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Improvement in Chemical Composition of Chlorella Vulgaris Isolated from Poompozhil Lake - A Rapid Method for Biomass Augmentation

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A B S T R A C T

Microalgae have emerged as promising biological resources owing to their high nutritional value, rapid growth, and diverse biotechnological applications. The present study aimed to evaluate the effect of superphosphate supplementation on the growth and biochemical composition of *Chlorella vulgaris* isolated from Poompozhil Lake, Thiruvallur District, Tamil Nadu. The algal culture was maintained under controlled laboratory conditions using dechlorinated tap water at 20°C with a 12 h light:12 h dark photoperiod. Superphosphate was employed as a nutrient supplement in the treatment group, while untreated cultures served as controls. Growth performance was assessed by hemocytometer-based cell counts, and biochemical constituents including carbohydrates, proteins, and lipids were estimated using standard analytical methods such as Anthrone, Lowry, and Bligh and Dyer techniques. The results demonstrated a significant enhancement in algal growth under superphosphate treatment. Cell density increased progressively from 472 × 10^4 to 770 × 10^4 cells mL^{-1} in treated cultures, compared to considerably lower counts in control conditions. The treated cultures also exhibited improved pH conditions and enhanced biomass accumulation. Biochemical analysis revealed increased concentrations of major cellular components, including carbohydrates (0.9938 mg g^{-1}), proteins (6.56 mg g^{-1}), and lipids (0.3278 mg g^{-1}) compared with control cultures. The findings indicate that superphosphate supplementation effectively stimulates cellular division, biomass production, and metabolite accumulation in *Chlorella vulgaris*. The study highlights a cost-effective approach for large-scale algal cultivation and suggests potential applications in biomass production, biofertilizer recycling, nutraceutical development, and sustainable biotechnology.

Introduction

Micro-algae are microscopic algae, typically found in freshwater and marine systems. They are unicellular species that exist individually, in chains, or in groups. Depending on the species, their sizes can range from a

few micrometers (µm) to a few hundred micrometers. Unlike higher plants, microalgae do not have roots, stems, and leaves. Microalgae, capable of performing photosynthesis, are important for life on Earth; they produce approximately half of the atmospheric oxygen and use the greenhouse gas carbon dioxide simultaneously to grow photoautotrophically. Algae are prominent in bodies of water, common in terrestrial environments, and are found in unusual environments, such as on snow and on ice. Seaweeds grow mostly in shallow marine waters, under 100 metres (330 ft); however, some have been recorded to a depth of 360 metres (1,180 ft). The various sorts of algae play significant roles in aquatic ecology. Microscopic forms that live suspended in the water column (phytoplankton) provide the food base for most marine food chains. In the open waters of ponds, lakes, and especially the vast oceans, the algal phytoplankton is the only means by which diffuse solar radiation can be fixed into biological compounds. In these open-water (or pelagic) habitats, the phytoplankton are consumed by small, grazing animals known as zooplankton, most of which are crustaceans (Lee *et al* 1987). The zooplankton are, in turn, fed upon by larger zooplankton or by small fish (these predators are known as planktivores), which may then be eaten by larger fish (or piscivores). At the top of the open-water food web may be fish-eating birds, seals, whales, very large fish such as sharks or bluefin tuna, or humans (Andersen, 2005). Therefore, the possibility of all of the animals occurring higher in the food webs, including the largest of the top predators, is ultimately dependent on the productivity of the microscopic phytoplankton of the pelagic marine ecosystem.

Microalgae can play an important role in the future. They are one of the potential sources of food and feed provided by Nature with the potential to feed an ever-growing and affluent population. Microalgae are the photosynthetic organisms in the first levels of the aquatic food chains, on which an ever-growing part of our food will have to come. Our challenge is to domesticate these plants, as we have done with higher plants, to allow us to manage their large-scale production for a wide range of applications, including feeds.

