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Research Article

Enhancing Algal Growth by Stimulation with LED Lighting and Ultrasound

Shao-Yi Hsia[1] and Shiuh-Kuang Yang[2]

[1]*Department of Mechanical and Automation Engineering, Kao Yuan University, Kaohsiung City 82151, Taiwan*
[2]*Department of Mechanical and Electro-Mechanical Engineering, National Sun Yat-sen University, Kaohsiung City 80424, Taiwan*

Correspondence should be addressed to Shao-Yi Hsia; syhsia@cc.kyu.edu.tw

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Algae are not only rich in natural nutrients, but are also a high-priced health food. An important constituent called "growth factor" is extracted from algae and used as an ingredient in medical drugs, foods, cosmetics, and other products. Its enormous potential market should not be taken lightly. Algae are mostly found near coastal areas and their habitats are limited by a number of natural factors, leading to large labor and financial expenditures to harvest. This report describes our study of indoor algae production using LED lights and ultrasound and manipulating other growth factors at different temperatures. Ultrasound treatment at the alga's natural resonant frequency was varied to determine optimal algal growth using the Taguchi method to plan and to analyze the experiments. The results were very satisfying, showing an 8.23% increase in the growth rate by the fifth day due to ultrasound treatment and an amazing 27.01% growth rate due to biomechanical stimulation.

1. Introduction

Algae present themselves to many substantial potential markets. They contain rich nutritive elements that can be extracted from fronds for use in medical drugs, foods, and cosmetics. As food, algae are considered an ideal and balanced healthy food because of their protein, vitamin B_{12}, β-carotene, unsaturated fatty acids, various minerals, and other bioactive substances. In medicine, algae can generate large quantities of unique extracts during cell division that are effective at maintaining health and curing diseases.

Spolaore et al. [1] discussed the first utilization of microalgae in China more than 2000 years ago, when fat choy was used during famine. However, microalgae biotechnology was not actually developed until the last century, and now there are many commercial applications. For instance, they play a critical role in aquaculture and their cellular compounds are used in human food, animal feed, and cosmetics. What is more, microalgae contain high-value elements that are applied to products such as the polyunsaturated fatty acid oils that are added to infant formula milk powder and nutrition supplements. Future research should focus on improving production systems and genetically modifying bacterial strains. Chiu [2] cultivated algal cells in low (8×10^5 cells/mL) and high (8×10^6 cells/mL) concentrations and showed that high initial cell concentrations under a carbon dioxide environment present faster growth rates and that the CO_2 tolerance of microalgae increases with increasing algal cell densities. To understand growth and CO_2 removal efficiencies, three semicontinuous culture strategies were tested for biomass capacity: (1) a quarter of the culture media being replaced every two days, (2) a third of the culture media being replaced every three days, and (3) half of the culture media being replaced every eight days. These experiments showed that microalgae biomass capacity increased to 0.61 g/L/d when one quarter of the culture media was replaced every two days.

The application of light to research on cultivation can be traced back to 1961, when Pipes and Koutsoyannis [3] established a *Chlorella* family cultivation model and proved that cell density is proportional to irradiation time. From this study, the irradiated *Chlorella* appeared with the amount

of growth 0.275 mL, confirming theoretical predictions. Tamulaitis et al. [4] used LED lamps with four wavelengths for comparison with traditional experiments using high-pressure sodium lamps for cultivating radish and lettuce seedlings: (1) 455 nm, which is closely related to phototropism, (2) 660 nm for photosynthesis, (3) 735 nm (far infrared) for changing plant growth type, and (4) newly produced 640 nm LED lamps. Their experiments revealed that LED light sources outperformed high-pressure sodium lamps at photosynthesis and growth. Advantageously, the new LEDs cost much less than early LED lamps. The experiment proved the critical effects of wavelengths 640 nm and 660 nm on photosynthesis, and plant shape showed obvious changes under far infrared irradiation. Fu et al. [5] utilized an LED photo bioreactor in 2012 to enhance the biological production and cell density of *Chlorella*. Such research proved the feasibility of using LED lighting and carbon dioxide in microalgae biotechnology to further improve biological productivity and other benefits.

Reviewing the previous research of ultrasound on biological effects, Wang et al. [6] cultivated *Chlorella* cells in Petri dishes under ultraviolet irradiation for 0, 20, 40, 60, 80, 100, and 120 sec in order to study the effects of ultraviolet irradiation on *Chlorella* growth, reproduction, and chlorophyll content, finding that irradiation for 20–60 sec appeared to stimulate monocell division and enhance cell growth. Nevertheless, when ultraviolet irradiation was prolonged for more than 80 sec, cell density was sharply reduced. Ultraviolet irradiation for 20–80 sec did not reveal obvious effects on the *Chlorella* chlorophyll content, but obvious and reduced effects did occur after 100 sec. Increasing the solar ultraviolet irradiation time apparently directly affects algal growth, reproduction, and chlorophyll content and indirectly influences photosynthetic efficiency. Rajasekhar et al. [7] utilized 20 kHz ultrasound for irradiating poisonous cyan bacteria and microcystisaeruginosa in a lagoon to reduce the algal growth rate, finding that it inhibited the growth of three types of microalgae (*circinalis* > microcystisaeruginosa > *Chlorella*) and that the highest reduction rate occurred during the initial 5 min. Their study proved that ultrasound can selectively remove harmful cyanobacteria from a lagoon. Hsia and Chou [8] attempted to combine dark-fermentation biohydrogen production and ultrasound by determining the number of experiments with Taguchi methods to establish the factors of the experiment and achieve optimization conditions to develop rapid and effective biohydrogen production. They discovered that ultrasound energy and frequency, irradiation time, and starch concentration affect the biohydrogen production system by changing the hydrogen production efficiency and hydrogen production rate. Under optimization conditions, dark-fermentation biohydrogen production efficiency increased by 32.29%.

In this study, freshwater *Chlorella* was used to evaluate LED lighting, cultivation temperature, and ultrasound exposure using the Taguchi method orthogonal array to search for optimal conditions for enhancing freshwater *Chlorella* production and further contribute to cultivation techniques and environmental protection. A schematic depicting algal growth under stimulation by LEDs and ultrasound is shown in Figure 1, and the experimental flowchart is noted in Figure 2.

