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Research article

Effects of Ultrasonic Stimulation and Light Intensity on the Growth Rate and Biomass Productivity of *Chlorella ellipsoidea* in a Closed-Batch Cultivation System

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Abstract: Microalgae exhibit high nutritional value as animal feed in aquatic animal nurseries. Therefore, approaches to enhance biomass productivity are of great economic significance. This study aimed to evaluate the effect of ultrasonic wave stimulation on the specific growth rate and biomass yield of *Chlorella ellipsoidea* strain TISTR 8260. *C. ellipsoidea* stimulated by ultrasonic waves at 50 Hz for 1, 5, and 10 min were cultivated in a closed-batch cultivation system with varying light intensities. Data revealed that stimulation for 1 min and rearing at 71.43 μmol m^{-2} s^{-1} resulted in the highest biomass productivity and specific growth rates, with averages of 0.89±0.008 g/L/day and 0.59±0.009 per day, respectively. The findings of this study emphasize the usefulness of ultrasonic waves in enhancing the biomass productivity of microalgae.

Keywords: Biomass; *Chlorella ellipsoidea*; Growth rate; Microalgae; Ultrasonic stimulation

1. Introduction

Ultrasonic sound waves, operating within the high-frequency range of 20 to 20,000 hertz, pose risks to small animals but are safe for humans. "acoustic cavitation" refers to the formation and subsequent collapse of bubbles within a liquid when exposed to an ultrasonic field [1-2]. This phenomenon gives rise to various physical effects, including shock waves, microjets, and turbulence, as these cavitation bubbles oscillate and implode [3-4]. Consequently, cavitation finds applications in diverse areas, such as cleaning, extraction, and emulsification. Ultrasonic sound waves can also generate nanoscale zinc oxide, enhancing green light emission. In medicine, ultrasonic sound waves are employed to create a device for parental gender diagnosis, producing embryo images with frequencies ranging from 1 to 20 KHz during echocardiogram procedures for fetuses [5-6]. Similarly, ultrasonic waves between 1 and 3 MHz can detect bone fractures. Furthermore, ultrasonic sound waves, particularly at 20 KHz, can disrupt microalgae cell walls and extract fats, a process referred to as lipoproteinization [7-8]. Additionally, ultrasonic waves have effectively preserved the color of unripe

mangoes by inhibiting chemical oxidation and the accumulation of phenol and malondialdehyde. Utilizing ultrasonic waves in irrigation water for pest management is environmentally friendly, as they leave no chemical residues. Moreover, ultrasonic waves enhance the extraction of fats from green microalgae [9]. These waves induce chemical and physical changes through the cavitation phenomenon, encompassing compression (molecule clumping) and wave expansion. Sound can separate molecules by generating rhythmic changes that mimic vigorous agitation, facilitating molecular diffusion. Consequently, the extraction rate increases due to the heightened contact area between the solvent and the sample and reduced extraction time [10-11]. Ultrasonic therapy has emerged as a potential alternative to enhancing microbial growth and chemical production. In the case of small, detectable algae, it has the potential to augment either endogenous chemical synthesis or cell development. The efficacy of this technique depends on the specific microorganisms involved and the operational conditions employed. Employing ultrasound to combat detrimental algal blooms carries significant ecological implications and threatens drinking water sources. The study examined the mortality of *Microcystis aeruginosa* cells and the release of intracellular organic substances by utilizing ultrasonic frequencies at 20 kHz, 740 kHz, and 1120 kHz during the cultivation of the small green algae *M. aeruginosa* in freshwater in Wuhan, China [12]. Light is another crucial factor that significantly influences the algae growth rate, serving as the primary energy source for autotrophic organisms like microalgae. Natural light, when direct, exhibits significant variation in intensity based on seasonal and geographical factors. As the algae population expands, light penetration becomes limited due to cell growth, especially for bottom-dwelling algae, impacting their access to adequate light. Consequently, this limitation affects cellular photosynthesis efficiency. To address this, a Light-emitting diode (LED) offers the flexibility to adjust wavelength, light intensity, and illumination duration, providing tailored lighting conditions for the algae [13]. Light intensity and spectral characteristics shape cell growth and biomass composition. Light intensities of 100, 250, and 500 μmol m^{-2} s^{-1}, along with three different light sources such as fluorescent lamps, RGB LEDs, and white LEDs, affect three green algae species: *Chlamydomonas reinhardtii*, *Desmodesmus quadricauda*, and *Parachlorella kessleri* both the growth rate and biomass productivity [14]. Moreover, light intensity plays a pivotal role in synthesizing various crucial compounds within algae, offering advantages in both their growth and utilization [15].

