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Optimization of Ultrasonication for *Chlorella* sp. Growth: Impacts on Biomass Productivity and Nutrient Utilization

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Authors' contributions

This work was carried out in collaboration between both authors. Author AM designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author SKP analyzed the study and approved the manuscript. Both authors read and approved the final manuscript.

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ABSTRACT

Aims: This study aimed to investigate the effect of ultrasonic treatment of different durations on *Chlorella* sp. culture to analyze biomass concentration, biomass productivity and chlorophyll and macronutrients, such as nitrate and phosphate, which were added to the *Chlorella* sp. culture.
Study Design: This investigation sought to optimize the duration of exposure of live microalgal culture to ultrasonic treatment to estimate economically significant biomass and pigments.

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Microalgal biomass has potential applications in biofuels, pharmaceuticals, and nutraceuticals, and is highly sought after by industry. Consequently, it is imperative to optimize this process for large-scale production. Chlorophyll pigment is not only crucial in the photosynthesis process but also serves as a high-value component in the food, cosmetic, and health industries. Furthermore, utilizing the optimized duration of ultrasonication treatment, residual nitrate and phosphate in the medium were analyzed, providing key insights into the nutrient consumption of *Chlorella* sp. under ultrasonication stress.

Place and Duration of Study: The present investigation was conducted over 36 days to estimate the effect of ultrasonication on biomass, chlorophyll, nitrate and phosphorus concentration in *Chlorella* culture at the laboratory of Synthetic Ecology and Environment Biotechnology, Department of Environmental Science, School of Earth Sciences, Central University of Rajasthan, Bandarsindri, Ajmer.

Methodology: In this study, the effects of ultrasonication on biomass and chlorophyll content were evaluated by treating *Chlorella* sp. cultures for 5, 10, and 15 min in addition to an untreated control group. Based on these results, an optimized ultrasonication duration of 5 min was selected for further analysis. During this phase, the nitrate and phosphorus contents in the culture medium were measured to investigate the influence of ultrasonication on these nutrient levels. All treatments were conducted in triplicate to ensure reproducibility of the results, with the mean and standard deviation calculated as measures of statistical reliability. The use of mean and standard deviation in triplicate tests ensure that the results are consistent and reproducible, hence providing a measure of statistical dependability.

Results: The biomass concentration and biomass productivity concentration increased from 29.9 ± 0.00240 mg/L (control) to 66.8 ± 0.01485 mg/L, 1.275 ± 0.5033 (control) to 3.71 ± 0.4163 mg/L/day respectively. The highest total chlorophyll concentration was observed in the exponential phase (9 days of culture) which was 3.7772 ± 0.0500. There was a decrease in the concentrations of nitrate and phosphate throughout the culture period, with a total nitrate consumption of 38.5% and total phosphate consumption of 24%.

Conclusion: Chlorophyll and biomass concentrations were maximized after 5 min of ultrasonic treatment, but declined with longer exposure. Nutrient analysis revealed a significant decrease in nitrate and phosphate concentrations over time, consistent with nutrient uptake by the growing cells and the subsequent accumulation of metabolites.

Keywords: Chlorella; microalgae; ultrasonication; biomass; cell biovolume; macronutrients.

1. INTRODUCTION

Microalgae are considered a highly innovative and promising source of biomass for biorefinery applications, especially for producing biofuels such as biodiesel from microalgal oil [1]. Beyond biofuels, microalgae are a rich source of valuable biproducts [2], such as pigments, proteins, carbohydrates etc. [3]. These products have significant applications in the food and feed industries, demonstrating the versatility of microalgae for various biotechnological applications [4]. During the growth cycle of microalgae, they possess an incredible capacity to accumulate a variety of macromolecules. Due to this unique characteristic which endows them in versatility in the composition which rarely seen in the other organism [5,6,7]. The growth conditions in the microalgae play an important role in determining the macromolecular profile of microalgae for example, during nutrient starvation conditions in microalgae its metabolism shifts and enhances the accumulation of carbohydrates, which are used as energy reserves in the late phase.

Chlorella is an important nutritional source for human consumption and contains high levels of proteins and other important nutrients [8]. The cell wall of *Chlorella* is very compact and its disruption requires a greater amount of energy. The proximate composition of microalgae may be altered significantly during the stationary phase, as nitrate starvation causes an increase in carbohydrate levels [9]. A major challenge in utilizing micro algal biomass in biorefinery facilities is the requirement for downstream processes that are sufficiently effective to extract important chemicals in a sustainable manner. Disruption of the micro-algal cell wall is an important step in increasing product recovery in the later stages of algal biorefineries, which aim to use algal biomass as a sustainable resource. To disrupt the algal cell wall, various physical-

mechanical and bio- chemical technologies have been applied to increase the intracellular microalgae content [10]. Ultrasonic treatment ruptures the microalgal cell wall, allowing the direct extraction of internal chemicals for commercial purposes [11]. During ultrasonic treatment, there was an increase in the surface area of the algal cells, which indicated the breakdown of the cell wall and its disintegration. There is a change in the spherical shape of *Chlorella* to an asymmetrical shape under the release of ultrasonic energy and intracellular components revelation of ultrasonic energy and intracellular components released in the surrounding medium [12].

