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Research Article

Impact of light quality on a native Louisiana *Chlorella vulgaris/Leptolyngbya* sp. co-culture

Light effect on cultures of microalgae has been studied mainly on single species cultures. Cyanobacteria have photosynthetic pigments that can capture photons of wavelengths not available to chlorophylls. A native Louisiana microalgae (*Chlorella vulgaris*) and cyanobacteria (*Leptolyngbya* sp.) co-culture was used to study the effects of light quality (blue–467 nm, green–522 nm, red–640 nm and white–narrow peak at 450 nm and a broad range with a peak at 550 nm) at two irradiance levels (80 and 400 μmol m^{-2} s^{-1}) on the growth, species composition, biomass productivity, lipid content and chlorophyll-a production. The co-culture shifted from a microalgae dominant culture to a cyanobacteria culture at 80 μmol m^{-2} s^{-1}. The highest growth for the cyanobacteria was observed at 80 μmol μmol m^{-2} s^{-1} and for the microalgae at 400 μmol m^{-2} s^{-1}. Red light at 400 μmol m^{-2} s^{-1} had the highest growth rate (0.41 d^{-1}), biomass (913 mg L^{-1}) and biomass productivity (95 mg L^{-1} d^{-1}). Lipid content was similar between all light colors. Green light had the highest chlorophyll-a content (1649 μg/L). These results can be used to control the species composition of mixed cultures while maintaining their productivity.

Keywords: Co-culture / Cyanobacteria / Light attenuation / Light quality / Microalgae productivity

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1 Introduction

Microalgae and cyanobacteria are photosynthetic organisms that can use CO_2 as a source of carbon and produce valuable bioproducts, such as pigments, oil, proteins, pharmaceuticals and nutraceuticals. The interest for the culture of these organisms has increased in the last years for the production of third generation biofuels. Global demand of petroleum fuels is projected to increase by 40% by 2025; therefore alternative energy sources will be needed to help support this growth. Biofuels, fuels derived from biomass, are an option as a renewable carbon neutral energy source [1]. The potential biodiesel from microalgae is significantly higher (58 700–136 900 L/ha) than the next highest land based crop, oil palm (5950 L/ha) [2]. Other advantages of using microalgae as a biofuel sources are: higher growth rates than crops currently used, including corn and palm; oil content in microalgae can exceed 80% of the dry weight of biomass; they do not compete for land with food crops; can be grown in environments unsuitable for other crops utilizing CO2 as their carbon source and are capable of growing year round [2, 3].

Since microalgae are photosynthetic organisms, light, particularly light quality (wavelength distribution) and light intensity have been studied to determine its effects on growth, biomass and lipid content [4–8]. Traditional methods of testing light intensity used fluorescent light, with a broad wavelength distribution [7]. More recently light emitting-diodes (LEDs) are being used light sources because they provide a narrow wavelength distribution, are more efficient and dissipate less heat [9]. In most studies either red or blue light produced the best results [4, 5, 8, 10–12]. However, some studies have found other colors are more favorable. In some studies, green light at high intensities was found to promote the highest biomass productivity and concentration in *Spirulina platensis* [6], while other found that blue light resulted in the highest growth rate and biomass productivity [12]. Mohsenpour et al. found that orange light produced the highest growth rate in *Chlorella vulgaris* as compared to red [13]. The optimum wavelength varies not only by species, but also by strain and culture conditions [13, 14].

It has been established that different environmental factors such as nitrogen levels, temperature and salinity can significantly affect lipid content in microalgae [3, 15–17]. Another environmental factor that increases lipid content is light. Blue light has been found to induce higher lipid content in *Chlorella*

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Abbreviations: LED, light emitting-diode; **MFI**, mean fluorescent intensity

species [8, 11]. An increase light intensity has been shown to increase lipid content in *Dunaliella salina* and *Phaeodactylum tricornutum* [18, 19].

While most studies look at single strains, mixed cultures of microalgae have been shown to produce higher biomass and lipid content [20, 21]. Advances in co-cultures of microalgae and bacteria have produced promising results. For example bacteria *Azospirillum brasilense* grown with *Chlorella vulgaris* showed an increase in pigment and lipid content, as well as increased growth [22, 23]. Mixed cultures of photosynthetic organisms can result in increased biomass productivity, resistance to predation and contaminants, and reduction of energy inputs through mutualism [24]. Additionally, use of species natives of the target culture area, removes the problems associated with the introduction of non-native species.

In this study, a Louisianan native co-culture of microalgae and cyanobacteria was used. This co-culture has been found to be resistant to changing conditions such as extreme pH, salinity and temperature shifts. These conditions are common in coastal areas, such as the Gulf of Mexico's north coast. In previous work [25], it has been found that this co-culture has higher growth rates and resistance to contamination than the microalgae grown in monoculture. Additionally, the co-culture was able to use organic carbon sources in heterotrophic and mixotrophic regimes [26]. This study also found that under certain conditions the co-culture can shift its species composition from microalgae dominant to cyanobacteria dominant. The shift in species was observed at lower irradiance levels or when the culture density increases.

Suppressive subtractive hybridization of the *Chlorella vulgaris* in co-culture compared with the microalgae alone [27], showed differential expression of photosystems I and II, upregulation of stress response and oil globule associated genes.

This study focuses on the effects of different wavelength distributions (blue–467 nm, green–522 nm, red–640 nm and white–narrow peak at 450 nm and a broad range with a peak at 550 nm) on the growth and species ratio of the Louisiana co-culture. No attempt was made to optimize the productivity (mg L^{-1} d^{-1}) of biomass or lipids.

2 Materials and methods

The impact of different wavelength distributions (blue, green, red and white) at two irradiance levels on the major growth factors of a microalgae and cyanobacteria co-culture was studied in batch cultures.

