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# Algal biomass as a food and feed supplement: Process engineering analysis

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**ABSTRACT**

**Background:** The global market for algae-based food and feed supplements is well-established and growing rapidly. The sector has grown fivefold since 2000. While microalgae products still hold a modest market share, the large-scale commercial production of microalgae as a primary source of proteins, lipids, carbohydrates, and other compounds remains in its early developmental stages. If successfully mass-produced, microalgae could make a significant contribution to global food and feed security.

**Objective:** The study aims to compare the potential of selected algal strains as sustainable sources of high-value biomass for food and feed using a low-cost, solvent-free analytical method based on Near-infrared spectroscopy.

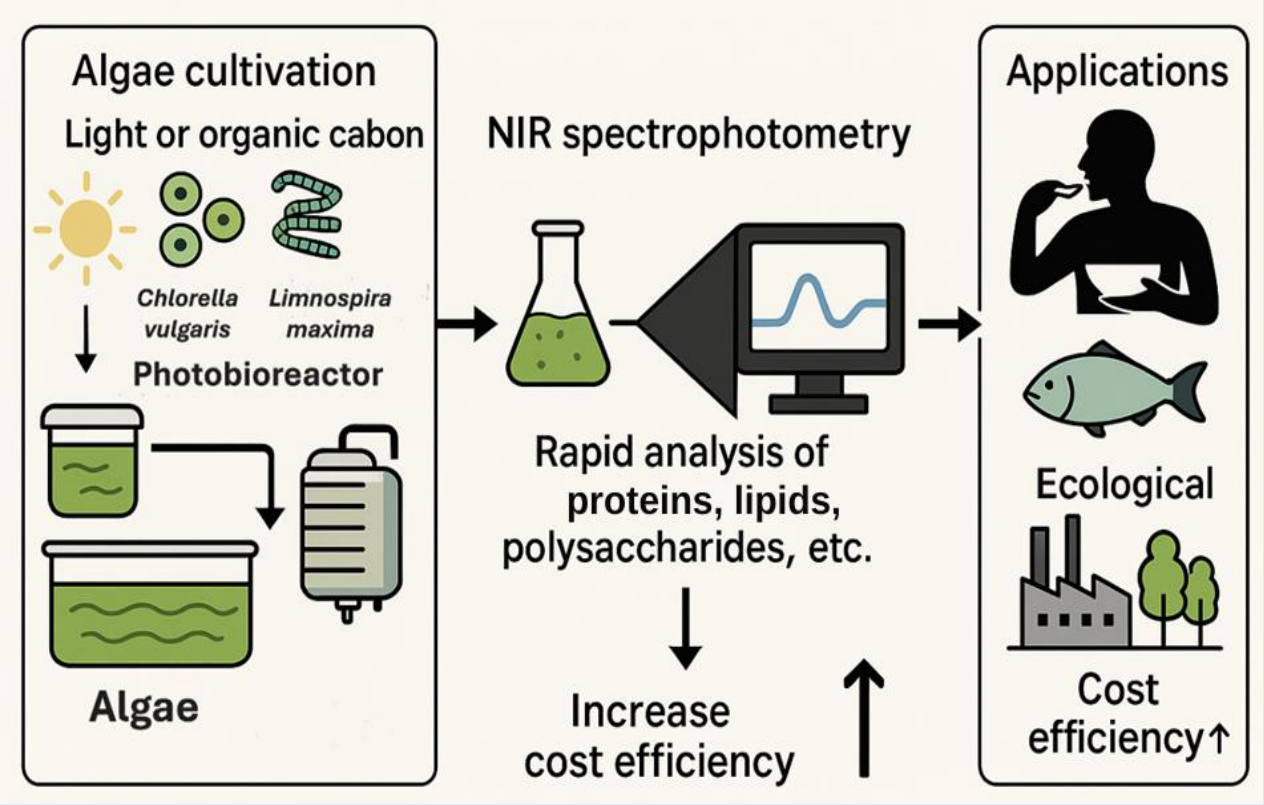
**Methods:** Green unicellular microalgae *Chlorella vulgaris* Pa-023, *Neochloris oleoabundans* Pa-022, and cyanobacterium *Limnospira maxima* Pc-02 were selected and cultivated in bubble column photobioreactors using nitrogen-rich media under LED illumination with red and blue light spectrums. Near-infrared spectroscopy was applied as a non-destructive method to monitor protein, lipid, and carbohydrate content during cultivation.

**Results:** Quantitative analysis revealed distinct biochemical profiles among *C. vulgaris, N. oleoabundans*, and *L. maxima*, with protein contents of 49.2, 40.0, and 59.0 g/100 g dry weight, respectively. *L. maxima* showed the highest protein content, while *N. oleoabundans had the highest lipid content (26.0 g/100 g dry weight), suggesting their suitability for protein-rich products and lipid-based applications*. A cost-efficiency assessment of *L. maxima* cultivation in a 100-L photobioreactor (PUEVIT GmbH, Germany) yielded 98 g dry biomass in 7 days, with production costs estimated at €43.56.

**Novelty:** The study uniquely integrates comparative biochemical profiling of algal and cyanobacterial biomass with a cost-efficiency analysis of semi-industrial biomass production. Key innovations include: (i) species-specific application potential; (ii) the first techno-economic assessment of *L. maxima* cultivation in a controlled photobioreactor; (iii) a practical demonstration of economically viable microalgal biomass production.

**Conclusion:** Near-infrared spectroscopy has proven to be an effective method for non-destructive biomass analysis. However, improving model accuracy requires a broader calibration set with well-characterized standards. Expanding this dataset will enhance the predictive power and reliability of the method.