The increased biomass production of single-celled algae holds particular importance, given their pivotal role as a primary component of animal feed in aquatic animal breeding facilities [16-17]. The economic significance of these tiny green algae in the fish farming industry is underscored by their remarkable nutritional content, especially their rich protein content, which is crucial for fish growth. *Chlorella* sp. is a green microalgae renowned for its nutrient-rich profile, featuring high protein and fiber content, boasting an impressive protein level of 60%. Moreover, *Chlorella* sp. contains alpha-linolenic acid, which is associated with reducing cholesterol levels in blood vessels. It is also abundant in essential elements such as vitamin B12, A, beta-carotene, and nucleic acid. In Thailand, using algae in aquaculture farms as a beneficial dietary supplement has been instrumental in safeguarding economically significant aquatic species like fish and shrimp from diseases. These advantages, including rapid production and enhanced biomass productivity, have the potential to impact the local economy [18-19] significantly. This study aimed to investigate the impact of ultrasonic waves on the specific growth rate and biomass productivity of *Chlorella ellipsoidea* strain TISTR 8260, a microalga. To conduct this research, we utilized a closed-batch culture system capable of regulating various light intensities, including 14.29, 42.86, and 71.43 μmol m^{-2} s^{-1}, in addition to fluorescent light (114.29 μmol m^{-2} s^{-1}).

2. Materials and Methods

2.1 Experimental design

A completely randomized design (CRD) was employed to study the specific growth rate and biomass productivity of the green microalga *C. ellipsoidea* strain TISTR 8260. The growth parameters of *C. ellipsoidea* stimulated by ultrasonic waves at 50 Hz for 1, 5, and 10 min were compared to the untreated control group. The treatments were cultured in the BG-11 medium with light intensities of 14.29, 42.86, and 71.43 μmol m^{-2} s^{-1}, as well as fluorescent light (114.29 μmol m^{-2} s^{-1}). Each experimental set included three replicates.

2.2 Test strain and composition of the culture medium

The *C. ellipsoidea* strain TISTR 8260 was obtained from the Biotechnology Department, Thailand Institute of Scientific and Technological Research (TISTR). The microalga was grown in the BG-11 culture medium at a nutrient-to-alga ratio of 9:1. One-liter volumes of the BG-11 medium (HiMedia, India) were prepared in 2L-flasks and sterilized by autoclaving at 121 °C for 15 min. The nutrient formula of BG-11 was as follows: $NaNO_3$ (15.0 g/L), K_2PHO_4 (0.4 g/L), $MgSO_4.7H_2O$ (0.75 g/L), $CaCl_2$ $2H_2O$ (0.36 g/L), citric acid (0.06 g/L), C_6H_5+4yFexNyO_7 (0.06 g/L), EDTA disodium magnesium salt (0.01 g/L), Na_2CO_3 (0.02 g/L), and 1.0 mL trace metal mix A5 (consisting of H_3BO_4 28.6 g/L, $MnCl_2{\cdot}4H_2O$ 18.1 g/L, $ZnSO_4{\cdot}7H_2O$ 2.22 g/L, $NaMoO_4{\cdot}2H_2O$ 3.9 g/L, $CuSO_4{\cdot}5H_2O$ 0.079 g/L, and $Co(NO_3)2{\cdot}6H_2O$ 0.494 g/L) and the pH was adjusted to 7.0 [20]. The starter culture of the microalga was cultivated for 14 days with shaking at 150 rpm in a closed, intelligently controlled cabinet exposed to a light intensity of 42.86 μmol m^{-2} s^{-1} for 12 h per day. The starter culture was used at an optical density at 600 nm (OD_{600} nm) of 0.244.

2.3 The rearing experiment

The starter culture of *C. ellipsoidea* TISTR 8260 was used for the rearing experiment. Different light intensities were applied, and the specific growth rates and biomass productivities were measured. The rearing experiment was conducted in a closed-shift intelligent control cabinet at a temperature of 28 ± 1 °C and light intensities of 14.29, 42.86, and 71.43 μmol m^{-2} s^{-1} form the light emitting diode (LED) (9 watts, B1, Thailand), as well as fluorescent lamp (36 watts, Silver, Thailand) at 114.29 μmol m^{-2} s^{-1} for 12 h per day over 14 days.

