Effect of Carbon Dioxide Concentration on the Growth Response of *Chlorella vulgaris* Under Four Different Led Illumination

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Abstract: This experiment examined the growth response of *Chlorella vulgaris* exposed to CO_2 concentrations increasing from ambient to 8.5% and under white, blue, red and red-blue lights after 15 days incubation. Biomass production increased with increasing CO_2 concentrations under all light sources. The highest biomass production, 1.59 g L^{-1}, was obtained when the algae were supplied with 8.5% CO_2 and exposed to white light. Biomass production under blue, red and red+blue light was 1.53 g L^{-1}, 0.45 g L^{-1} and 1.27 g L^{-1}, respectively. The research suggests that *C. vulgaris* is not able to adapt production of its photosynthetic pigments to absorb light sources different that it is normally has evolved to.

Keywords: *Chlorella vulgaris*, Photobioreactor, Biomass production, CO_2 concentration, Artificial light.

1. INTRODUCTION

Phototrophic algal growth requires light, mineral nutrients, water and an inorganic source of carbon (CO_2). While there are algae, such as *Chlorella sp*, that can grow relatively rapidly under the ambient air concentration of CO_2 (0.037%) [1, 2], maximizing biomass production for the purpose of CO_2 capture requires that higher concentrations of CO_2 be provided. Algae have been grown in closed atmospheres at high CO_2 concentrations (10-20% and higher) with the objective of CO_2 fixation [3-5]. The alga *C. vulgaris* is a good candidate for biomass production under high CO_2 concentrations because it is able to fix up to 74% of the original CO_2 with only 2 seconds of CO_2 residence time [6].

Recently designs of closed photo-bioreactors for the purpose of enhancing light-use efficiency and CO_2 fixation, as well as biomass production, has received more attention because algae are efficient photosynthetic organisms [7] and have potential to reduce atmospheric CO_2 levels [8], or reduce emissions from a gas or coal power plant. Thus *C. vulgaris* produce compounds of economical value such as antioxidant (β-carotenes), natural colorants, oils such as omegas 3, 6 and 9, and proteins and carbohydrates. This study evaluated standing biomass production of the alga *C. vulgaris* growing under normal and elevated CO_2 concentrations and exposed to 4 different light sources.

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2. MATERIALS AND METHODS

2.1. Algal Source

The algal specie, *Chlorella vulgaris* (UTEX 26), was obtained from the culture collection of algae at the University of Texas at Austin (UTEX, 205 W, 24th St., Austin, Texas, TX 78712, USA). The culture was established in 1 L batch for 15 d and then transferred to 4 L reactors. The population density was established using direct microscopic counting techniques. The population density at the start of the experiment was set at 4.5- 5*10^5 cells mL^{-1} by adjusting the volume of medium in the reactors.

2.2. Light Sources

Sources providing white, blue, red, and red-blue light were evaluated. They were 0.1 W LEDs obtained from ALAS™ (Shanghai, China) with the following emission parameters: red, 620-625 nm wavelength at 1345 µmol m^{-2} s^{-1} intensity; blue, 425-430 nm wavelength at 2143 µmol m^{-2} s^{-1}; white, 380-760 nm wavelength at 1838 µmol m^{-2} s^{-1} intensity. Each lighting system contained 52 LEDs fixed into a clear square plexiglass tube (dimension 4×4 cm and 33 cm in length) and placed in the centre of the 4-L culture flask (internal diameter 12 cm). The illumination system was powered by a transformer (Model SA 201-3485, ASTEC, CA, USA) supplying 12 V and 8 Amp. To ensure that external illumination would not affect the experiment, the laboratory was kept dark (0 µmol m^{-2} s^{-1}) throughout the incubation period.

Light intensity was monitored during the incubation using 2 different light meters: photosynthetic light was

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measured using a quantum meter (Model MQ output in μmol m^{-2} s^{-1}, Apogee Instruments Inc., Logan, UT, USA) and visible light was measured using a lux meter (Model MS6610 output in Lux, VIA Instruments, Shanghai, China).