Chlorella is a whole food because each cell of this organism is a self-sufficient organism containing all the life forces within. It contains phytonutrients and cofactors in a balanced amount that is beneficial for the overall restoration and Maintenance of health. Its high content of vitamins, minerals, protein, amino acids, and other vital nutrients enables it to supply a wide range of nutrients that the human body needs. Chlorella is regarded as a functional food in countries like Japan due to the fact that it contains all the essential nutrients needed by the human body and can carry out many therapeutic functions in the body. This is made possible because the nutrients in Chlorella interact with the body's system in such a way as to promote the body's ability to heal, balance, and revitalize.

Chlorella is able to provide vital nutritional support that is lacking in the modern diet consumed by many individuals today. With its rich nutrient content, it can help fill the gap in the nutritional requirements of people. Augmentation was attempted in the present investigation with a Chlorella sp. Isolated from a nearby locality to choose the indigenous Chlorella sp. The specific environmental profile of the Poompozhil Nagar Lake (located in the Avadi/Thiruvallur region of Tamil Nadu) exposes the microalgae to unique seasonal nutrient and UV conditions. The objective of the present study is to evaluate the influence of different media apart from the traditionally used one, on the biochemical growth content of *Chlorella vulgaris,* to find out the best defined inorganic media for the cultivation of *Chlorella vulgaris*

Materials and Methods

Micro algae *Chlorella* sp was obtained from the culture collection of Poompozhil Lake, Thiruvallur District, were employed in the present investigation. The algal culture was grown in dechlorinated tap water. The flasks were then placed in a culture rack for two weeks at 20°C, with a 12 h light (200 m E-2 s-1):12 h dark illumination regime. Two grams of super phosphate, a commercial fertilizer, was added to the Treated flasks as a source of nutrients. The Control flasks, with tap water that is dechlorinated, were also inoculated with algae, but otherwise treated identically with all treatment conditions. The treatment conditions were provided for 30 days.

Growth Measurement

Microalgal growth was monitored by enumerating cells using an improved Neubauer hemocytometer, and the growth data were plotted on a semi-logarithmic scale to determine the growth pattern. The specific growth rate (divisions day^{-1}) was calculated using the following equation (Guillard, 1973):

$$K = \frac{\log N_t - \log N_0}{\log 2 \times t}$$

where:

N = Number of cells mL^{-1} at the end of the logarithmic growth phase or biomass concentration (mg L^{-1})

N_0 = Initial cell concentration (cells mL^{-1}) or initial biomass concentration (mg L^{-1})

t = Duration of the logarithmic growth phase (days)

For dry weight determination, algal cultures were harvested by centrifugation at 7,500 rpm for 15 min using a refrigerated microcentrifuge. The cell pellets were washed with glass-distilled water to remove residual medium components and centrifuged again under identical conditions. The washed biomass was dried in a hot-air oven at 60°C for 24 h or until a constant weight was achieved, and the dry biomass was subsequently recorded.

Biomass production was estimated by periodically collecting culture samples, harvesting the cells by centrifugation, and determining their dry weight. The harvested biomass was briefly washed to eliminate adhering culture medium. For extraction, 2 mL of the respective solvent was added to the biomass and incubated for 15 min, followed by mild sonication for 5 min to facilitate cellular disruption. The resulting mixture was centrifuged at 2,000 rpm for 15 min to separate cellular debris, and the supernatant was further clarified by centrifugation at 5,000 rpm for 15 min prior to subsequent biochemical analyses.

The algal extract was collected in sterile storage vials and allowed to undergo complete solvent evaporation. The dried extract was subsequently re-suspended in 1 mL of sterile distilled water and stored under refrigerated conditions until further analysis. Total carbohydrate content was estimated using the Anthrone method (Hedge & Hofreiter, 1962), total protein content was determined following the method described by Lowry *et al*. (1951), and total lipid content was analyzed according to the procedure of Bligh and Dyer (1959).