2.4 Determination of specific growth rates and biomass productivity

To assess the microalga growth, the OD_{600} nm of different treatments was measured using a spectrophotometer (Brand Peak, model C-7100, USA). Aliquots of 1 mL of the cultures were collected and measured daily to generate a linear regression model. The specific growth rates were calculated using Equation 1, and the biomass productivity using Equation 2 [21]. The daily algal biomass measurement is calculated by employing a linear regression equation that compares the OD_{600} of the current sample to that of the initial cultivation.

$$P = \frac{X_1 - X_0}{t_1 - t_0} \quad (1)$$

$$\mu = \frac{\mathrm{In}(X_1 - X_0)}{t_1 - t_0} \quad (2)$$

where P is the mass productivity (biomass productivity; g/L/day),
μ is the specific growth rate (per day), and
X_1 and X_0 are the alga mass (g/L) at day T_1 and T_0, respectively

2.5 Statistical analysis

Each experiment was repeated three times for each sample (n = 3), and the standard deviations (SD) were calculated. The differences between groups were determined using the R program's one-way analysis of variance (ANOVA), followed by Tukey's multiple comparison analysis. At 95% confidence, a *p*-value of 0.05 was considered statistically significant.

3. Results and Discussion

3.1 The effect of ultrasonic waves on biomass productivity

To evaluate how high-frequency sound waves at 50 Hz affect the growth of *C. ellipsoidea* strain TISTR 8260, we exposed the microalgae to different durations of ultrasonic treatment while keeping the light intensity at various levels. These experimental conditions were implemented following ultrasonic treatment intervals of 1, 5, and 10 minutes, as opposed to the control group, which did not undergo ultrasonic stimulation. The results revealed that at 14.29 μmol m^{-2} s^{-1}., the mean biomasses were 0.64 ± 0.028, 0.77 ± 0.007, and 0.72 ± 0.012 g/L/day at 1, 5,

and 10 min, respectively. Among the three time points, the biomass at 1 min was significantly less, with a *p*-value of 0.0001 (DF = 3, F(3, 52) = 133.5), while the control group had an average biomass of 0.70 ± 0.009 g/L/day (Figure. 1a).

At 42.86 μmol m^{-2} s^{-1}, the 10-min ultrasonic stimulation yielded the highest average biomass productivity (0.79 ± 0.015 g/L/day). This value was significantly (*p*-value = 0.0001; DF = 3, F(3, 52) = 12.58) higher than the 1-min and 5-min ultrasonic stimulation and the control group, with average biomass productivity of 0.75 ± 0.020, 0.75 ±0.023, and 0.76 ± 0.011 g/L/day, respectively (Figure. 1b). In addition, the ultrasonic stimulation for 1 min and 5 min at 71.43 μmol m^{-2} s^{-1} produced higher biomass productivity than the 10 min stimulation and the control groups with a *p*-value of 0.0001 (DF = 3, F(3, 52) = 322.5). The *p*-value was 0.0020 when comparing the mean values at 1 and 5 min. The average biomass productivity values of *C. ellipsoidea* stimulated by ultrasonic waves were 0.89 ±0.008, 0.87 ± 0.008, and 0.80 ± 0.012 g/L/day at 1, 5, and 10 min, respectively, while the biomass of the control group was 0.82 ± 0.006 g/L/day. At the fluorescent light intensity, the average biomass productivity of *C. ellipsoidea* strain TISTR 8260 resulting from ultrasonic stimulation at the different time points was higher than that of the non-stimulated control group. The biomass values were 0.66 ± 0.023, 0.69 ± 0.020, 0.67 ± 0.025, and 0.53 ± 0.003 g/L/day at 1, 5, and 10 min of stimulation and the control, respectively. The *p*-value was 0.0001 (DF = 3, F(3, 52) = 185.1) compared to the control group. When comparing the 1 min and 5 min biomass, the *p*-value was 0.0015, while it was 0.0151 when comparing the 5 min and 10 min values.