**Keywords:** algae, high-value biomass, NIR spectrophotometry, food and feed supplement, cost-efficiency.




**Graphical Abstract:** Cost-efficient algae biomass production

## INTRODUCTION

The global market for microalgae-based food and feed supplements is well-established. Microalgae are currently exploited as dried whole biomass and as a source of high-value compounds, including nutraceuticals and natural colorants. Though the total production volume and market share of microalgae-derived food and feed supplements remain relatively modest compared to conventional alternatives, the sector exhibits significant growth potential. Production has increased fivefold since the early 2000s. Moreover, several applications of microalgae in this sector have already gained popularity [1-2]. Nevertheless, the large-scale commercial production of microalgae for macronutrient-rich outputs (e.g., proteins, lipids, carbohydrates) that could significantly impact global food and feed security remains in its early stages [3].

Among commercially cultivated species, green unicellular microalgae *Chlorella* and blue-green algae (cyanobacteria), such as *Arthrospira platensis* and *L. maxima,* dominate the market, primarily in the form of dried whole biomass. Their annual global production volumes are approximately 5,000 and 2,000 metric tons of dry matter, respectively, with an estimated market value of around $40 million per year each [4-5]. In contrast, algae-derived high-value biomolecules such as astaxanthin, β-carotene, and omega-3 fatty acids (e.g., DHA and EPA) have lower production volumes but significantly higher economic value. For instance, the global production of DHA/EPA from algae is estimated at only 240 metric tons per year. Yet, the market value, historically dominated by marine fish-derived sources, exceeds $300 million annually [4,6]. Accordingly, algae are emerging as a sustainable and scalable source of bioactive compounds. Algae-based proteins and lipids poised to become increasingly important in food ingredient markets [7-9].

Recent studies have underscored the nutritional and functional value of algae-derived compounds, particularly in the context of human health. For instance, Capelli et al. [10] reviewed the suitability of microalgal astaxanthin for nutritional and therapeutic use, while Grimmig et al. [11] and Viola et al. [12] explored the neuroprotective potential of algal antioxidants. Furthermore, seaweed and microalgae have been shown to provide essential vitamins [13], modulate the immune system [14], and support gut microbiota balance [15], reinforcing their role as foundational ingredients in the development of functional foods.

Among the major microalgal groups, green microalgae (class Chlorophyceae) are particularly abundant. Microalgae, broadly defined, encompass a diverse range of unicellular, photosynthetic eukaryotes that possess a nucleus and membrane-bound organelles, such as chloroplasts and mitochondria. Cyanobacteria (class Cyanophyceae), often referred to as blue-green algae, also fall under the operational definition of microalgae. However, they are phylogenetically distinct prokaryotes belonging to the domain Bacteria, lacking a nucleus and membrane-bound organelles [2,5].

Currently, the most widely cultivated phototrophic microalgal species include the genera of *Arthrospira*, *Chlorella*, *Dunaliella*, and *Haematococcus*, all of which utilize solar or artificial light as their primary energy source. Some *Chlorella* species have also been found to grow heterotrophically. Additionally, certain marine heterotrophic microalgae, such as *Crypthecodinium*, *Schizochytrium*, and *Ulkenia*, are cultivated under dark, controlled conditions using organic carbon sources, similar to yeast fermentation, for the production of omega-3 fatty acids. In aquaculture, microalgae such as *Chlorella*, *Isochrysis*, *Pavlova*, *Phaeodactylum*, *Chaetoceros*, *Nannochloropsis*, *Skeletonema*, *Thalassiosira*, *Haematococcus*, and *Tetraselmis* are

widely used to support the nutrition of larval fish [1, 16-17].

Despite the promising applications, the technological maturity of microalgae cultivation remains limited. While considerable research and development efforts have been directed toward improving cultivation systems, a transformative shift is still required. Production scale-up must be coupled with cost-reduction strategies to enable broader industrial adoption.

Analytical advancements, such as near-infrared (NIR) spectroscopy, have gained significant traction for rapid compositional analysis. NIR spectroscopy has been successfully applied to various agricultural matrices, with over 30 parameters accurately predicted in cereals and legumes, often with greater precision and speed than conventional reference methods [18].

Traditional techniques for determining the macronutrient content, including proteins, carbohydrates, and lipids, are accurate but inefficient. They are typically destructive, labor-intensive, and time-consuming, particularly when processing large sample sets. NIR spectroscopy offers a non-destructive and rapid alternative. The technique relies on the absorption of overtone and combination vibrations of hydrogen-containing functional groups (X-H, where X = C, N, O) in the near-infrared region (800–2500 nm). Chemometric tools, particularly partial least squares (PLS) regression, are employed to develop predictive models. PLS regression iteratively correlates the spectral data (X variables) with reference values (Y variables), making it highly suitable for full-spectrum analyses.

Although wavelength selection is not strictly required for PLS modeling, model accuracy can be significantly enhanced by excluding non-informative variables. Automated variable selection methods, such as interval partial least squares (iPLS) and the successive projections algorithm (SPA), have demonstrated superior performance over manual selection, particularly in mitigating issues related to multicollinearity, spectral overlap, and variable interactions [19-21].

NIR spectroscopy is considered a secondary analytical method because it necessitates the development of robust calibration models based on a representative sample set with known composition. This "calibrate–collect–predict" cycle is a cornerstone of NIR spectroscopy applications across various domains. The calibration dataset must encompass the whole concentration range and compositional variability of the analytes of interest to ensure predictive accuracy in unknown samples [22-23].

The emergence of compact, portable, and cost-effective NIR spectrometers has further expanded the scope of application. As these devices become more accessible, they hold the potential to be deployed across multiple stages of biomass processing chains--ranging from raw material evaluation to final product quality control. This versatility makes NIR spectrometry applicable in diverse industries, including petrochemical refining, pharmaceutical synthesis, food manufacturing, and biofuel production. The development of portable NIR technologies is advancing rapidly, underscoring their growing relevance in decentralized and real-time compositional analysis [24].