2.1 Co-culture species

This Louisiana co-culture that was isolated from College Lake on the Louisiana State University campus in Baton Rouge, LA. The co-culture was a mix of *Chlorella vulgaris* (microalgae) and *Leptolyngbya* sp. (cyanobacteria). The co-culture roughly 97% microalgae and 3% cyanobacteria, was maintained in the laboratory under fluorescent lighting with an approximate light intensity of 250 μmol m^{-2} s^{-1}. Stock co-cultures were grown in sterilized Type I water (nanopure) with Bold's Basal Medium (BBM) [28] containing $NaNO_3$ (2.94 mM), $CaCl_2$•$2H_2O$ (0.17 mM), $MgSO_4$•$7H_2O$ (0.3 mM), K_2HPO_4 (0.43 mM), KH_2PO_4 (1.29 mM), NaCl (0.43 mM), P-IV metal solution (2 mM $Na_2EDTA{\cdot}2H_2O$, 0.36 mM $FeCl_3{\cdot}6H_2O$, 0.21 mM $MnCl_2{\cdot}4H_2O$, 0.0037 mM $ZnCl_2$, 0.0084 mM $CoCl_2{\cdot}6H_2O$, 0.017 mM $Na_2MoO_4{\cdot}2H_2O$), vitamin B_{12}, biotin vitamin and thiamine vitamin.

2.2 Experimental design

The two irradiance levels (80 and 400 μmol m^{-2} s^{-1}) and four wavelength distributions (blue–467 nm, green–522 nm, red–640 nm and white–narrow peak at 450 nm and a broad range with a peak at 550 nm) were tested. These irradiance levels were chosen based on previous research with this co-culture that showed a maximum growth rate for the co-culture at 400 μmol m^{-2} s^{-1} and a shift from microalgae dominant to cyanobacteria dominant at or below 80 μmol m^{-2} s^{-1} [25]. The wavelength distributions were found using a Jaz Ocean Optics spectrometer. Light-emitting diodes were used as light sources. Light intensity was measured using a light sensing logger LI-COR 1400 with a LI-193 Underwater Spherical Quantum Sensor attached to it. LED lights were coiled around plastic mesh and placed inside cylindrical chambers made from galvanized steel flashing. Irradiance levels were adjusted by blocking excess light with aluminum foil.

Triplicate 2 L Erlenmeyer flasks with 1100 mL of BBM were used to maintain the co-culture. The initial cell count was about 300 ± 75 cells/μL. The temperature was maintained at 25 ± 2°C with a recirculating water bath. The water bath was custom made to maintain the circulation at the bottom of the flask, while allowing light penetration to the culture. Continuous aeration at 0.24 LPM was supplied to each experimental flask. The pH was controlled by adding CO_2 at 2% (v/v) to the air supply to maintain a pH between 7.5 and 8. Initial and final measurements of nitrate (mg/L), nitrite (mg/L), orthophosphates (mg/L) and biomass (mg/L) were obtained. Daily measurements were cell counts, optical densities, temperature and pH. Cell counts (cells/μL) measured with a BD Accuri C6 Flow Cytometer (excitation wavelength of 488 nm and the fluorescence emission filter wavelength of 670-nm-long pass). Optical density absorbance was taken at a wavelength of 664 nm by using a HACH DR4000 spectrophotometer.

The irradiance level that was more favorable for microalgae growth based on cell counts was analyzed to determine the optimum wavelength distribution. The optimum wavelength distribution was based on growth rates, biomass, biomass productivity, chlorophyll-a and lipid content. Biomass for each day was calculated by using an optical density to biomass calibration curve. These curves were prepared every three days with samples of one of the replicates for each condition. Growth curves were prepared (days vs. biomass) to determine the maximum growth rate of the co-cultures during the exponential phase. Growth rates were calculated using the biomass found from the optical density to biomass calibration curve. The growth based on biomass was calculated as follows:

$$\mu = \frac{ln\frac{X_1}{X_2}}{(t_1 - t_2)} \quad (1)$$

where X_1 is the initial biomass during the exponential phase, X_2 is the final biomass during the exponential phase at time t_1 and t_2, respectively.

Chlorophyll-a content was determined following the Standard Methods for the Examination of Water and Wastewater method number 10200 H using a Turner TD4000 flurometer [29]. Lipid content was evaluated by using the Nile Red method utilizing the flow cytometer to determine the lipid content by mean fluorescent intensity (MFI) as described by Cirulis, Strasser [30]. Briefly, a stock Nile Red stain was made by dissolving Nile Red powder with acetone for a concentration of 1 mg/mL. This stock was then diluted further with acetone to make a solution of Nile red at a concentration of 200 μg/mL. This 200 μg/mL is used to stain co-culture samples at a working concentration of 2 μg/mL with a cell concentration of about 2000 cells/mL and measuring the MFI 30 min after being stained. Nile red is excited by a blue laser at 488 nm and its fluorescence is detected using a filter with a peak emission of 585 $\pm$ 20 nm. The lipid content was calculated from the MFI using a calibration curve. A five point calibration curve (min: 15.5 % lipid content max: 37% lipid content) was made with lipid content found using the Folch [31] method and its corresponding MFI. The calibration curve produced an $r^2 = 0.9649$.

Light attenuation was studied to determine how the light intensity changed with respect to the co-culture biomass between the different wavelength distributions. Light attenuation was measured at 400 μmol m^{-2} s^{-1}. Five biomass concentrations (72, 209, 447, 646 and 832 mg/L) were used, with DI water being used as biomass concentration of 0.0 mg/L. The light intensity was measured with a LI-COR 1400 and a LI-193 USQS. To measure the light intensity the sensor was placed in the center of the flask.