Several studies have been conducted to enhance the extraction yield of microalgal products using ultrasonication, but no study has reported the accumulation of value-added products using ultrasonication on live microalgal cultures to ultrasonic treatment. In this study, the duration of exposure of live microalgal culture was optimized to estimate economically important biomass and pigments. Furthermore, using the optimized duration of ultrasonication treatment, residual nitrate and phosphate in the medium were also analyzed, which provides key insights into the nutrient consumption of *Chlorella* sp. under ultrasonication stress.

2. MATERIALS AND METHODS

2.1 Micro Algae Cultivation and Medium Composition

Microalgal samples (*Chlorella* sp.) were collected from the Laboratory of Synthetic Ecology and Environment Biotechnology, Department of Environmental Science, School of Earth Sciences, Central University of Rajasthan, Bandarsindri, Ajmer, India. In this study, microalgae samples were cultivated in bold basal medium (BBM). The initial pH of the BBM medium was 6.8-7.2. Microalgae cultivation was carried out using a 1 L flask and kept at room temperature (27 ± 0ºC). The culture was maintained at 24:0 (Day/ Night) under light conditions until it reached the stationary phase.

2.2 Biomass Harvesting

Ultrasonic treatment at an intensity of 45 kHz was applied to the cultures using an Athena Technology probe sonicator for durations of 1, 5, and 10 min, excluding the control group. Each treatment was conducted in triplicate. Following ultrasonication, centrifugation was performed for biomass harvesting at 4500 rpm for 15 minutes, and the dry cell weight was subsequently calculated**.** Biomass was harvested by centrifugation. In this centrifugation method, the culture was centrifuged at 4500 rpm for 15 minutes. The pellet was centrifuged once more at 4500 rpm for an additional 15 minutes after washing with distilled water and the top layer was removed. The biomass pellet was transferred to the biomass petri plates and dried overnight in a hot air oven at 30^0C. After oven drying petri plates containing biomass were kept in a desiccator for 20-30 minutes so that extra moisture was absorbed. Biomass was calculated by subtracting the weight of empty Petri plates from the dry weight of the plates. The dry cell weight was calculated by using equation 2.1 and biomass productivity was calculated by equation (2.2)

$$\text{Dry cell weight} = W_2 - W_1 \quad (2.1)$$

W1 = Initial weight of petri plate before oven drying
W2 = Final weight of petri plate after oven drying Biomass

$$\text{Productivity} = \text{Biomass} \div \text{age of culture} \quad (2.2)$$

2.3 Chlorophyll Estimation

Chlorophyll content was determined using the 80% acetone method: 10 ml of algal sample was utilized to quantify the chlorophyll content. Ultrasonic treatment was applied for durations of 1, 5, and 10 minutes prior to chlorophyll content determination. The culture was centrifuged (Neuation IFUGE UC02) at 3500 rpm for 10 minutes to obtain the pellet. The pellet was collected and resuspended in 1 ml of 80% acetone; the mixture was thoroughly homogenized by vortexing and centrifuged at 3500 rpm for 10 minutes. This process was repeated until the pellet became colorless. The absorbance of the supernatant was measured at wavelengths of 664 nm and 647 nm.The readings were converted into of chlorophyll a, b, and total chlorophyll content using the equation described by Jeffrey and Humprey (1975) [13]:

$$\text{Chlorophyll a} = (11.93 * A_{664}) - (1.93 * A_{647}) \quad (2.3)$$

$$\text{Chlorophyll b} = (20.36 * A_{647}) - (5.50 * A_{664}) \quad (2.4)$$

$$\text{Total Chlorophyll} = (\text{Chlorophyll}_A + \text{Chlorophyll}_B) \quad (2.5)$$

2.4 Macronutrients Analysis

Total and Particulate nitrate were analyzed by the salicylate method (TRI- reagent method). Total and particulate phosphorous were analyzed using the ascorbic acid method (APHA,2007) [14].

2.4.1 Nitrate analysis

A stock solution of potassium nitrate (KNO_3) was prepared at a concentration of 100 mg/L, and nitrate standards ranging from 1 to 40 ppm were generated for calibration of the spectrophotometric (Agilent Technologies Cary-100) determination of nitrate concentration at 410 nm absorbance. A 20 mL sample of algal culture (*Chlorella* sp.) was subjected to ultrasonic treatment at 45 kHz for 5 minutes using an Athena Technology probe sonicator to disrupt the cells and enhance the release of intracellular compounds. The treated culture was subsequently centrifuged at 4500 rpm for 10 minutes to separate the biomass from the supernatant, from which a 1 mL aliquot was extracted for nitrate analysis. For particulate nitrate analysis, the supernatant was filtered using a Whatman filter paper with a pore size of 2.5 microns.

