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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

In response to the global demand for sustainable protein sources, this study investigates the potential of microalgae as a renewable biomass for protein production using biochemical methods. Indigenous algal strains were isolated and cultivated under controlled conditions to optimize biomass yield and protein content. A combination of spectrophotometric assays, microscopy, and biochemical extraction techniques were employed to characterize and quantify protein levels. Parameters such as pH, light intensity, temperature, and nutrient availability were optimized to enhance productivity. The results demonstrated that under ideal growth conditions, microalgae could produce a significant amount of protein with high bio-sustainability and minimal environmental impact. This work highlights the applicability of algae-derived protein as a viable, eco-friendly alternative to conventional animal and plant-based protein sources, supporting the advancement of sustainable food systems and environmental biotechnology.

Introduction

Background

Environmental concerns and rapid population growth are made up increasing the demand for sustainable and more environmentally friendly protein sources, which finds for exploring ways to meet the world's nutritional demands and fulfills words' needs . Conventional protein sources, such as those which are derived from crops and livestock produces high amount of greenhouse gas emissions, have a more negative impact on the environment. They use excessive water and extensive land. Algae, microalgae, have become a different substitute in this regard because of their high protein content, quick growth rates, and low resource needs. By the United Nations findings by 2050, there will be 9.7 billion people on the planet. So, to feed them food production should be double [1]. Nowadays, more than 3.5 million

deaths are caused by maternal and child malnutrition annually. According to the World Health Organization (WHO), around 45% of child mortalities are caused by malnutrition, and over one billion people have inadequate protein intake [2]. For these reasons, there is a need to find new, nutrient-rich protein sources. Microalgae are a diverse group of microorganisms known as phytoplankton and their classification is under constant revision due to new genetic evidence [4]. Nevertheless, most microalgae are unicellular, photosynthetic microorganisms producing oxygen and assimilating carbon dioxide by obtaining macro- and micronutrients from aquatic environments. The term 'microalgae' applies to both eukaryotic microalgae and prokaryotic cyanobacteria [5] and they play an important role in the marine food chain as the primary source of omega-3 fatty acids [6]. Microalgae can survive in wastewater, ocean water and brine water; they have a 13 high growth rate and productivity, they do not compete with agricultural land, and they have high CO2-fixing efficiency [7]. Microalgae, such as "Spirulina, Chlorella, and Dunaliella," are well-recognized for their nutritional profiles and ability to thrive in diverse environmental conditions. For instance, Spirulina comprises 60-70% protein by dry weight and is rich in essential amino acids, while Chlorella offers up to 60% protein content along with vital vitamins and minerals. These properties position algae as a sustainable, scalable solution to addressing food security challenges.

The research includes laboratory-scale studies of algal cultivation, protein extraction, and characterization. It also includes metabolic pathway analysis and sustainability assessments to establish algae as a mainstream protein source.

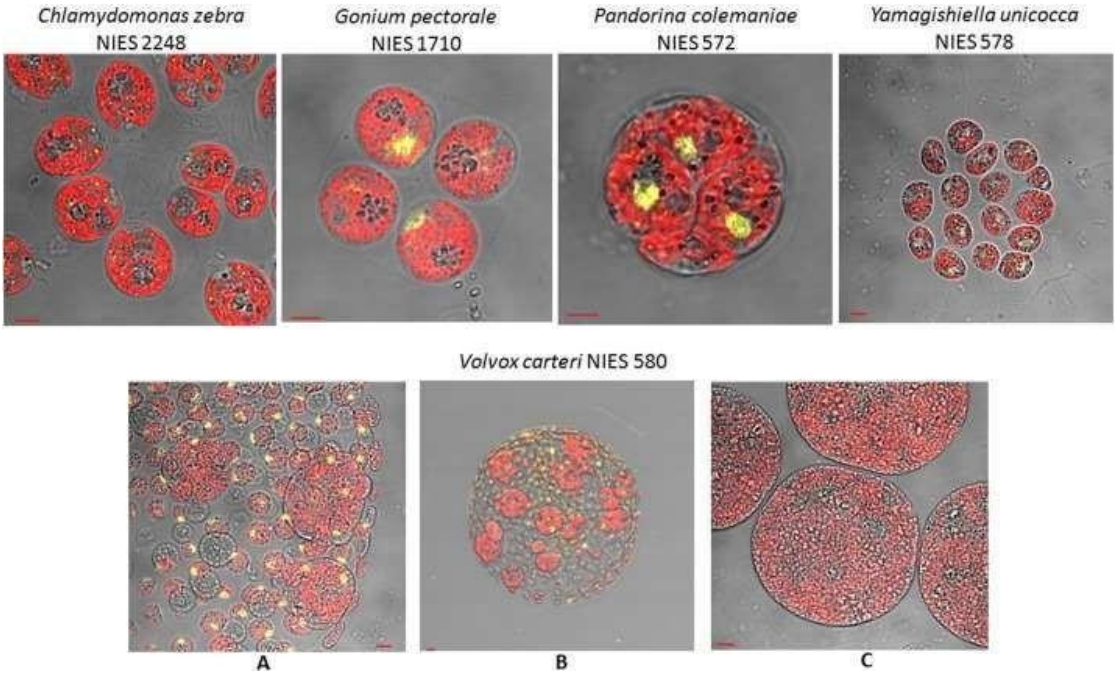

Figure 1: Confocal laser scanning images merged with differential interference contrast microscopic images.

Algae as a Protein Source

Algae are highly efficient organisms for protein production due to their rapid growth, high photosynthetic efficiency, and ability to utilize non-arable lands. Among algae, microalgae have gained significant attention. Studies highlight the high protein content in Spirulina (60-70%), Chlorella (50-60%), and Dunaliella (40-50%), ma3king them suitable for human and animal consumption.

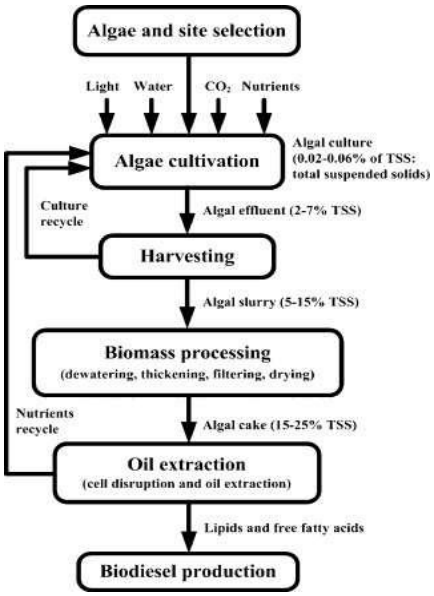

Figure 2: Microalgae biodiesel value chain stages.