2.3. Light Dispersion

The LEDs light emissions are very powerful; a bulb of 0.1 W produces light emissions in the photosynthetic spectra from 1300 to 2140 μmol m^{-2} s^{-1} depending in the wavelength. This amount of energy is higher than the optimal photosynthetic light absorption by algae, which is 50-250 μmol m^{-2} s^{-1}, and can cause photo-inhibition and photo-damage [9-12]. Since the dispersion of this light occurs within a short distance, the algal specie in the study received a lower light emission intensity than the maximum value for photosynthetic growth.

The distance from the bulbs to the algal suspension was 7 mm, sufficient distance to reduce the light intensity to below 300 μmol m^{-2} s^{-1} and minimize photo inhibition. The resulting light intensity is optimal for algal phototropic growth under artificial conditions [13]. See Table **1** for more details about light dispersion versus distance in water measured during the course of this experiment with the Apogee quantum meter.

2.4. Photobioreactor and CO_2 Supply

The photobioreator is a bubble-column glass container of 36 cm length, 12 cm diameter, and volume 4 L. Carbon dioxide at concentrations of 0.035% (350 ppm ± 50), 1.1%, 3.7% and 8.5% ± 0.18% was supplied at a rate of 864 mL min^{-1} L^{-1} of algal suspension from a compressed air tank equipped with a mass flow regulator (Model 191 AR-60, Gentec Corporation, Shanghai, China). Two turbines, (Model ACO-9720, Hailea, Hailea Industrial Zone, Guangdong, China) each with an output of 30 L min^{-1}, were used to mix the algal suspension. The concentration of dissolved CO_2 in the inlet gases was measured at 5 s intervals with a CO_2 monitor (Model 7001, Telaire-General Electric, California, USA). Room temperature was measured with a temperature monitor (Model 7001, Telaire-General Electric, CA, USA). Periodically during the experiment the algal suspension temperature was measured with an infrared thermometer (Model Fluke 62, Fluke Corporation, Everett, Washington, USA), Both room temperature and algae suspension temperature were about 22°C $\pm$ 0.9.

2.5. Culture Medium

The culture medium was prepared using a commercial synthetic fertilizer (Solucat 25-5-5, Atlantica Agricola, Villena, Spain) by dissolving 0.8 g in 1 L distilled water. At the start of each experiment the culture medium contained 114 mg L^{-1} ammonium-N, 86.4 mg L^{-1} nitrate-N, 40 mg L^{-1} phosphate, 40 mg L^{-1} potash, 160 μg L^{-1} iron, 80 μg L^{-1} manganese, 80 μg L^{-1} boron, 16 μg L^{-1} zinc, and 16 μg L^{-1} copper; the electrical conductivity was 0.95 ± 0.01 mS measured using a EC sensor (Model HI 991301, Hanna, MI, USA).

2.6. Experimental Design

Each experiment was set out in 24-4 L photobioreactors, exposed to four different light treatments (white, blue, red and red-blue) and 6 replicates, and incubated for 15 d. Each photobioreactor contained 3 L of culture medium. The experiment was repeated for the four different CO_2 concentrations.

2.7. Biomass Analysis

Colorimetric determination methodology was used to calculate the standing biomass production at the end of the incubation. Light absorption of each sample was measured at 680 nm with a spectrophotometer

Table 1: LED Light Intensity and Distance from the Light Source

Light dispersion from the source			
	Light intensity (μmol m^{-2} s^{-1})		
Distance from light source (mm)	Blue	Red	White
0	2143	1345	1828
1	568	225	413
5	206	173	186
10	95	72	103