2.4.2 Phosphorous analysis

A stock solution of 50 ppm potassium phosphate (K_2HPO_4) was prepared. Phosphorus standards were prepared through serial dilution, ranging from 0.04 to 3 mg/L. For subsequent analysis, 5 mL of each standard was combined with 0.8 mL of a mixed reagent, and the mixture was allowed to develop a blue color for 10 minutes. The absorbance of the resulting solutions was measured at 880 nm to quantify phosphorus concentrations. For culture analysis, 40 mL of algal culture *(Chlorella* sp.) was subjected to ultrasonic treatment to facilitate cell disruption. Following treatment, the culture was centrifuged at 4500 rpm for 5 minutes to separate the biomass from the supernatant. The supernatant subsequently analyzed for the total phosphorus content. For particulate phosphorus determination, the supernatant was filtered through Whatman filter paper of size 2.5 microns.

3. RESULTS AND DISCUSSION

3.1 Biomass Concentration

The biomass concentration in the control group was 29.9 ± 0.00240 mg/L. An increase in biomass concentration was observed when the culture underwent ultrasonication for 1 min. Maximum biomass was recorded after 5 min of ultrasonic treatment. However, when ultrasonication was applied for > 5 min, a slight decrease in the biomass was noted. After 10 min of ultrasonic treatment, the biomass content was 56.3 ± 0.00566 mg/L. The highest biomass productivity was observed after 5 min of ultrasonication treatment with a total biomass of 66.8 mg/L, which was 2.91 times greater than the control (22.9 mg/L). In the ultrasonic pre-treatment for biomass extraction, the biomass concentration increased when ultrasonic pre-treatment was applied for 1 and 5 min. However, with an increase in the duration of ultrasonic pre-treatment (10 min), the biomass concentration decreased. One-minute ultrasonic pretreatment resulted in a brief exposure, which caused a minor disruption that led to a small increase in the biomass. Five minutes of ultrasonication treatment was more extensive and caused greater cell disruption, which increased the mass transfer of the intracellular components present in the medium [15,16]. Ultrasonication initially stimulates biomass production by disrupting cell walls and releasing intracellular contents, while also increasing metabolic activity in the short term (1 to 5 minutes). However, extended exposure (10 minutes) results in heat accumulation and structural damage, which compromises cells and reduces biomass [17].

Table 1. Effect of ultrasonic treatments on Biomass (Mean SD N=4)

Treatments	Biomass (mg/l)	Biomass Productivity (mg/L/day)
Control	22.9 ± 0.0024	1.275 ± 0.5033
1 minute	47.3 ± 0.0016	2.62 ± 0.2886
5 minutes	66.8 ± 0.0148	3.71 ± 0.4163
10 minutes	56.3 ± 0.0056	3.310 ± 0.6557

**Ultrasonic treatment of different duration to analyze biomass concentration*

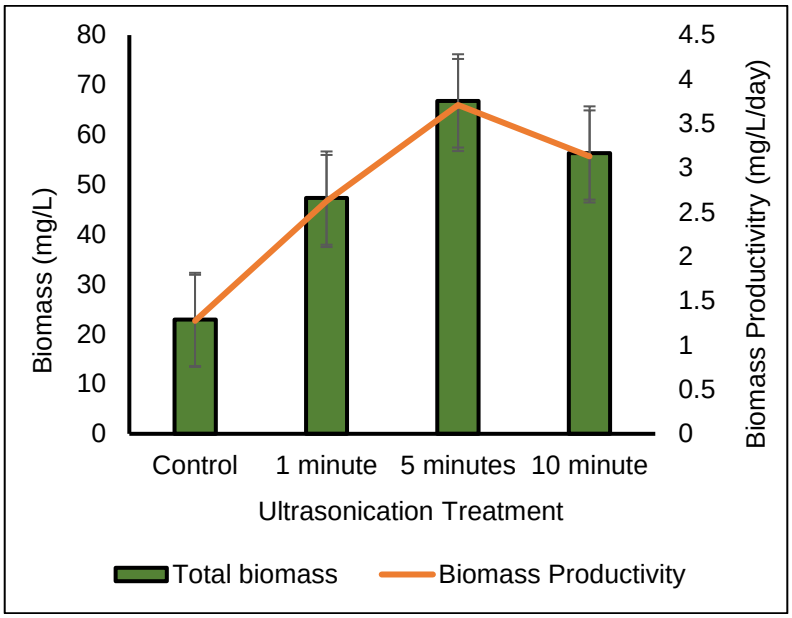

Fig. 1. Effect of ultrasonic treatment on biomass and biomass productivity

3.2 Chlorophyll Estimation

The Chlorophyll concentration increased when the culture was optimized for 1 and 5 minutes. The higher concentration of chlorophyll was observed after 5 minutes of ultrasonic optimization from the induction phase to the stationary phase. It was observed that the chlorophyll concentration decreased when the culture was optimized for 10 minutes. Table 2 and Fig. 2 illustrate that in the exponential phase, the chlorophyll b concentration was higher than that of chlorophyll a on days 9 and 12 of the culture, which were 1.9593 ± 0.00433 and 1.7764 ± 0.03126, respectively. During the optimal growth phase, active cell division and photosynthesis occur, and adequate nutrient availability increases the concentration of chlorophyll b. Additionally, *Chlorella* may metabolically adapt to environmental conditions, enhancing its photosynthetic efficiency during this period, which accounts for the increase in chlorophyll b concentration on the 9th and 12th days of *Chlorella* culture [18,19]. The highest total chlorophyll concentration was observed in the exponential phase (day 9 of culture), which was 3.7772 ± 0.0500. As the culture aged, there was a decrease in the chlorophyll content in the stationary phase due to the lower availability of nutrients, whereas in the logarithmic phase, the chlorophyll content was higher because of the availability of nutrients [20]. Chlorophyll a decreased with increasing concentrations of nitrate in the medium. Nutrient availability affects the chlorophyll content in microalgal species.