Cultivation Techniques

Micro algal cultivation can be conducted in open ponds or closed photo bioreactors. Closed systems offer controlled environments, ensuring higher biomass yields and reduced contamination risks. Optimal growth conditions, including light intensity, photoperiod, temperature, CO2 concentration, and nutrient availability, significantly influence protein production.

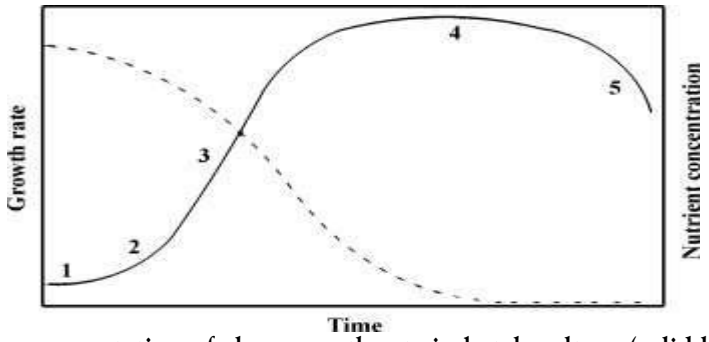

Figure 3: Schematic representation of algae growth rate in batch culture (solid line) and nutrients concentration (dashed line)

Metabolic Engineering

Metabolic engineering strategies aim to enhance protein biosynthesis in algae by targeting pathways such as mTOR and eIF2. Techniques like CRISPR-Cas9 allow genetic modifications to up regulate protein synthesis enzymes or suppress competing pathways, thereby improving yield.

Table 1: Culture systems for microalgae

Culture systems for microalgae	Closed systems (PBRs)	Open systems (Ponds)
Contamination control	Easy	Difficult

Contamination risk	Reduced	High
Sterility	Achievable	None
Process control	Easy	Difficult
Species control	Easy	Difficult
Mixing	Uniform	Very poor
Operation regime	Batch or semi-continuous	Batch or semi-continuous
Space required	A matter of productivity	PBRs ~ Ponds
Area/volume ratio	High (20–200 m^{-1})	Low (5–10 m^{-1})
Population (algal cell) density	High	Low
Investment	High	Low
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Investment	High	Low

Environmental Sustainability

Comparative studies demonstrate that algae-based protein production has lower water, land, and carbon footprint requirements than conventional sources such as soy or livestock. For example, algae can grow using saline or wastewater, reducing freshwater dependency.

Table 2: Lipid content and productivities of different microalgae species

Marine and freshwater microalgae species	Lipid content (% dry weight biomass)	Lipid productivity (mg/L/day)	Volumetric productivity of biomass (g/L/day)	Areal productivity of biomass (g/m^2/day)
***Ankistrodesmus* sp.**	24.0–31.0	–	–	11.5–17.4
Botryococcus Baruni	25.0–75.0	–	0.02	3.0

Chaetoceros muelleri	33.6	21.8	0.07	–
Chaetoceros calcitrant	14.6–16.4/39.8	17.6	0.04	–
Chlorella emersonii	25.0–63.0	10.3–50.0	0.036–0.041	0.91–0.97
Chlorella protothecoides	14.6–57.8	1214	2.00–7.70	–
Chlorella sorokiniana	19.0–22.0	44.7	0.23–1.47	–

As non-diastrophic animals, algae depend on environmental nitrogen and phosphorus for growth. Algae under extreme stress from prolonged shortage of macronutrients like carbon, nitrogen, phosphorus, and sulfur experience growth slowdown and eventually cell death.

Algal Strain Selection

The study begins with selecting micro algal species based on protein content and growth characteristics. Species such as Spirulina platensis, Chlorella vulgaris, and Tetraselmis Chui are prioritized for their high protein yields and robust cultivation protocols.

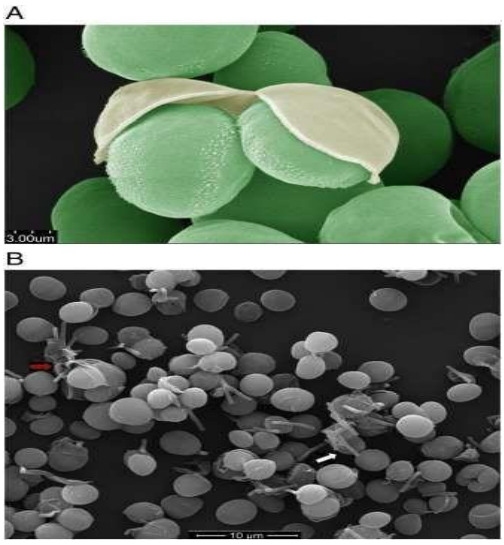

Figure 5: The algal photosphere and its components. Recolored SEM images of axenic C. vulgaris.

Studies from our laboratory [1] and elsewhere [2] suggest that photosphere is one of the most ignored and distinctive habitat for bacteria. Scanning [3](SEM) images show axenic C. vulgaris and xenic C. vulgaris with bacteria on the cell wall and beneath the cell sheath. The photosphere is equivalent to an oasis for heterotrophic bacteria, where high concentrations of fixed organic carbon is excreted for consumption, compared with the vast oligotrophic surroundings in ocean and freshwater.

Cultivation Conditions

Algal strains are cultivated in laboratory-scale photo bioreactors. Key parameters optimized include:

- Light intensity and photoperiod: Adjusted to enhance photosynthesis.
- Temperature: Maintained within optimal ranges for growth.
- Nutrient availability: Ensured by providing adequate nitrogen and phosphorus.
- pH: Monitored and adjusted to maintain suitable growth conditions.

Table 3: A summary of current understanding of evolutionary and ecological roles to harness biotechnological potential of algal–bacterial interactions.

1.	**Strain selection**	**Algae harboring PGPB are known to evade pathogens like harmful bacteria and fungi.**	**Help maintain desirable microbial community and avoid frequent cultivation crashes.**	[1]
2.	Cultivation	Bacteria aid algal growth by supplementing various major and minor nutrients in oligotrophic environments.	Enhanced growth rate and algal productivity. Reduced dependence on supplied nutrients.	[5]
3.	Harvesting	Bacteria initiate algal flocculation possibly for two reasons. Firstly, large algal– bacterial flocs help algae evade predators like zooplankton as large flocs are difficult to consume. Secondly, bacteria willingly settle algae resulting in algal death and subsequent bacterial degradation.	Harvesting accounts for 30% of overall costs in algal bio- product industry. Large algal– bacterial flocs settle readily, resulting in reduced use of flocculants and costs, and better yield.	[1]
4.	Extraction	Pathogenic bacteria weaken algal cell wall resulting in disruption and cell death, playing a leading role in decomposition.	A study showed enhanced lipid recovery from bacteria infested algae thereby reducing the conic solvent extraction.	[5]

Protein Extraction and Analysis

Protein extraction is conducted using mechanical, solvent-based, and enzymatic methods. Extracted proteins are analyzed for:

- Amino acid composition: Determined using high-performance liquid chromatography (HPLC).
- Functional properties: Includes solubility, emulsifying capacity, and gel formation.