The primary objective of this investigation was to comprehensively assess the biotechnological potential of the green unicellular microalgal species Chlorella vulgaris Pa-023 and Neochloris oleoabundans Pa-022, as well as the cyanobacterium *Limnospira maxima* Pc-02, as sustainable sources of high-value biomass for applications in food and animal feed. This evaluation was

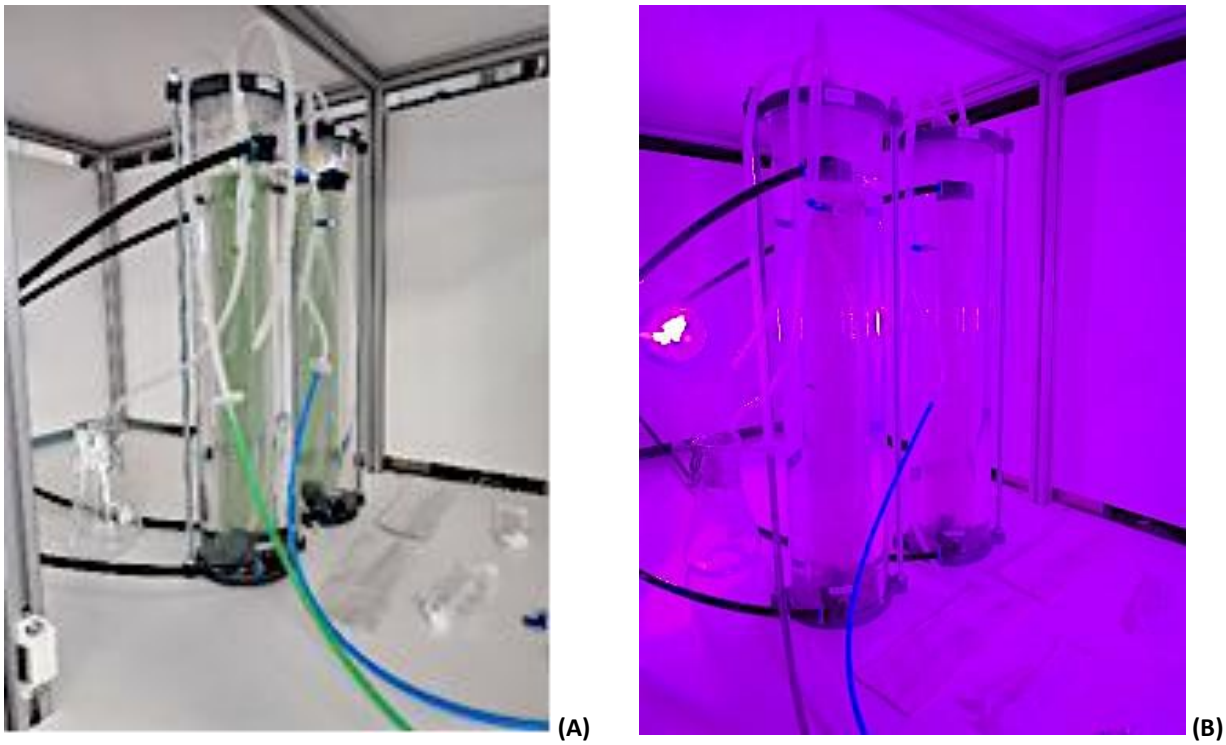
conducted through the integration of a cost-effective, high-throughput, and solvent-free analytical approach capable of delivering rapid, non-destructive, and accurate profiling of biomass composition. The study aimed to establish the biochemical and nutritional profiles of the selected strains, with a particular focus on protein, lipid, and polysaccharide contents. Additionally, the research examined the feasibility of implementing scalable cultivation systems under optimized conditions to maximize biomass yield and compositional quality, while aligning with principles of environmental sustainability and circular bioeconomy.

## METHODS

**Microorganisms and cultivation media:** The strains of green unicellular microalgae *Chlorella vulgaris* Pa-023 and *Neochloris oleoabundans* Pa-022, along with the cyanobacterium strain *Limnospira maxima* Pc-02, were initially isolated from various Armenian fresh water samples. These waters were obtained from the culture collection of the Laboratory of Alternative Energy Sources ("Armbiotechnology" Scientific and Production Center, NAS RA), and then cultivated in a bubble column photobioreactor. For the cyanobacteria strain, the waters were cultivated with modified standard Zarrouk's medium [25,26] under light-emitting diode (LED) illumination with red and blue light spectrums. For the green microalgae strains, the waters were cultivated with nitrogen-rich PUE I nutrient medium (the detailed composition remains proprietary at this stage of development).

**Cultivation of *Limnospira maxima* in bubble column photobioreactor under blue and red LED light spectra:** The cultivation of cyanobacterium was conducted in a bubble column photobioreactor equipped with two controllable LED light sources (Figure 1). Illumination was provided using red and blue spectral ranges to optimize photosynthetic activity. The light parameters were as follows: Photosynthetically Active Radiation (PAR): 2495.010 µW/cm²; Photosynthetic Photon Flux Density (PPFD): 116.399 µmol/m²/s; PPFD-UV: 0.115 µmol/m²; PPFD-Blue (B): 40.378 µmol/m²/s; PPFD-Green (G): 1.377 µmol/m²/s; PPFD-Red (R): 74.644 µmol/m²/s. Light intensity and spectral distribution were set, monitored, and validated using a Hopoocolor OHSP-350P spectroradiometer (www.hopoocolor.com). The photobioreactor was loaded with 1 liter of modified Zarrouk's medium and inoculated with 10% (v/v) of a culture suspension. Cultivation conditions in the photobioreactor were maintained as follows: an airflow rate of 200 L/h, a temperature of 29°C, and a pH adjusted to 9.0. During cultivation, 1 mL samples were collected at regular intervals and analyzed spectrophotometrically at a wavelength of 750 nm. After 216 hours of cultivation, the culture was filtered through a 51 µm filter to remove the supernatant, and the biomass was collected and dried in a moisture analyzer at 40°C for 3 hours. The dried biomass was then stored at 5 °C for subsequent analysis. Determination of dry biomass and moisture content was performed using a moisture analyzer operated at 105 °C until a constant weight was achieved.