Results and Discussion

A low-cost cultivation methodology employing a High Rate Algal (HRA) pond system was previously investigated by Sivasubramaniam (2006), who demonstrated that the biochemical composition of *Chlorella vulgaris* increased significantly during mass cultivation in open algal ponds. The phycoremediation potential of *Chlorella vulgaris* has also been extensively reported, highlighting its ability to remove nutrients and pollutants from aquatic environments (Sivasubramanian, 2006). Large-scale cultivation technologies and utilization processes for microalgae have been successfully developed in several countries, and similar approaches may provide significant benefits for developing nations (Venkataraman & Becker, 1985). In recent decades, microalgal biotechnology has gained considerable importance because of its applications in food, feed, pharmaceuticals, and environmental management (Sivasubramanian, 2006).

Chlorella vulgaris is recognized for its exceptionally high chlorophyll content, which may constitute up to 7% of its dry weight, exceeding the chlorophyll levels found in *Spirulina* and alfalfa. Owing to its rich chlorophyll content, it is often referred to as the “Supreme Whole Food Concentrate.” The organism contains all essential amino acids required for human nutrition and serves as an excellent source of high-quality protein with superior bioavailability (Mohan *et al*., 2009). Additionally, *Chlorella* is rich in essential minerals such as potassium, magnesium, calcium, and iron, which contribute to cardiovascular health, hematopoiesis, and metabolic functions.

The presence of zinc, selenium, iodine, carotenoids, lutein, and xanthophyll further enhances its nutritional value. High levels of arginine stimulate growth hormone secretion, while Chlorella Growth Factor (CGF), rich in nucleic acids, supports cellular growth and regeneration. The presence of glucosamine in the cell wall contributes to the maintenance of cartilage, tendons, and ligaments. Furthermore, antioxidants such as chlorophyll, vitamins A, C, and E, carotenoids, and selenium may help reduce oxidative stress and alleviate symptoms associated with arthritis and chronic inflammation (Steenblock, 1987).

The present investigation demonstrated that superphosphate supplementation significantly enhanced biomass production in *Chlorella vulgaris*. Increased biomass accumulation resulted from rapid cell division stimulated by phosphorus availability under favorable culture conditions. This was evidenced by progressive increases in cell density determined through

hemocytometric analysis. The increase in pH values observed during cultivation may be attributed to enhanced photosynthetic activity and greater algal biomass production.

Moreover, significant increases in major cellular constituents, including carbohydrates, proteins, and lipids, were observed in treated cultures. These biomolecules represent essential nutritional components and indicate improved physiological status of the algae. Similar findings were reported by Molina *et al*. (1999), who observed enhanced biochemical composition under nutrient-enriched culture conditions. The application of superphosphate provided an adequate phosphorus source, promoting nucleic acid synthesis, cell division, and overall biomass productivity. Consequently, rapid cell multiplication resulted in increased cell counts at regular intervals, confirming the effectiveness of superphosphate supplementation in enhancing the growth and biochemical composition of *Chlorella vulgaris*.