Microalgae as food products are like a future that will come soon, but the truth is that microalgae are old allies of [6] and Spirulina varieties have a historical record as food before 1900. In recent decades, microalgae biomass has been applied as food with healthy claims once the main products based on microalgae are food supplements, additives, and ingredients. For instance, DHA) is present in fortifications of juices, milk, and other beverages, especially for infants and children [7] Worldwide, between 2015 and 2019, approximately 13.090 new food products were reported to contain algae or derived components. These new products included 79% in food and 21% in beverages.

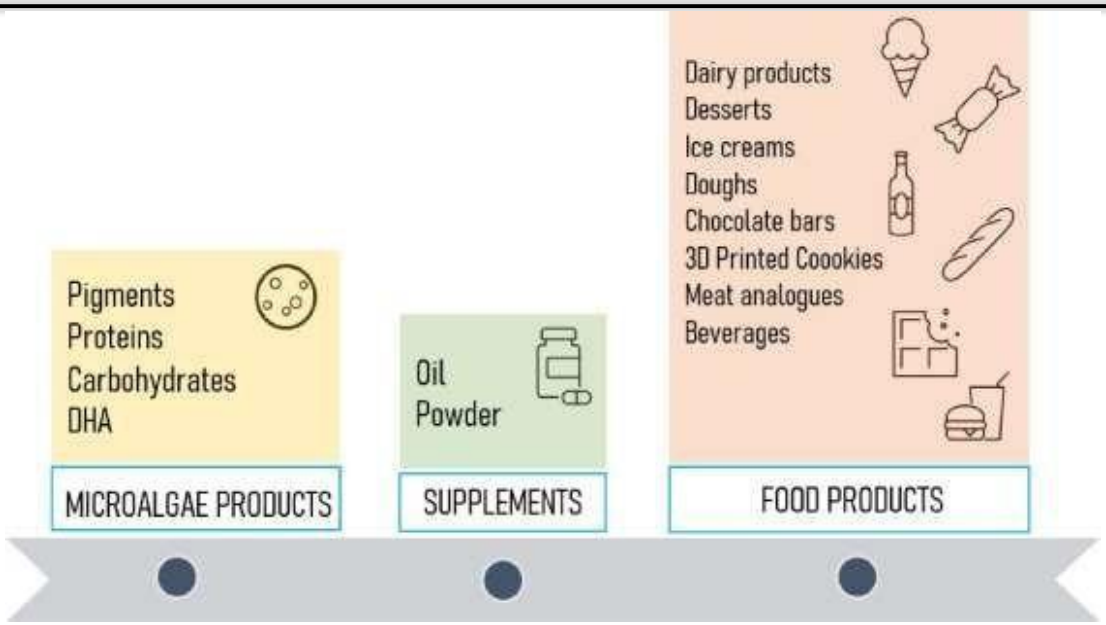

Figure 6: Algae and microalgae-based food products.

Materials and Method

Strain and Medium

A single-celled green alga Chlorella spp, which is known due to its high protein content and chlorophyll, is the algal strain used for this research. The cultures were grown in BG-11 medium; a well-known synthetic medium made specially for freshwater microalgae and including vitamins, phosphates, calcium, magnesium, trace elements, and nitrogen (in the form of ₃). Because of its ability to increase the best possible algal development or growth, as evidenced by the body of current literature, BG-11 was finally considered . [3].

Medium Preparation: Analytical-grade chemicals were used to make BG-11, which was then sterilized for 15 minutes at 121°C.

Culture Vessels: 200 mL working volumes were used in 500 mL sterile Erlenmeyer flasks.

Inoculum Preparation: Pre-cultures were used as inoculum at 5–10% v/v after being cultivated for 7 days.

Environmental Conditions: The culture was then kept at 25 ± 2°C with cool white, fluorescent light (~3000 lux) with a 16:8-hour light: dark cycle.

Agitation: To avoid the sedimentation, flasks were physically spun to provide even light exposure after 2 days. [4].

Figure 8: BG-11 Media

The main aim of this experimental arrangement was to find the maximum nutrient availability, and to get rid of contaminants, and mimic natural development circumstances.

Growth Monitoring: Tools and Techniques

Daily monitoring of the following parameters was performed:

- Optical Density (OD_{680}): By measuring the amount of light absorbed by chlorophyll pigments, this measurement which does not damage the samples used to determine algal biomass.
- pH Monitoring: Both digital meters and visual pH strips were employed to track changes in the acidity or alkalinity of the culture through pH levels, offering to see the metabolic variations.

Along with these readings, microscopic examinations should also done on Days 1, 3, and 6 to see the cellular structure, density, and chlorophyll production. Additionally, visual observations regarding color changes and biomass settling were documented using tube-based techniques [4].

Observations from the Research

Growth Curve Phases: Detailed Breakdown

There are five different phases in the growth curve of Chlorella species, each of which represents a different physiological and metabolic role in the culture of algal biomass. The daily measurement of optical density (OD_{680}), pH levels, and done microscopic analysis helps in the observation of progress through these stages. Below is a comprehensive explanation of every stage.

Lag Phase (Day 0–1)

The very first time the Chlorella cells acclimate to unique environment in the BG- 11 medium is known as the lag phase. As metabolic processes like enzyme and protein synthesis start, there is can see small cell division. Because of the dissolved CO_2, the pH remains at 5.5 but the OD_{680} gradually increases. When viewed under a microscope Cells appear tiny, sparse, and have underdeveloped chloroplast. The cells are completely ready for active expansion in subsequent phases during this phase.[1].

Figure 9: Log Phase

Log (Exponential) Phase (Day 1–3)

During the log phase Chlorella cells divide rapidly, which pushes the algal biomass to grow exponentially. Cells divide through mitosis more quickly during this stage, increasing the biomass of the culture. As number of cells increases, the optical density (OD_{680}) also begins to grow noticeably [6]. The intake of CO_2 for photosynthesis and the release of oxygen are indicated by the pH level rising from 5.5 to roughly 6.3. Increased photosynthetic activity is shown by a higher chlorophyll content as the culture attains brighter. When viewed under a microscope, the cells appear more consistent in size and shape indicating synchronized division. In respect to maximize biomass production and get ready for the best development and metabolic processes, this phase is particularly important.

Experimental Growth Phase (Day 3–10)

The peak of Chlorella's physiological and metabolic processes appears during the experimental growth phase. During the cultivation period, the cells are in good health and produce the maximum biomass due to their fast proliferation. Around Day 10, the pH increases to about 8.0 and the OD_{680} values peak, which indicate that CO_2 is almost depleted and photosynthetic activity is at its peak. The algal cells seem big, packed, consistent and highly pigmented under a microscope, which shows that the chloroplasts were completely formed and functioning well. Since the cells are actively accumulating proteins and their metabolic processes are fully powered, this stage is crucial for protein development [7]. To produce high-quality biomass for subsequent protein extraction, the growth rate during this phase is crucial.