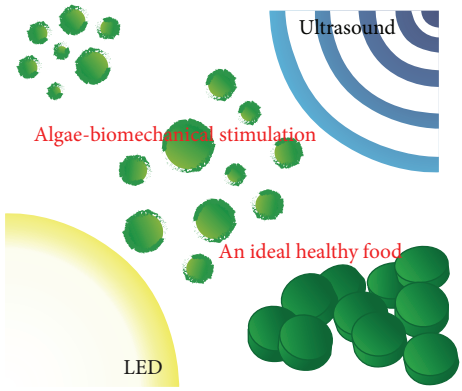


Figure 1: Schematic of algae growth with stimulation by LEDs and ultrasound.

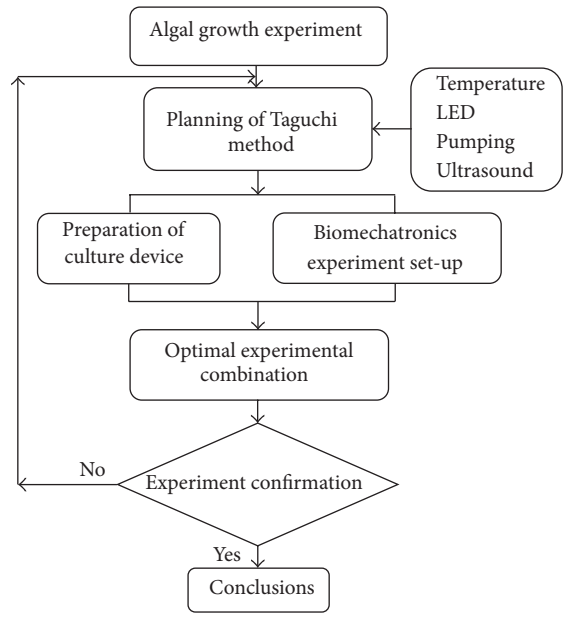


Figure 2: Flowchart of algal growth experiment.

2. Research Methodology

2.1. Brief Introduction to Chlorella and Its Cultivation. *Chlorella* (*Chlorella* sp.) is a spherical or oval-shaped microorganism with a diameter of 2–12 μm, depending on variety. Cell size changes with variety, cultivation time, nutrition, and environment. *Chlorella* is 40% protein, 10% fat, 10% carbohydrate, 6% ash, and 34% chlorophyll and *Chlorella* extract.

Freshwater *Chlorella*, which is classified into the kingdom Protista, phylum Chlorophyta, class Trebouxiophyceae, order Chlorellales, and family Chlorellaceae, was cultivated in this study. Cells were spherically shaped with diameters of 2–10 μm, flagellate, and required sunlight, water, and carbon dioxide for growth. Its great reproductive ability allows

Chlorella to divide into four cells every 20 h. By converting solar energy in the cell, large amounts of oxygen are released during reproduction and photosynthesis is 10 times higher than in other plants. Because *Chlorella's* photosynthesis achieves 8% efficiency, *Chlorella* could be a potential source of food and energy like other efficient crops such as sugarcane. It is also attractive for its potential as a food source due to its high content of protein and other necessary nutrients. In the dry form, it contains about 45% protein, 20% fat, 20% carbohydrate, 5% fiber, and 10% vitamins and minerals. Therefore, *Chlorella* is suggested as a cheap protein food supplement to control weight, prevent cancers, and reinforce immune systems.

Chlorella cultivation is divided into outdoor and indoor cultivation. Outdoor cultivation suffers more from weather, so indoor cultivation is utilized to overcome that. Generally speaking, many nutritional sources could be used to grow algae. For instance, Bold's Basal Medium, Walne's medium, and the recipe provided by Tungkang Biotechnology Research Center are used as culture media. The cultivation process can be divided into two stages. First, a conical flask or serum bottle is pasteurized before adding the cultivated algal source. Initial culturing takes 1-2 weeks, being artificially stopped before cell density gets too high. Secondly, the water is disinfected with bleach and then neutralized with sodium sulfite. Algae are then cultivated in a larger container until the desired density is reached.

2.2. Culture Media. Culture media generally contain a carbon source, nitrogen source, inorganic salts, and microelements. A carbon source provides the carbon required for growth and reproduction. Nitrogen, which can be organic or inorganic, was used in this research. There are nine major elements: carbon, hydrogen, oxygen, nitrogen, phosphorus, potassium, sulphur, calcium, and magnesium. The microelements boron, manganese, zinc, molybdenum, cobalt, iodine, and copper are also required by *Chlorella*.

Nutrient salts in culture media have great effects on algal growth. A lack of nutrient salts results in unexpected intermediate products during cell metabolism and photosynthesis. Nutrient salts contain (a) the major elements of nitrogen, phosphorus, iron, silicon, magnesium, and sodium, (b) ions of copper, manganese, zinc, molybdenum, and cobalt, and (c) vitamins. The percentages and varieties of carbon and nitrogen sources directly affect algal growth and composition. Previous research shows that algae largely store fat when limited by nitrogen. The culturing fertilizer used in our experiments was Walne's medium (Table 1).

2.3. Development of the LED and Its Application to Bioindustry [9]. Light-emitting diode (LED) products were developed early in 1968, although they had low light intensity and were not available in a full palette of colors. LEDs have since been developed in various colors. Their usage has expanded greatly in automobiles, communication products, information products, traffic lights, irradiation, and bioindustry, among which the latter has recently become popular in agricultural production and biomedicine.

Table 1: Walne's medium.