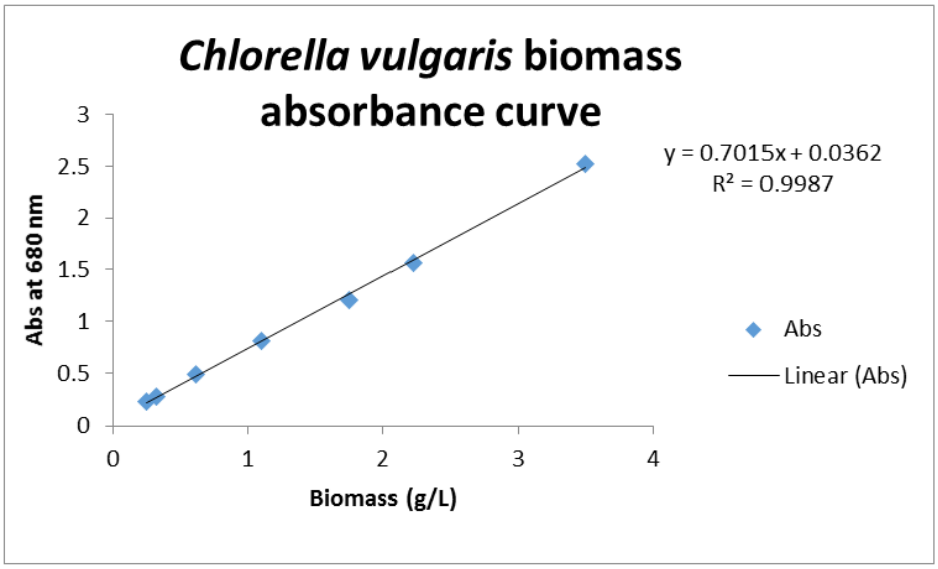

Figure 1: Relationship between *C. vulgaris* biomass and light absorbance (680 nm).

(Shimadzu UV-1600, Shimadzu Scientific Instruments Inc, Columbia, USA). For the calibration of this methodology seven samples with different biomass concentrations of *C. vulgaris*, ranging from 0.25 to 3.49 g L^{-1} were used to establish a relationship between standing biomass an absorbance. The resulted equation Y = 0.7015x + 0.0362 with R^2 of 0.9987 was used to determine the biomass from 96 replicates in this study, the regression and the equation is shown in Figure **1**.

2.8. Statistical Analysis

The results were analyzed using one way ANOVA test with the software Biostatistics 1.0 at α≤ 0.01. We performed one way ANOVA test for different CO_2 concentration as well as different light treatments.

3. RESULTS AND DISCUSSION

3.1. Standing Biomass Production Under Increasing CO_2 Concentrations

Standing biomass of *C. vulgaris* increased with increasing concentrations of CO_2 under the four different light sources (Figure **2**). The highest biomass production, 1.59 g L^{-1}, was found when the algal culture were supplied with 8.5% CO_2 and exposed to white light. Biomass production under blue, red and red-blue light was 1.53 g L^{-1}, 0.45 g L^{-1} and 1.27 g L^{-1}, respectively. An experiment growing *Chlorella sp* under increasing concentrations of CO_2 found that the standing biomass increased from 0.5 to 5.7 g L^{-1} reaching the highest standing biomass when the CO_2 increase to 10% [14]. In a similar study growing *Chlorella sp* at different CO_2 concentrations, resulted in standing biomass of 2 g L^{-1} at 10% CO_2 [15]. Other study with *Chlorella* showed a standing biomass of 3 g L^{-1} when the algal was grown at 10% CO_2, also good growth was reported with *Chlorella sp* at CO_2 concentrations from 10 to 50% [16] reaching 2 g L^{-1} when the CO_2 concentration range from 5 to 40% [17]. Standing biomass of 2 g L^{-1} also was obtained growing *Chlorella sp* at 5% CO_2 [18] concentration of 2% and 10% CO_2 has resulted in biomass synthesis of 1.67 to 1.5 g L^{-1} after 6 days of incubation [19]. Several studies conducted with *C. vulgaris* have reported a typical standing biomass between 0.25 g L^{-1} and 1.7 g L^{-1} under phototropic growth after 12-15 days of incubation [20,21], growing *Chlorella sp* under digested manure had produced standing biomass of 1.7 g L^{-1} after 21 days of incubation [22, 23]. The lowest levels of standing biomass 0.21 g L^{-1} were obtained growing *C. vulgaris* in waste water [24], however using artificial waste water *C. vulgaris* had developed standing biomass of 1.6 g L^{-1} after 11 days of incubation [25].