**Cultivation of *Chlorella vulgaris* Pa-023 and *Neochloris oleoabundans* in bubble column photobioreactor under blue and red LED light spectra:** The cultivation of microalgal strains was conducted in a bubble column photobioreactor under continuous illumination at an intensity of 116.399 µmol/m²/s and a controlled temperature of 29 °C for a total duration of 216 hours (Figure 1). Two LED light sources emitting in the red and blue spectral ranges were used, and their illumination parameters were consistent with those applied in the cultivation of *Limnospira maxima*.



**(A)** **(B)**

**Figure 1.** Cultivation of microalgae and cyanobacteria in a bubble column photobioreactor under **(A)** standard lighting and **(B)** blue-red LED light spectra.

The photobioreactor was charged with 1 liter of PUE I medium and inoculated with 10% (v/v) of actively growing culture. The inoculum (100 mL) was pre-cultivated in PUE I medium on a rotary shaker at 200 rpm and 29 °C for 7 days. The cultivation parameters were maintained as follows: an airflow rate of 200 L/h, a temperature of 29°C, and a pH of 8.0.

Throughout the cultivation period, 1 mL samples were collected at regular intervals to monitor biomass concentration via spectrophotometric measurements at 750 nm. After 192 hours, the cultures were harvested by centrifugation at 6000 rpm for 10 minutes. The resulting biomass was dried using a moisture analyzer at 40°C for 3 hours and then stored at 5°C for subsequent analyses. Determination of dry biomass and moisture content was performed at 105 °C until a constant dry weight was achieved.

The specific growth rate (μ) of the microalgae and cyanobacteria was calculated according to the formula (1):

(1) $\mu = \ln (N_2 / N_1) / (t_2 - t_1)$,

where μ is the specific growth rate ($h^{-1}$), and $N_1$ and $N_2$ represent the biomass concentrations at times $t_1$ and $t_2$, respectively.

### NIR Spectrophotometry Analysis

**Spectral acquisition:** Before spectral data collection, the samples were equilibrated at room temperature (21°C) for 12 hours to stabilize their moisture content and temperature. For spectral acquisition, 0.5 g of biomass was placed in 4 mL glass cuvettes with a 10 mm path length. NIR spectra were acquired by measuring the diffuse reflectance of the biomass samples in the wavelength range of 900–1700 nm. Each sample was scanned in duplicate, and two replicate measurements

were performed per sample. The average spectrum of each sample was used for subsequent data analysis.

**Sample pre-processing method:** Spectral pre-processing aims to eliminate physical interferences in the spectra, thereby enhancing the performance of subsequent multivariate regression and improving the final predictive model through the careful selection of appropriate pre-processing techniques. Multiplicative scatter correction (MSC) was applied to reduce the influence of scattered light on the diffuse reflectance NIR spectra. Additionally, spectral smoothing and baseline shifts were addressed using the Savitzky-Golay derivative method, as described by Savitzky and Golay [27].

**Variable selection:** Interval Partial Least Squares (iPLS) and Successive Projections Algorithm (SPA) were employed for variable selection. Models were developed using a reduced subset of wavelengths selected by SPA, which enhances model performance by minimizing multicollinearity issues in MLR, as reported by [27]. The variable selection process was conducted in the MATLAB environment (MathWorks, USA).

**Calibration and validation:** The contents of protein, total carbohydrates, and lipids were quantified using the PLS algorithm for predicting chemical component concentrations. Calibration and validation procedures were conducted in MATLAB using the PLS Toolbox (Eigenvector Research, Inc., USA). For calibration, three commercial powdered samples of *Chlorella pyrenoidosa* and *Limnospira maxima*, with known concentrations of protein, carbohydrates, and lipids, were utilized according to Naturwaren-Niederrhein GmbH methodology (https://www.naturwaren-niederrhein.de), as referenced by [27]. The known contents of proteins, total carbohydrates, and fat in the standard samples are presented in Table 1:

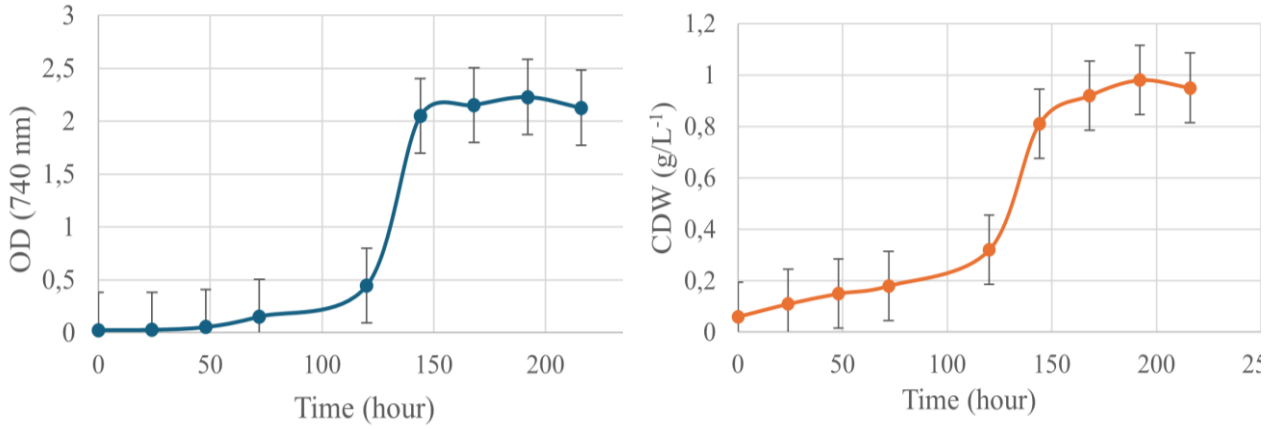
**Table 1**. Protein, carbohydrate, and lipid content of standard samples

| Content (g/100g) | *Chlorella pyrenoidosa* | *Limnospira maxima* | |
|---|---|---|---|
| | | Standard 1 | Standard 2 |
| Proteins | 57.3 | 60.4 | 64 |
| Carbohydrates | 11 | 20.4 | 18 |
| Lipids | 10.3 | 4.1 | 5.9 |