3.3 Nitrate and Phosphorous Concentration

The initial concentration of the total nitrate in the media before inoculation was 49.97 mg/L, and after inoculation, the total nitrate concentration was 53.6 ± 0.3214 mg/L. The total nitrate consumption in the *Chlorella* culture without ultrasonic optimization was 38.5% from the lag phase to the stationary phase. (Tables 3 and 4 and Figs. 3 and 4 illustrate the total nitrate and particulate nitrate concentrations without ultrasonic optimization and with ultrasonic optimization, respectively). It was observed that there was fluctuation in the total nitrate concentration during the exponential phase, and particulate nitrate concentration decreased from the lag phase to the stationary phase. Similarly, the total phosphate concentration in the medium before inoculation was 30 mg/L. There was a significant decrease in the concentration of phosphate from the lag phase to the stationary phase in *Chlorella* sp. culture. Total phosphate consumption was 24% in the *Chlorella* sp. culture. There was also a decrease in the concentrations of the total and particulate phosphate from the induction phase to the stationary phase of growth. (Tables 5, 6 and Figs. 5, 6 illustrate total and particulate phosphate concentrations in the culture supernatant without and with ultrasonic optimization.) In the present study, it was observed that there was a decrease in the concentration of nitrate and phosphate in the

culture due to nutrient uptake by the cells for their growth. It was also observed that there was a decrease in the nutrient concentration inside the cell, as nitrate and phosphate are essential nutrients utilized by the microalgae for various metabolic activities, protein synthesis, nucleic acid synthesis, and energy production. Nitrogen is an important element required for the synthesis of nucleic acids, proteins, amino acids (including enzymes and coenzymes), and chlorophyll for metabolic activities. The reduction in the concentration of nitrogen leads to slow growth and produces stress conditions, which trigger the microalgae to accumulate more lipids and carbohydrates. Like nitrate, phosphorus is also an essential constituent of nucleic acids, cell energy carriers, and biomembrane systems. The limitation of phosphorus also leads to stress conditions that allow the microalgae to accumulate energy storage metabolites [21,22].