3.2. Statistical Analysis of the Standing Biomass

The present statistical analysis is a one way ANOVA comparing the four CO_2 concentration treatments at one specific light. For example; the algae growing in four CO_2 concentration treatments under white light to analysis possible differences in the standing biomass. The second analysis is comparing the same CO_2 concentration under different light treatment with the objective of determines if the wavelength of the light affects standing biomass. An ANOVA was used to compare individual treatments of

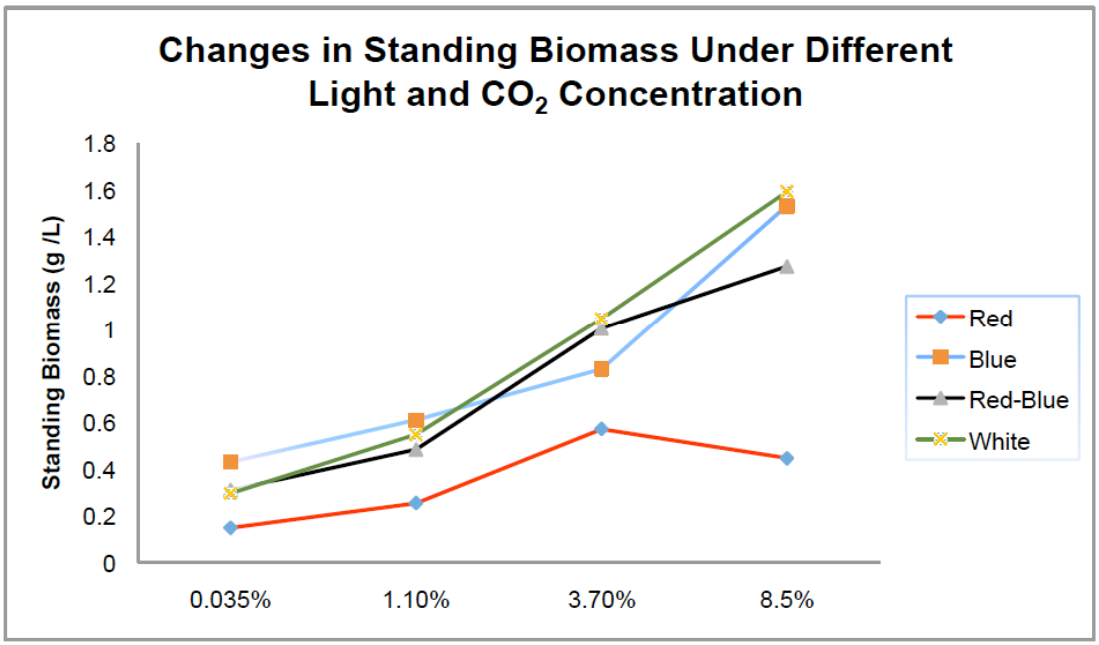

Figure 2: Standing biomass production with increasing CO_2 concentrations and exposed to different light sources. Mean standard deviation; red 0.007, blue 0.010, red-blue 0.008, and white 0.012.

CO_2 and light to one another. Table **2** compares the effect of CO_2 concentration in the standing biomass in four different type of illumination.

The ANOVA shows a statistical difference at α = 0.01 in the standing biomass of the algae *C. vulgaris* growing under increasing CO_2 concentrations (0.035%, 1.1%, 3.7% and 8.5% CO_2) and 4 different light wavelengths. The F value from the experiment are between 1117 and 13256 and the F of the table at α = 0.01 is 4.94. The statistical analysis confirms that the differences in standing biomass production with increasing CO_2 concentrations and under the four different light sources are significant. For the next ANOVA test (Table **3**) we leave the CO_2 concentration constant to see changes in the standing biomass if the light wavelength changes and the light power remains constant.

The ANOVA for light source shows statistical differences at α = 0.01. The tests for the four CO_2 concentrations gave an F ranging from 740 to 3982 and the F of tables is 4.94. There is statistical difference in *C. vulgaris* standing biomass when is grown under different concentration of CO_2 and exposed to light sources of different wavelength.

3.3. Light Source Effects on Biomass Production

Exposure of the algae to white LED light and supplied with 8.5% CO_2 concentration resulted in the highest standing biomass of this study 1.6 g L^{-1}, even

Table 2: Analysis of Variance of the Standing Biomass for Four Different CO_2 Concentration Under Constant Light Source

ANOVA different CO_2 concentration at α=0.01					
Light source	**Red**	**Blue**	**Red-Blue**	**White**	**F table**
Different CO_2	3966.07	12466.36	13256.17	11174.18	4.94

Table 3: Analyses of Variance of the Standing Biomass if the Light Source Changes and the CO_2 Concentration Remains Constant

ANOVA different wave length illumination at α=0.01					
CO_2 concentration	**0.0350%**	**1.10%**	**3.70%**	**8.5%**	**F table**
Different light source	1578.89	3982.81	2308.04	7404.78	4.94