Light attenuation coefficients, which represent the rate at which light intensity decreases as the culture density increases, were calculated as follows:

$$I_D = I_w e^{-k_b X} \quad (2)$$

where I_D is the light intensity (μmol m^{-2} s^{-1}) at a specific culture density, I_w is the light intensity (μmol m^{-2} s^{-1}) of clear water with no culture, X is the biomass concentration (mg/L) of the co-culture and k_b is the light attenuation coefficient (L/mg). In this study, the value of I_w was 400 μmol m^{-2} s^{-1}.

2.3 Distinguishing between microalgae and cyanobacteria

Microalgae and cyanobacteria cells were distinguished by their different pigments using a flow cytometer with two fluorescence filters, one to detect chlorophyll-a (emission wavelength >640 nm) and one to detect phycocyanin (emission wavelength of 650 nm). Both microalgae and cyanobacteria have chlorophyll-a; however cyanobacteria also has phycocyanin, which microalgae does not. The chlorophyll-a filter is excited by a blue laser at 488 nm and detects fluorescence at 670 nm and above. The phycocyanin filter is excited by a red laser at 640 nm and detects fluorescence at 675 $\pm$ 12.5 nm. Chlorophyll-a is also excited by the red laser and fluoresces in the red region which is why the microalgae cells in Fig. 2d have a high fluorescence intensity in both the *x*- and *y*-axes.

2.4 Statistics

Statistical analysis was performed using SAS 9.4. The light intensity that was chosen as the best for the growth of microalgae was analyzed for significant differences in growth rates, biomass, lipid content and chlorophyll-a content using a single factor ANOVA. Significant differences between the different colors were determined using the Tukey test with a $p < 0.05$ being considered as a significant difference.

3 Results and discussion

3.1 Growth under irradiance level 80 and 400 μmol m^{-2} s^{-1}

Results from batch cultures grown at 80 μmol m^{-2} s^{-1} showed a species composition shift from microalgae dominant to cyanobacteria dominant. Figure 1 shows the growth curves, according to cell counts, for the various wavelength distributions. For each wavelength distribution *C. vulgaris* (microalgae) was the dominant species at Day 1 (inoculation) and after Day 6 *Leptolyngbya* sp. (cyanobacteria) became the dominant species (Fig. 1).

The dominance of cyanobacteria under low intensities is consistent with the research of others [32, 33]. The shift to a cyanobacteria dominant species under low light follows the work found by Källqvist [34] that studied the diatoms *Asterionella, Diatoma* and *Synedra* and the cyanobacteria *Planktothrix* in a Norwegian lake. Källqvist [34] found that at depths less than 1 meter the diatoms grew faster than the cyanobacteria; however at depths below 3 m, where there is low light intensity only the cyanobacteria grew. A study by Van Liere [35] also showed that low light intensities cyanobacteria outcompetes microalgae. In that study, low light intensity of 1 W/m^2 the cyanobacteria *Planktothrix agardhii* resulted in higher growth rate than the microalgae *Scenedesmus protuberans*. When the light intensity increased to 24 W/m^2 *S. protuberans* had a higher growth rate than *P. agardhii*.

Growth under 400 μmol m^{-2} s^{-1} showed no shift species composition because the higher light intensity was more favorable for the growth of *C. vulgaris* than *Leptolyngbya* sp. Growth curves of the co-culture at 400 μmol m^{-2} s^{-1} under the different colors can be seen in Fig. 2. Microalgae cell counts were consistently higher than the cyanobacteria cell counts at 400 μmol m^{-2} s^{-1}. Towards the end the cyanobacteria cell count started to increase, most likely due to the high biomass concentration causing low light penetration. Although the increase in cyanobacteria cells could be due to the decline in nitrate concentration as the media final nitrate concentrations were below 1.0 mg/L, which can inhibit microalgae growth, the increase of the cyanobacteria occurred well before the nitrogen was limiting for the microalgae. Cyanobacteria may be able to fix nitrogen (N_2) from the air to use as a nitrogen source. By fixing N_2 the cyanobacteria are able to continue growing when the nitrates are low. Based on cell