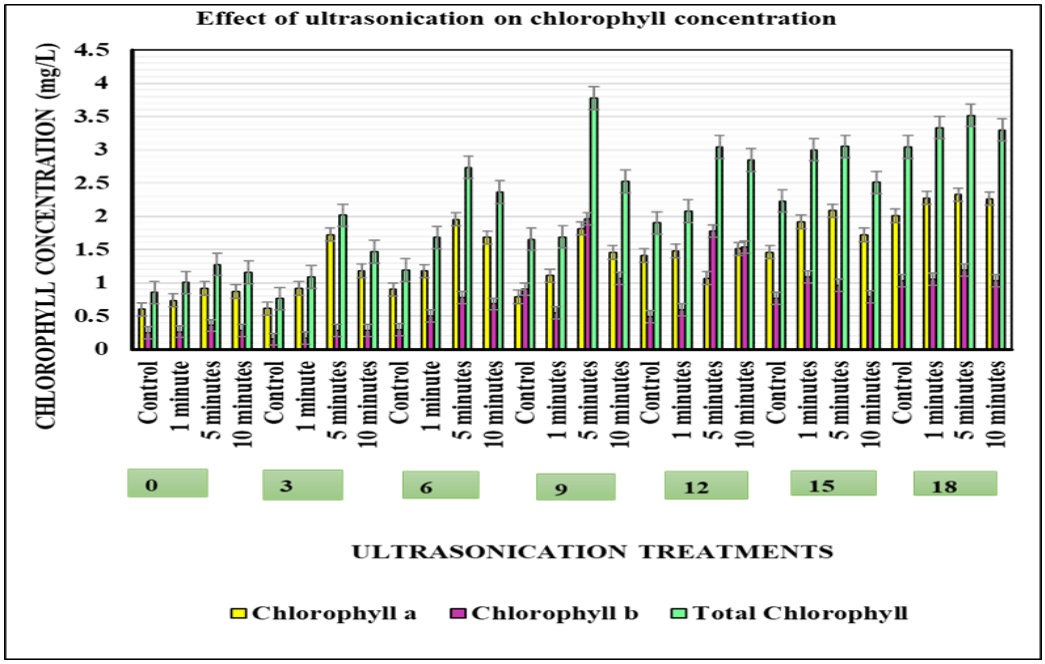

Fig. 2. Chlorophyll concentration in different ultrasonic treatments

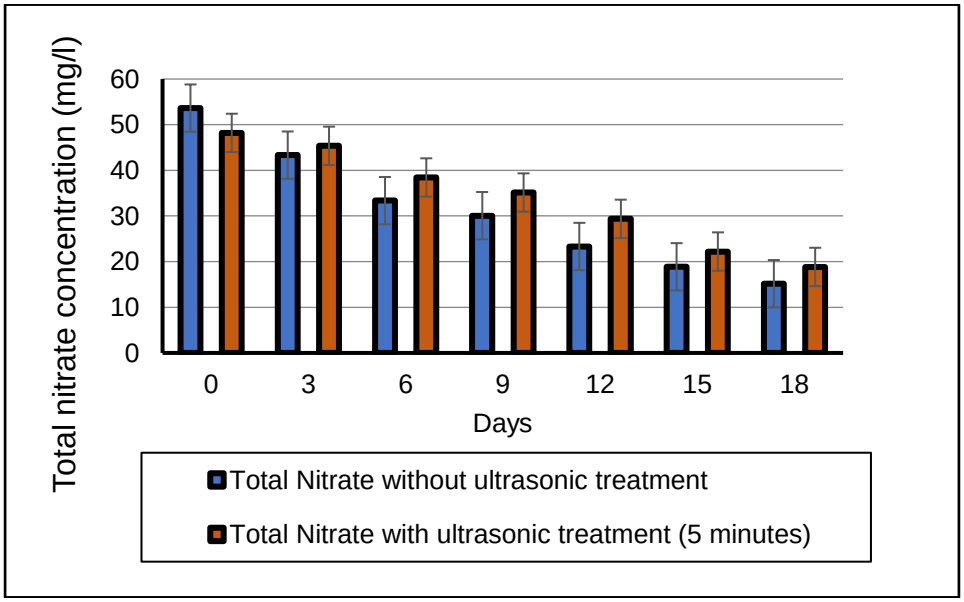

Fig. 3. Total nitrate concentration was analyzed with and without ultrasonic treatment in culture supernatant

Table 2. Chlorophyll concentrations in growing cultures of *Chlorella* sp.

Days of culture	Treatments	Chlorophyll a	Chlorophyll b	Total Chlorophyll
0 day	Control	0.6028 ±0.135	0.2521 ± 0.006	0.8548 ± 0.129
	1 minute	0.7371 ±0.044	0.2663 ± 0.0076	1.0033 ± 0.051
	5 minutes	0.9174 ±0.002	0.3598 ± 0.062	1.2771 ± 0.064
	10 minutes	0.8685 ±0.027	0.289 ± 0.041	1.15841 ± 0.014
3 days	Control	0.6123 ± 0.012	0.1527 ± 0.072	0.7650 ± 0.084
	1 minute	0.9166 ± 0.084	0.1721 ± 0.058	1.0887 ± 0.142
	5 minutes	1.7258 ± 0.302	0.2876 ± 0.046	2.0135 ± 0.348
	10 minutes	1.1790 ± 0.204	0.2870 ±0.192	1.4660 ± 0.397
6 days	Control	0.8999 ±0.007	0.2919 ± 0.005	1.1918 ± 0.001
	1 minute	1.1751 ± 0.005	0.5058 ± 0.057	1.6808 ± 0.063
	5 minutes	1.9546 ± 0.050	0.7792 ± 0.069	2.7338 ± 0.019
	10 minutes	1.6821 ± 0.285	0.6809 ±0.154	2.3630 ±0.440
9 days	Control	0.7901 ± 0.050	0.9006 ± 0.021	1.6536 ± 0.054
	1 minutes	1.1080 ± 0.003	0.5455 ± 0.057	1.6907 ± 0.071
	5 minutes	1.8179 ± 0.045	1.9593 ± 0.004	3.7772 ± 0.050
	10 minutes	1.4586 ± 0.035	1.0667 ± 0.013	2.5252 ±0.021
12 days	Control	1.4083 ± 0.068	0.4932± 0.033	1.9015 ± 0.101
	1 minutes	1.4789 ± 0.032	0.5962 ± 0.069	2.0751 ± 0.102
	5 minutes	1.0695 ± 0.052	1.7764 ± 0.031	3.0411 ± 0.073
	10 minutes	1.5090 ± 0.032	1.5321 ± 0.041	2.8459 ± 0.084
15 days	Control	1.4601 ± 0.028	0.7680 ± 0.054	2.2281 ±0.025
	1 minutes	1.9153 ± 0.096	1.0854 ± 0.060	3.0007 ± 0.096
	5 minutes	2.0823 ± 0.014	0.9666 ± 0.149	3.0489 ± 0.163
	10 minutes	1.7209 ± 0.017	0.7896 ± 0.118	2.5104 ± 0.136
18 Days	Control	2.0067± 0.000	1.0352 ± 0.272	3.0419 ± 0.271
	1 minutes	2.2751± 0.022	1.0578 ± 0.746	3.3329 ± 0.768
	5 minutes	2.3263± 0.014	1.1890 ± 0.523	3.5153 ± 0.538
	10 minutes	2.2656 ± 0.017	1.0329± 0.013	3.2985 ± 0.004

[*]Concentration of chlorophyll a, b and total chlorophyll in Chlorella sp. culture

Table 3. Total nitrate concentration (mg/L) (Mean SDN=4)

