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Production of Bio-Sustainable Protein from Algae Using Biochemical Approaches: A Green Alternative to Conventional Protein Sources

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Abstract

The escalating global demand for sustainable protein sources necessitates the exploration of alternative biomass resources. This study investigates the potential of microalgae, specifically *Chlorella* species, as a renewable source for protein production using biochemical extraction methods. Indigenous algal strains were isolated and cultivated under controlled laboratory conditions to optimize biomass yield and protein content. The research employed a combination of spectrophotometric assays, microscopy, and biochemical extraction techniques to characterize and quantify protein levels throughout the growth cycle. Key parameters, including pH, light intensity, temperature, and nutrient availability, were systematically optimized to enhance productivity. The growth dynamics of *Chlorella* spp. were monitored over 20 days, revealing distinct lag, exponential, stationary, and decline phases. Protein extraction was performed using alkaline lysis with 0.5 N NaOH, followed by heat treatment at 80°C. The investigation demonstrated that an optimal protein yield of 3.03 mg/mL was achieved at pH 6 with 2.0 g/L sodium bicarbonate supplementation. The results indicate that microalgae can produce substantial quantities of high-quality protein with minimal environmental impact, requiring significantly less land and water resources compared to conventional animal and plant-based protein sources. This work establishes algae-derived protein as a viable, eco-friendly alternative supporting the advancement of sustainable food systems and environmental biotechnology.

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Introduction

The convergence of rapid population growth and escalating environmental concerns has intensified the search for sustainable protein alternatives. Conventional protein sources, including livestock and crop-based systems, contribute significantly to greenhouse gas emissions, require extensive land use, and consume substantial freshwater resources [1]. According to United Nations projections, the global population will reach 9.7 billion by 2050, necessitating a doubling of food production to meet nutritional demands [2]. Currently, over one billion people worldwide experience inadequate protein intake, with maternal and child malnutrition causing more than 3.5 million deaths annually [3].

Microalgae represent a diverse group of photosynthetic microorganisms that have emerged as promising candidates for sustainable protein production. These organisms exhibit rapid growth rates, high photosynthetic efficiency, and the ability to thrive in non-arable lands and wastewater environments [4]. Species such as *Spirulina* (60-70% protein), *Chlorella* (50-60% protein), and *Dunaliella* (40-50% protein) have demonstrated exceptional nutritional profiles suitable for human and animal consumption [5]. Furthermore, microalgae cultivation does not compete with agricultural land use and offers high carbon dioxide fixation efficiency, contributing to carbon sequestration efforts [6]. The biochemical composition of microalgae includes essential amino acids, vitamins, minerals, and bioactive compounds that confer functional properties valuable for food applications [7]. Previous studies have established that algal proteins possess favourable solubility, emulsifying capacity, and gel-forming characteristics [8]. However, the optimization of cultivation conditions and extraction methodologies remains critical for maximizing protein yield and quality [9].

The choice between open pond systems and closed photobioreactors (PBRs) significantly influences biomass productivity and protein content. As summarized in, closed systems offer superior contamination control and process optimization capabilities, while open systems require lower capital investment [10]. Understanding these trade-offs is essential for designing cost-effective production systems. This study focuses on *Chlorella* species, a unicellular green alga recognized for its high protein content and chlorophyll concentration. The growth dynamics in batch culture demonstrate the characteristic phases of microalgal cultivation that must be understood for optimal harvest timing [11]. The research objectives include: (1) characterizing the growth dynamics of *Chlorella* spp. under controlled laboratory conditions; (2) optimizing pH and carbon supplementation parameters for maximum protein production; (3) developing an efficient alkaline-based protein extraction protocol; and (4) evaluating the potential of algae-derived protein as a sustainable alternative to conventional sources.

Materials and Methods

Strain and culture medium

Chlorella spp. was selected as the experimental organism due to its established high protein content and robust growth characteristics. The algal strain was cultivated in BG-11 medium, a synthetic freshwater medium formulated specifically for cyanobacteria and microalgae [12]. The medium composition included sodium nitrate ($NaNO_3$) as the nitrogen source, dipotassium phosphate (K_2HPO_4), magnesium sulfate ($MgSO_4 \cdot 7H_2O$), calcium chloride ($CaCl_2 \cdot 2H_2O$), citric acid, ferric ammonium citrate, EDTA, sodium carbonate (Na_2CO_3), and trace elements including boron, manganese, zinc, copper, molybdenum, and cobalt. All chemicals were of analytical grade. The medium was prepared by dissolving specified quantities of each component in distilled water and sterilized by autoclaving at 121°C for 15 minutes. Culture vessels consisted of 500 mL sterile Erlenmeyer flasks containing 200 mL working volume. Pre-cultures grown for 7 days were used as inoculum at 5-10% (v/v).