Table.1 Growth of *Chlorella vulgaris* under mass cultivation

Days	No of cells/ml (x10^4)
4	265
8	327
12	348
16	396

Fig.1 Growth of Chlorella vulgaris under mass cultivation

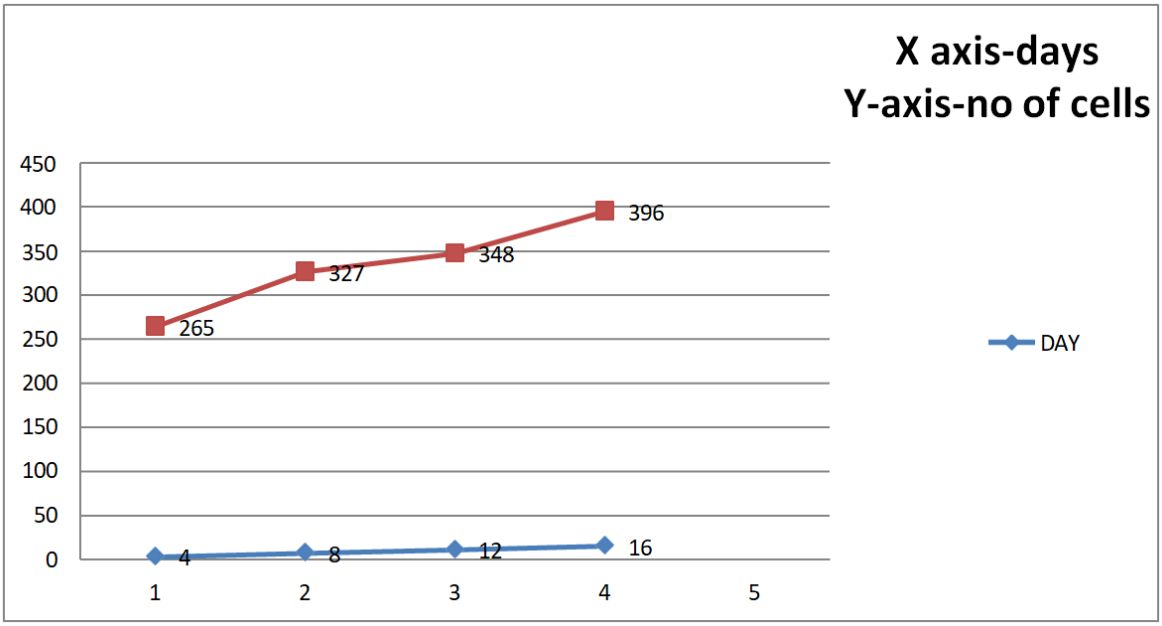


Table.2 Growth pattern of *Chlorella vulgaris* under control conditions (in normal water)

pH	No of cells/ml (x10^4)
8.72	86
8.61	162
8.53	215
8.38	267
8.27	292

Fig.2 Growth pattern of Chlorella vulgaris under control conditions

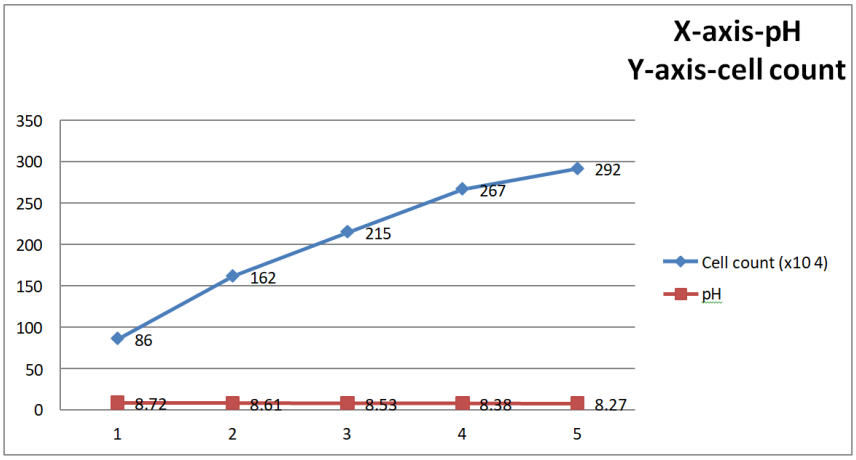


Table.3 Growth pattern of chlorella vulgaris under treated condition (super phosphate)

pH	Cell count
9.89	472
9.68	514
9.35	570
8.41	685
8.12	770

Fig.3 Cell Count analysis and pH values of *Chlorella sp*

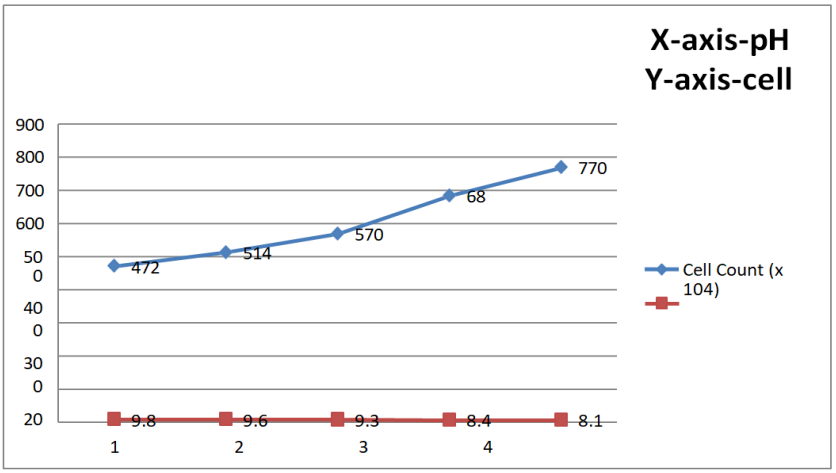


Table.4 Comparison of cell counts of Chlorella vulgaris under control and treated conditions