Days of Culture	Total Nitrate without optimization (mg/L)	Total nitrate with ultrasonic optimization (mg/L)
0 day	53.6 ± 0.3214	48.2 ± 0.4582
3 days	43.3± 0.2516	45.3 ± 0.1527
6 days	33.3±0.2645	38.4 ± 0.2086
9 days	38.06 ± 0.6082	29.1 ± 0.2081
12 days	29.3 ± 0.4256	23.3 ± 0.2516
15 days	18.8 ±0.3511	22.2 ± 0.1905
18 days	15.1 ± 0.3785	18.8 ± 0.0577

[*]Total nitrate concentration was estimated for 18 days (every 3 day) with and without ultrasonic optimization

Table 4. Particulate nitrate concentration (mg/L)

Days of culture	Particulate Nitrate without ultrasonic optimization (mg/L)	Particulate Nitrate with ultrasonic optimization (mg/L)
0 day	14.5 ± 0.2081	16.6 ± 0.1527
3 days	12.10 ± 0.1732	15.3 ± 0.2645
6 days	10.4 ± 0.1527	11.2 ±0.0577
9 days	5.73± 0.2086	6.33 ±0.1527
12 days	4.87±0.1154	6.03±0.0577
15 days	3.17 ±0.0577	5.43±0.3214
18 days	2.13± 0.0580	4.8 ± 0.200

[*]Particulate nitrate concentration was estimated for 18 days (every 3 day) with and without ultrasonic optimization

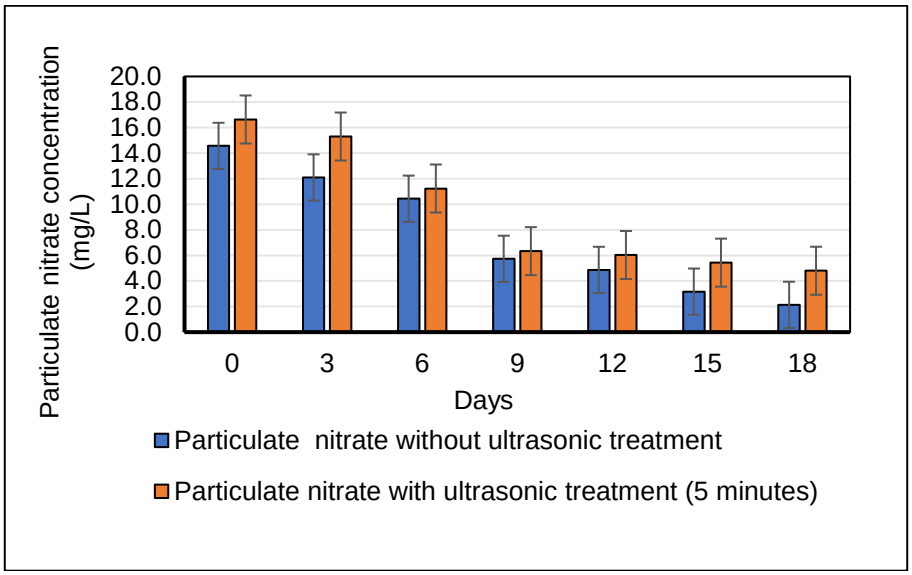

Fig. 4. Particulate nitrate concentration in culture supernatant with and without ultrasonic treatment

Table 5. Particulate phosphate concentration in culture supernatant (mg/L) (Mean SDN =4)

Days of culture	Total Phosphate without optimization (mg/L)	Total Phosphate with ultrasonic optimization (mg/L)
0 day	27.2 ± 0.2645	29.5 ± 0.2309
3 days	19.7 ± 0.5777	22.2 ± 0.0577
6 days	17.6 ± 0.5507	20.1 ± 0.1527
9 days	15 ± 0.3464	19.6 ± 0.1537
12 days	5.58 ± 0.8036	10.1 ± 0.2081
15 days	4.13 ± 0.1154	6.7 ± 0.1732
18 days	2.96 ± 0.1527	3.3 ± 0.1527