**Statistical analysis:** All experiments were conducted in five biological replicates (n = 5). Data are presented as mean ± standard deviation (SD). Statistical analysis was performed using Microsoft Excel (version 2504). Differences between experimental groups were evaluated using the Student's (William Sealy Gosset) t-test criteria. A p-value of less than 0.05 was considered statistically significant.

## RESULTS

**Cultivation of *Limnospira maxima* in a bubble column photobioreactor:** The growth performance of *Limnospira maxima* Pc-02 was evaluated in a 1-liter bubble column photobioreactor under continuous illumination with a combination of blue and red LED light, using a modified Zarrouk's medium. Biomass development was assessed by monitoring optical density (OD) and dry weight (DW) over time, enabling the determination of the specific growth rate (Figure 2).




**(A)** **(B)**

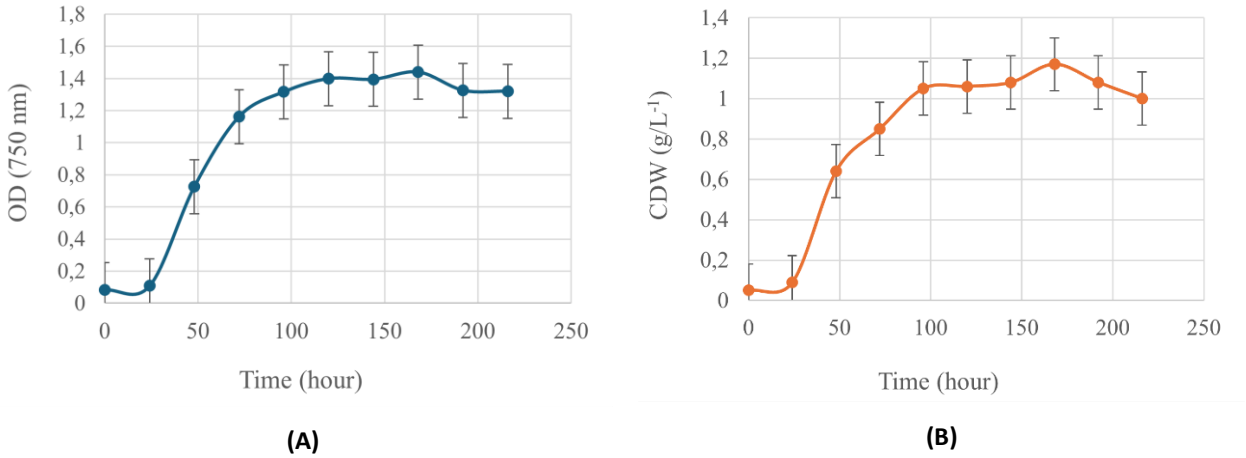
**Figure 2.** Changes in optical density **(A)** and dry biomass **(B)** during the cultivation of *Limnospira maxima* cyanobacterium. The error bars represent the standard deviation from the mean of the replicate data (n = 5).

Figure 2 illustrates the temporal progression of optical density and dry biomass concentration during the cultivation period. Upon completion of cultivation, the filtration of the culture yielded 10.70 g of wet biomass per liter. Moisture analysis indicated a water content of 92.03%. A 1.07 g subsample was subjected to thermal drying for 6 minutes and 51 seconds, resulting in a final dry weight of 0.086 g. Based on these results, the solid content was calculated to be 7.97%, with a wet weight increase of 1,254.65% and a regained value of 1,154.65%.

The maximum dry biomass concentration of 0.98 $g/L^{-1}$ was achieved after 192 hours of cultivation. The specific growth rate (μ) was calculated to be 0.34 $day^{-1}$. However, a noticeable decline in growth rate was observed beyond day 8, likely due to increased culture density, reduced light penetration, and depletion of essential nutrients, particularly nitrate.

**Cultivation of *Chlorella vulgaris* and *Neochloris oleoabundans* in a bubble column photobioreactor:** The strains *Chlorella vulgaris* Pa-023 and *Neochloris oleoabundans* Pa-022 were cultivated in parallel under identical conditions in a 1-liter bubble column photobioreactor with constant blue and red LED light, using PUE I nutrient medium. Growth kinetics were evaluated through measurements of OD and biomass DW.