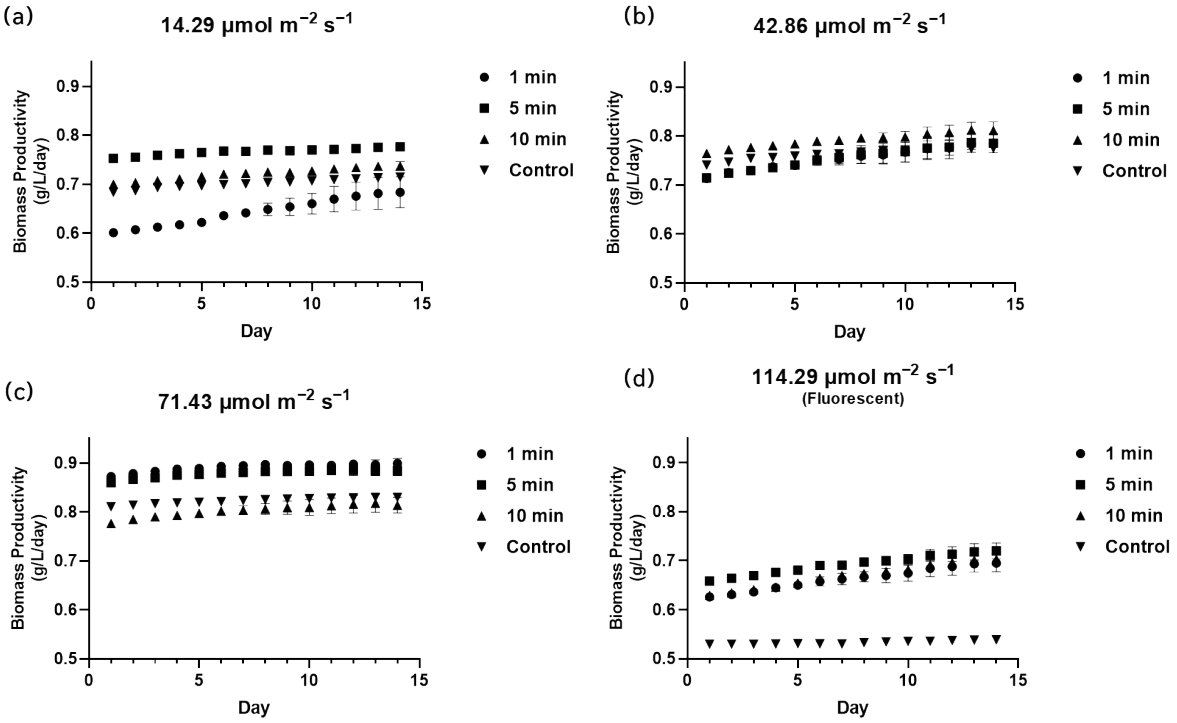

Figure 1. The biomass productivity according to the light intensity culture (a): 14.29 μmol m^{-2} s^{-1}, (2): 42.86 μmol m^{-2} s^{-1}, (3): 71.43 μmol m^{-2} s^{-1} and (4): Fluorescent after ultrasonic stimulation at 1, 5, and 10 min and control.

3.2 The impact of ultrasonic stimulation on the specific growth rates

The specific growth rate is an important parameter to evaluate the quality of the cultivation process. We further assessed the effect of ultrasonic stimulation on *C. ellipsoidea* growth by determining the specific growth rates of strain TISTR 8260 when stimulated by ultrasonic waves for 1, 5, and 10 min, compared to the control group. The mean specific growth rates at 14.29 μmol m^{-2} s^{-1} were 0.26 ± 0.044, 0.44 ± 0.009, 0.37 ± 0.017, and 0.35 ± 0.014 per day for the 1-, 5-, and 10-min stimulation groups and the control group, respectively (Figure. 2a). The highest specific growth rate value was recorded for the 5-min stimulation group with a *p*-

value of 0.0001 (DF = 3, F(3, 52) = 120.8). The *p*-value was 0.0246 when comparing the mean specific growth rates of the 10-min ultrasonic wave exposure with the control group. At a light intensity of 42.86 μmol m^{-2} s^{-1}, the exposure to ultrasonic waves for 10 min resulted in the highest specific growth rate (0.47 ± 0.018), with a *p*-value of 0.0001 (DF = *3*, F(3, 52) = 12.28) compared to the other treatment groups and the control. Exposure to sound waves for 1 and 5 min led to specific growth rates similar to the control group, with values of 0.42 ± 0.030, 0.42 ± 0.031, and 0.44 ± 0.014 per day, respectively (Figure. 2b). The results also revealed that at 71.43 μmol m^{-2} s^{-1}, higher specific growth rate values were recorded for the 1- and 5-min stimulation groups compared to the 10-min and control groups, with a *p*-value of 0.0001 (DF = *3*, F(3, 52). = 318). The mean specific growth rates were 0.59 ± 0.009, 0.57 ± 0.009, 0.48 ± 0.016, and 0.56 ± 0.007 per day for the stimulation groups of 1, 5, and 10 min. The control group, respectively (Figure. 2c). In addition, at the fluorescent light intensity, the three ultrasonic exposure times resulted in mean specific growth rates significantly higher than the control group, with a *p*-value of 0.0001 (DF = 3, F(3, 52) = 226.4). The 1-min and 10-min stimulation groups had similar growth rates. The mean specific growth rate values were 0.29 ±0.035, 0.34 ±0.029, 0.30 ± 0.037, and 0.07 ± 0.006 per day for the 1-, 5-, and 10-min stimulation groups and the control, respectively (Figure. 2d).