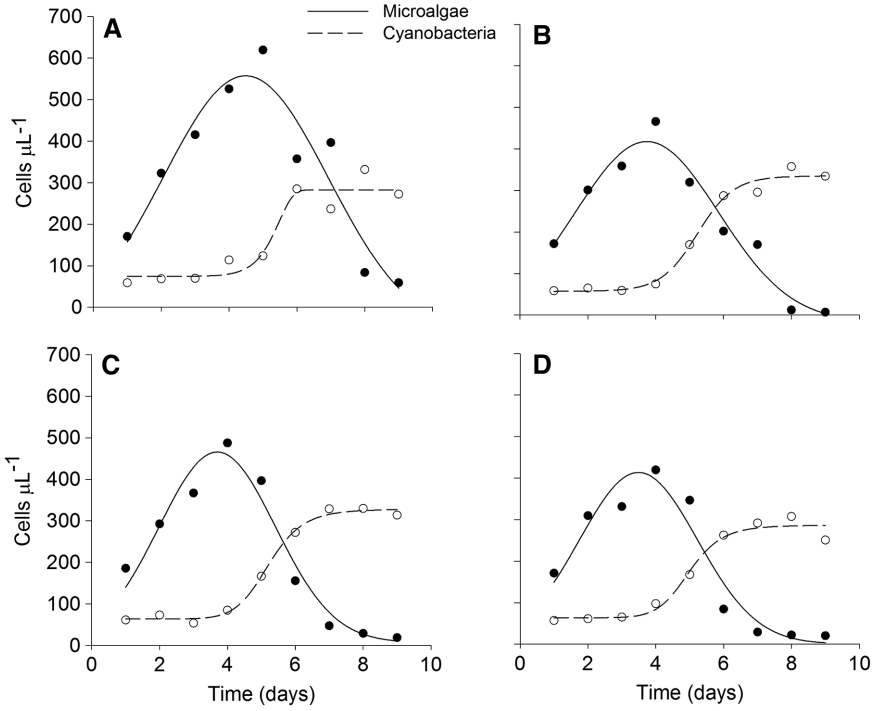

Figure 1. Growth curves of *C. vulgaris* (microalgae) and *Leptolyngbya* sp. (cyanobacteria) at 80 μmol m^{-2} s^{-1} under the different wavelength distributions, 1(A) blue light, 1(B) green light, 1(C) red light, 1(D) white light. Microalgae growth curves were fitted with a Gaussian curve and cyanobacteria growth curves were fitted with a sigmoidal curve.

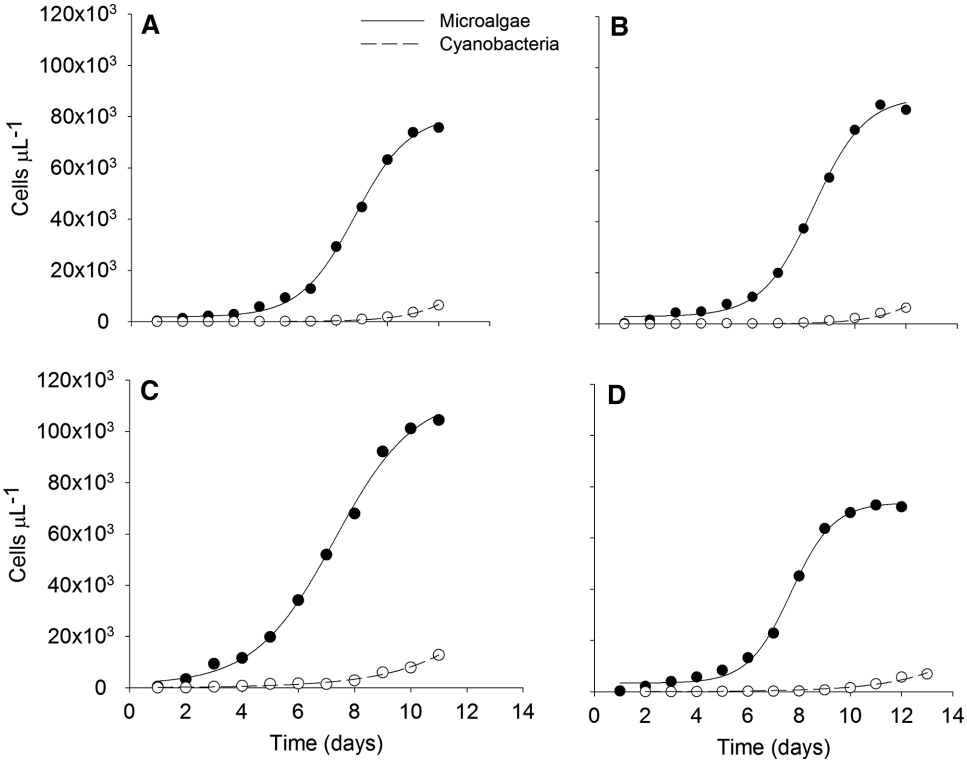

Figure 2. Growth curves of *C. vulgaris* (microalgae) and *Leptolyngbya* sp. (cyanobacteria) at 400 μmol m^{-2} s^{-1} under the different wavelength distributions, 1(A) blue light, 1(B) green light, 1(C) red light, 1(D) white light. Microalgae growth curves were fitted with sigmoidal curves and cyanobacteria curves were fitted with exponential growth curves.

counts it was determined that microalgae production was higher at irradiance of 400 μmol m^{-2} s^{-1}. Therefore, co-culture samples at 400 μmol m^{-2} s^{-1} were evaluated further to determine the optimum wavelength distribution.

3.2 Effects of light quality at 400 μmol m^{-2} s^{-1}

Growth curves, based on biomass (mg/L), for the various wavelength distributions are shown in Fig. 3. There are many types

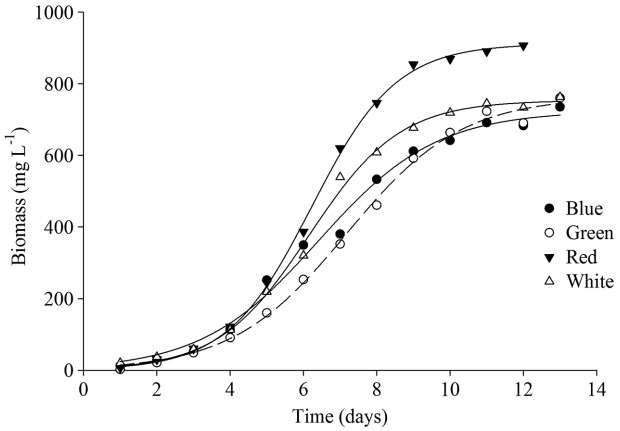

Figure 3. Biomass growth curves for the co-culture at 400 μmol m^{-2} s^{-1} under four wavelength distributions fitted with a logistic growth curve. The dashed line represents the growth curve for the green light that was not found using the logistic model.

of growth models that are used to describe the growth of microorganisms [36]. Two very common models are the logistic and Gompertz models. The logistic growth model was applied to the growth curves of the co-culture grown under the different color of lights. The logistic model used is represented as follows:

$$X = \frac{X_{\max}}{1 + e^{a-\mu t}} \quad (3)$$

where X (mg L^{-1}) is the co-culture biomass at time t (day), $X_{\max}$ (mg/L) is the maximum biomass achieved (carrying capacity), a is a constant value in the model which dictates the relative position from the origin and μ (d^{-1}) is the growth rate of the co-culture. The maximum biomass calculated from Eq. (3) can be found in Table 2. Interestingly the logistic model produced a very low r^2 value for the green light growth curve compared to $r^2 > 0.98$ for the other colors. This is why in Table 2 for green light the $X_{\max}$ is "N/A". The Gompertz model was also applied to the green light growth curve and produced a very low r^2 value as well. This may be due to the fact that there is no clear distinction between the exponential phase of the graph and the stationary phase. The logistic model defines three distinct sections: the lag phase at the beginning, the exponential phase in the middle and the stationary phase at the end of the curve.