**Total phosphate concentration was estimated for 18 days (every 3 day) with and without ultrasonic optimization*

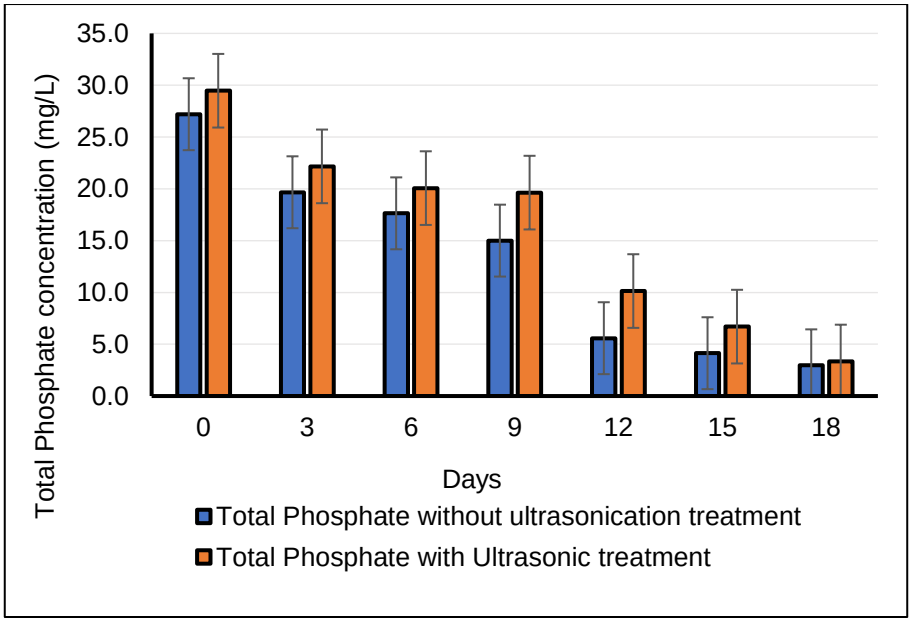

Fig. 5. Total phosphate concentration in culture supernatant

Table 6. Particulate phosphate concentration in culture supernatant (mg/L) (Mean SDN =4)

Days of culture	Particulate Phosphate without optimization (mg/L)	Particulate Phosphate with ultrasonic optimization (mg/L)
0 day	16.7 ± 0.5196	16 ± 0.5032
3 days	10.1 ± 0.3214	10.8 ± 0.2516
6 days	8.8 ± 0.2516	10.7 ± 0.2886
9 days	7 ± 0.5033	9.7 ± 0.4163
12 days	6.2 ± 0.2645	9.1 ± 0.6557
15 days	5.2 ± 0.2081	7.1 ± 0.1154
18 days	4.4 ± 0.2645	6.3 ± 0.1527

**Particulate phosphate concentration was estimated for 18 days (every 3 day) with and without ultrasonic optimization*

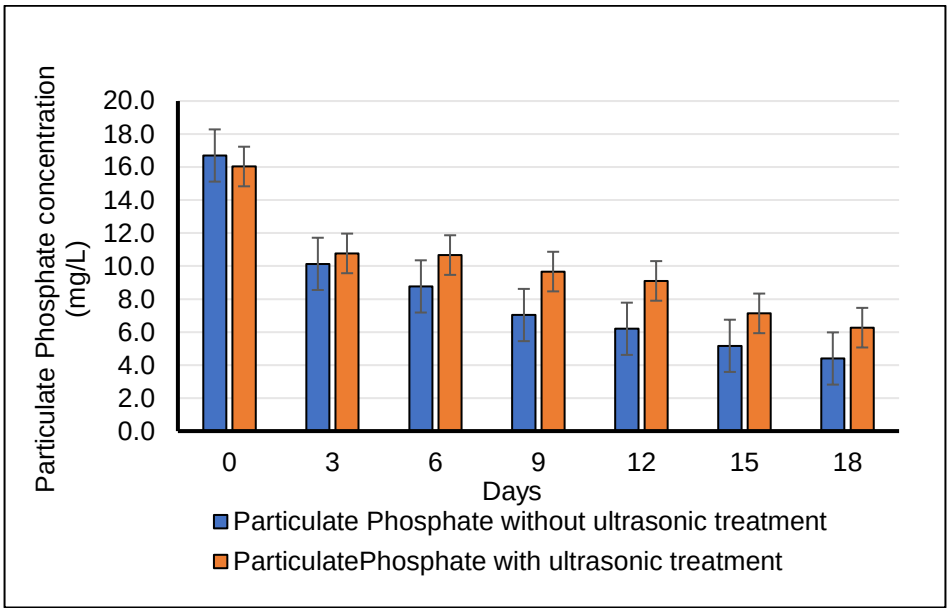

Fig. 6. Particulate phosphate concentration in culture supernatant

4. CONCLUSION

Ultrasonication induces stress in *Chlorella* cultures, which enhances the accumulation of secondary metabolites. This investigation demonstrated that ultrasonic treatment exerted a significant effect on major physiological and biochemical parameters in *Chlorella* sp. The chlorophyll concentration reached its maximum after 5 minutes of ultrasonic treatment; however, a decline in chlorophyll concentration was observed with prolonged exposure, indicating a potential trade-off between cell disruption and pigment preservation. Nutrient analysis revealed a significant decrease in nitrate and phosphate concentrations over time, consistent with nutrient uptake by the proliferating cells and the subsequent accumulation of metabolites. Overall, ultrasonic treatment effectively enhanced biomass productivity and nutrient utilization in microalgal cultures, offering a valuable approach for optimizing algal biomass production and metabolic profiles in various biotechnological applications. Further investigations could explore the long-term effects and optimize treatment parameters for improved industrial scalability.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

The authors declare that generative AI technologies, specifically ChatGPT (version 3) by OpenAI, were utilized in the writing and editing of this manuscript. The use of this AI tool was aimed at enhancing the clarity and quality of the content.