Cell count (control)	Cell count (treated)
86	472
162	514
215	570
267	685
292	770

Fig.4 Growth pattern of *Chlorella vulgaris* under control conditions

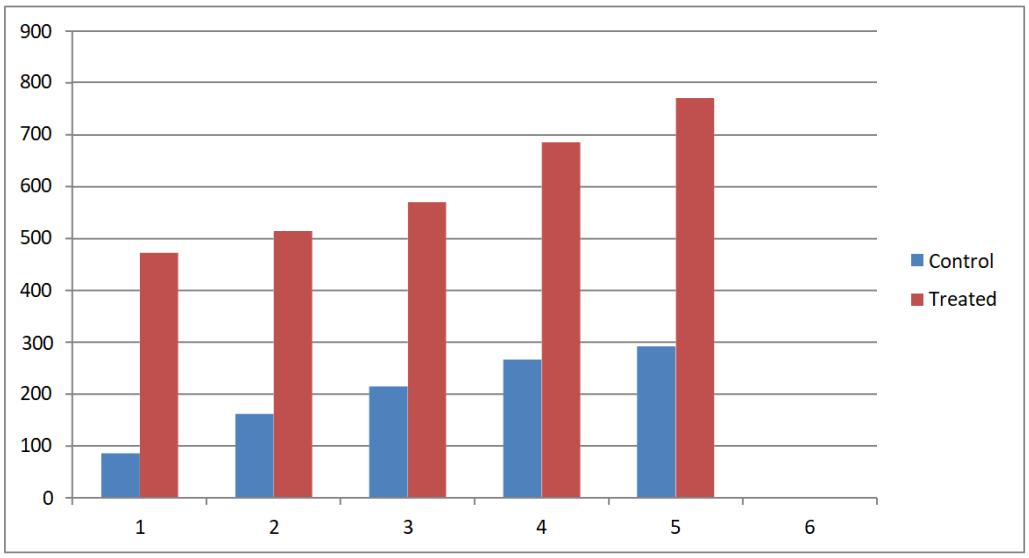
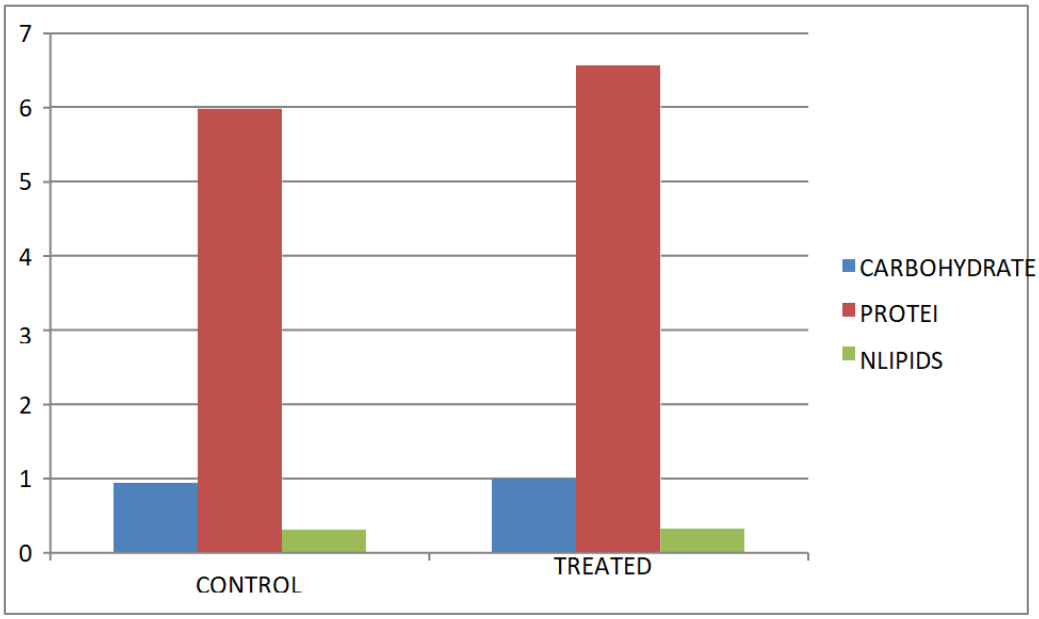