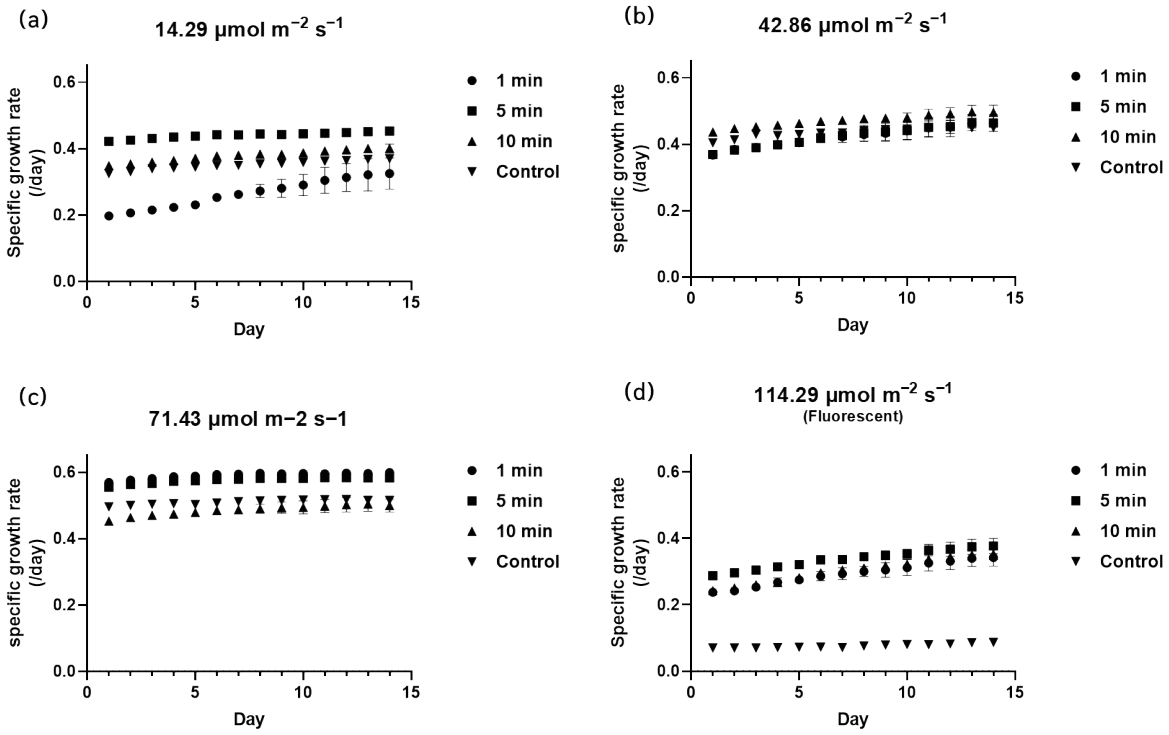

Figure 2. The specific growth rate according to the light intensity culture (a): 14.29 μmol m^{-2} s^{-1}, (2): 42.86 μmol m^{-2} s^{-1}, (3): 71.43 μmol m^{-2} s^{-1} and (4): Fluorescent after ultrasonic stimulation at 1, 5, and 10 min and control.

In this study, the biomass productivity resulting from the stimulation of ultrasonic growth at 50 Hz for 5 and 10 min ranged from 0.65 to 0.89 g/L/day. Previous research on the semi-continuous cultivation of *C. vulgaris* for lipid production tested four ultrasonic wave frequencies and recorded the highest biomass yield at 20 Hz [22]. Other studies have reported varying optimal frequencies for different algae strains: *C. sorokiniana* SDEC-18 achieved its highest biomass (0.63 g/L/day) with 15 Hz stimulation, increasing lipid production from 50.53% to 64.38%. *Scendesmus* sp., when stimulated at a frequency of 20 Hz for 2 minutes, yielded a maximum biomass of 0.68 g/L/day [23]. In this investigation, the highest average biomass (0.89 g/L/day) of *C. ellipsoidea* strain TISTR 8260 was attained when the algae were cultivated at 71.43 μmol m^{-2} s^{-1} with ultrasonic wave stimulation at 50 Hz for 1 min [24]. Notably, this study employed a frequency twice as high as previous research, which might account for the shorter time needed to enhance the desired growth rate. Furthermore,

the biomass productivity in this study surpasses last year by approximately 23%. The exposure to high-frequency ultrasonic waves may have activated the cell walls of algae, potentially leading to accelerated cell division [12].