Details of the AI usage is given below:

1. **Name:** ChatGPT
2. **Version:** 3
3. **Model:** GPT-3
4. **Source:** OpenAI

5. **Inputs:** “summarizing effects of ultrasonication on biomass “
   “use of algal biomass for various purposes”

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Dolganyuk V, Belova D, et al. Microalgae: A promising source of valuable bioproducts. Biomolecules. 2020;10(8): 11531.
2. Zhang S, Zhang L, et al. A review on biodiesel production from microalgae: Influencing parameters and recent advanced technologies. Frontiers in Microbiology. 2022;13:970028.
3. Nair A, Ahirwar A, et al. Astaxanthin as a king of ketocarotenoids: Structure, synthesis, accumulation, bioavailability and antioxidant properties. Marine Drugs. 2023;21(3):176.
4. Geada P, Moreira C, et al. Algal proteins: Production strategies and nutritional and functional properties. Bioresource Technology. 2021;332:125125.
5. Masojídek J, Ranglová K, et al. Variables governing photosynthesis and growth in microalgae mass cultures. Processes. 2021;9(5):820.
   Available:https://doi.org/10.3390/pr9050820
6. Yang L, Chen J, et al. Growth and lipid accumulation by different nutrients in the microalga *Chlamydomonas reinhardtii*. Biotechnology for Biofuels. 2018;11:1-12.
7. Mourya M, Khan M, et al. Latest trends and developments in microalgae as potential source for biofuels: The case of diatoms. Fuel. 2022;314:122738.
8. Lorenzo K, Santocildes G, et al. Bioactivity of macronutrients from chlorella in physical exercise. Nutrients. 2023;15(9):2168.
9. Ambati RR. Nitrogen, phosphorus and microalgal: In Encyclopedia; 2021.
   Available:https://encyclopedia.pub/entry/7701
10. Di Caprio F, Altimari P, Pagnanelli F. Ultrasound-assisted extraction of carbohydrates from microalgae. Chemical Engineering Transactions. 2021;86:25-30.
11. Chemat F, Rombaut N, et al. Review of green food processing techniques. Preservation, transformation, and extraction. Innovative Food Science & Emerging Technologies. 2017;41:357-377.
12. Huang Y, Yang L, et al. unexpected formation of hydrides in heavy rare earth containing magnesium alloys. Journal of Magnesium and Alloys. 2016;4(3):173–180.
13. Jeffrey SW, Humphrey GF. New spectrophotometric equations for determining chlorophylls a, b, c1 and c2 in higher plants, algae and natural phytoplankton. Biochem Physiol Pflanzen. 1975;167:191–194.
14. Available:https://r.search.yahoo.com/_ylt=Awr984mc8OZm0v8K3NpXNyoA;_ylu=Y29sbwNncTEEcG9zAzMEdnRpZAMEc2VjA3Ny/RV=2/RE=1727620509/RO=10/RU=https%3a%2f%2farchive.org%2fdetails%2fgov.law.apha.method.2320.1992/RK=2/RS=n6oQf0rttSEH2WzzZqJo5IyTPPw-
15. Naveena B, Armshaw P, Tony Pembroke J. Ultrasonic intensification as a tool for enhanced microbial biofuel yields. Biotechnol Biofuels. 2015;8:140.
   Available:https://doi.org/10.1186/s13068-015-0321-0
16. Lasta P, da Silva PA, Caetano PA, Pinheiro PN, Zepka LQ, Jacob-Lopes E. Microalgae application in chemicals, enzymes, and bioactive molecules. In: Inamuddin, Ahamed, M.I, Prasad, R. (eds) Application of Microbes in Environmental and Microbial Biotechnology. Environmental and Microbial Biotechnology. Springer, Singapore; 2022.
   Available:https://doi.org/10.1007/978-981-16-2225-0_14
17. Sarwer A, Hamed SM, Osman AI, et al. Algal biomass valorization for biofuel production and carbon sequestration: A review. Environ Chem Lett. 2022;20:2797–2851.
   Available:https://doi.org/10.1007/s10311-022-01458-1
18. Tavares L, Nudi MH, Arroyo PA, et al. Effect of different concentrations of

phosphorus and nitrogen on the growth of the microalgae *Chlorella vulgaris*. Int J Energy Environ Eng. 2023;14:563–572. Available:https://doi.org/10.1007/s40095-022-00535-z

19. Piasecka A, Baier A. Metabolic and proteomic analysis of *Chlorella sorokiniana*, *Chloroidium saccharofilum*, and *Chlorella vulgaris* cells cultured in autotrophic, photoheterotrophic, and mixotrophic cultivation modes. Molecules. 2022;27(15):4817. Available:https://doi.org/10.3390/molecules27154817
20. Fiz Da Costa, Fabienne Le Grand, Claudie Quere, Gael Bougaran, Jean Paul Cadoret, et al. Effects of growth phase and nitrogen limitation on biochemical composition of two strains *Tisochrysis lutea*. Algal Research - Biomass, Biofuels and Bioproducts. 2017;27:177-189.
21. Ratomski P, Hawrot-Paw M. Influence of nutrient-stress conditions on *Chlorella vulgaris* biomass production and lipid content. Catalysts. 2021;11(5):573. Available:https://doi.org/10.3390/catal11050573
22. Estevam BR, Pinto LFR, Filho RM, et al. Growth and metabolite production in *Chlorella* sp.: analysis of cultivation system and nutrient reduction. Bioenerg. Res. 2023;16:1829–1840. Available:https://doi.org/10.1007/s12155-022-10532-z

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