Co-cultures grown under red light had the highest final biomass (913 mg/L) and it was significantly higher than final biomass production under blue, green and white light (Table 1). Red light also had the highest biomass productivity (95 mg L^{-1} d^{-1}) and this was significantly higher ($p = 0.0016$) than productivity at the other wavelength distributions (Table 1). White, blue and green had very similar biomass productivities, because their respective final biomass concentrations and growth durations were all relatively similar.

ANOVA results showed that the different wavelength distributions had a significant effect ($p = 0.0005$) on the specific growth rates. Co-cultures grown under red light had the highest specific growth rate (0.41 d^{-1}) and it was significantly higher than growth rates under white, green and blue light (Table 1). Blue light had the lowest growth rate, biomass and biomass productivity.

These results coincide with those of other authors [37, 38] that found that red light promoted the highest growth rate and produced the highest biomass in *C. Vulgaris* compared to the other wavelength distributions. Wang et al. [12] also found that red light was the optimal wavelength for the growth of *Spirulina platensis.* Red light being the optimum wavelength for growth in this study may be due to the chlorophyll in green microalgae strong absorption peak in the red region between 630−675 nm [39]. The peak wavelength emission of the red LED, 640 nm, used in this study falls within this absorption region. In contrast, Blair et al. [40] found that blue light produced the highest growth rate and biomass productivity in *Chlorella vulgaris,* but the optimum light for growth and biomass productivity varied by days of culture and media.

Table 1 shows the energy per mole of photon for each wavelength (peak wavelengths were used for the white light). The energy was calculated based on Planck's constant and the wave's equation [41]. Red light has the lowest energy at 187 kJ (mol/photon); whereas blue has the highest at 256 kJ (mol photon)$^{-1}$. Energy from white and green light are in the middle. Energy from light is important for the start of photosynthesis. To initiate photosynthesis a photon of light must excite an electron in a chlorophyll-a molecule from the ground state to the first excited energy state [42]. The energy associated in the red region (680–700 nm) is optimal for photosynthesis because it has the required energy to excite an electron from the ground state to the first excited energy state. A photon excited to the first energy state has enough energy to initiate photosynthesis [42, 43]. Shorter wavelengths, less than 450 nm, will excite an electron from the ground state to the second excited energy level. At this second energy level, the electron will need to lose energy in the form of heat or fluorescence to move down to the first energy level to start photosynthesis [42]. The peak wavelength emission of the red light (640 nm) used in this study is very close to the wavelength energy range (680–700 nm) needed to initiate photosynthesis. Whereas, the blue and white light max peaks (467 and 450 nm, respectively) used in this study are associated with higher energy causing a delayed start to photosynthesis. The higher biomass and growth rates under red light found in this study support the idea that red light is the optimum light for photosynthesis.

The lipid content between the different wavelength distributions was not significantly different ($p = 0.7349$), although red light produced the highest lipid content (19.71%). The results show that green light promotes more chlorophyll-a content. ANOVA results showed that the different wavelength distribution had a significant effect ($p = 0.0019$) on the chlorophyll-a (chl-a) content. Green light produced the highest chl-a content (1649 μg/L) and was significantly higher than the other wavelength distributions (Table 1). Blue and white light have very similar average chl-a content and were not significantly different. This may be due to white light having a strong peak in the blue region, which has a similar peak to the blue light. Red light produced the lowest chl-a content and was not significantly different than blue or white light. These results coincide with Mohsenpour and Willoughby [44] that found that *C. vulgaris* grown under green light had higher chl-a production than other

Table 1. Final biomass (X_{final}) maximum biomass (X_{max}) found from Eq. (3), biomass productivity ($X_{prod,}$), growth rate (μ), lipid content, chlorophyll-a content (Chl-a) and energy per photon for each light color at 400 μmol m^{-2} s^{-1}

	Blue	Green	Red	White
X_{final} (mg/L)*	$747 \pm 95^{b)}$	$767 \pm 31^{b)}$	$913 \pm 31^{a)}$	$760 \pm 35^{b)}$
X_{max} (mg/L)	724	N/A	912	754
$X_{prod.}$ (mg L^{-1} d^{-1})*	$70 \pm 9^{b)}$	$72 \pm 3^{b)}$	$95 \pm 4^{a)}$	$73 \pm 3^{b)}$
μ (d^{-1})*	$0.27 \pm 0.012^{b)}$	$0.28 \pm 0.029^{b)}$	$0.41 \pm 0.023^{a)}$	$0.33 \pm 0.035^{b)}$
Lipid Content* (% dry biomass)	$19.57 \pm 1.38^{a)}$	$18.08 \pm 0.87^{a)}$	$19.71 \pm 2.39^{a)}$	$18.11 \pm 2.06^{a)}$
Chl-a (μg/L^{-1})*	$1108 \pm 191^{b)}$	$1649 \pm 197^{a)}$	$729 \pm 61^{b)}$	$1155 \pm 228^{b)}$
Energy (kJ (mol photon)$^{-1}$)	256	229	187	266 218