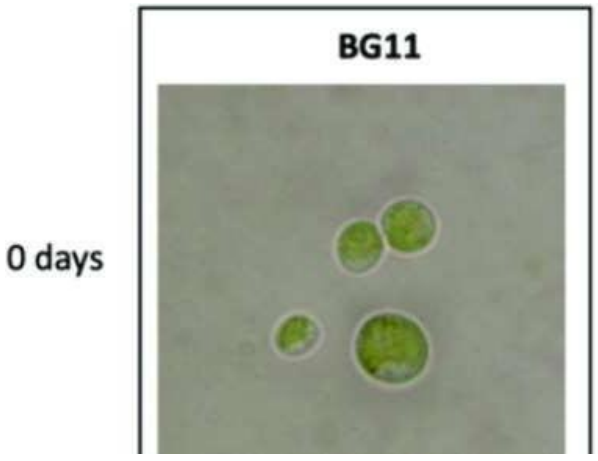

Figure 10: Exponential Phase

Stationary Phase (Day 10–14)

During this phase, the rate of cell division first remains constant and then slows down, but the total biomass remains constant as the culture enters the stationary phase. Various limiting factors, including nutrition depletion, light reduction from self- shading, and the accumulation of metabolic waste products, are considered for this decrease. Growth reaches a plateau when the optical density at 680 nm stabilizes between 1.1 and 1.2. The pH will be relatively constant, dropping slightly to between
7.5 and 7.8, which indicates that metabolic processes are slowing down but not entirely ceasing. In respect of the reduction in cell division, the cells remain alive and find a way to store proteins. Because the cells maintain their structural integrity and protein content during this phase, they are ideal for protein extraction, which makes it important for biomass collection.

Decline Phase (Day 14–20)

The decline phase is the last stage in Chlorella growth, during which time waste accumulation and nutrient depletion cause the culture to degenerate. Reduced photosynthetic activity is indicated by a decline in cell viability, a plateau in pH at 7.0, and a drop occur in OD_{680} to about 0.964. Cells look warped under a microscope, have less chloroplasts, and many of them sink to the bottom of the flask because their membranes are shattered. Autolysis, in which cells broke internally, results in lower- quality biomass throughout this phase. So this phase tell us it's not the best time for harvesting, but it gives insight into how Chlorella behaves when grown for a long-time membrane integrity [2].

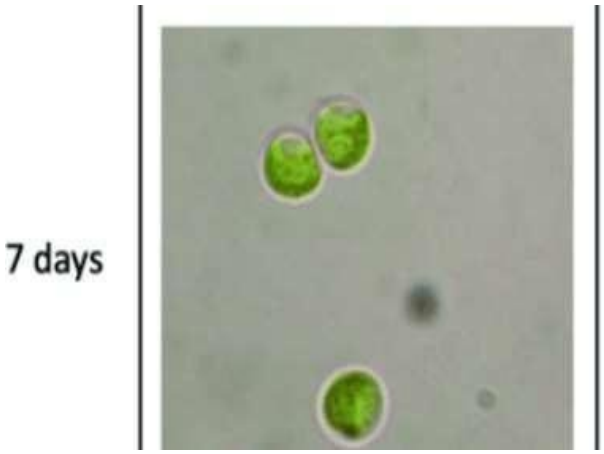

Figure 11: Decline Phase

To understand the dynamics of Chlorella spp. growth is essential for optimizing biomass yield for protein extraction and usages for sustainable protein production. The careful monitoring of pH, OD_{680}, and microscopic observations has produced valuable data that will help in the research's next phases.

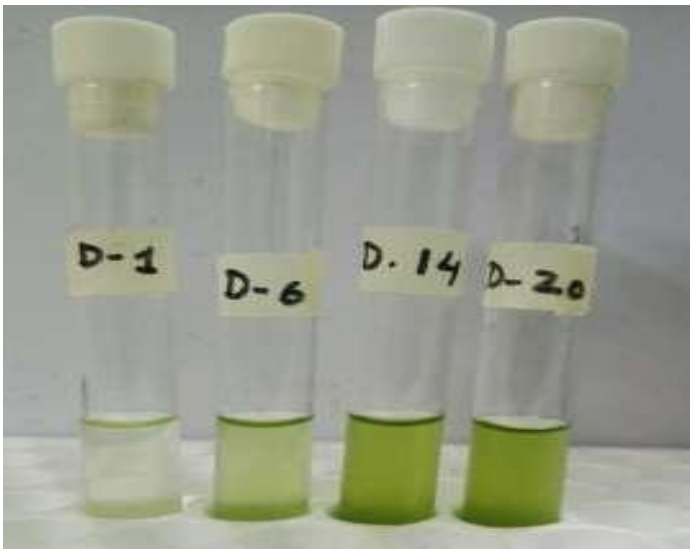

Figure 12: Quantitative Analysis of Algal Growth Over 20-Day Period

pH Monitoring Using Visual Tools

pH indicator strips were used as a simple, affordable, and easy method to monitor metabolic variations in the Chlorella culture, In addition to digital pH meters that provide accurate numerical readings. These strips were especially useful for quickly finding out the general pH trend without the need for specialized equipment, and they were a great way to visually evaluate more complex data. Over the evaluation of six days, a unique pH monitoring experiment was conducted to see the metabolic progress of the culture. In order to confirm the overall health and metabolic activity of the culture, the color changes of the pH indicator strips were connected to the optical density (OD_{680}) and microscopic analyses during this research.

The pH strip shows the given variations:

- **Day 2:**

pH strip has an orange tint for pH of about 5.0 . The early phases of algae growth are characterized by this acidic environment, where the culture medium absorbs dissolved CO_2 from the air and made carbonic acid. The early growth phase, when photosynthetic activity is still at optimum level and CO2 absorption has not been increased noticeably, is showed by this acidification

- **Day 6:**

On the sixth day yellow-green tinge appeared on the pH strip , which indicate a pH of roughly 7.5. The colony had entered a more active phase of photosynthesis, as shown by this pH shift from neutral to slightly basic [7]. For growth, Chlorella was now efficiently using CO_2 which made it into organic materials and generating oxygen as a byproduct. The shift to a more alkaline pH shows that photosynthetic activity was progressing, as indicated by the faster rate of growth in CO_2 uptake and oxygen production.