Ultrasonic stimulation with varying light intensity levels significantly influences growth rate and biomass productivity. The peak yield is achieved at a light intensity of 71.43 μmol m^{-2} s^{-1}, whereas the lowest product is observed at 114.29 μmol m^{-2} s^{-1} in the culture. This phenomenon resembles findings in the batch cultivation of *Squama quadricauda*, where the highest biomass yield of 0.68 g/L/day was recorded at a light intensity of 42.86 μmol m^{-2} s^{-1} for 5 min. Similarly, *Scenedesmus obliquus* (FACHB12) displayed a biomass production of 0.69 g/L/day when cultured at 14.29 μmol m^{-2} s^{-1} after 3 min of sound wave stimulation at 23 Hz [25]. Based on the results, lower light intensity is optimal for accelerating the growth rate and biomass production of algae in this study. Offering excess light intensity may lead to cell overexposure rather than promoting accelerated algae proliferation. Employing an ultrasonic pulse enhances membrane permeability and induces changes in enzyme structure, facilitating improved aeration and the release of cell clusters during cultivation. These alterations also extend to cellular and genetic components, as illustrated in Figure 2, showcasing the impact of this application on microalgae growth. These transformative effects arise from cavitation events. Ultrasonic sound waves induce metabolic changes, increasing transmembrane permeability and enzyme structural modifications. This process introduces air and releases cellular clusters, consequently affecting cell count and producing elevated chemicals [26].

In contrast to our findings, the cultivation of *B. braunii*, stimulated by sound waves at a frequency of 40 Hz every four days, resulted in a maximum specific growth rate of 0.089 per day and a biomass output of 0.043 g/L/day [27]. In our study, we employed ultrasonic sound waves at 50 Hz and allowed the algae to grow for 14 days, with specific growth rates and biomass under these conditions. The highest specific growth rate recorded was 0.59 per day when *C. ellipsoidea* was exposed to ultrasound waves for 1 min and cultivated under a light intensity of 71.43 μmol m^{-2} s^{-1}. In comparison, *Nannochloris* sp., stimulated at 20 Hz for 2 min and cultured in the Bold medium, exhibited the highest specific growth rate of 0.42 per day and a biomass yield of 0.78 g/L. Interestingly, the exposure of *C. vulgaris* to sound waves at the same frequency as in our study did not result in an enhanced growth rate; instead, it achieved a specific growth rate of 0.65 per day and a biomass output of 0.772 g/L/day [28]. This entails initiating ultrasonic stimulation at the onset of the growth phase, thereby sustaining a continuous increase in biomass productivity through repeated algae stimulation.

4. Conclusions

In this study, we evaluated the effect of ultrasonic stimulation on the growth of *C. ellipsoidea* strain TISTR 8260 using a batch-cultivation system. *C. ellipsoidea* was exposed to ultrasonic waves at 50 Hz for 1, 5, and 10 min and cultivated at different light intensities. They stimulated *C. ellipsoidea* with ultrasonic waves for 1 min and rearing at a light intensity of 71.43 μmol m^{-2} s^{-1} improved biomass productivity and specific growth rates. Beyond creating the optimal conditions to stimulate growth and enhance algae biomass production in our experiment, these methods could be extended to other commercially significant algae strains. Leveraging ultrasonic sound waves to accelerate the cultivation of economically valuable green microalgae, thereby reducing their growth cycle and increasing biomass output, presents considerable promise. This strategy may yield economic advantages for entrepreneurs within the marketing ecosystem.

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Author Contributions: A short paragraph specifying their contributions must be provided for research articles with several authors. The following statements should be used "Conceptualization, S.T. and S.S..; methodology, S.T..; software, S.S..; validation, S.T., S.R.., and S.S..; formal analysis, X.X.; investigation, S.R..;

resources, S.T..; data curation, S.T..; writing-original draft preparation, S.T..; writing-review and editing, S.S..; visualization, S.R..; supervision, S.S..; project administration, S.R. and S.S.. All authors have read and agreed to the published version of the manuscript." Please turn to the CRediT taxonomy for the term explanation. Authorship must be limited to those who have contributed substantially to the work reported.

