Comparative Analysis of Open Pond and Photobioreactor Cultivation for Enhanced Microalgal Biomass Production

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ABSTRACT

This study focuses on isolation, identification and comparative cultivation of microalgae in open pond and closed photobioreactor systems. Water samples were collected from various freshwater sources such as ponds, lakes and river of in and around Tamil Nadu, Andhra Pradesh and Telangana. The isolation of collected algal samples and followed by purification using serial dilution techniques. Cultivation was carried out in BG11 medium under controlled conditions. Growth performance was assessed by UV-Visible Spectrophotometer. Molecular characterization was performed through *rbcL* gene amplification and phylogenetic analysis to confirm species identity. Comparative study was conducted using open pond and closed photobioreactor systems are used to cultivate algal biomass and provides a controlled environment that can enhance micro algal growth and lipid accumulation. Factors such as light, temperature, pH and nutrients that promote higher lipid yields such as omega-3 fatty acids. Results revealed that the closed photobioreactor system exhibited significant production of biomass yield when compared to the open pond system. This research highlights the importance of precise strain identification and cultivation system optimization for efficient microalgal biomass production. Furthermore, the extraction of bioactive compounds from the identified algal sample taken into consideration for commercial applications, particularly in cosmetics (for skin repair and anti-aging) and cancer therapy, due to their antioxidant property and presence of omega-3 fatty acid content.

Keywords: Microalgae, Molecular identification, Bioactive compounds, Photobioreactor, Anticancer compounds.

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INTRODUCTION

Microalgae are unicellular, photosynthetic organisms found in diverse aquatic environments, playing a key ecological role in carbon cycling and oxygen production. Their ability to synthesize valuable compounds such as lipids, proteins, pigments and bioactive metabolites combined with rapid growth and adaptability makes them a

promising renewable resource for sustainable biomass production [1]. Unlike terrestrial crops microalgae can grow on non-arable land using wastewater or flue gases and many species produce commercially valuable molecules like omega-3 fatty acids and carotenoids. However, large-scale application is limited by challenges in cultivation, harvesting and processing [2]. While open ponds are cost-effective but prone to contamination, closed photobioreactors offer higher productivity and quality at greater expense. Recent advances emphasize optimizing cultivation systems to balance productivity and economic feasibility. Open pond systems, though widely used, face issues such as contamination, inconsistent light penetration and temperature fluctuations that hamper biomass yield [3]. Photobioreactors provide controlled environments with improved light distribution and reduced contamination risk but require higher capital investment and operational costs. Innovative hybrid systems combining benefits of both are being explored to enhance scalability and cost-effectiveness [4]. Current research on biomass production in microalgae under distinct cultivation strategies focuses on optimizing growth conditions and processing technologies to enhance biomass yield and fully exploit the potential of microalgae in energy, nutrition, pharmaceutical and environmental applications.

2. Materials and Methods

2.1. Collection of Water Samples

Water samples were collected from a total of twelve different sites, including ponds, lakes, rivers and other relevant aquatic environments. Samples were obtained from various depths and locations using sterile bottles for the isolation of microalgae [5].

2.2. Cultivation and Enrichment of Microalgae Present in the Environmental Water Samples

The collected water samples were centrifuged at 3700 rpm for 10–15 minutes, allowing the algal cells to settle and form a pellet at the bottom of the tube. After discarding the supernatant, the concentrated algal pellet was transferred into sterile flasks containing growth medium, then incubated under continuous shaking at 150 rpm and a temperature of 25–30°C for 7–14 days [6].

2.3. Isolation, Culturing and Microscopic Examination of Microalgae

Serial dilution of the enriched cultures was performed to reduce the diversity and concentration of organisms in the samples. Standard plating techniques were then used to isolate individual microalgal strains by spreading the diluted samples onto BG11 agar plates. The plates were incubated at 28°C for 7 to 14 days to allow colony development [7]. Microscopic observation was done using a 40x or 100x lens to check the purity and identify the microalgae based on their cell shape and colony appearance [8].