The pH indicator strip's color changes were visually stunning and present as a simple and easy way to verify that photosynthesis actively occurring in the culture. The observation that the Chlorella culture was shifting from initial development to increased metabolic activity was further supported by the change in pH from acidic to neutral, which matched the OD_{680} measurements and microscopic analyses. These visual representations also provided a quick and inexpensive and sustainable way to monitor the culture's metabolic changes in lab settings with limited resources. This method's simplicity makes it a good choice for real-time algal culture monitoring, enhancing more complex techniques and providing researchers with a rapid understanding of the development of culture's circumstances.[4].

Biomass Development in Micro centrifuge Tubes.

I collected samples of the Chlorella culture at different intervals of times and kept them in 1.5 mL micro centrifuge tubes so I could visually course the biomass growth. I was able to observe how biomass changed over time using this method, paying careful attention to sedimentation, coloration, and total density. Moreover, the quantitative information obtained from OD_{680} and pH measurements, the samples

provided a unique qualitative perspective on the culture's development.

Figure 13: pH at different Days (Lab Scale)Day 2 Sample: Early-stage development was shown by the culture's turbid and light green appearance. Since the cells were still small and dispersed at this point, there was no noticeable pellet at the tube's bottom after centrifugation. Although there were other some cells present, the low turbidity shows that they had not yet started to integrate to form a dense biomass.

• Day 6 Sample: The culture had become opaque and considerably deeper to a bright green by Day 6, which indicates greater chlorophyll development and improved photosynthesis. A clear pellet had developed at the bottom of the tube after centrifugation, indicates that the cells had grown in both quantity and biomass density. An important indication that the culture was finally entering its exponential growth stage that was the most rapid accumulation phase of biomass, which is seen by the increased turbidity and the presence of sediment.

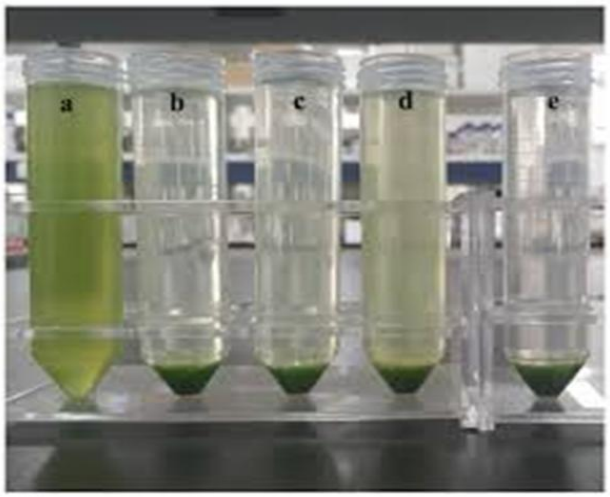

Figure 14: Biomass samples on different days

Chlorella was rapidly growing and accumulating biomass, as demonstrated by the color and sedimentation changes that were seen. When choosing the best time to harvest the culture for protein extraction, this visual information is crucial. The later samples' development of a noticeable pellet suggested that the biomass had reached a density appropriate for the further steps of protein extraction, which will be examined in more detail during the next experiment's stages.[8].

Microscopic Analysis

On Days 1, 3, and 6, a 400x magnification microscopic analysis was done to obtain the structure, health, and development of Chlorella cells throughout

time. This complete research made it possible to seek the changes in cell structure, pigmentation, and general growth in greater detail, providing important information on the microbiological state of the culture and supporting the findings from pH and OD_{680} measurements.

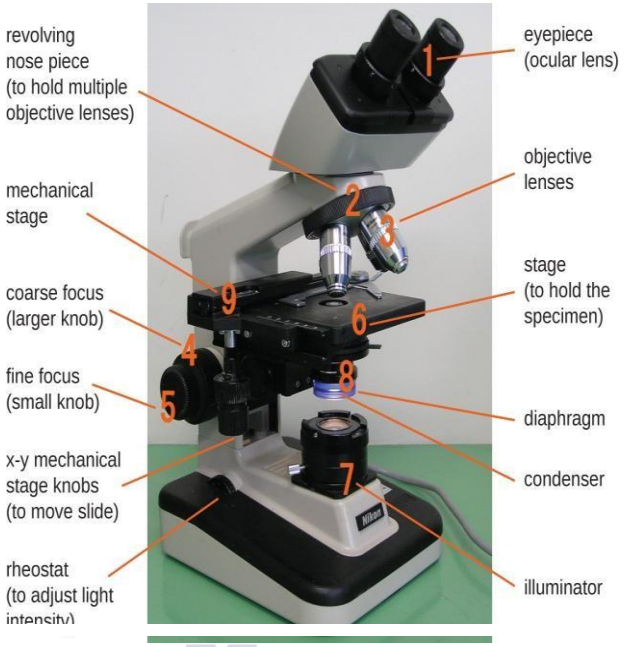

Figure 15: Compound Light Microscope

• Day 1: The cells were tiny, sparse, and lacked completely developed chloroplasts on the first day. At this early stage, the cells were initially spherical or oval shaped and isolated, with small pigmentation. The unshowy growth rate and comparatively acidic pH observed on Day 1 are become the evidence of the maturation of the chloroplasts, which is important for photosynthesis. These evaluations supported the findings from OD measurements and pH monitoring, which confirms that the culture was still in the lag period, during which the cells were adapting to their pristine environment and starting metabolic processes.

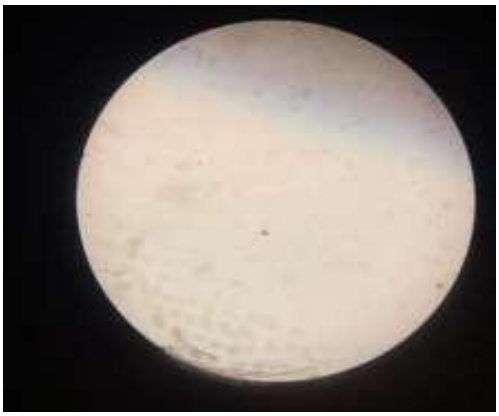

Figure 16: Cell Division at Day

• Day 3: The cell density had grown by the third day. The cells increased their integrity through size and shape which suggest that their division was timed. As the cells had more involved in photosynthesis, the green color became more intense, indicating an increase in chlorophyll content. At this point, the progression of the chloroplasts was also more considerable, which indicates that the change from the lag phase to the log (exponential) phase. This increase in cell division and pigmentation matched

the upward trend in the OD_{680} measurements and the rising pH, indicating that the culture was actively expanding or growing and using CO_2 for photosynthesis.