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References

[1] Zhang, H.; Qing-Kai, F.; Fan, C. et al. Effect of pulsed powder ultrasound on plasma morphology and its changing mechanism. *Int J Adv Manuf Technol*, 2021; 116, 1225–1232. https://doi.org/10.1007/s00170-021-07540-2.

[2] Valero, D.; Zhang, G.; Bung, D.B.; Chanson H. On the estimation of free-surface turbulence using ultrasonic sensors. *Flow Measurement and Instrumentation*, 2018; 60, 171-184. https://doi.org/10.1016/j.flowmeasinst.2018.02.009.

[3] Qifa, Z.; Sienting, L.; Dawei, W.; Kirk S. Piezoelectric films for high frequency ultrasonic transducers in biomedical applications. *Progress in Materials Science*, 2011; *56*(2), 139-174. https://doi.org/10.1016/j.pmatsci.2010.09.001.

[4] Bulliard-Sauret, O.; Berindei, J.; Ferrouillat, S.; Vignal, L.; Memponteil, A.; Poncet, C.; Leveque, J.M.; Gondrexon N. Heat transfer intensification by low or high frequency ultrasound: Thermal and hydrodynamic phenomenological analysis. *Experimental Thermal and Fluid Science*, 2019; 104, 258-271, https://doi.org/10.1016/j.expthermflusci.2019.03.003

[5] Xiong, J.; Wang, Y.; Ma, G-M.; Zhang, Q.; Zheng S-S. Field Applications of Ultra High Frequency, Techniques for Defect Detection in GIS. *Sensors*, 2018; *18*(8), 2425 pp.https://doi.org/10.3390/s18082425.

[6] Deffieux, T.; Konofagou, E. E. Numerical study of a simple transcranial focused ultrasound system applied to blood-brain barrier opening. *in IEEE Transactions on Ultrasonics, Ferroelectrics, and Frequency Control*, 2010; *57*(12), 2637-2653. https://doi: 10.1109/TUFFC.2010.1738.

[7] Shahram, N.; Seyed, H.H.; Meysam, M.R. CFD simulation of acoustic cavitation in a crude oil upgrading sonoreactor and prediction of collapse temperature and pressure of a cavitation bubble. *Chemical Engineering Research and Design*, 2014; *92*(1), 166-173. https://doi.org/10.1016/j.cherd.2013.07.002

[8] Muthupandian, A. The characterization of acoustic cavitation bubbles – An overview. *Ultrasonics Sonochemistry*, 2011; *18*(4), 864-872, https://doi.org/10.1016/j.ultsonch.2010.11.016.

[9] Khalesi, B.; Sohani, B.; Ghavami, N.; Ghavami, M.; Dudley, S.; Tiberi G. A Phantom Investigation to Quantify Huygens Principle Based Microwave Imaging for Bone Lesion Detection. *Electronics*, 2019; *8*(12), 1505. https://doi.org/10.3390/electronics8121505.

[10] Glacio, S.; Araujo, L.; Matos, J.B.L.; Jader, O.; Fernandes, S.; Cartaxo J.M.; Luciana, R.B.; Gonçalves, F.; Fernandes, A.N.; Wladimir, R.L. Extraction of lipids from microalgae by ultrasound application: Prospection of the optimal extraction method, *Ultrasonics Sonochemistry*, 2013; *20*(1), 95-98. https://doi.org/10.1016/j.ultsonch.2012.07.027.

[11] Mubarak, M.; Shaija, A.; Suchithra, T.V. A review on the extraction of lipid from microalgae for biodiesel production. *Algal Research*, 2015; 7, 117-123. https://doi.org/10.1016/j.algal.2014.10.008.

[12] Peng, Y.; Zhang, Z.; Kong, Y., Li, Y.; Zhou, Y.; Shi, X.; Shi, X. Effects of ultrasound on Microcystis aeruginosa cell destruction and release of intracellular organic matter, *Ultrasonics Sonochemistry*, 2020; *63*, 1350-4177, https://doi.org/10.1016/j.ultsonch.2019.104909.

[13] Xiaoying, L.; Shirong, G.; Taotao, C.; Zhigang, X.; Tezuka, T. Regulation of the growth and photosynthesis of cherry tomato seedlings by different light irradiations of light emitting diodes (LED). African journal of biotechnology, 2012; *11* (Suppl. 22), 6169-6177)

[14] Bialevich, V.; Zachleder, V.; Bišová, K. The Effect of Variable Light Source and Light Intensity on the Growth of Three Algal Species. *Cells* 2022; *11*, 1293. https://doi.org/10.3390/cells11081293.