Cultivation conditions

Cultures were maintained under controlled environmental conditions: temperature of 25±2°C, cool white fluorescent light at approximately 3000 lux with a 16:8-hour light: dark photoperiod. Flasks were manually agitated every 2 days to prevent sedimentation and ensure uniform light exposure.

Growth monitoring

Algal growth was monitored daily using the following parameters: Optical density (OD_{680}) was Measured using a spectrophotometer at 680 nm to estimate biomass concentration based on chlorophyll absorption [13]; pH Monitoring was done using a digital pH meters and visual indicator strips were employed to track metabolic changes in the culture medium; Cell morphology, density, and chloroplast development were examined at 400× magnification

on Days 1, 3, and 6 using a microscope; and color changes and biomass settling were documented using tube-based techniques.

Protein extraction protocol

Protein extraction was performed using an alkaline-based method with the following procedure: Algal biomass was harvested during the exponential growth phase by centrifugation at 5,000 rpm for 5 minutes. Pellets were washed 2-3 times with distilled water to remove residual medium components. Cell lysis was achieved using 0.5 N NaOH to break down cell walls and release intracellular proteins. Heat treatment at 80°C for 10 minutes was applied to enhance protein solubility. Samples were cooled on ice for 5 minutes and centrifuged at 13,000 rpm at 4°C for 10 minutes. The extraction procedure was repeated using the residual pellet to maximize protein recovery.

Protein quantification

Protein concentration was determined using a NanoDrop spectrophotometer at 280 nm, based on the absorbance of aromatic amino acids (tryptophan, tyrosine, and phenylalanine). The method required only 2 µL of sample without additional reagents. Results were cross-validated using the Bradford assay to ensure accuracy [14].

Parameter optimization

To evaluate the effect of pH on protein production, cultures were adjusted to pH 6, 8, and 10 using NaOH and HCl. For carbon supplementation studies, sodium bicarbonate ($NaHCO_3$) was added at concentrations of 0.5, 1.0, and 2.0 g/L. All experiments were conducted in triplicate with appropriate controls.

Results

Growth curve analysis

The growth dynamics of *Chlorella* spp. over the 20-day cultivation period exhibited the characteristic sigmoidal pattern of microbial growth, comprising five distinct phases. The lag phase (Fig. 1) represents the initial adaptation period where cells acclimate to the BG-11 medium environment [15]. Metabolic processes, including enzyme and protein synthesis, were initiated with minimal cell division. The pH remained at approximately 5.5 due to dissolved CO_2, while OD_{680} showed a gradual increase. Microscopic examination revealed small, sparse cells with underdeveloped chloroplasts. During the log (exponential) phase (Fig. 2), rapid cell division through mitosis resulted in exponential biomass increase. OD_{680} values rose significantly as cell numbers increased. The pH increased from 5.5 to approximately 6.3, indicating active CO_2 fixation and oxygen production through photosynthesis. Enhanced chlorophyll content produced a brighter green culture color. Microscopic analysis showed more uniform cell size and shape, indicating synchronized division.

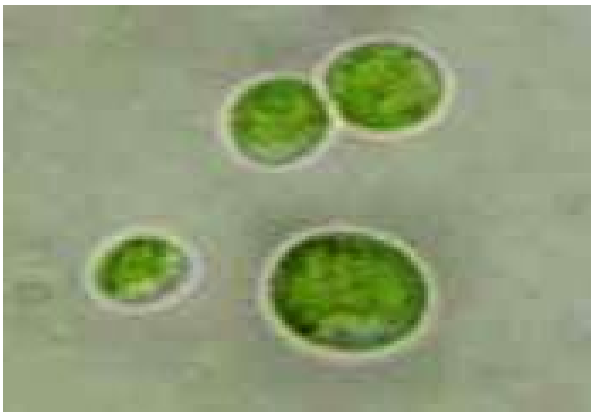

Fig. 1 Lag phase of *Chlorella* growth. Microscopic observation showing sparse cell distribution during the initial adaptation period (Day 1).

Fig. 2 Exponential growth phase. Dense cell population observed during peak growth (Day 3-6), characterized by rapid cell division and chlorophyll accumulation.

The experimental growth phase (Day 3-10) represented peak physiological and metabolic activity. By day 10, pH reached approximately 8.0 and OD_{680} values peaked, indicating maximum photosynthetic activity and near-complete CO_2 depletion. Cells appeared large, densely packed, and highly pigmented with fully developed chloroplasts. This phase was optimal for protein accumulation as

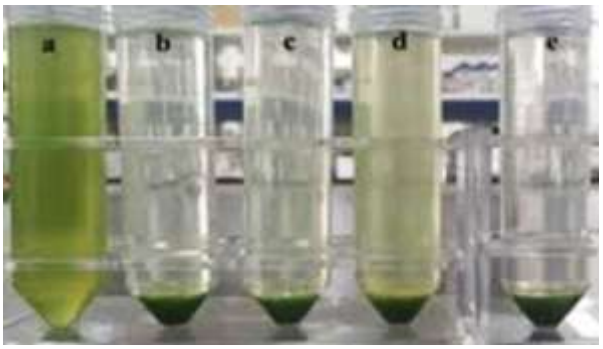

Fig. 3 Decline phase characteristics. Microscopic image showing cell deterioration and reduced viability during the late cultivation period (Day 14-20).