2.4. Molecular Identification of Microalgae

Genomic DNA was isolated from the culture using a commercial extraction kit and the DNA quality was verified on a 1.2% agarose gel, showing a distinct high–molecular-weight band indicating purity and integrity. The *rbcL* gene fragment was amplified using gene-specific primers in a WEE 32-well thermal cycler under optimized PCR conditions, producing a single discrete amplicon of approximately ~850 bp [9]. The amplified product was bead-purified and subjected to Sanger sequencing reactions using both forward and reverse primers with the BigDye Terminator v3.1 Cycle Sequencing Kit on an ABI 3731xL Genetic Analyzer. The resulting chromatograms were analyzed to obtain high-quality reads, which were assembled into consensus sequences of about~ 600 bp using alignment software such as Geneious or Chromas [10]. The consensus sequence was then BLAST-searched against the NCBI GenBank database to confirm the taxonomic identity, and phylogenetic relationships were established using neighbor-joining analysis in MEGA v11 software [11].

2.5. Selection of Growth Medium for Mass Cultivation

The growth media were selected to optimize microalgae development during bulk production. BG11 medium, Bold's Basal Medium and Modified Hoagland's medium were prepared according to their compositions transferred into 250 mL Erlenmeyer flasks and sterilized at 121℃ for 15 minutes. The selected microalgal isolate was separately added to each growth medium and its

growth was monitored by measuring the optical density using a UV-Vis spectrophotometer on 5^{th},10^{th},15^{th}, and 20^{th} day following inoculation [12].

2.6. Construction of Compact and Portable Bioreactor

The bioreactor system was optimized to precisely control critical growth parameters for maximizing microalgal biomass production. Environmental factors including pH (maintained at 7.9–10.5), temperature (15–40°C), and light intensity (provided by a 10W LED emitting 1500–2000 lux with controlled photoperiods to prevent photoinhibition) were continuously monitored and regulated by integrated sensors [13]. Operational parameters such as aeration, ensured by blower mechanism, facilitated uniform mixing and effective gas exchange, providing adequate CO_2 and oxygen supply [14]. Nutrient concentrations, comprising essential macro- and micronutrients, were carefully managed to prevent toxicity and support sustained microalgal growth and biomass accumulation [15]. This integrated system allowed for optimized cultivation conditions, enhancing microalgal biomass yield, culture stability and downstream processing efficiency.

2.7. Comparative Evaluation of Open Pond and Bioreactor Systems for Microalgae Cultivation

Microalgae cultivation was conducted in two distinct systems: an open pond and a bioreactor. The open pond system used a glass tank exposed to natural environmental conditions, while the bioreactor enabled precise control of temperature, pH, light intensity and aeration. Both systems employed the same microalgal strain for batch cultivation over a fixed period [16]. Environmental parameters including temperature, pH and light intensity were continuously monitored and maintained within optimal ranges. Nutrient levels were standardized to ensure comparability between systems. Growth performance was assessed by regularly measuring optical density and biomass productivity, while contamination risks and system stability were monitored throughout cultivation [17]. This comparative study provided valuable insights into the productivity, culture purity and contamination susceptibility of each system, highlighting their respective strengths and limitations for research and industrial microalgae applications.

2.8. Harvesting and Drying of Algal Biomass

The microalgal biomass obtained after mass cultivation was harvested by centrifugation at 4000 rpm for 10 minutes, during which the supernatant was discarded and the pellet collected on a glass plate [18]. The collected biomass was then dried in a hot air oven at 46°C for 10 minutes to remove excess moisture while maintaining cell integrity, after which it was finely ground using a clean mortar and pestle to produce a uniform powder suitable for subsequent extraction and analytical procedures [19].

3. Results and Discussion

3.1. Collection of Water Samples

Water samples collected from each site showed visibly distinct characteristics such as variations in colour, turbidity and odor, indicating differences in nutrient content and algal presence. All the samples were properly labelled and stored under sterile conditions for further studies.

Figure1: Collection of water samples

Table 1: Sample collection areas

S.no	Location	Source
1	Ayapakkam	Lake

2	Thiruvallur	Pond
3	Red hills	Lake
4	Madhavaram	Lake
5	Tirupathi	Tank
6	Tirumala	Pond
7	Ahobilam	Tank
8	Alampur	River
9	Somasila	River
10	Singavatnam	Lake
11	Kondraopalle	Canal
12	Banjarahills	Well

3.2. Cultivation and Enrichment of Microalgae from Environmental Water Samples

Centrifugation of water samples led to efficient concentration of algal cells, which were then transferred to fresh growth medium and incubated under controlled shaking and temperature conditions. After 14 days growth was observed in most cultures, the culture medium gradually changed from light green to deep green indicating active algal proliferation. This healthy growth confirmed that the chosen conditions were optimal for the maintenance and multiplication of environmental microalgae.