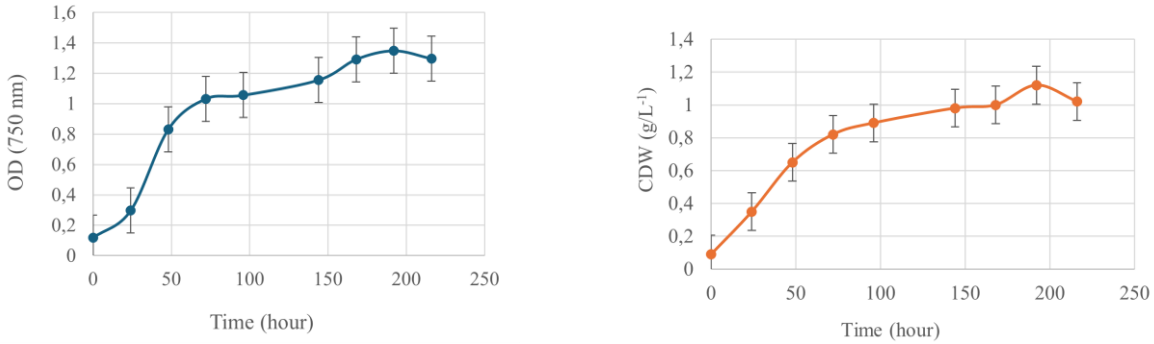
As illustrated in Figure 3, the highest biomass concentration for *Chlorella vulgaris* Pa-023 was achieved after 168 hours of cultivation, reaching 1.17 $g/L^{-1}$. Centrifugation (6000 rpm, 10 minutes) yielded 8.9g of wet biomass per liter. The sample (1.006 g) exhibited a moisture content of 90.56%, with a drying duration of 5 minutes and 7 seconds, resulting in a dry weight of 0.095 g. The solid content was 9.44%, with a wet weight increase of 1058.95% and a regain of 958.95%. The specific growth rate was calculated to be 0.384 $day^{-1}$. A slight decline in growth rate was observed after 7 days, likely due to increased cell density, light attenuation, and nutrient depletion.




**(A)** **(B)**

**Figure 3.** Changes in optical density **(A)** and dry biomass **(B)** during the cultivation of *Chlorella vulgaris* algae. The error bars represent the standard deviation from the mean of the replicate data (n = 5).

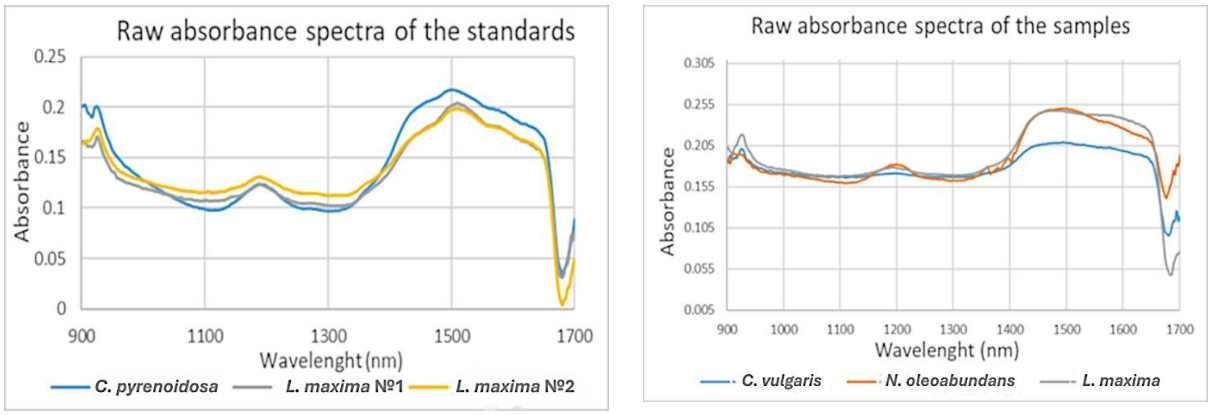
According to Figure 4, maximum biomass accumulation for *Neochloris oleoabundans* Pa-022 was observed at 192 hours, reaching 1.12 g/L$^{-1}$. Centrifugation of 1 liter of culture yielded 9.7g of wet biomass. The sample (1.056g) had a moisture content of 91.41%. After 4 minutes and 58 seconds of drying, the dry weight was measured as 0.091 g, corresponding to a solid content of 8.59%, a wet weight increase of 1,116.74%, and a regain of 1,163.74%. The specific growth rate was determined to be 0.315 day$^{-1}$. A gradual decrease in growth rate was observed after day 8, presumably due to self-shading and nutrient limitation.




**(A)** **(B)**

**Figure 4.** Changes in optical density **(A)** and dry biomass **(B)** during the cultivation of *Neochloris oleoabundans* algae. The error bars represent the standard deviation from the mean of replicate data (n = 5).

**Spectral analysis:** The raw absorbance spectra of standard (*Chlorella pyrenoidosa, Limnospira maxima*) and experimental (*Chlorella vulgaris, Neochloris oleoabundans, Limnospira maxima*) samples of algal powders exhibit comparable overall trends and feature distinct absorption bands near 920nm, 1200nm, and within the 1450-1650nm range. These spectral features are attributed to overlapping overtone and combination bands, primarily arising from C–H, O–H, and N–H vibrational modes. Such overlaps are characteristic of complex biological matrices and reflect the presence of key functional groups in the biomass (Figure 5).




**(A)** **(B)**

**Figure 5.** Raw absorbance spectra of **(A)** standard and **(B)** experimental microalgal and cyanobacterial powders

**Prediction of protein, lipid, and carbohydrate contents:** The predicted contents of protein, lipids, and carbohydrates for *Chlorella vulgaris*, *Neochloris oleoabundans*, and *Limnospira maxima* are presented in Table 2. The protein content was found to be highest in *Limnospira maxima* (59 ± 2.4g/100g), followed by *Chlorella vulgaris* (49.2 ± 2.2g/100g), then *Neochloris oleoabundans* (40 ± 2.1g/100g). Carbohydrate content ranged from 15.8 ± 1.6g/100g in *Chlorella vulgaris* to 21 ± 1.9g/100g in *Limnospira maxima*. In terms of lipid content, *Neochloris oleoabundans* exhibited the highest value (26 ± 1.9g/100g), which is approximately 27% higher than that of *Chlorella vulgaris* (19.6 ± 1.7g/100g) and about 76% higher than that of *Limnospira maxima* (6 ± 1.1g/100g).

