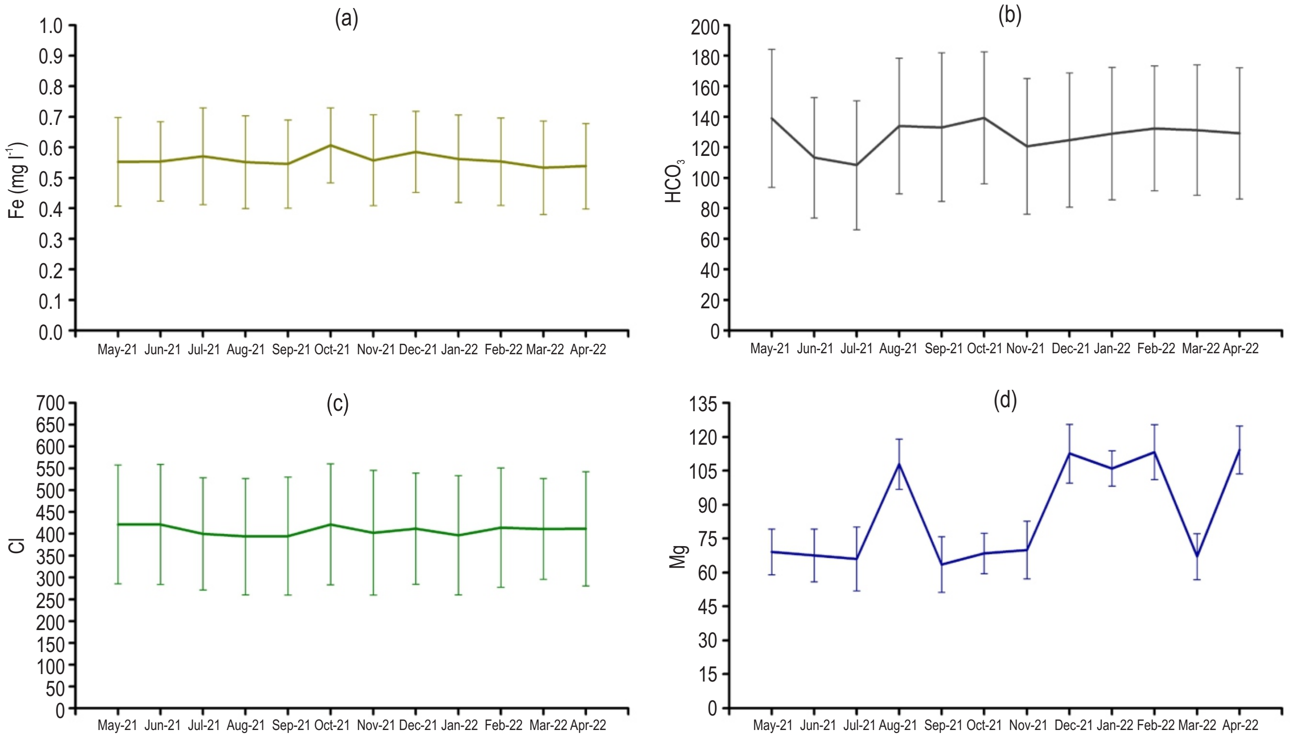

Fig. 5: Monthly variations in selected physicochemical parameters of water in *Spirulina* raceway ponds from May 2021 to April 2022. The line plots with error bars represent mean values ± S.D. for (A) iron (Fe, mg l^{-1}), (B) bicarbonate (HCO_3-), (C) chloride (Cl^-), and (D) magnesium (Mg^{2+}). All numbers reported are derived from the average of three replicates. These parameters reflect the ionic balance and micronutrient availability within the culture medium, which are important for maintaining stable water chemistry and supporting optimal *Spirulina* growth throughout seasonal fluctuations.

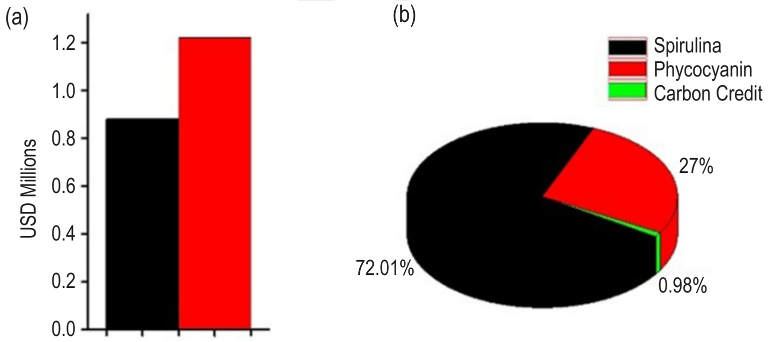

Fig. 6: Estimated revenue potential of the *Spirulina* production facility under diversification strategies. (A) Bar graph showing projected revenue (USD millions) before diversification (black bar) and after implementation of product diversification and carbon credit monetization (red bar). (B) Pie chart illustrating the proportional contribution of different revenue streams following diversification: *Spirulina* biomass (72.01%), phycocyanin extraction (27%), and carbon credit earnings (0.98%). These projections highlight the economic benefits of expanding product portfolios and integrating carbon sequestration monetization into *Spirulina* production systems.

et al., 2023). A multicenter study would offer more robust and generalizable insights into scalability and policy alignment by capturing regional variability in environmental conditions, resource availability, and market dynamics. Such an approach would also provide stronger evidence for policymakers and stakeholders, ensuring that recommendations for investment planning, infrastructure development, and market diversification are adaptable across diverse production settings. Nonetheless, this study provides a foundational perspective on how strategic reconfiguration of existing production facilities can significantly enhance industry performance and revenue generation.

The present investigation underscores the dual potential of phycocyanin extraction and carbon dioxide sequestration in enhancing both the economic and environmental outcomes of *Spirulina* production. Integration of a biorefinery approach enables nearly 41% additional revenue, primarily drivenby high-value phycocyanin and monetized carbon credits. Beyond profitability, the industrial-scale cultivation of *Spirulina* presents a viable strategy for climate change mitigation by sequestering atmospheric CO_2 into algal biomass under controlled, low-emission conditions. This aligns with emerging carbon offset mechanisms and contributes to the broader decarbonization agenda. These findings offer a scalable model for small- to medium-sized units to boost profitability while aligning with global sustainability goals. Strategic reinvestment of this revenue into infrastructure and quality systems can elevate India's competitiveness in premium international markets.

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Ethical approval: The study did not involve any human participants or animals requiring ethical clearance. All experimental procedures, incBuding CHNS estimation from Spirulina powder, pigment extraction and quantification, and water quality analysis, were conducted in accordance with standard analytical protocols. The authors confirm that all methods were performed in compliance with relevant institutional, national, and international guidelines and standards.

Conflict of interest: The authors declare that is there is no conflict of interest.

Data availability: The datasets supporting the findings of this study, including Spirulina biomass production records, CHNS elemental analysis, pigment estimation data, and water quality parameters, are available from the corresponding author upon reasonable request. These data have been compiled and archived in accordance with institutional policies and can be provided for academic or research purposes upon request.

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