[15] Tran-Nguyen, Q. A.; Tran, T. T. V.; Trinh-Dang, M. Effects of Light on the Growth and β-carotene Accumulation in the Green Algae Dunaliella salina. *Asian Journal of Biology*, 2023; *18*(1), 1–10. https://doi.org/10.9734/ajob/2023/v18i1332

[16] Parniakov, O.; Apicella, E.; Koubaa, M.; Barba, F.J.; Grimi, N., Lebovka, N.; Pataro, G.; Ferrari, G.; Vorobiev E. Ultrasound-assisted green solvent extraction of high-added value compounds from microalgae *Nannochloropsis* spp. *Bioresource Technology*, 2015; 198, 262-267. https://doi.org/10.1016/j.biortech.2015.09.020.

[17] Ulker, D.; Keris-Sen, U.; Sen, G.; Soydemir, M.; Gurol, D. An investigation of ultrasound effect on microalgal cell integrity and lipid extraction efficiency. *Bioresource Technology*, 2014; 152, 407-413, https://doi.org/10.1016/j.biortech.2013.11.018.

[18] Seyfabadi, J.; Ramezanpour, Z.; Amini Khoeyi, Z. Protein, fatty acid, and pigment content of Chlorella vulgaris under different light regimes. *Journal of Applied Phycology*, 2011; *23*: 721–726. https://doi.org/10.1007/s10811-010-9569-8.

[19] Dawczynski, C.; Schubert, R.; Jahreis, G. Amino acids, fatty acids, and dietary fibre in edible seaweed products. *Food Chemistry*, 2007; *103*(3): 891-899. https://doi.org/10.1016/j.

[20] Carvalho, D.V.; Pereira, E.M.; Cardoso, J.S. Machine learning interpretability: a survey on methods and metrics. *Electronics*, 2019; 8, 832. https://doi.org/10.3390/electronics8080832.

[21] Tang, D.; Han, W.; Li, P.; Miao, X.; Zhong, J. CO_2 biofixation and fatty acid composition of Scenedesmus obliquus and *Chlorella Pyrenoidosa* in response to different CO_2 levels. *Bioresource Technol*, 2011; 102, 3071-3076.

[22] Xiaotong, Z.; Kaiwei, X.; Wenjuan, C.; Yanhui, Q.; Yanpeng, L. 2021. Rapid extraction of lipid from wet microalgae biomass by a novel buoyant beads and ultrasound assisted solvent extractionmethod. *Algal Research*, 2021; 58, https://doi.org/10.1016/j.algal.2021.102431

[23] Ramachandran, S.; Aran I. Low power ultrasound treatment for the enhanced production of microalgae biomass and lipid content. *Biocatalysis and Agricultural Biotechnology*, 2019; 20. https://doi.org/10.1016/j.bcab.2019.101230.

[24] Zhen, X.; Haiyan, P.; Lijie, Z.; Zhigang, Y.; Changliang, N.; Qingjie, H.; Ze Y. Accelerating lipid production in freshwater alga *Chlorella sorokiniana* SDEC-18 by seawater and ultrasound during the stationary phase. *Renewable Energy*, 2020; 161, 448-456. https://doi.org/10.1016/j.renene. 2020.07.038.

[25] Qun, W.; Jinjie, Y.; Ruge, C.; Shangru, Y.; Yonghe, T.; Xiangmeng, M. Low-frequency ultrasound and nitrogen limitation induced enhancement in biomass production and lipid accumulation of *Tetradesmus obliquus* FACHB-12. *Bioresource Technology*, 2022; 358. https://doi.org/10.1016/j.biortech.2022.127387.

[26] Pereira, R.N.; Jaeschke, D.P.; Mercali, G. et al. Impact of ultrasound and electric fields on microalgae growth: a comprehensive review. *Braz. J. Chem. Eng*. 2023; *40*, 607–622. https://doi.org/10.1007/s43153-022-00281-z

[27] Xu, L.; Wang, SK.; Wang, F. et al. Improved Biomass and Hydrocarbon Productivity of *Botryococcus braunii* by Periodic Ultrasound Stimulation. *Bioenerg Res*, 2019; 7, 986–992. https://doi.org/10.1007/s12155-014-9441-9.

[28] Dianursanti, S.A.R.; Maeda, Y.; Yoshino, T.; Tanaka, T. The Effects of Solvents and Solid-to-Solvent Ratios on Ultrasound-Assisted Extraction of Carotenoids from *Chlorella vulgaris. International Journal of Technology*, 2020; *11*(5), 941-950. https://doi.org/10.14716/ijtech.v11i5.4331.