Stock solution I	$NaNO_3$	100 g
	$NaH_2PO_4 \cdot 2H_2O$	20 g
	Na_2EDTA	45 g
	H_3BO_3	33.6 g
	$MnCl_2 \cdot 4H_2O$	0.36 g
	$FeCl_3 \cdot 6H_2O$	1.3 g
	Stock solution II	1 mL
	H_2O	1 L
Stock solution II	$ZnSO_4 \cdot 7H_2O$	4.4 g
	$CoCl_2 \cdot 6H_2O$	2 g
	$(NH_4)_6Mo_7O_{24} \cdot 4H_2O$	0.9 g
	$CuSO_4 \cdot 5H_2O$	2 g
	H_2O	100 mL
In-use	H_2O	1 L
	Stock solution I	1 mL

Tubular fluorescent lamps (TFL) and high-pressure sodium lamps are the most common artificial light sources for agricultural production. The key research focus is on fill-in light, enhancing fill-in light uniformity, adjusting light quality, and developing artificial light sources having higher efficiencies. Progress in electrooptical technology has enhanced the brightness and efficiency of light-emitting diodes such that these light sources are feasible for agricultural production. Light-emitting diodes have high photovoltaic conversion efficiencies, small volumes, long service lives, fixed wavelengths, and low heat production and use only direct current. These advantages offer adjustable light intensity, adjustable light quality (percentages of red/blue or red/far-infrared), low cooling load, and the ability to enhance production per unit area in comparison to currently used systems that rely on fluorescent lamps or high-pressure sodium lamps as artificial light sources. As a result, it is a suitable artificial light source for indoor and controlled agricultural production environments (like plant tissue culture and plant growth chambers).

2.4. Biological Effects of Ultrasound [10, 11]. Many studies show that ultrasound transmits energy via particle vibration within propagation media to form an ultrasound field. When ultrasound irradiates biological media at a distinct frequency and intensity, various physical effects appear in ultrasound energy and matter particles. These are divided into thermal and nonthermal effects and are further divided into mechanical effects and cavitation effects. Cavitation effects are regarded as nonthermal effects and have the greatest impact on biological tissue, as ultrasound transmits a wave of condensation and rarefaction in liquids that results in cavitation. Cavitation happens in a liquid when the liquid generates tiny bubbles due to the positive and negative pressures of a moving ultrasound wave, or tiny bubbles occur in the liquid due to ultrasound vibration, growth, contraction, or crashing. When cells experience the high shear wave that is generated from vibrated bubbles or bubble crashing, a series of biological reactions occurs. Rayleigh-Plesset announced

a mathematical model of inner cavitation vibration of an incompressible liquid in 1949 [7]. Applying this theory to calculate the appearance of dark-fermentation hydrogen-producing rod bacteria and the internal tissue can result in a natural frequency by setting different pulse intensity for the vibration. The radius a, surface tension σ, heat capacity ratio γ, density ρ, viscosity coefficient η, and pressure P_0 of bacteria are substituted in the following equation to calculate natural frequency:

$$\left(\omega_r'\right)^2 = \frac{1}{\rho a^2}\left[3\gamma\left(P_0 + \frac{2\sigma}{a}\right) - \frac{2\sigma}{a}\right] - \left(\frac{2\eta}{\rho a^2}\right)^2. \quad (1)$$

2.5. Ultrasound Scattering within a Sphere [12]. Since *Chlorella* is sphere-like, irradiating *Chlorella* with ultrasound, it can be regarded as sonic irradiation within a sphere, and thus the equation for ultrasound within a sphere can be used. The incident wave is quantified as

$$p_i = \frac{A}{r}Be^{-ar/2}e^{ikr}, \quad (2)$$

where A and B are amplitude and beam of the wave, r is the displacement vector, and k is the wave number. All parameters except the displacement vector are assumed to be constant around the target object. The wave is scattered once it reaches the perimeter of the target because of the spherical shape of the target object. Generally speaking, a scattered wave does not show isotropy because the target object is an anisotropic sound distributor. From this viewpoint, the sound pressure distribution of a scattered wave at the perimeter of the target object is

$$p_s = \frac{A_s}{r_1}B_s e^{-ar_1/2}e^{ikr_1}. \quad (3)$$

Here, the lower case and 1 stand for the parameter and coordination is related to the target object. Solving target object characteristics with this equation is similar to solving parameters A_s and B_s (amplitude and beam of the scattered wave).

In regard to the interaction between the sound wave and scatter power, the ratio of scattered power (W_s) and incidence sound intensity (I_0) can be measured in consideration of the generated sound wave and the scattering efficacy of the scattered power:

$$\sigma_s = \frac{W_s}{I_0}. \quad (4)$$

The parameter σ_s is called the scattering cross section (expressed in m^2). The physical meaning of σ_s can be regarded as the effective scattering area between the scattered power and incidence sound wave. Apparently, σ_s and the object appear to have a close relationship with the orientation of the incidence sound wave.

Sphere circumference is far smaller than the wavelength ($ka_0 < 1$), and the function between the sound wave and sphere is scattering. When the sound wave is incident to a small rigid sphere with a radius a_0, the ratio of scattered sound intensity and incidence sound intensity is

$$\frac{I_s}{I_0} = \frac{\pi^2 V^2}{r_1^2\lambda^4}\left(1 - \frac{3}{2}\cos\theta_s\right)^2 = \frac{(ka_0)^4 a_0^2}{9r_1^2}\left(1 - \frac{3}{2}\cos\theta_s\right)^2, \quad ka_0 \ll 1. \quad (5)$$

$V = (4/3)\pi a_0^3$ represents sphere volume. The ratio of inverse scattering cross section and geometric cross-sectional area is

$$\frac{\sigma_{s,b}}{\pi a_0^2} = \frac{25}{9}(ka_0)^4, \quad ka_0 \ll 1. \quad (6)$$

This happens when $ka_0 \leq 0.5$, and the inverse scattering cross section sharply increases (ray scattering) by a fourth power equation and approaches 1 when $ka_0 \geq 10$. This presents complex oscillation changes in $1 < ka_0 < 10$ because of the creeping wave being diffracted along the surface. In the example of *Chlorella*, a_0 is 1~4 μm and the speed of sound in water is 1473 m/s. Substituting 1 MHz ultrasound exposure into the equation, sound wave velocity $c = f \times \lambda$ and the acquired wavelength λ is 1.473×10^{-6} m. When $ka_0 = (2\pi/\lambda)a_0$, the final $4.26 \times 10^{-3} < ka_0 < 1.71 \times 10^{-2}$ belongs to ray scattering, and the relationship of ka_0 versus cross-sectional area $\sigma_{s,b}$ is indicated in Figure 3.

