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Maximizing Algal Growth in Batch Reactors Using Sequential Change in Light Intensity

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Abstract Algal growth requires optimal irradiance. In photobioreactors, optimal light requirements change during the growth cycle. At low culture densities, a high incident light intensity can cause photoinhibition, and in dense algal cultures, light penetration may be limited. Insufficient light supply in concentrated algae suspensions can create zones of dissimilar photon flux density inside the reactor, which can cause suboptimal algal growth. However, growth of dense cultures can also be impaired due to photoinhibition if cells are exposed to excessively high light intensities. In order to simultaneously maintain optimal growth and photon use efficiency, strategies for light supply must be based on cell concentrations in the culture. In this study, a lipid-producing microalgal strain, *Neochloris oleoabundans*, was grown in batch photobioreactors. Growth rates and biomass concentrations of cultures exposed to constant light were measured and compared with the growth kinetic parameters of cultures grown using sequentially increasing light intensities based on increasing culture densities during batch growth. Our results show that reactors operated under conditions of sequential increase in irradiance levels yield up to a 2-fold higher biomass concentration when compared with reactors grown under constant light without negatively impacting growth rates. In addition, this tailored light supply results in less overall photon use per unit mass of generated cells.

Keywords Sequential change in light · Algal growth · *Neochloris oleoabundans* · Batch culture · Photobioreactor · Algae biofuels

Introduction

Microalgae can serve as feedstocks for a variety of products including biodiesel [1, 2], biohydrogen [3], pigments, polyunsaturated fatty acid [4, 5], and for environmental

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purposes such as capture of CO_2 from flue gases [6, 7] and remediation of environmental pollutants [8]. There are two primary options for mass production of algae—open systems such as raceway ponds or closed photobioreactors. Outdoor culture systems, while cheaper, have been shown to have lower long-term productivity due to limited options for light and carbon management as well as susceptibility to contamination [5, 9]. On the other hand, while initial capital investments for photobioreactors are higher than those for open ponds, better contaminant management and improved utilization of photosynthetically active radiation as well as carbon can lead to superior overall productivities [10].

Since phototrophic growth requires inputs of light and inorganic carbon (most often derived from dissolved carbon dioxide), algae culturing can potentially be limited by inadequate availability of either of these components to growing cells. When culture densities increase during growth, cellular absorption of light as well as other optically relevant effects such as scattering and shading results in a significant decrease in overall light penetration into culture vessels [11, 12]. This creates zones of dissimilar photon flux density (PFD) inside the reactor with light availability mostly restricted to regions near the illuminated surfaces. Consequently several reactor designs, including flat-plate and tubular-type, seek to maximize the ratio of illuminated surface to volume [13]. Mixing significantly improves photon distribution to the growing cultures in these reactors by assisting the periodic transport of cells into the lighted regions of the photobioreactors [14–16]. Higher growth yields are generally observed in well mixed reactors [17, 18]. Other designs integrate optics with growth reactors using sunlight collection and distribution devices [19, 20]. Such optical designs seek to deliver light to growing reactors based on photosynthetic needs and thereby improve efficiency of photon use as well as areal productivity [19].

When culture densities are low during early stages of logarithmic growth, light effectively penetrates through the entire culture medium, and a low light flux is adequate. In dense suspensions, higher intensity light is required to penetrate deeper and be more available to growing cultures. Thus, supply of light can be tailored to the requirements of algal cultures as they progress through their growth cycle to enhance light use while achieving high cell concentrations. Previous studies have shown that light control in photobioreactors can serve to improve growth rates [21, 22] as well as final biomass concentrations [22, 23]. However, these studies utilize sophisticated control methods that include frequent changes in light intensities. In large-scale systems processing several million gallons of algal cultures for biofuel production, such precise controls are likely to be difficult to implement, and perhaps unnecessary. Simpler methods that involve only periodic alteration of growth parameters are more practical. Based on this supposition, we hypothesized that phototrophic growth could be sustained to reach high algal culture densities by a "fed-batch" approach to light supply. In such a system, increase in incident intensities would serve to replenish light within the bioreactor when photon limitations became evident from a decline in growth rate.