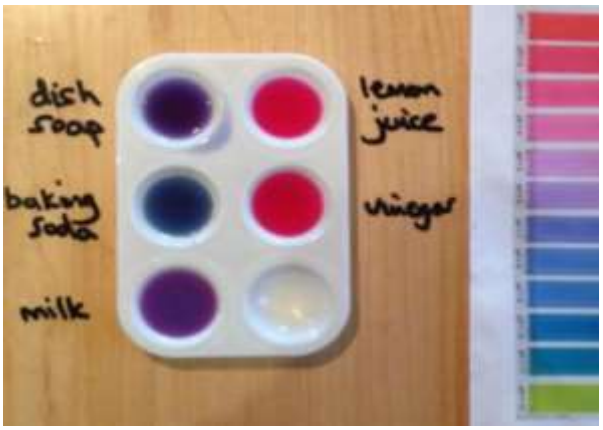

Fig. 4 pH indicator strips showing pH variation during cultivation. Visual documentation of metabolic changes from acidic (Day 2, orange) to alkaline (Day 6, green-yellow) conditions.

metabolic processes were fully activated. During the stationary phase (Day 10-14), cell division rate stabilized and subsequently declined due to nutrient depletion, light limitation from self-shading, and accumulation of metabolic waste products. OD_{680} stabilized between 1.1 and 1.2, while pH remained relatively constant at 7.5-7.8. Despite reduced division, cells maintained structural integrity and protein content, making this phase suitable for biomass harvesting. The decline phase (Day 14-20) (Fig. 3) was characterized by nutrient exhaustion and waste accumulation leading to culture deterioration. Cell viability decreased, OD_{680} dropped to approximately 0.964, and pH stabilized at 7.0. Microscopic examination revealed distorted cells with reduced chloroplast content and membrane damage. Autolysis resulted in lower quality biomass unsuitable for protein extraction. The pH monitoring using visual indicator strips (Fig. 4) confirmed the metabolic progression from acidic (Day 2, orange, pH ~5.0) to alkaline (Day 6, green-yellow, pH ~7.5) conditions. Biomass development in microcentrifuge tubes demonstrated the visual progression from turbid light green (Day 2) to opaque bright green with visible pellet formation (Day 6). Microscopic analysis on Days 1, 3, and 6 documented the progression from sparse small cells to dense healthy populations with fully developed chloroplasts.

Effect of pH on protein production

Protein synthesis exhibited strong pH dependence (Table 1). Maximum protein yield of 0.97 mg/mL was achieved at pH 6, representing optimal conditions for enzymatic processes involved in protein synthesis and photosynthesis. At pH 8, protein production decreased to 0.40 mg/mL due to reduced CO_2 availability and inefficient nitrogen assimilation. Minimal protein production (0.015 mg/mL) occurred at pH 10, where extreme alkaline conditions caused oxidative stress and enzyme degradation [16].

Effect of sodium bicarbonate supplementation

Carbon supplementation demonstrated a dose-dependent increase in protein yield (Table 2). At 0.5 g/L $NaHCO_3$, protein concentration was 0.315 mg/mL due to insufficient carbon availability limiting biosynthesis. Optimal protein yield of 3.03 mg/mL was achieved at 2.0 g/L $NaHCO_3$, were ample carbon supply enhanced photosynthetic output and protein synthesis. Bicarbonate ions were actively transported via membrane-bound channels and utilized in the Calvin cycle for carbon fixation and protein production [17].

Protein extraction efficiency

The alkaline lysis protocol with heat treatment successfully disrupted algal cell walls and released intracellular proteins. Enhanced lysis methods, including French Press, enzymatic lysis, and detergent treatments, may further improve protein recovery from recalcitrant species [18]. The second

Table 4 Effect of pH on algal protein production.

pH	Protein (mg/mL)	Biological Implications
6	0.965	Optimal enzymatic activity and nutrient solubility
8	0.404	Reduced CO_2 availability, inefficient nitrogen assimilation
10	0.015	High oxidative stress, enzyme degradation

Results from this study demonstrating optimal protein synthesis at pH 6.

Table 5 Effect of sodium bicarbonate concentration on protein yield.