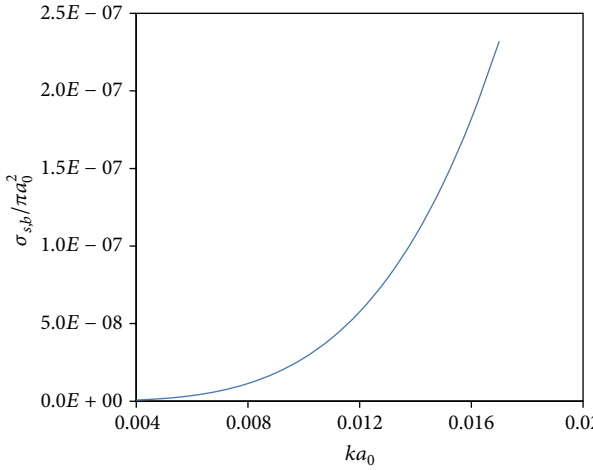


Figure 3: Relations of cross-sectional area $\sigma_{s,b}$ versus ka_0.

2.6. Taguchi Quality Method. In the Taguchi experimental design, quantified experimental results are called quality characteristics and can achieve an ideal by determining the controlling factors in the experimental design. In order to solve a problem, an engineer needs to fully understand the characteristics of product quality and problems and organize the levels of quality with a fishbone diagram or equivalent. To conduct the experiment at the least cost, an orthogonal array should be selected based on control factors and the levels needed to achieve the required quality with the most precision. The experimental data in this study were further

analyzed with a factor reaction analysis and analysis of variance to adjust the control factors, allowing the quality characteristics of the arranged function to enable an optimal design. Traditionally, the orthogonal array is named $L_b(c^d)$, representing d factors with c levels in each factor for b experiments.

2.6.1. Signal to Noise Ratio (S/N Ratio). Different S/N ratio equations are available according to the objectives of quality and are divided into Nominally the Best, Smaller is Better, and Larger is Better. Larger is Better was applied to our experiment [8, 13]:

$$S/N_{\mathrm{LB}} = -10 \log \left[\frac{\sum_{i=1}^{n} \left(1/\left(y_i\right)^2\right)}{n} \right], \tag{7}$$

where y_i is the measured value and n is the number of repeated measurements.

2.6.2. Response Table and Additive Equation. The factor response table and reaction diagram were constructed in order to understand the effects of the factors on the objective. A mean was calculated for the S/N ratio of each factor at each level and further transformed into the reaction diagram:

$$M_{ij} = \frac{\sum_{k=1}^{N} \left(S/N_{ijk}\right)}{N}. \tag{8}$$

M_{ij} is the mean of the S/N ratio containing i factors and j levels, k is the kth S/N ratio with i factors and j levels, and N is the number of experiments with i factors and j levels.

After completing the response table, the importance of the control factors at each level of quality can be evaluated by

$$\begin{aligned} &\eta\left(Ai, Bj, Ck, Dl, \ldots\right) \\ &\quad = \overline{\eta}_{Ai} + \overline{\eta}_{Bj} + \overline{\eta}_{Ck} + \overline{\eta}_{Dl} + \cdots - (n-1)\,\overline{\eta}, \end{aligned} \tag{9}$$

where η is the quality objective and $\overline{\eta}_{Ai}$ and $\overline{\eta}$ are the response value and the mean, respectively, in the response table. The additive equation (9) will be used as the prediction model to foresee any combination that we want to know.

3. Experimental Framework

3.1. Experimental Plan. Using a microscope, the number of freshwater *Chlorella* per mL was first calculated with a hemolytic meter. After dilution, the OD (optical density) relationship diagram and concentration were drawn. A high concentration of freshwater *Chlorella* was generated with mass culturing methods. A spectrophotometer was used for measuring daily OD, which was compared to the OD relationship diagram and concentration, and a growth curve drawn. Freshwater *Chlorella* was then observed with a microscope (Figure 4) at 1000x. The alga used in this experiment was purchased from Tungkang Biotechnology Research Center (Figure 5).

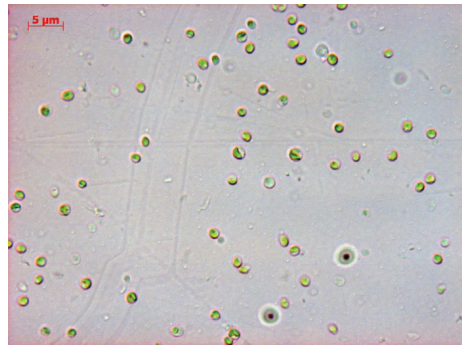


Figure 4: Microscope of the freshwater *Chlorella*.

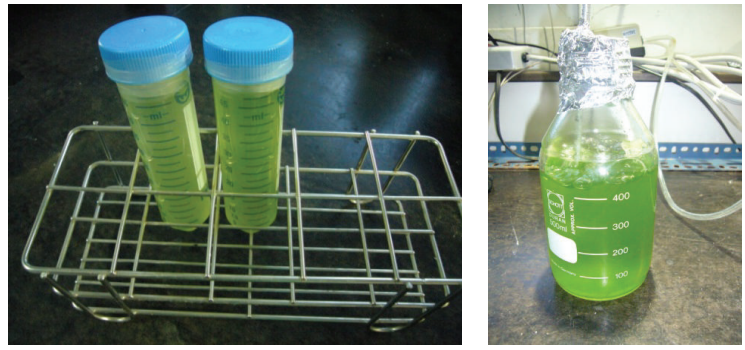
3.2. Cultivation Experiment

3.2.1. Culture Volume. Culture volume was of primary concern during cultivation, as the larger the volume, the smaller the percentage of the surface being irradiated. For this reason, algae were initially cultivated in small volumes and then moved to larger containers after 1-2 weeks.