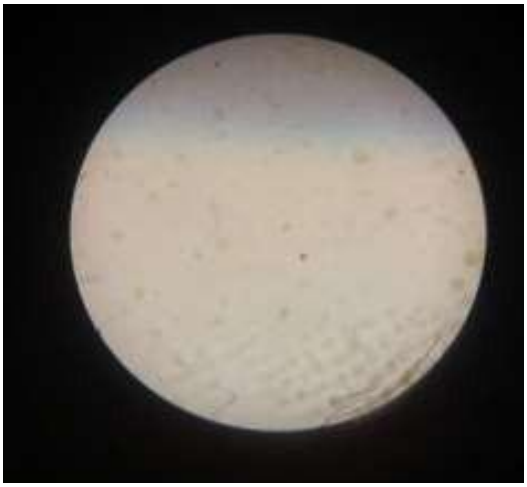

Figure 17: Cell Division at Day 3

• Day 6: By Day 6, the Chlorella cell population had grown substantially in size and chloroplast development, and it was densely integrated and tightly packed. With their vibrant green tint and robust appearance, the cells demonstrated that photosynthesis was function at its peak efficiency. The rising OD_{680} measurements and the steady pH levels furtherm o r e supported the high-density population's suggestion that the culture was in its peak development phase, indicating that it had reached its exponential growth phase. The chloroplasts maturation (division) and the cells general health at this point are the evidenced that the culture's successful progression and validated the experimental setup [5].

Figure 18: Cell Division at Day 6

Optimization and Quantification

Microalgae are becoming a viable source of high-quality proteins for use in medicinal, food, and feed products. They have the advantages of quick growth, a high protein content, and the capacity to use wastewater or very little other resources to grow on non-arable soil. To optimize protein recovery and guarantee economic viability, effective protein extraction and measurement techniques are crucial. Three main experimental components are covered in this chapter are:

(1)Protein extraction utilizing an alkaline-based approach

(2)The impact of pH on protein production; and

(3)The contribution of sodium bicarbonate ($NaHCO_3$) concentrations to protein yield enhancement. These studies main focus on reproducibility and cost-

effectiveness in order to optimize the process for use on an industrial scale.

Biomass Collection and Preparation

Cultivation and Harvesting

Under controlled laboratory setting BG-11 medium was used to produce the algal biomass. To increase the photosynthesis process and nutrient uptake, algal biomass was kept under constant light with enough aeration During the exponential phase 50 mm of algal suspension was obtained. Centrifugation was run for 5 minutes at 5,000 rpm. Without breaking the pellet cells, them, this speed was optimized. While lesser speeds result to inadequate biomass recovery, high speeds may promote premature lysis.

Washing and Clarification

The pellets first washed with distilled water by following the centrifugation to avoid any raw medium components. This washing step is crucial to obtain the maximum or optimized yield of protein without any contamination at the end. The washing procedure was repeated 2- 3 times to obtain the desired results.

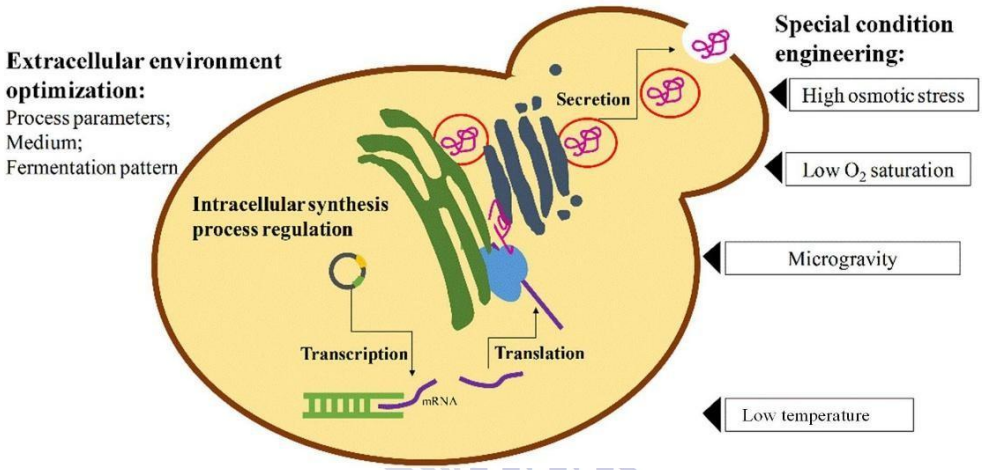

Figure 20: Protein Extraction Methodology

Cell Lysis and Solubilization

Alkaline lysis was done by using 0.5N NaOH. By breaking peptide and ester linkages, this durable base breaks down the micro algal cell walls and releases intracellular proteins .to prevent the breakdown of protein alkaline environment denatures natural proteases.

Heat Treatment

A 10-minute heat treatment at 80°C was given to lysate. Protein structures can unfold more easily because of this heat exposure, which increases solubility. The incubation period was constrained because extended heating can cause protein denaturation or aggregation.

Cooling and First Centrifugation

To prevent additional thermal damage, the samples were chilled on ice for five minutes after heating. To separate the solubilized protein in the supernatant from the cell debris in the pellet, centrifugation was performed for 10 minutes at 4°C and 13,000 rpm.

Second Extraction (Recommended)

The extraction procedure was repeated using the leftover pellet that had been suspended in new 0.5N NaOH. This step was a useful addition to optimize recovery because it raised the overall protein production by about 20–30%.

Protein Quantification

Nano drop A280 Measurement

The Nano drop spectrophotometer was first used to quantify the protein content at 280 nm. The absorbance of aromatic amino acids (such as tryptophan, tyrosine, and phenylalanine) a t 280 nm on basis o f this t e c h n i q u e . Advantages include

- Quick results with just 2 µL of sample.
- No need for further reagents because the condition is satisfied as per condition. Overestimation may result from interference from nucleic acids, which can likewise absorb at this wavelength.

Table 4: Typical Protein Measurement Techniques

Method	Sensitivity	Interferences	Cost
Bradford Assay	High	Detergents (e.g., SDS)	Low
BCA Assay	Very High	Reducing agents (e.g., DTT)	Medium
Lowry Assay	Moderate	Chemicals (e.g., Tris)	Low

Typical protein measurement techniques are contrasted in the above table. According to the analysis, reliability is increased by cross-validating Nano drop A280 data using the Bradford assay. DNase and RNase treatments were used in cases where nucleic acid interference was suspected.