*Mean ± standard deviation. Different letters represent significant difference ($p < 0.05$) among treatments from single factor ANOVA

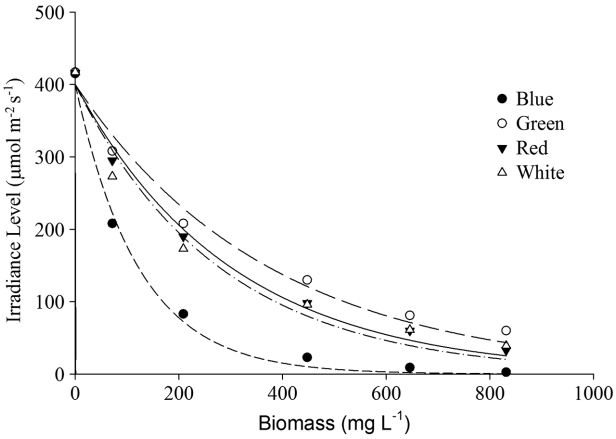

Figure 4. Light attenuation curves for the four wavelength distributions at 400 μmol m^{-2} s^{-1} fitted using Eq. 2.

wavelength distributions. Green light was also found to induce higher chl-a content in *Gloeothece membranacea* [13].

Chl-a has strong absorption peaks in the red and blue region of the visible light spectrum. Light outside these two absorption peaks are not effectively absorbed by this pigment. Different wavelength distributions can affect the amount of chl-a produced. Under red light the chl-a produced is lower due to the higher efficiency of the pigment in the utilization of photons of this energy. Green light is highly reflected by microalgae and has low penetration ratio of chlorophyll. Therefore, it has lower rates of absorption and photosynthetic efficiency [45], resulting in a need for higher levels of the pigment to capture the same amount of energy.

3.3 Light attenuation at 400 μmol m^{-2} s^{-1}

As microalgae grow and become more dense light penetration is reduced due to mutual shading [46]. Light attenuation was studied with the four wavelength distributions at 400 μmol m^{-2} s^{-1} (Fig. 4). Light attenuation coefficients (k_b) values from Eq. (3) for blue, white, red and green light can be found in Table 2. Blue light showed the largest decline in light intensity which corresponds to the largest k_b value. Green light showed the smallest decline in light attenuation and had the smallest k_b value. This supports the fact that green light is reflected most by green microalgae [47]. Red and white light have very similar light attenuation and k_b values.

Table 2. Light attenuation coefficients (k_b) for the different wavelength distributions at 400 μmol m^{-2} s^{-1}

Color	k_b (L mg^{-1}).
Blue	8.200×10^{-3}
Green	2.672×10^{-3}
Red	3.325×10^{-3}
White	3.594×10^{-3}

An absorption spectrum of this co-culture was measured using a HACH DR 6000 spectrophotometer to determine its absorption peaks. This co-culture has two large peaks corresponding to the blue and red region. The blue region absorbance is larger than the red region. As a result, blue light will have a higher light attenuation coefficient. The large peak in the blue region of the white light used in this work, explains the higher attenuation, compared with the red light.

4 Concluding remarks

Light is an important aspect of photosynthesis in microalgae. This study looked at the effects of two irradiance levels and four different wavelengths on a native Louisianan co-culture. It was found that at 80 μmol m^{-2} s^{-1} the co-culture species shifted from microalgae dominant to cyanobacteria dominant after 6 days in all four wavelength distributions. The growth of microalgae in terms of cell counts substantially higher at 400 μmol m^{-2} s^{-1} than 80 μmol m^{-2} s^{-1}. Red light had significantly a higher growth rate (0.41 d^{-1}), biomass (913 mg/L) and biomass productivity (95 mg L^{-1} d^{-1}) at 400 μmol m^{-2} s^{-1} compared to the other colors. This indicates that red light was the optimum wavelength to grow this co-culture and would be an ideal color to use for cultivation of this co-culture. Lipid content was similar under all wavelength distributions and it was found that there was no significant difference. Chl-a was highest under green light; whereas red light had the lowest. Blue light promoted the

largest light attenuation coefficient, while green had the lowest in cultures with 832 mg/L.

Practical application

Light quality and light quantity are important aspects when growing microalgae. Manipulation of light can yield different results that can produce valuable bioproducts. A co-culture of cyanobacteria and microalgae was used in this study. The microalgae in this co-culture has been shown to have higher productivity as well as growth than when maintained as monocultures. Low light intensities are favorable for cyanobacteria growth, which can produce the valuable pigment phycocyanin. Red light at high intensities is favorable for microalgae growth, which can be used to produce large amounts of biomass for biofuels or other bioproducts.

Nomenclature

Chl-a	[μg L^{-1}]	Chlorophyll- a concentration
I_D	[μmol m^{-2} s^{-1}]	Light intensity at D culture density
I_w		Light intensity with clear water without culture
k_b	[L mg^{-1}]	Attenuation coefficient due to biomass
μ	[d^{-1}]	Growth rate
X	[mg L^{-1}]	Biomass concentration
X_1	[mg L^{-1}]	Biomass at the start of the exponential growth phase
X_2	[mg L^{-1}]	Biomass at the end of the exponential phase
X_{final}	[mg L^{-1}]	Final biomass
X_{max}	[mg L^{-1}]	Maximum biomass concentration
X_{prod}	[mg L^{-1} d^{-1}]	Biomass productivity

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The authors have declared no conflict of interest.

5 References

[1] Dragone, G., Fernandes, B. D., Vicente, A. A., Teixeira, J. A., Third generation biofuels from microalgae. Current research, technology and education topics in applied microbiology and microbial biotechnology. 2010, *2*, 1355–1366.