3.3. Isolation, Culturing and Microscopic Examination of Microalgae

After serial dilution and plating on BG11 agar, distinct green colonies appeared within 7–14 days of incubation at 28°C. The colonies showed variation in size and color, indicating the presence of different microalgal species. Microscopic observation under 40x and 100x magnification confirmed the presence of uniform, spherical green cells characteristic of *Chlorella*. Pure and stable *Chlorella* cultures were successfully isolated and maintained for further studies.

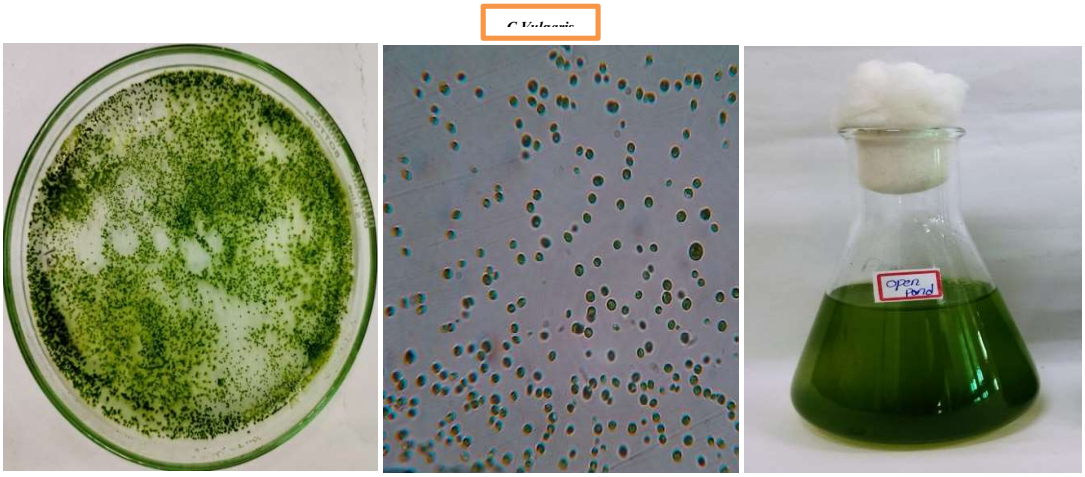

Figure 2: Pure culture of microalgae

3.4. Molecular Identification of Microalgae

The *rbcL* gene amplification and sequencing

produced clear, reliable results with high-quality DNA confirmed by a distinct band on agarose gel. A single ~850 bp amplicon was observed on a 1.2% agarose gel after PCR and the assembled consensus sequence (~600 bp) showed 98–100% identity with database sequences confirming accurate species identification. Phylogenetic analysis confirmed the correct species identification and validated *rbcL* as a reliable marker for genetic characterization. The isolate was specifically identified as *Chlorella vulgaris* isolate 1_1 ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (*rbcL*) gene, partial cds; chloroplast, confirming its identity at the molecular level.