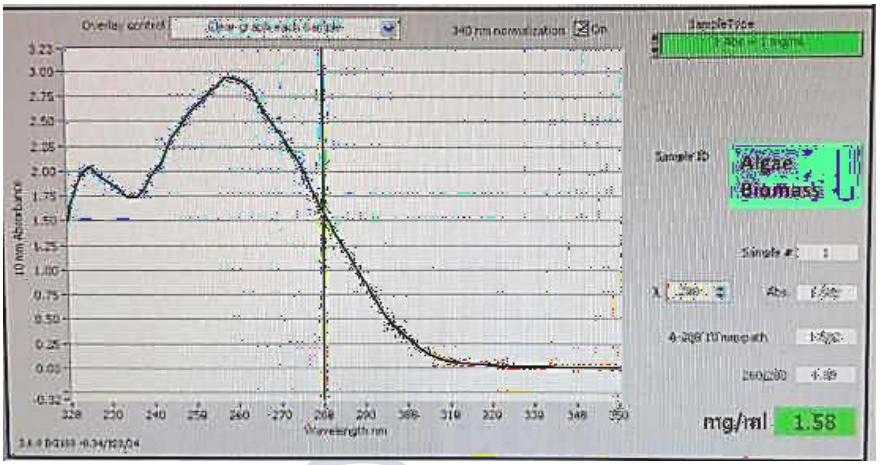

Figure 21: Wavelength Variance with Absorbance

Effect of pH on Algal Protein Production

Experimental Design

Using NaOH and HCl, the growth media was adjusted to pH 6, 8, and 10 in order to assess the impact of pH. Consistency was ensured by using BG-11 media in all circumstances. The light and aeration conditions used to develop the cultures were the same.

Results and Interpretation

Table 5: Biological Implications

pH	Protein (mg/mL)	Biological Implications
6	0.9655	Optimal enzymatic activity and nutrient solubility
8	0.4035	Reduced CO_2 availability, inefficient nitrogen assimilation
10	0.0150	High oxidative stress, enzyme degradation

Protein synthesis peaked at pH 6. This pH is ideal for the enzymatic processes involved in protein synthesis and photosynthesis. Protein production was low at pH 10 due to the severe alkaline environment that hampered cellular metabolism.

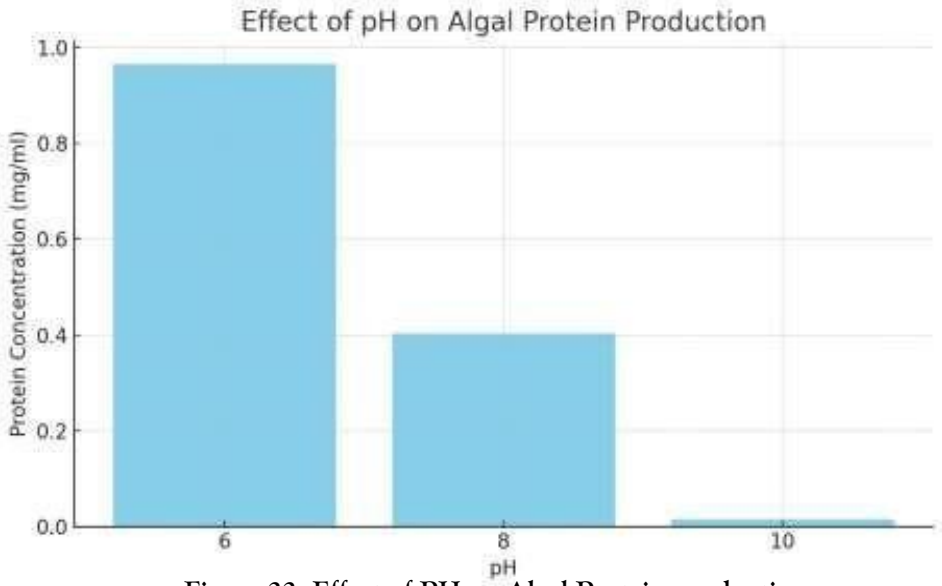

Figure 22: Effect of PH on Algal Protein production

Mechanistic Insights

The predominant form of inorganic carbon at pH 6 is CO_2, which facilitates effective photosynthesis. Additionally, the stability and functionality of algae transport systems and enzymes have improved. CO_3^{2-} , on the other hand, is the predominant and less accessible carbon form at pH 10. Additionally, high pH reduces the synthesis of proteins by causing oxidative damage and ammonia toxicity.

was assessed.

Results and Analysis

Table 6: Dose Implications

$NaHCO_3$ (g/L)	Protein (mg/mL)	Implications
0.5	0.305	Insufficient carbon, limited biosynthesis

The table demonstrates a dose-dependent increase in protein yield. At 2.0 g/L, $_3$ provided ample carbon, which enhanced photosynthetic output and protein synthesis.

Mechanism

Bicarbonate ions are actively transported by algae via membrane-bound channels. After entering the cell, the bicarbonate fixes carbon and creates proteins through the Calvin cycle. Increased $NaHCO_3$ availability boosts protein and biomass output. The results repeatability is confirmed by a low standard error across replicates.

Effect of Sodium Bicarbonate ($_3$) on Protein Yield

Carbon Supplementation

In addition to providing dissolved inorganic carbon, $NaHCO_3$ buffers pH variations in the growth media. By introducing varying concentrations (0.5, 1.0, and 2.0 g/L) into the cultures, its impact on protein production

Optimization Strategies and Industrial Considerations

Enhanced Lysis Methods

Protein release, particularly from harder microalgae, can be increased by other methods including the French Press, enzymatic lysis using lysozyme or

cellulose, and the application of detergents like Triton-X100 or CHAPS.

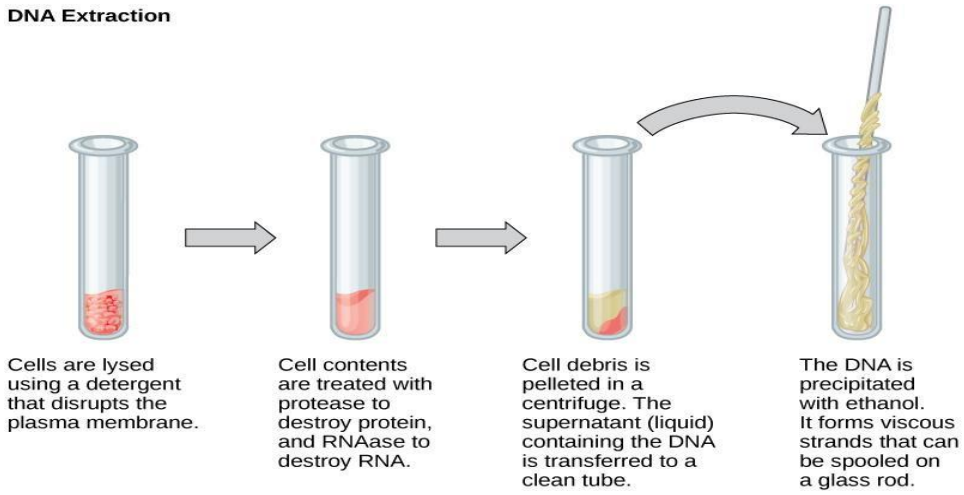

Figure 23: Enhanced Lysis Method

Improved Quantification Accuracy

When using A280 values, it is recommended to use DNase/RNase enzymes to eliminate interfering nucleic acids. It is advised to cross-validate using the Bradford or BCA assays for increased quantification precision.