[2] Chisti, Y., Biodiesel from microalgae. *Biotechnol. Adv.* 2007, *25*, 294–306.

[3] Brennan, L., Owende, P., Biofuels from microalgae—A review of technologies for production, processing, and extractions of biofuels and co-products. *Renew. Sustain. Energy Rev.* 2010, *14*, 557–577.

[4] Atta, M., Idris, A., Bukhari, A., Wahidin, S., Intensity of blue LED light: A potential stimulus for biomass and lipid content in fresh water microalgae Chlorella vulgaris. *Bioresour. Technol.* 2013, *148*, 373–378.

[5] Hultberg, M., Jönsson, H. L., Bergstrand, K.-J., Carlsson, A. S., Impact of light quality on biomass production and fatty acid content in the microalga Chlorella vulgaris. *Bioresour. Technol.* 2014, *159*, 465–467.

[6] Ravelonandro, P. H., Ratianarivo, D. H., Joannis-Cassan, C., Isambert, A. et al. Influence of light quality and intensity in the cultivation of Spirulina platensis from Toliara (Madagascar) in a closed system. *J. Chem. Technol. Biotechnol.* 2008, *83*, 842–848.

[7] Shu, C.-H., Tsai, C.-C., Liao, W.-H., Chen, K.-Y. et al. Effects of light quality on the accumulation of oil in a mixed culture of Chlorella sp. and Saccharomyces cerevisiae. *J. Chem. Technol. Biotechnol.* 2012, *87*, 601–607.

[8] Teo, C. L., Atta, M., Bukhari, A., Taisir, M. et al. Enhancing growth and lipid production of marine microalgae for biodiesel production via the use of different LED wavelengths. *Bioresour. Technol.* 2014, *162*, 38–44.

[9] Schulze, P. S. C., Barreira, L. A., Pereira, H. G. C., Perales, J. A. et al. Light emitting diodes (LEDs) applied to microalgal production. *Trends Biotechnol.* 2014, *32*, 422–430.

[10] Chen, H.-B., Wu, J.-Y., Wang, C.-F., Fu, C.-C. et al. Modeling on chlorophyll a and phycocyanin production by Spirulina platensis under various light-emitting diodes. *Biochem. Eng. J.* 2010, *53*, 52–56.

[11] Das, P., Lei, W., Aziz, S. S., Obbard, J. P., Enhanced algae growth in both phototrophic and mixotrophic culture under blue light. *Bioresour. Technol.* 2011, *102*, 3883–3887.

[12] Wang, C.-Y., Fu, C.-C., Liu, Y.-C., Effects of using light-emitting diodes on the cultivation of *Spirulina platensis*. *Biochem. Eng. J.* 2007, *37*, 21–25.

[13] Mohsenpour, S. F., Richards, B., Willoughby, N., Spectral conversion of light for enhanced microalgae growth rates and photosynthetic pigment production. *Bioresour. Technol.* 2012, *125*, 75–81.

[14] Herron, H., Mauzerall, D., The development of photosynthesis in a greening mutant of Chlorella and an analysis of the light saturation curve. *Plant Physiol.* 1972, *50*, 141–148.

[15] Silaban, A., Bai, R., Gutierrez- Wing, M. T., Negulescu, I. I. et al., Effect of organic carbon, C:N ratio and light on the growth and lipid productivity of microalgae/cyanobacteria co-culture. *Eng. Life Sci.* 2014, *14*, 47–56.

[16] Converti, A., Casazza, A. A., Ortiz, E. Y., Perego, P. et al. Effect of temperature and nitrogen concentration on the growth and lipid content of Nannochloropsis oculata and Chlorella vulgaris for biodiesel production. *Chem. Eng. Process.: Process Intensification* 2009, *48*, 1146–1151.

[17] Wahidin, S., Idris, A., Shaleh, S. R. M., The influence of light intensity and photoperiod on the growth and lipid content of microalgae Nannochloropsis sp. *Bioresour. Technol.* 2013, *129*, 7–11.

[18] Kurpan Nogueira, D. P., Silva, A. F., Araújo, O. Q. F., Chaloub, R. M., Impact of temperature and light intensity on triacylglycerol accumulation in marine microalgae. *Biomass Bioenergy* 2015, *72*, 280–287.

[19] Weldy, C. S., Huesemann, M., Lipid production by Dunaliella salina in batch culture: Effects of nitrogen limitation and light intensity. *US Department of Energy J. Undergraduate Res.* 2007, *7*, 115–22.

[20] Prathima Devi, M., Venkata Subhash, G., Venkata Mohan, S., Heterotrophic cultivation of mixed microalgae for lipid accumulation and wastewater treatment during sequential growth and starvation phases: Effect of nutrient supplementation. *Renewable Energy* 2012, *43*, 276–283.

[21] Venkata Mohan, S., Prathima Devi, M., Mohanakrishna, G., Amarnath, N. et al. Potential of mixed microalgae to harness biodiesel from ecological water-bodies with simultaneous treatment. *Bioresour. Technol.* 2011, *102*, 1109–1117.

[22] de-Bashan, L. E., Bashan, Y., Moreno, M., Lebsky, V. K. et al. Increased pigment and lipid content, lipid variety, and cell and population size of the microalgae Chlorella spp. when co-immobilized in alginate beads with the microalgae-growth-promoting bacterium Azospirillum brasilense. *Can. J. Microbiol.* 2002, *48*, 514–521.

[23] Gonzalez, L. E., Bashan, Y., Increased growth of the microalga Chlorella vulgaris when co-immobilized and cocultured in alginate beads with the plant-growth-promoting bacterium Azospirillum brasilense. *Appl. Environ. Microbiol.* 2000, *66*, 1527–1531.