**Table 2.** Predicted protein, lipid, and carbohydrate contents of experimental algal biomass samples (g/100g dry weight).

| Content (g/100 g) | *Chlorella vulgaris* | *Neochloris oleoabundans* | *Limnospira maxima* |
|---|---|---|---|
| Protein | 49.2 ± 2.2 | 40 ± 2.1 | 59 ± 2.4 |
| Lipids | 19.6 ± 1.7 | 26 ± 1.9 | 6 ± 1.1 |
| Carbohydrates | 15.8 ± 1.6 | 20 ± 1.8 | 21 ± 1.9 |

The errors represent the standard deviation from the mean of the replicate data (n = 5).

Given their high protein and relatively low carbohydrate and lipid contents, the biomass of *Chlorella vulgaris* and *Limnospira maxima* may be considered promising candidates for food and feed applications. In contrast, the biomass of *Neochloris oleoabundans*, characterized by a significantly higher lipid content and comparatively lower protein and carbohydrate levels, appears more suitable for lipid-oriented applications, such as components in functional lipid supplements. However, further analysis of fatty acid profiles is required to evaluate the nutritional and industrial value of the lipids present.

**Process engineering analysis--cost estimation for cultivation of *Limnospira maxima* in a 100-liter photobioreactor:** Cost efficiency plays a critical role in the optimization of microalgal cultivation systems. A cost analysis was performed for the 7-day cultivation of *Limnospira maxima* in a 100-liter photobioreactor to assess the economic feasibility of large-scale biomass

production. The study considered the consumption of chemicals, water, and electricity, with cost estimations based on EU (Germany) market prices (Table 3).

**Table 3.** Estimated costs of the cultivation cycle of *Limnospira maxima* in a 100-liter photobioreactor.

| Item | Average price per unit (€)* | Quantity used per 100-Liter | Cost per 100-Liter (€) |
|---|---|---|---|
| **Sodium nitrate ($NaNO_3$)** | 1.28/kg | 0.02 kg | 2.56 |
| **Sodium bicarbonate ($NaHCO_3$)** | 1.56 kg | 0.8 kg | 1.25 |
| **PUE II (nutrient solution**)** | 7.13/L | 0.05 L | 0.35 |
| **Electricity** | 0.32/kWh | 0.72 kWh | 39.14 |
| **Water (industrial price)** | 2.6/1000 L | 100 L | 0.26 |
| **Total Cost** | - | - | 43.56 |

*Comment:* * Bulk average prices in the EU as of Q1 2025. ** The detailed composition of PUE II remains proprietary at this stage of development.

**Table 4.** Electrical power consumption of 100-liter photobioreactor components.

| Component | Power Consumption (W) |
|---|---|
| **Lighting system** | 288 |
| **Pump (50% load)** | 40 |
| **Heater** | 400 |
| **Total** | 728 W |

Electricity consumption was calculated based on the power requirements of the photobioreactor components over a 7-day operational period, as shown in Table 4. The total energy consumption over 7 days was 0.72 kWh, accounting for the continuous operation of the lighting, temperature regulation, and circulation systems.

At the end of the cultivation period, the biomass yield was 1070 g (wet weight), corresponding to 98.0 g of dry biomass (Table 5).

**Table 5.** Biomass yield of *Limnospira maxima* after 7 days of cultivation.

| Biomass Type | Yield (g) |
|---|---|
| **Wet biomass** | 1070 |
| **Dry biomass** | 98.0 |

Based on the total input cost of €43.56, the net production cost is approximately €43.56 per 1,070 g of wet biomass or €43.56 per 98 g of dry biomass. This analysis underscores the relatively high proportion of electricity costs (approximately 89.5% of total operational expenses), highlighting the need for process optimization. Further efforts should focus on enhancing biomass productivity and reducing energy demand, particularly through the integration of renewable energy sources (e.g., solar or wind power). This would significantly improve the economic viability and sustainability of large-scale *Limnospira maxima* cultivation systems.

### Analysis of Costs in the EU Market Context

**Chemical inputs:** The cost of sodium nitrate (€1.28/kg) reflects the typical pricing for laboratory-grade reagents, which significantly inflates operational costs. For

industrial-scale applications, bulk procurement or the use of alternative nitrogen sources (e.g., urea or ammonium compounds) may reduce this expense. Sodium bicarbonate, a common buffering agent and carbon source, is reasonably priced in the EU at approximately €1.56/kg. Its high usage volume (0.8 kg per 100 L) results in a modest cost contribution (€1.25). The PUE II nutrient solution is used in small quantities and contributes minimally to overall costs (€0.35), suggesting it is economically feasible for use in mass production.

**Electricity:** The cost of electricity (€0.32/kWh) is notably high and accounts for 89.5% of the total production cost in this setup. This represents the average household or commercial electricity tariffs in Germany and other high-income EU countries, which typically range from €0.28 to €0.35/kWh, depending on the provider and contract conditions.

The photobioreactor's total power demand (728 W) over 7 days significantly impacts the operational budget, emphasizing the need for energy-efficient equipment and potential transition to renewable energy sources (e.g., solar panels or heat recovery systems) for economic and environmental sustainability.

**Water:** Water prices in the EU vary widely, but generally remain low for non-potable, or industrial, water. The applied cost of €2.26 per 1000 liters is consistent with regional rates and contributes minimally (€0.26) to total production costs.

### Implications and Recommendations

**Cost distribution:** The majority of the total cultivation cost is driven by electricity, followed by the high cost of sodium nitrate. Optimizing these two factors could substantially reduce production expenses.

**EU funding and policy opportunities:** Given the EU's support for green technologies and bioeconomy, producers may benefit from subsidies or funding schemes aimed at integrating renewable energy and promoting sustainable bioproduction.