Table 4: Mean Standing Biomass Production Under Increasing CO_2 Concentrations and Exposed to Four Different Light Sources

Standing Biomass (g L^{-1})				
CO_2 /Light source	**0.035%**	**1.10%**	**3.70%**	**8.5%**
Red	0.148± 0.009	0.254± 0.008	0.571± 0.006	0.446± 0.005
Blue	0.429± 0.007	0.610± 0.006	0.828± 0.013	1.531± 0.014
Red-blue	0.309± 0.003	0.483± 0.003	1.003± 0.009	1.271± 0.016
White	0.296± 0.006	0.548± 0.006	1.045± 0.014	1.592± 0.021

Note: ± Standard deviation.

higher than 0.16 g L^{-1} obtained in a study growing *Scenedesmus dimorphus* at 10% CO_2 [26]. Different results were obtained growing *Nannochloropsis* sp under LED illumination, the results of this study show that blue 470 nm developed the highest growth rate follow by white, green 550 nm and red 680 nm [27]. Contrary to the rest of the lights treatments, biomass production under red light was highest 0.57 g L^{-1} when the cultures were exposed to 3.7% CO_2 and not at the highest CO_2 concentration 8.5% where biomass was 0.44 g L^{-1}.

The red 625 nm always developed less biomass than the rest of the lights treatments, these results confirm the study made in *Isochrysis galbana* which demonstrated that a shorter wavelength such as blue 460 nm is more photosynthetic efficient than a longer wavelength as red 670 nm [28]. However a study made with *C. vulgaris* growing in synthetic waste water and under different LED illumination, conclude that red 660 nm developed higher biomass 0.28 g L^{-1} than white 0.25 g L^{-1}, yellow 590 nm 0.21 g L^{-1}, purple 410 nm 0.16 g L^{-1} blue 460 nm 0.15 g L^{-1} and green 550 nm 0.1 g L^{-1} [29]. Red 660-680 nm match better the *C. vulgaris* absorption peak than red 625 nm, however it was expected *C. vulgaris* adaptation to this light spectra by modifying its chlorophylls or by producing more complementary pigments such as carotenoids in the absorption peak of 620-630 nm, little evidence of light spectra adaptation is observed from the results of this study.

The highest biomass production in cultures exposed to blue light 1.5 g L^{-1} were obtained when the algal grew at 8.5% of CO_2. However biomass production under blue light was higher than the other light sources tested in this study for CO_2 concentrations of 1.1% and lower when the standing biomass is less than 0.6 g L^{-1}. Since the photosynthetic part of algae are the chloroplast light reaching the rest of the algae body is not been used for photosynthesis, therefore increases in standing biomass increase light shadowing. The shadowing effect is light blocked and not used for photosynthetic process by algae located near to the light source resulting in less light reaching those algae farther away from the light source. This effect cause loss of photons, therefore the amount of energy lost is greater at lower wavelength when the light photon has more energy [27]. Since blue 425 nm light has more energy per photon ($6.68*10^{-19}$ J) than do the other light sources, for example red 625 nm ($3.18*10^{-19}$ J), losses of light energy caused by light shadowing would be greatest under blue light exposure. From the data in Table **4**, the limit where the biomass production response less to blue light switches between 0.6 to 0.8 g L^{-1}. At this algal biomass concentration, the light source combination of red-blue performed better than blue light alone because the red light losses for shadowing effect are less than blue, and red light complements the blue light resulting in a higher standing biomass. White light travels further and would have reached more distant algae, therefore would perform better when the standing biomass is greater than 0.8 g L^{-1}.

4. CONCLUSIONS

Production of *C. vulgaris* biomass increased when supplied with increasing CO_2 concentrations up to 8.5% under the four light sources. Growth of the algae was better under blue light when algae were supplied with lower CO_2 concentrations and the standing biomass was low. The results of this study show that *C. vulgaris* does not adapt production of their photosynthetic pigments to absorb light from a wavelength spectrum different from one that they would normally be exposed to.

Further studies with more CO_2 concentration are recommended to establish the limit where CO_2 is no longer a factor for increasing biomass production. It is

further recommended to compare *C. vulgaris* growth under red light at 660 nm, 670 nm with that under red at 625 nm.

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