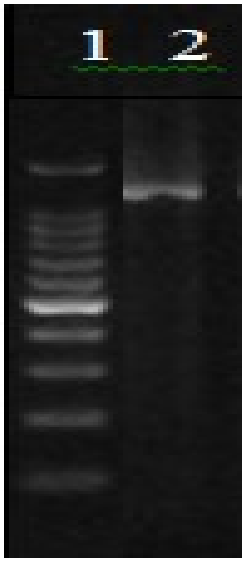

Figure 3. 1.2% Agarose gel showing single ~850 bp of *rbcL amplicon*

>0924/HA/001_rbcl_F
CCTCAACCAGGTGTTCCGCCAGAAGAAGCTGGTGCAGCGGTAGCAGCGGAATCATCAACTGGTA
CTTGGACGACTGTATGGACTGACGGTTTAACTAGCTTAGATCGTTACAAAGGTCGTTGTTATGATA
TTGAGCCAGTTCCAGGTGAAGAAAACCAATACATTGCATACATTGCATATCCTTTAGATCTTTTTG
AAGAAGGTTCTGTAACAAACTTATTTACTTCAATCGTAGGTAACGTATTTGGTTTCAAAGCTCTTC
GTGCTTTACGTTTAGAAGATCTTCGTATTCCACCAGCTTACGTAAAAACTTTCCAAGGTCCTCCTC
ACGGTATTCAAGTAGAACGTGATAAACTTAACAAATACGGTCGTGGTTTATTAGGTTGTACAATTA
AACCAAAATTAGGTCTTTCAGCTAAAAACTACGGTCGTGCTGTATATGAATGTTTACGTGGTGGTC
TTGACTTTACTAAAGATGATGAAAACGTAAACTCTCAACCATTTATGCGTTGGAGAGATCGTTTCT
TATTTGTAGCTGAAGCTATTTATAAATCTCAATCAGAAACTGGTGAAATTAAAGGGCACTATTTAA
ACGCAACTGCAGCAACAGCTGAAGAAATGCTTAAAC
> 0924/HA/001_rbcl_R
GTGCTGAGTGTGCTAAAGATTTAGGTGTACCTATTATCATGCACGACTACTTAACAGGTGGTTTCA
CAGCAAATACAAGTTTAGCTCACTACTGTCGTGATAATGGTCTTCTTTTACACATTCACCGTGCTAT
GCACGCTGTAATTGACCGTCAAAGAAATCATGGTATTCACTTCCGTGTTTTAGCAAAAGCTCTTCG
TTTATCTGGTGGTGACCACTTACACTCTGGTACTGTTGTAGGTAAATTAGAAGGTGAACGTGAAG
TAACATTAGGTTTCGTTGACTTAATGCGTGATGACTACGTTGAAAAAGATCGTAGTCGTGGTATTT
ACTTCACTCAAGACTGGGTTTCTTTACCTGGTACAATGCCAGTAGCTTCTGGTGGTATTCACGTAT
GGCACATGCCAGCTCTTGTTGAAATTTTCGGTGATGATGCTTGTTTACAATTCGGTGGTGGTACTT
TAGGACACCCTTGGGGTAATGCTCCAGGTGCGGCTGCAAACCGTGTTGCTTTAGAAG

Table 2: Nucleotide sequences of *Chlorella* species obtained using *rbcL* gene

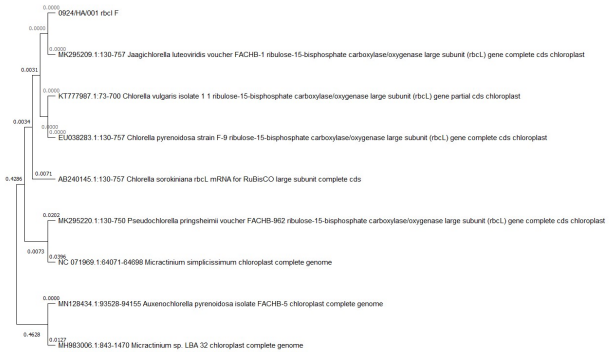

Figure 4: Phylogenetic tree based on *rbcL* gene of *Chlorella* species

3.5. Selection of Growth Medium for Mass Cultivation

The isolated *Chlorella vulgaris* strain exhibited variable growth in the three tested culture media BG11, Bold's Basal Medium (BBM) and Modified Hoagland's Medium over a 20days incubation period under controlled conditions. Optical density measurements using a UV–Vis spectrophotometer indicated that BG11 medium supported the highest growth, with OD680nm reaching 0.89 ± 0.02, followed by BBM at 0.76 ± 0.04, and Modified Hoagland's Medium at 0.43 ± 0.05. Visual observation also confirmed these results, as cultures in BG11 appeared darker green and denser compared to the other media. These findings demonstrate that BG11 medium provides optimal nutrient conditions for the mass cultivation of *Chlorella vulgaris* making it the preferred medium for subsequent biomass production.

S.NO	DAYS	BG11	BBM	MHM
1	Day 5	0.236	0.212	0.193
2	Day 10	0.397	0.349	0.248
3	Day 15	0.539	0.488	0.317
4	Day 20	0.892	0.764	0.435

Table 3: Growth readings in various media

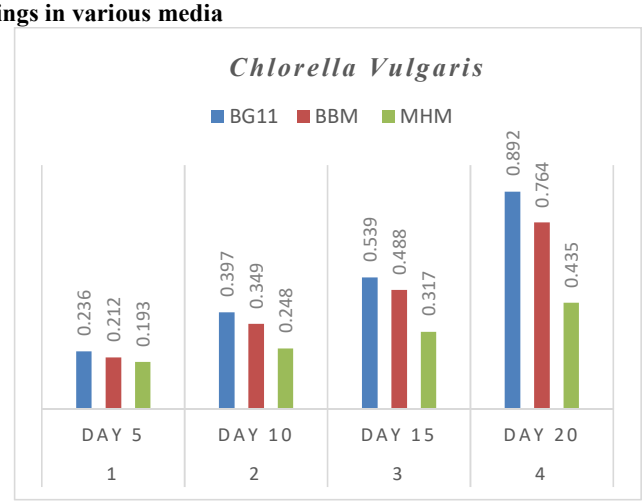

Figure 5: Optical density of isolate in various media

3.6. Construction of Compact and Portable Bioreactor

The construction of a compact and portable bioreactor successfully enabled controlled cultivation of *Chlorella vulgaris* under laboratory conditions. Designed with a transparent glass vessel