3.2.2. Cultivation Methods. Cultivation is generally classified into static culture, shaking culture, and pumping culture. Static culture is used when there is no shaker or when a pumping culture is not needed. Shaking culture is often utilized for high concentrations of algae which will not be experimented on in short periods of time; hence, the algae culture is shaken for slow growth. Pumping culture is generally used to add new algae sources into a culture environment when growth to the target concentration in the shortest time is required. At the same time, freshwater *Chlorella* with three initial concentrations was cultivated in Walne's medium with ODs of 0.02, 0.04, and 0.06 for five days. Growth was recorded, and the growth curve shows that algae were not easily cultivated in ODs below 0.06. Algae in Walne's medium with ODs above 0.06 were used for the Taguchi experiment.

3.3. LED Irradiation Experiment. The light source in the experiment was white LEDs (replacing the original white fluorescent lights) in a constant-temperature shaking incubator equipped with an irradiation timer. A 12 hr irradiation period tends to imitate the general situation for freshwater *Chlorella* growth. A 24 hr irradiation period is also discussed herein for comparison.

3.4. Ultrasound Exposure Experiment. The mechanical effects of ultrasound exposure were stimulated on the experiment to discuss the effects of natural frequency and nonnatural frequency of freshwater *Chlorella*, sound intensity, and irradiation time on the cultivation. In the ultrasound experiment, the natural ultrasound frequency of freshwater *Chlorella* was first calculated in order to set the transducer frequency so that the freshwater *Chlorella* would resonate optimally for biological effect. Considering freshwater *Chlorella* as a bacterial microorganism, the data were selected according to



(a) Freshwater *Chlorella* (b) Mass culture

Figure 5: Original freshwater *Chlorella* and after mass culture.

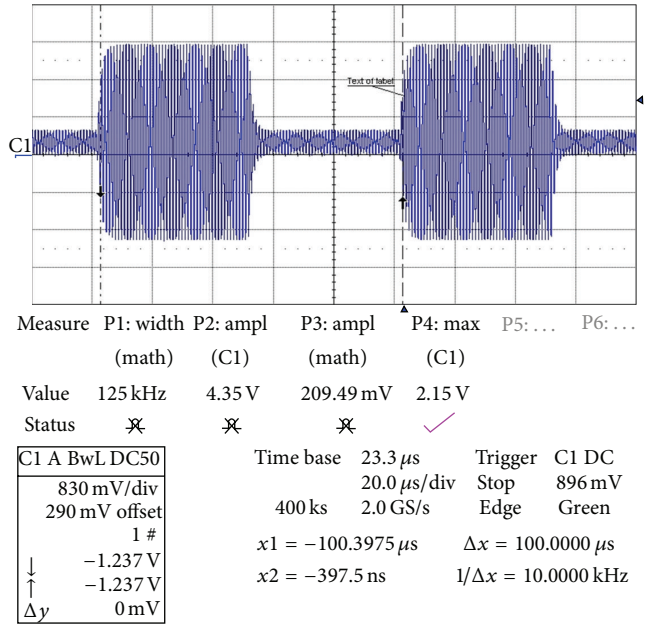


Figure 6: Ultrasound waveform induced by function generator.

σ = 72.75 dyn/cm, γ = 1.4, ρ = 1 g/cm^3, η = 9.197 × 10^{-3} g/cm·sec, P_0 = 760 torr, and a = 2–8 μm. Finally, the above parameters were substituted into (1), resulting in the natural resonance frequency of freshwater *Chlorella* being calculated as 0.5–3.5 MHz.

Although the natural frequency of freshwater *Chlorella* was 0.5–3.5 MHz, the frequency calculated by mean cell size was 1 MHz. The 0.5 and 1.0 MHz single-crystal straight-beam longitudinal-wave immersion transducers were therefore selected as the natural frequency irradiation transducers. A power amplifier was utilized in the ultrasound exposure experiment for amplifying the modulated waveform generated from the signal generator so as to control the ultrasound intensity of the induced transducer for better parameter control. Figure 6 shows the induced ultrasound waveform, which is the modulated waveform combined with a 10 kHz square wave and sine wave. Based on the irradiation frequency of the transducer, the sine wave frequency was adjusted to 0.5 and 1 MHz. Since the ultrasound transducer presents Gaussian distribution, differences in irradiation intensity are inevitable. However, in order to observe the freshwater *Chlorella* change under irradiation, possibly overlapped intensities should be avoided when setting irradiation intensity. Moreover, in consideration of the irradiation intensity consistency when frequencies are 0.5 and 1 MHz in the Taguchi experiment, ultrasound exposure intensities of 5.5 and 18.5 volts were utilized in this study.

There has been no related research on the effects of ultrasound exposure time on freshwater *Chlorella*. Hsia and Chou [8] utilized ultrasound to irradiate anaerobic sludge for generating hydrogen in 2014 and discovered that an on/off schedule of 15/15 min was best in comparison with no irradiation and full-time irradiation and that full-time irradiation results in inhibition. Shao-Yi et al. [14] used ultrasound for generating hydrogen in 2012 in order to enhance biomass hydrogen production efficiency and found

Table 2: Seven factors and two levels of the orthogonal array $L_8(2^7)$.

Factors	Specifics	Level 1	Level 2
A	Temperature (°C)	25	30
B	LED irradiation intensity (lux)	3000	8000
C	LED irradiation time (hr)	12	24
D	Pumping intensity (c.c./min)	0	2000
E	Ultrasound frequency (MHz)	0.5	1
F	Ultrasound voltage (Volt)	5.5	18.5
G	Ultrasound exposure time	10 s	15 min

that the best enhancement occurred with irradiation for 10 sec every 8 h. Irradiation times for this experiment were therefore set at 10 sec and 15 min every 8 h.