**Scalability and industrial adaptation:** For industrial-scale production, replacing high-purity laboratory chemicals with industrial-grade alternatives, along with integrating energy-efficient or renewable systems, is essential for economic viability. PUEVIT GmbH, operating under its franchise brand ALGENWERK, has successfully scaled up its operations. It is currently establishing multiple external, decentralized production modules with capacities ranging from 30,000 to 48,000 liters. This modular approach allows for highly flexible, scalable, and location-adaptable deployment across diverse operational environments.

## DISCUSSION

Cultivation experiments conducted on Limnospira maxima, Chlorella vulgaris, and Neochloris oleoabundans in bubble column photobioreactors revealed notable differences in growth kinetics, biochemical profiles, and biomass yield potential under controlled laboratory conditions. Among these, *L. maxima* Pc-02 exhibited a high protein content (59 ± 2.4 g/100 g DW), making it an attractive candidate for food and feed applications. However, its relatively low lipid content (6 ± 1.1 g/100 g DW) may limit its suitability for lipid-based biofuel or nutraceutical markets. In contrast, *N. oleoabundans* produced the highest lipid content (26 ± 1.9 g/100 g DW), indicating a strong potential for applications in biodiesel production or as functional lipid supplements.

From a cultivation standpoint, *C. vulgaris* Pa-023 demonstrated the highest specific growth rate ($\mu$ = 0.384 $day^{-1}$), followed closely by *L. maxima* ($\mu$ = 0.34 $day^{-1}$), then *N. oleoabundans* ($\mu$ = 0.315 $day^{-1}$). These growth rates reflect a generally favorable adaptation of all three species to the photobioreactor environment, albeit with

diminishing returns observed beyond 7–8 days of cultivation. This trend is likely attributable to increased culture density, light attenuation, and nutrient limitation factors, which are well-documented constraints in dense phototrophic systems.

The application of NIR spectroscopy proved effective for the non-destructive estimation of macromolecular composition in algal biomass. Nonetheless, the accuracy and reliability of spectral predictions were limited by the number and diversity of reference samples. The substantial spectral overlap in the NIR region, particularly due to the combination bands of C–H, O–H, and N–H bonds, emphasizes the need for expanded calibration datasets encompassing a wider range for biochemical variability to improve multivariate modeling and predictive capability.

From an economic perspective, the cost analysis of *L. maxima* cultivation in a 100-liter photobioreactor underscores a key bottleneck in current microalgal biotechnology: the disproportionately high share of electricity costs (in this case, 89.5% of total operating expenses). This finding aligns with existing literature that emphasizes the energy-intensive nature of photobioreactor operation, particularly when artificial lighting and temperature control are required. While laboratory-grade sodium nitrate, sodium bicarbonate, and micronutrient supplementation (via PUE II nutrient solution) contribute relatively little to total cost, strategic substitution with industrial-grade or alternative nutrient sources could yield further savings.

Water costs were minimal and unlikely to pose a barrier to scale-up in the EU context. However, regional differences in water pricing and access, particularly for large-scale operations, must be considered in future techno-economic assessments.

To enhance the sustainability and economic viability of large-scale cultivation systems, future strategies should prioritize the integration of renewable energy sources (e.g., solar PV systems, passive solar heating, biogas cogeneration, or energy storage) and the adoption of high-efficiency lighting and pumping technologies. Additionally, exploring circular economic approaches, such as wastewater valorization and the utilization of agro-industrial by-products, could significantly offset input costs while enhancing environmental performance.

Finally, given the EU's policy emphasis on bioeconomy, circularity, and green energy, producers may be eligible for targeted subsidies or innovation funding. These funds could significantly accelerate the development of economically viable and environmentally sustainable microalgae-based production systems. The ALGENWERK business model already meets these criteria and has demonstrated economic feasibility, with the first customers currently in the planning stages of their production sites.

## CONCLUSION

Near-infrared spectroscopy was successfully employed as a non-destructive analytical tool for estimating protein, carbohydrate, and lipid contents in green microalgae (*Chlorella vulgaris* and *Neochloris oleoabundans*) and cyanobacteria (*Limnospira maxima*). Calibration was performed using commercially available powders of *Chlorella pyrenoidosa* and *Limnospira maxima*, with known biochemical compositions. The results highlight the necessity for a broader set of standard reference materials with accurately quantified macromolecular content. This would enhance multivariate regression models, improve the correlation between spectral data and chemical composition, and enable more precise compositional predictions.

Among the evaluated strains, *Limnospira maxima* Pc-02 exhibited particularly favorable biochemical characteristics, including a high protein content and moderate carbohydrate levels, as well as the potential for significant biomass yield in controlled photobioreactor cultivation. These attributes position *L. maxima* Pc-02 as a promising candidate for sustainable food and feed production. Nevertheless, process optimization is crucial for enhancing biomass productivity and reducing operational costs, particularly through the integration of renewable energy technologies that aim to minimize electricity consumption. Continued refinement of cultivation protocols and analytical calibration will be critical for advancing the commercial viability of microalgal and cyanobacterial biomass production.

**List of Abbreviations:** DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; NIR, near-infrared spectroscopy; PLS, partial least squares; iPLS, interval partial least squares; LED, light-emitting diode; PPFD, photosynthetic photon flux density; MSC, multiplicative scatter correction; SPA, successive projections algorithm; MLR, multiple linear regression; OD, optical density; DW, dry weight; Federation of European Microbiological Societies, FEMS; EU, European Union.

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**Authors' contributions:** All authors have accepted responsibility for the entire content of this manuscript and consented to its submission to the journal, reviewed all the results, and approved the final version of the manuscript. BH was involved in project supervision and investigation, data analysis and validation, and writing the original draft. GM, LS, and GH were responsible for data curation, analysis, and methodology development. RH, TS, and SH also dealt with methodology and data analysis. AS, AG, AZ, and GK performed formal analysis and investigation. VG handled supervision, methodology, data curation, analysis, writing support, and review.

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