$NaHCO_3$ (g/L)	Protein (mg/mL)	Implications
0.5	0.305	Insufficient carbon, limited biosynthesis
1.0	0.892	Adequate carbon supply
2.0	3.030	Optimal carbon for maximum protein synthesis

Results from this study show a dose-dependent increase in protein yield with $NaHCO_3$ supplementation.

extraction step improved overall protein recovery by 20-30%. The baseline protein concentration from a standard 50 mL culture was 1.58 mg/mL, which was enhanced to 3.030 mg/mL under optimized conditions (pH 6, 2.0 g/L $NaHCO_3$). Quality assessment techniques, including SDS-PAGE and amino acid profiling, will confirm the nutritional value of extracted proteins in future studies [19].

Discussion

The present study demonstrates the feasibility of *Chlorella* spp. as a sustainable source of high-quality protein through optimized cultivation and extraction protocols. The growth curve analysis revealed that Chlorella exhibits typical microbial growth kinetics with clearly defined phases, consistent with previous reports on microalgal cultivation [20]. The exponential phase (Day 1-10) represents the critical window for biomass production, where maximum cell division and protein accumulation occur. The correlation between pH changes and metabolic activity provides valuable insights into culture health monitoring. The shift from acidic (pH 5.5) to alkaline (pH 8.0) conditions during active growth reflects the balance between CO_2 dissolution (forming carbonic acid) and photosynthetic CO_2 fixation (consuming carbonic acid and releasing oxygen). This pH progression serves as an effective indicator of photosynthetic efficiency and can be utilized for real-time culture monitoring using simple visual methods [21].

The pH optimization results align with established physiological understanding of microalgal metabolism. At pH 6, CO_2 is the predominant form of inorganic carbon, readily available for photosynthetic carbon fixation via ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO). The stability and functionality of transport systems and enzymes are optimized under mildly acidic conditions [22]. Conversely, at pH 10, carbonate (CO_3^{2-}) becomes the dominant carbon species, which is less bioavailable and can cause oxidative damage and ammonia toxicity, explaining the severely reduced protein synthesis. The dose-dependent response to sodium bicarbonate supplementation confirms carbon availability as a limiting factor in algal protein production. Bicarbonate serves dual functions: as a carbon source for photosynthesis and as a pH buffer stabilizing the culture medium. The 2.0 g/L concentration provided sufficient inorganic carbon to support maximum photosynthetic rates without inducing osmotic stress or pH instability [23].

Comparative analysis with conventional protein sources highlights the sustainability advantages of algal protein. Microalgae cultivation requires approximately 10-50 times less land than soybean production and 100-400 times less than beef production per unit protein [24]. Additionally, algae can utilize saline or wastewater, reduce freshwater dependency, and provide wastewater treatment benefits through nutrient removal. The alkaline extraction method employed in this study offers several advantages for industrial scale-up: simplicity, cost-effectiveness, and avoidance of toxic solvents. The heat treatment step enhances protein solubility while the alkaline environment denatures native proteases, preventing protein degradation [25]. The 20-30% yield improvement from secondary extraction justifies the additional processing step for industrial applications. Future research directions should include advanced protein characterization using SDS-PAGE and mass spectrometry to evaluate amino acid composition and functional properties. Comparative cost analysis between bicarbonate and gaseous CO_2 supplementation would inform economic feasibility. Extension of the optimized protocol to other microalgal species, such as *Scenedesmus* and *Spirulina*, may reveal species-specific advantages for protein production.

Conclusion

This study successfully demonstrates the potential of Chlorella microalgae as a sustainable protein alternative through systematic optimization of cultivation parameters and extraction protocols. The integration of optical density measurements, pH monitoring, and microscopic analyses provided a comprehensive understanding of growth dynamics, identifying the exponential phase (Day 3-10) as optimal for biomass harvesting. Key findings include: (1) maximum protein yield of 3.030 mg/mL was achieved at pH 6 with 2.0 g/L sodium bicarbonate supplementation, representing a 92% improvement

over baseline conditions; (2) the alkaline lysis protocol with heat treatment effectively disrupted cell walls and preserved protein integrity; and (3) secondary extraction improved overall recovery by 20-30%. The results establish algae-derived protein as a viable, environmentally sustainable alternative to conventional protein sources. Microalgae cultivation offers significant advantages, including rapid growth, minimal land and water requirements, carbon sequestration capability, and compatibility with wastewater utilization. These characteristics position algal protein as a critical component of future sustainable food systems. The optimized methodology provides a foundation for scale-up to industrial production, with recommendations for maintaining culture pH at 6, supplementing with 2-4 g/L $NaHCO_3$, and implementing downstream processing, including ultrafiltration for concentration and freeze-drying for preservation.

Acknowledgments

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Conflict of Interest

The authors declare no conflict of interest regarding the publication of this manuscript.

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