3.5. Taguchi Experiment. Temperature, LED irradiation intensity, LED irradiation time, pumping intensity, ultrasound frequency, ultrasound voltage, and ultrasound exposure time were the factors selected for the Taguchi experiment (Table 2). Referring to the $L_8(2^7)$ orthogonal array, the control factors and levels required for this experiment are also included in Table 2. The temperature of the algal growth culture experiments was 25~30°C. The experimental flowchart in Figure 2 depicts the experimental process. First, the algal culturing device was constructed and the ultrasound experiment was set up. The factors and parameters were then selected and Taguchi methods utilized for planning the experiment. Experimental data was used to look for the optimal combination, assessed with analysis of variance, and finally the experiment was confirmed. The Larger is Better S/N in equation (7) was used for the optimization experiment to search for maximum freshwater *Chlorella* production. Based on the control factors within given levels, the mean of S/N ratios was calculated to assess the effects of the factors/levels on quality and then construct a response table. An analysis of variance was utilized to evaluate experimental error, evaluate which control factors were meaningful, and compensate for the insufficiency of the reaction diagram with statistics. Finally, control factors with low importance values were considered to be part of the experimental error.

4. Results and Discussion

4.1. Freshwater Algae Experiment. In order to determine the initial concentration suitable for freshwater *Chlorella* cultivation, three concentrations were used for the experiment: OD 0.02 (106×10^6cells/cc), OD 0.041 (209×10^6cells/cc), and OD 0.063 (320×10^6cells/cc). Figure 7 shows the growth curves resulting from each concentration, which indicate that stable growth appeared at an OD of 0.063 but growth did not proceed when OD was < 0.041. Although slight growth appeared on the second day, concentrations dropped from the third day. The following experiments are therefore discussed based on the initial OD concentration of 0.063 being optimal.

4.2. Taguchi Experiment. Freshwater *Chlorella* in Walne's medium was used in this experiment. Eight sets of experimental data (Table 3) on the amount of growth and

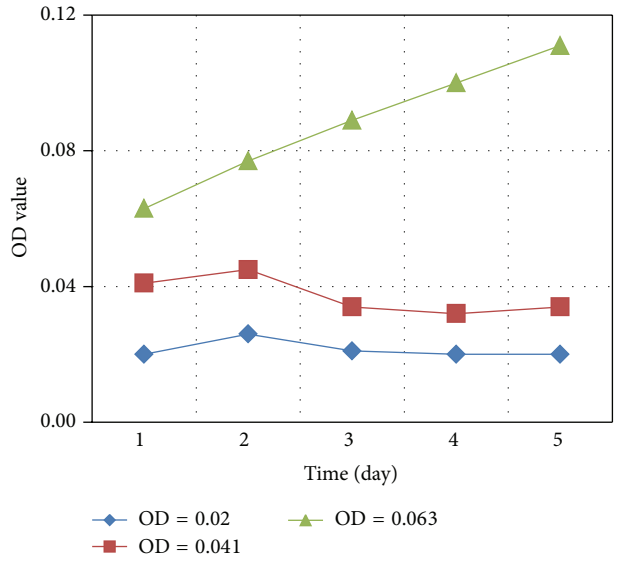


Figure 7: Freshwater *Chlorella* growth curves for different initial concentrations.

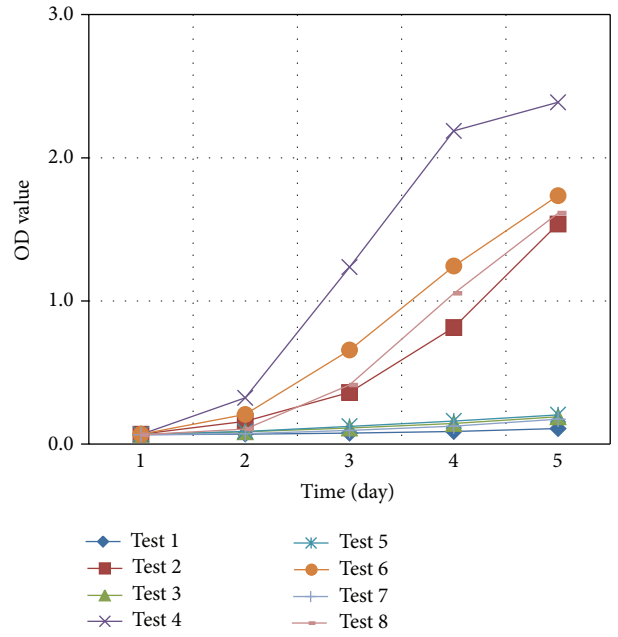


Figure 8: Freshwater *Chlorella* growth curves for eight experiments.

the growth rate over five days were used to prepare growth trend diagrams (Figures 8 and 9). OD in the eight sets of experiment data increased with increasing numbers of days, with the fourth set showing the most growth followed by the sixth set. The second and eighth sets presented similar growth levels, while the first, third, fifth, and seventh sets grew the least (Figure 8). Growth rates increased in the fourth, sixth, and eighths sets on the second, third, and fourth days but decreased on the fifth day (Figure 9). The fourth set decreased the most, the second continued growing, and the first, third, fifth, and seventh sets barely increased. The fourth set, which

Table 3: S/N ratios and maximal amounts of freshwater chlorella growth using the Taguchi method.

Exp.	y_1	y_2	y_3	Average (10^6 cells/c.c.)	Standard deviation	S/N (dB)
1	218.63	213.66	203.72	212.00	7.59	46.52
2	7284.26	7304.14	7289.23	7292.54	10.34	77.26
3	606.19	601.22	606.19	604.54	2.87	55.63
4	11537.55	11517.68	11492.83	11516.02	22.41	81.23
5	675.76	675.76	685.69	679.07	5.74	56.64
6	8268.08	8278.02	8282.99	8276.36	7.59	78.36
7	541.60	536.63	541.60	539.94	2.87	54.65
8	7681.76	7686.73	7691.70	7686.73	4.97	77.71
			Ave.=	4600.90	Ave.=	66.00

Table 4: Growth response of freshwater chlorella for different S/N ratios.