for uniform light distribution, the system incorporated strategically arranged with LED light for continuous illumination and an integrated CO_2 supply to maintain carbon availability. A mechanical pump ensured uniform mixing and efficient gas exchange, preventing cell sedimentation. Sensors monitored critical parameters including temperature, pH and light intensity for real-time control. The entire system was powered using a standard 220V AC supply, making it both portable and user-friendly. The constructed bioreactor provided stable and reproducible conditions suitable for the mass cultivation of microalgae.

3.7. Comparative Evaluation of Open Pond and Bioreactor Systems for Microalgae Cultivation

The comparative evaluation of open pond systems and bioreactors revealed significant differences in microalgal growth performance, biomass productivity and contamination risk. Cultures grown in the open pond exhibited moderate growth, with an average biomass concentration of approximately **0.64 g/L** but were frequently affected by contamination from other microorganisms and environmental fluctuations, including variations in temperature and light intensity.

In the bioreactor-cultivated cultures demonstrated enhanced growth and productivity, with biomass concentrations reaching **1.29 g/L** attributed to precise control of temperature, pH, light intensity and nutrient availability. The closed system of the bioreactor minimized contamination risk and allowed for reproducible cultivation conditions. Despite these advantages, bioreactors incurred higher operational costs and required careful maintenance, while open ponds remained more cost effective for large-scale cultivation.

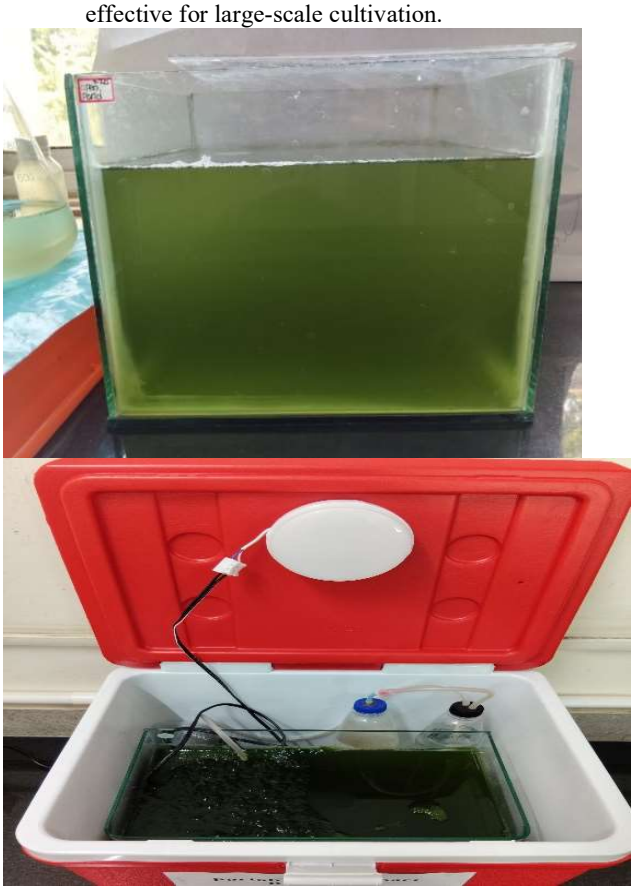

Figure 6: Open pond and Bioreactor

Table 4: Comparative table for open pond and bioreactor

Parameter	Open Pond System	Bioreactor System
System Type	Open	Closed
Cost	Low	High
Scalability	High	Limited
Environmental Control	Poor	Excellent
Contamination Risk	High	Very low
Productivity	0.64 g/L	1.29 g/L
Water and Gas Exchange	Natural	Controlled
Maintenance	Simple, frequent	Complex, stable
Application Suitability	Large-scale, low-cost biomass production	High-value product or research use

3.8. Harvesting and Drying of Algal Biomass

After centrifugation at 4000 rpm for 10 minutes, dense dark green pellets of *Chlorella vulgaris* formed at the bottom of the tubes, indicating successful cell concentration and effective separation from the culture medium. The pellets were washed twice with distilled water to remove impurities, and the wet biomass was collected for drying. The harvested biomass was dried in a hot air oven at 46°C for 10 minutes, yielding a dry, dark green material with reduced moisture content and no signs of thermal degradation. The dried biomass was then ground into a fine, uniform green powder using a clean mortar and pestle, producing a homogeneous sample suitable for further biochemical extraction and analysis while maintaining the integrity and quality of the algal biomass.