Table.7 Cellular components level under control and treated:

Cellular Components	Control	Treated
Carbohydrate	0.9376	**0.9938**
Protein	5.98	**6.56**
Lipids	**0.3156**	**0.3278**

Fig.5 Levels of Protein, Carbohydrate, and Lipids in the experimental studies



The above observations were recorded under mass cultivation conditions. In cultures maintained in dechlorinated tap water, pH and cell density were periodically measured using a digital pH meter and an improved Neubauer hemocytometer, respectively. These measurements were essential for comparing the growth performance of *Chlorella vulgaris* under control and superphosphate-treated conditions. The obtained data clearly demonstrated the extent of cell division and biomass enhancement following superphosphate supplementation.

The growth pattern of *Chlorella vulgaris* under treated conditions revealed a rapid increase in cell density accompanied by changes in pH. The addition of superphosphate significantly enhanced the growth of *Chlorella* species, indicating the importance of phosphorus availability in algal metabolism. Superphosphate, a commonly used agricultural fertilizer, supplied essential nutrients required for cellular division and biomass production. The primary objective of fertilization in phycoculture is to stimulate algal productivity, improve natural food production, optimize production costs, and maintain favorable growth conditions for cultured organisms.

A comparison between control and treated cultures clearly showed that cell numbers increased substantially under treated conditions. The enhanced growth observed in the treated cultures may be attributed to increased nutrient availability, which promoted rapid cell division and biomass accumulation.

Biochemical analysis of the harvested biomass revealed significant increases in carbohydrate, protein, and lipid contents, with maximum accumulation observed on the sixteenth day of cultivation. The altered biochemical composition of the biomass may be associated with the additional nutrient supply provided through superphosphate treatment.

Chlorella vulgaris is recognized as a highly nutritious microalga containing 10–30% lipids, 10–25% carbohydrates, essential amino acids, vitamins, and minerals. Previous studies have reported that nutrient composition, particularly nitrogen availability, influences fatty acid composition and biochemical constituents in freshwater microalgae (Yongmanitchai & Ward, 1991; Lee *et al*., 1989). Similar findings were observed in the present study, where treated cultures exhibited enhanced biomolecular contents. The increased availability of phosphorus likely promoted nucleic acid synthesis, cellular metabolism, and rapid cell division, ultimately resulting in greater biomass production and accumulation of major cellular constituents.

In Conclusion, the present investigation demonstrates a simple and cost-effective methodology for enhancing algal biomass production through superphosphate supplementation. This approach offers potential applications in renewable biomass generation and fertilizer recycling. *Chlorella vulgaris* may serve as an effective algal scrubber capable of utilizing nutrients released from agricultural runoff, thereby reducing environmental pollution while simultaneously producing valuable biomass. The enhanced production of carbohydrates, proteins, and lipids suggests potential applications in nutraceutical, pharmaceutical, and biotechnological industries. Further investigations using large-scale raceway pond systems are recommended to optimize biomass productivity and evaluate the commercial feasibility of this cultivation strategy for sustainable algal biotechnology.

Author Contributions

S. Haripriya: Investigation, formal analysis, writing—original draft. T. M. Vijayalakshmi: conceptualization, data curation, investigation, methodology, supervision; S.Mahalekshmi: Validation, methodology, writing—reviewing. R. Murali: writing, reviewing and editing;

Data Availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethical Approval Not applicable.

Consent to Participate Not applicable.

Consent to Publish Not applicable.

Conflict of Interest The authors declare no competing interests.

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