Industrial Scale-Up

Maintaining a culture pH of 6 and adding 2–4 g/L $_3$ are crucial at the industrial level. Additionally, the product is appropriate for storage and transportation thanks to downstream processing including freeze-drying for protein preservation and ultrafiltration for concentration.

Future Prospects

Future study plans include advanced protein profiling with SDS-PAGE and mass spectrometry. Compare the costs of bicarbonate and gaseous CO_2 supplementation was particularly important to done. Lastly, this procedure must be applied to additional microalgae species, like Scenedesmus and Spirulina, may give result of higher protein profiles.

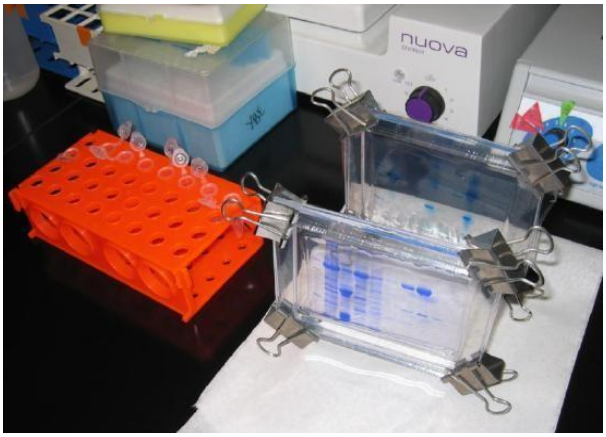

Figure 24: Quality Techniques

The quality procedures like SDS-PAGE, UV-Vis spectrophotometry, and amino acid profiling was done to obtain the protein concentration, composition, and purity. These methods ensure the extracted algal protein must fulfill the world's nutritional and functional standards and demands.

Conclusion, Discussion

Correlations and Discussion of Result

Referred from the chapter 4 By combining OD_{680}, pH values, ocular evaluations, and microscopic inspection, it was possible to fully comprehend the stages of Chlorella growth and validate the culture's progress. Every measure provided further information about the physiological state of the culture, supporting the experiment's findings.

• OD_{680} as a Measure of Biomass: It has been determined that OD_{680} is a reliable and effective indicator of biomass concentration. OD_{680} measurements increased dramatically during the culture's development phases, particularly during the exponential phase. The accumulation of algal cells and their chlorophyll content coincided with this rise in OD_{680}, confirming that biomass density was increasing. OD_{680} served as a quantitative measure of the culture's growth and established a clear link between cellular proliferation and the absorption of light by chlorophyll pigments.

• pH Variations and Metabolic Processes: Monitoring the culture's metabolic activity during the cultivation stage required careful attention to pH variations. Because CO_2 dissolved and formed carbonic acid, the pH stayed low during the lag phase. The pH steadily increased as the culture entered the exponential phase, indicating CO_2 fixation and the start of photosynthetic oxygen production. Since Chlorella was efficiently using CO_2 and producing oxygen through photosynthesis, this pH shift was a direct reflection of the metabolic processes taking place inside the cells. The correlation between growth stages and pH changes provided valuable information on the metabolic shifts from nutrient adaptation to maximum biomass output and photosynthesis.

• Visual and Microscopic Analysis: Color changes and sedimentation are examples of visual indicators that provide rapid and simple qualitative assessments of the culture's development. The presence of biomass silt in the bottom of the culture vessels throughout the exponential phase showed rapid growth and biomass buildup, and the green color of the culture became more intense as the chlorophyll content rose. These visible changes were confirmed by microscopic analyses, which showed the cell shape, density, and chloroplast growth at each observation site. As seen in the graph below, a dense cluster of healthy cells with mature chloroplasts was seen by Day 6 after a sparse population of tiny cells was seen on Day 1. These microscopic results confirmed that the culture was successfully developing because they were consistent with the higher OD_{680} and the pH alterations. [6].

• For the complete understanding of Chlorella's growth dynamics, integrating all of these measurements allowed. The change in pH shows that metabolic activity which includes photosynthesis and CO_2 fixation. A steady OD_{680} increase helps in biomass culture OD_{680}. These results were supported by microscopy and visual evaluations, which verified biomass buildup, growth patterns, and cell viability. This comprehensive strategy improved the experimental setup and offered a solid foundation for understanding the biological evolution of Chlorella as well as prospects for further protein extraction [7].

Conclusion

In this chapter the growth dynamics of Chlorella spp was done, which gives valuable information about the various stages of cultivation and the variables which affect biomass growth output. We obtained a comprehensive physiological growth of the culture by integrating optical density (OD_{680}), pH monitoring, ocular evaluations, and microscopic analyses.

The growth curve showed a discrete phase's typical microbial pattern, such as the exponential phase, which is characterized as by increasing photosynthetic activity and rapid cell division, and the lag phase, which is when cells adapted to their pristine environment. The maximum OD_{680} at Day 10, when the biomass culture reached its highest at its highest concentration, suggested the best time to harvest. Visual and microscopic analyses showed notable alterations in cell density, color, and chloroplast development.

Chlorella metabolic activity was found out by the experiment's pH variations, which tells us the effective amount of CO_2 fixation and oxygen production during the exponential development phase. Microscopic analyses supported the pH and OD_{680} results and shows a healthy cell growth. These findings show Chlorella's strong protein-obtaining ability and provide a reliable method of tracking its growth. The data given in this chapter provides information of the next stage, which consists of protein extraction and characterization, and tells a great method for future harvesting techniques.

As a conclusion, during my thesis research, I viewed the potential of Chlorella microalgae as a sustainable protein alternative. I learnt about the characteristics of algae, which shows that it does not require proper land, and it has rapid growth, low resource requirements, environmentally friendly and sustainable ways of getting protein.

BG-11 medium was used during cultivation and adding sodium bicarbonate (₃) at three different concentrations (0.5 g/L, 1.0 g/L, and 2.0 g/L) and adjusting the pH (6, 8, 10). The highest growth and maximum protein synthesis were seen at pH 6 with 2.0 g/L ($NaHCO_3$). Both biomass and protein output were improved by these circumstances. During the protein extraction. The algal biomass was treated by heat treatment at 80°C for 10 minutes and along with 0.5N NaOH. By using this technique, the algae cells were lysed successfully. then protein which contains supernatant gathered after centrifuging the lysate. The second extraction was done to improve the yield and improve the protein content by repeating the procedure exactly 2 to 3 times. To measure the protein concentration, a Nano drop spectrophotometer set to 280 nm was used. The 3.030 mg/ml was the ideal condition to obtain the maximum yield of protein. The baseline protein production of a standard 50 mL culture was 1.58 mg/ml. all the above discussing things helped us in lab scale to enhance the yield of protein extraction. When we used the proper heat treatment, NaOH lysis, optimal pH, and carbon supply conditions, a better result was obtained, and these characteristics give us strong Foundation.

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