	A	B	C	D	E	F	G
Level 1	65.16	64.69	64.03	53.36	64.55	65.52	65.19
Level 2	66.84	67.30	67.96	78.64	67.44	66.47	66.81
Range	1.68	2.61	3.93	25.28	2.89	0.95	1.62
Rank	5	4	2	1	3	7	6

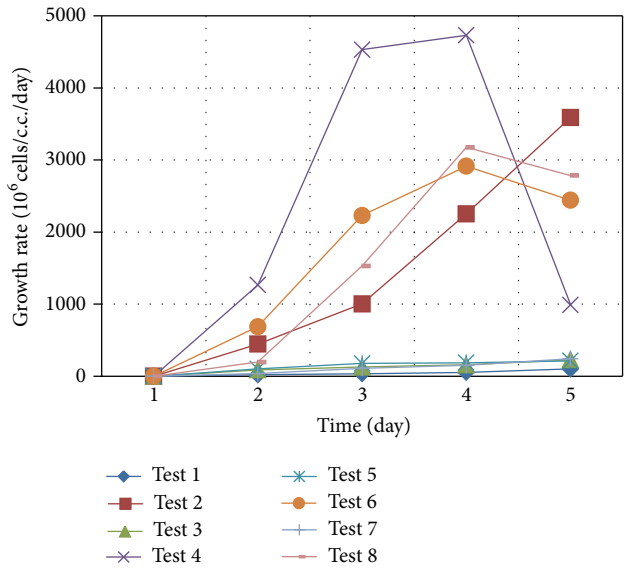


Figure 9: Freshwater *Chlorella* growth rates for eight experiments.

increased the most, had the largest growth rate in the first four days but slowed down on the fifth day, possibly because of cell density saturation (Figures 8 and 9). The primary objective of the experiment was to reduce the variability in freshwater *Chlorella* cultivation and only then consider other characteristics. In this case, analysis of variance for S/N was first taken into account, that is, analysis of variance of the mean of freshwater *Chlorella*, followed by the quality characteristics. Each of the previous experiments was repeated three times. Growth was organized for calculating the S/N ratios for control factor levels. The Larger is Better S/N ratio was used to determine the maximal amount of growth with the equation (Table 3). Table 4 shows the factor reaction, which

Table 5: Initial analysis of variance for different S/N ratios.

Factors	Sum of square	Degree of freedom	Variance
A	5.66	1	5.66
B	13.65	1	13.65
C	30.87	1	30.87
D	1278.34	1	1278.34
E	16.68	1	16.68
F	1.80	1	1.80
G	5.27	1	5.27

integrated the S/N ratio of the factors within the same level. For instance, the factor reaction of factor A at level 1 was the mean of the S/N ratio of the first four experiments. When determining the analysis of variance for S/N ratios, S/N data were repeated only once ($r = 1$) and substituted into the variance equation so that the error vector variability was 0. To estimate error vector variability, control factors with smaller variabilities were assumed to be unimportant and classified as the quadratic sum of error vector so as to continue the analysis of variance. Temperature variability was 5.66, ultrasound voltage variability 1.80, and ultrasound exposure time variability 5.27, all of which were smaller than other control factors (Table 5). Temperature, ultrasound voltage, and ultrasound exposure time were therefore pooled into an error vector for the next analysis of variance.

After integrating the above control factors into the experimental error, the analysis of variance for the amount of freshwater *Chlorella* growth and S/N ratio was recalculated (Table 6). LED irradiation time and pumping intensity combined totaled >80% of the confidence index and ultrasound frequency was closed. Nevertheless, key control factors were set so that the index of confidence was >75%

Table 6: Analysis of variance for different S/N ratios.

Factors	Sum of square	Degree of freedom	Variance	F value	Probability	Confidence level	Significance*
A	5.66	1	5.66	0.90	41.33%	58.67%	No
B	13.65	1	13.65	2.16	23.76%	76.24%	Yes
C	30.87	1	30.87	4.90	11.38%	88.62%	Yes
D	1278.34	1	1278.34	202.74	0.08%	99.92%	Yes
E	16.68	1	16.68	2.65	20.23%	79.77%	Yes
F	1.80	1	1.80	0.29	63.01%	36.99%	No
G	5.27	1	5.27	0.84	42.80%	57.20%	No
Error	18.92	3	6.31				
Total	**1352.26**	**10**	**135.23**				

*Note: at least 75% confidence level.

Table 7: Final analysis of variance for different S/N ratios.

Factors	Sum of square	Degree of freedom	Variance	F value	Probability	Confidence level	Significance*
A				Pooled			
B	13.65	1	13.65	2.16	23.76%	76.24%	Yes
C	30.87	1	30.87	4.90	11.38%	88.62%	Yes
D	1278.34	1	1278.34	202.74	0.08%	99.92%	Yes
E	16.68	1	16.68	2.65	20.23%	79.77%	Yes
F				Pooled			
G				Pooled			
Error	18.92	3	6.31				
Total	**1352.26**	**10**	**135.23**				

*Note: at least 75% confidence level.

Table 8: Growth response of freshwater chlorella based on quality characteristics.

	A	B	C	D	E	F	G
Level 1	4906.28	4114.99	3932.81	508.89	4194.91	5023.46	5136.08
Level 2	4295.53	5086.81	5269.00	8692.92	5006.89	4178.35	4065.72
Range	610.75	971.81	1336.19	8184.03	811.98	845.11	1070.36
Rank	7	4	2	1	6	5	3

and control factor variability was reduced. An analysis of variance of the key control factors was then completed (Table 7), from which LED irradiation intensity, LED irradiation time, pumping intensity, and ultrasound frequency (which presented a higher index of confidence) were regarded as important control factors in the S/N analysis. Pumping intensity appeared to have the most importance, followed by LED irradiation time, ultrasound frequency, and LED irradiation intensity. According to the maximal reaction of control factor levels to S/N ratios (Table 4), the optimized parameters contained $B2$, $C2$, $D2$, and $E2$; that is, LED irradiation intensity 8000 lux, LED irradiation time 24 hr, pumping intensity 2000 cc/min, and ultrasound frequency 1 MHz had the best freshwater *Chlorella* productivity. This combination of treatments reduced the variability in freshwater *Chlorella* growth.