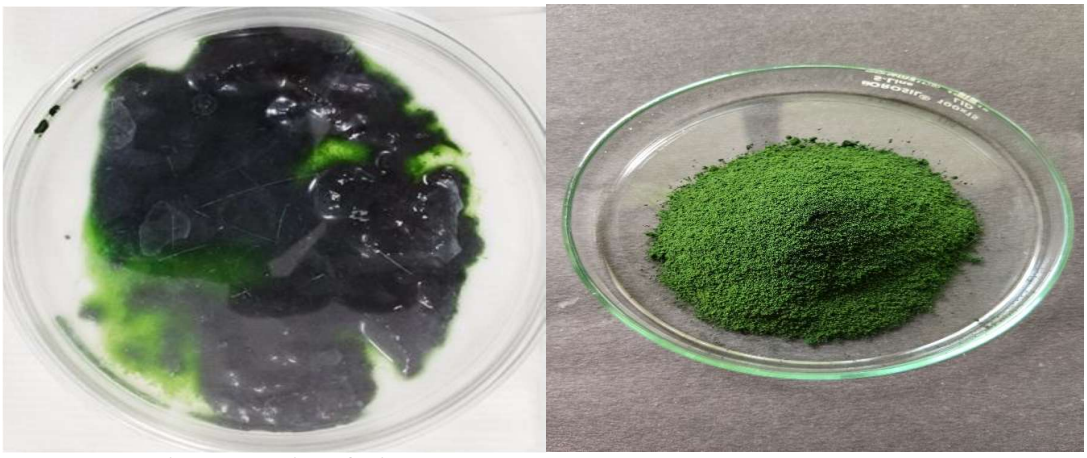

Figure 7: Harvesting and drying of microalgae

4. Discussion

The cultivation study of *Chlorella vulgaris* in open pond and compact bioreactor systems showed clear differences in growth performance, environmental

control and biomass yield. The open pond system was cost-effective and easy to scale up, but its productivity was moderate, with an average biomass concentration of 0.64 g/L. Because the culture was exposed to changes in weather and contamination from other microorganisms, it was difficult to maintain consistent results. Variations in temperature, pH and light intensity caused fluctuations in algal growth which is a common limitation of open cultivation systems [20].

In the compact bioreactor provided a well-controlled and stable environment that supported better algal growth. The system achieved a higher biomass yield of 1.29 g/L. The use of LED lighting ensured uniform illumination, while the regulated CO_2 supply and continuous mixing improved gas exchange and nutrient distribution. Real-time monitoring using sensors for temperature, pH and light intensity helped maintain optimal culture conditions. This closed setup reduced contamination risks and allowed reproducible results over multiple cultivation cycles. Although the bioreactor required higher investment and operational control, the gain in efficiency justified its use [21].

During downstream processing, centrifugation at 4000 rpm effectively concentrated the biomass. Low temperature drying preserved the natural pigments and biochemical quality, producing a fine green powder suitable for extraction and further analysis.

Overall, the study demonstrated that compact bioreactors offer major advantages over open pond systems. Their controlled environment, efficient gas exchange and stable conditions nearly doubled biomass productivity. These features make bioreactor systems highly suitable for producing high value microalgae products used in nutraceuticals, cosmetics, bioenergy, where consistency and quality are essential.

4. Conclusion

The study showed that the compact bioreactor performed much better than the open pond system for growing *Chlorella vulgaris*. The bioreactor-controlled environment helped maintain stable growth conditions, resulting in higher biomass yield and consistent results. On the other hand the open pond was cheaper and easier to run but often faced problems due to weather changes and contamination. Overall, the compact bioreactor proved to be a more efficient and dependable option for producing *Chlorella vulgaris* and valuable bioactive compounds for use in nutraceutical, cosmetic and bioenergy products.

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Conflict of interest:

The authors declare no conflict of interests

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