[24] Kazamia, E., Riseley, A. S., Howe, C. J., Smith, A. G., An engineered community approach for industrial cultivation of microalgae. *Ind. Biotechnol. (New Rochelle, N.Y.)* 2014, *10*, 184–190.

[25] Silaban, A. G., Growth rate and productivity of a Louisiana native microalgae/cyanobacteria co-culture: Feasibility for use in industrial biotechnology applications. Master of Science thesis. Civil and Environmental Engineering, Louisiana State University, Baton Rouge, Louisiana 2013.

[26] Silaban, A., Bai, R., Gutierrez-Wing, M. T., Negulescu, I. I. et al. Effect of organic carbon, C:N ratio and light on the growth and lipid productivity of microalgae/cyanobacteria coculture. *Eng. Life Sci.* 2014, *14*, 47–56.

[27] Tate, J. J., Gutierrez-Wing, M. T., Rusch, K. A., Benton, M. G., Gene expression analysis of a Louisiana native Chlorella vulgaris (Chlorophyta)/Leptolyngbya sp. (Cyanobacteria) coculture using suppression subtractive hybridization. *Eng. Life Sci.* 2013, *13*, 185–193.

[28] Bold, H. C., The morphology of Chlamydomonas chlamydogama, Sp. Nov. *Bull. Torrey Bot. Club.* 1949, *76*, 101–108.

[29] Rice, E. W., Baird, R. B., Eaton, A. D., Clesceri, L. S. (Ed.), *Standard Methods for the Examination of Water and Wastewater, American Public Health Association.* American Water Works Association, Water Environment Federation 2005.

[30] Cirulis, J. T., Strasser, B. C., Scott, J. A., Ross, G. M., Optimization of staining conditions for microalgae with three lipophilic dyes to reduce precipitation and fluorescence variability. *Cytometry A* 2012, *81*, 618–626.

[31] Folch, J., Lees, M., Sloane-Stanley, G., A simple method for the isolation and purification of total lipids from animal tissues. *J. Biol. Chem.* 1957, *226*, 497–509.

[32] Mur, R., Skulberg, O. M., Utkilen, H., Cyanobacteria in the environment, in: Chorus, I. and Bartram, J. (Eds.) *oxic Cyanobacteria in Water: A guide to their public health consequences, monitoring and management.* World Health Organization, London 1999.

[33] Havens, K. E., Phlips, E. J., Cichra, M. F., Li, B. l., Light availability as a possible regulator of cyanobacteria species composition in a shallow subtropical lake. *Freshwater Biol.* 1998, *39*, 547–556.

[34] Källqvist, T., Hydroecological field experiment, in: *Incubation of Natural Phytoplankton in Lake Gjersjøen.* Norwegian Institute for Water Research, F-80402, Oslo 1981.

[35] Mur, L., Gons, H., Van Liere, L., Some experiments on the competition between green algae and blue-green bacteria in light-limited environments. *FEMS Microbiol. Lett.* 1977, *1*, 335–338.

[36] Zwietering, M., Jongenburger, I., Rombouts, F., Van't Riet, K., Modeling of the bacterial growth curve. *Appl. Environ. Microbiol.* 1990, *56*, 1875–1881.

[37] Yan, C., Zhao, Y., Zheng, Z., Luo, X., Effects of various LED light wavelengths and light intensity supply strategies on synthetic high-strength wastewater purification by Chlorella vulgaris. *Biodegradation* 2013, *24*, 721–732.

[38] Xu, B., Cheng, P., Yan, C., Pei, H. et al. The effect of varying LED light sources and influent carbon/nitrogen ratios on treatment of synthetic sanitary sewage using Chlorella vulgaris. *World J. Microbiol. Biotechnol.* 2013, *29*, 1289–1300.

[39] Richmond, A., *Handbook of Microalgal Culture: Biotechnology and Applied Phycology*, Wiley, Hoboken, NJ, USA 2008.

[40] Blair, M. F., Kokabian, B., Gude, V. G., Light and growth medium effect on Chlorella vulgaris biomass production. *J. Environ. Chem. Eng.* 2014, *2*, 665–674.

[41] Held, G., *Introduction to Light Emitting Diode Technology and Applications*, CRC Press, Boca Raton, FL, USA 2016.

[42] Carvalho, A., Silva, S., Baptista, J., Malcata, F. X., Light requirements in microalgal photobioreactors: An overview of biophotonic aspects. *Appl. Microbiol. Biotechnol.* 2011, *89*, 1275–1288.

[43] Matthijs, H. C., Balke, H., Van Hes, U. M., Kroon, B. et al. Application of light-emitting diodes in bioreactors: Flashing light effects and energy economy in algal culture (Chlorella pyrenoidosa). *Biotechnol. Bioeng.* 1996, *50*, 98–107.

[44] Mohsenpour, S. F., Willoughby, N., Luminescent photobioreactor design for improved algal growth and photosynthetic pigment production through spectral conversion of light. *Bioresour. Technol.* 2013, *142*, 147–153.

[45] Park, J., Seo, J., Kwon, E. E., Microalgae production using wastewater: Effect of light-emitting diode wavelength on microalgal growth. *Environ. Eng. Sci.* 2012, *29*, 995–1001.

[46] Suh, I. S., Lee, C.-G., Photobioreactor engineering: design and performance. *Biotechnol. Bioprocess Eng.* 2003, *8*, 313–321.

[47] Yeh, N., Chung, J.-P., High-brightness LEDs—Energy efficient lighting sources and their potential in indoor plant cultivation. *Renewable Sustain. Energy Rev.* 2009, *13*, 2175–2180.