It is not enough to merely reduce variability; the quality, that is, the amount of growth, should also be taken into account. The variability of quality was further analyzed. In other words, analysis of variance was done to determine the mean amount of growth in order to discuss whether temperature, ultrasound exposure intensity, and ultrasound exposure time can be used to adjust quality. First, the mean amount of growth (Table 3) was calculated to the factor reaction according to the quality characteristics (Table 8). Analysis of variance of the mean (Table 9) was then calculated. Key control factors were set for an index of confidence >85%. LED irradiation intensity, LED irradiation time, pumping intensity, and ultrasound frequency were applied to reduce experimental variability, but only temperature, ultrasound exposure intensity, and ultrasound exposure time were considered in the analysis of variance of quality characteristics. Indices of confidence for temperature, ultrasound exposure intensity, and ultrasound exposure time were >85% and were regarded as the key control factors of quality characteristics (Table 9). The control factor reactions for the amount of freshwater *Chlorella* growth, temperature, ultrasound exposure intensity, and ultrasound exposure time were further selected as the optimal level combination. The maximal amount of growth occurred at 25°C, 5.5 v ultrasound

Table 9: Analysis of variance of quality characteristics.

Factors	Sum of square (10^6)	Degree of freedom	Variance (10^6)	F value	Probability	Confidence level	Significance*
A	0.75	1	0.75	2.86	11.05%	88.95%	Yes
B	1.89	1	1.89	7.23	1.61%	98.39%	Yes
C	3.57	1	3.57	13.67	0.20%	99.80%	Yes
D	133.96	1	133.96	512.73	0.00%	100.00%	Yes
E	1.38	1	1.38	5.05	3.91%	96.09%	Yes
F	1.43	1	1.43	5.47	3.27%	96.73%	Yes
G	2.29	1	2.29	8.77	0.92%	99.08%	Yes
Error	4.18	16	0.26				
Total	**145.20**	**23**	**6.31**				

*Note: at least 85% confidence level.

Table 10: Comparison of predictions with and without ultrasound treatments.

Predicted amount of growth	Without ultrasound (10^6 cells/c.c.)	With ultrasound (10^6 cells/c.c.)	Enhanced efficiency (%)
Day 1	347.86	347.86	0.00
Day 2	1318.23	1613.25	22.38
Day 3	4754.77	6144.80	29.23
Day 4	8763.35	10875.09	24.10
Day 5	10500.15	11863.88	12.99

Table 11: Comparison of experiments with and without ultrasound treatments.

Experimental amount of growth	Without ultrasound (10^6 cells/c.c.)	With ultrasound (10^6 cells/c.c.)	Enhanced efficiency (%)
Day 1	347.86	347.86	0.00
Day 2	1331.83	1613.25	21.13
Day 3	4838.13	6144.80	27.01
Day 4	9113.25	10875.09	19.33
Day 5	10961.71	11863.88	8.23

exposure intensity, and 10 s ultrasound exposure time ($A1$, $F1$, and $G1$ in Table 8). The control factors selected on the basis of S/N ratios and quality characteristics were then combined to conclude that the maximal amount of freshwater *Chlorella* growth was at $A1$, $B2$, $C2$, $D2$, $E2$, $F1$, and $G1$.

To understand the effects of ultrasound on freshwater *Chlorella* growth, additive equation (9) was first utilized for estimating the effects of an optimized combination with or without ultrasound on the amount of freshwater *Chlorella* growth (Table 10). The amount of freshwater *Chlorella* growth with ultrasound was enhanced with 12.99% by the fifth day and was the highest (29.23%) by the third day. The optimized combination was further tested in another experiment (Table 11). The amount of growth increased by 8.23% after five days of ultrasound, with the highest (27.01%) occurring on the third day. Finally, comparing the difference between the prediction and the experiment, the error of the highest amount of growth was about 2.22% and the difference in the amount of growth on the fifth day was 4.76%. The enhancement observed in the experiment was slightly lower than the prediction but tended to be consistent (Tables 10 and 11). Maximal enhancement appeared on the third day but gradually decreased afterward possibly because of cell density saturation (freshwater *Chlorella* cannot increase in an unlimited fashion within a limited volume).

Most current biodiesel materials are made from plant oils like soybean oil, grape seed oil, and palm oil, whose supplies are restricted because they are also used as cooking oils. The search for sustainable and stable sources of biodiesel feedstocks that can satisfy the large demand is essential. Researchers understand the feasibility of using microalgae as a biodiesel source [15]. The oil content of microalgae varies among species, and, in fact, a lot of microalgae do not produce oil and general oil production microalgae contain oil at 20–50% of cell weight. The oil production rate depends on the alga's growth rate, so species with high oil production rates are first choices. We proved in this study that the additive equation did not exhibit a large error during the experiment confirmation. Consequently, it is feasible to utilize the additive equation with biomechatronics to increase freshwater *Chlorella* growth.

5. Conclusion

This study aimed to enhance indoor freshwater *Chlorella* cultivation techniques. Biomechatronics with Taguchi methods were utilized to systematically look for the optimal combination of treatments to increase algal production and growth rates. Our conclusions are as follows.

(1) Small-volume cultivation is required initially to enhance culture and success rates. Pumping can greatly increase the culture rate, but the resultant water pollution needs attention.

(2) In a 300 mL freshwater *Chlorella* culture volume, shaking culture had the lowest culture concentration OD of 0.06 (320×10^6cells/cc); this grew 1.76x after five days of cultivation.

(3) Optimal culture conditions, based on Taguchi methods, are 25°C, 8000 lux LED irradiation intensity,

24 hr LED irradiation time, 2000 cc/min pumping intensity, 1 MHz ultrasound frequency, 5.5 v ultrasound exposure intensity, and ultrasound exposure time of 10 s every 8 hr.

(4) The predicted additive equation showed that the amount of freshwater *Chlorella* growth with ultrasound increased 12.99% after five days and optimal (29.23%) growth appeared on the third day. Confirmation of the experiment revealed that the amount of freshwater *Chlorella* growth treated with ultrasound increased 8.23% by day five and was best (27.01%) on the third day. The highest error of the amount of growth was about 2.22% with the predicted additive equation and confirmation.

Research results were optimized with the additive equation from Taguchi methods and biomechatronics to estimate the amount and rate of freshwater *Chlorella* growth. It can be used for increasing the amount of *Chlorella* or biooil that can be extracted from *Chlorella* by contributing to the development of better cultivation techniques with adequate environmental protection.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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