In this study, we tested our hypothesis using a lipid-producing microalgal strain *Neochloris oleoabundans* [24, 25] grown in externally illuminated stirred tank reactors. We observed that after the cultures stopped growing at a low incident photon flux, a step increase in illumination resulted in resumption of growth. Overall, better biomass concentrations were observed when growth started at low light intensities and progressed higher in comparison to cultures illuminated at higher initial levels. Kinetic parameters related to growth and nitrogen consumption were measured and are compared between the different phototrophic growth regimes tested.

Materials and Methods

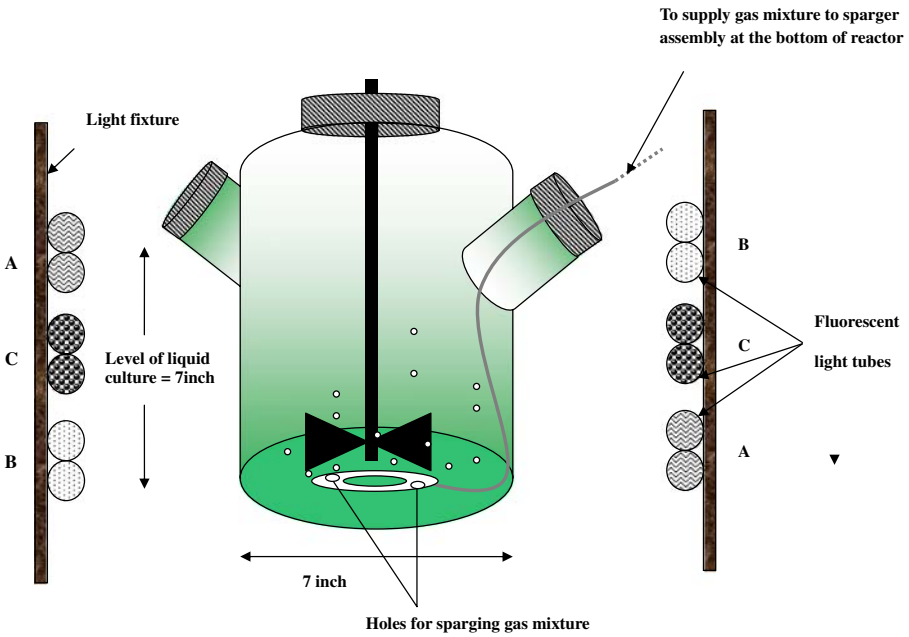
Organism, Media and Growth Conditions

N. oleoabundans (UTEX # 1185) cultures were grown in a modified Bristol media that contained the following: $NaNO_3$ (9 mM), KH_2PO_4 (1.4 mM), $MgSO_4.7H_2O$ (0.3 mM), $CaCl_2.2H_2O$ (0.17 mM), NaCl (0.43 mM), and ferric ammonium citrate (15 mg/L). Media pH was adjusted to 7.0 prior to autoclaving (121 °C, 20 min). The 100-mL cultures were first grown in 250-mL Erlenmeyer flasks on an illuminated shaker table (120 rpm), and log phase cultures were transferred to 6-L Cytostir® reactors (Kimble/Kontes, Vineland, NJ, USA) (working volume=5 L) that were used to perform growth studies reported here. All experiments were performed at room temperature (20 °C).

Experimental Setup

To determine the effect of different light intensities on algal biomass accumulation under constant illumination and the range of light intensities supporting maximal algal growth, *N. oleoabundans* cultures were grown at six different levels of irradiance. The irradiance was provided by fluorescent light tubes placed around the bioreactors, and different light levels were attained by changing the distance between fluorescent tubes and the bioreactors. A schematic of the experimental setup that depicts an end-on view of the arrangement is shown in Fig. 1. For the "sequential light change experiments," the reactors were illuminated by a bank of 12 (six on each side of bioreactor) Ecolux Sunshine 40 W fluorescent tubes (GE Lighting, Cleveland, OH, USA) set on a frame such that the lights were 3 in. away from the vessel walls. The length of the frame was 40 in. and using this setup, light could be supplied to three reactors when placed adjacent to each other (3 in. apart). For our experiments, three levels of light intensity were created by switching on four, eight, or 12 (two, four, or six each on both sides) light tubes. The location of the light tubes that remained illuminated at each intensity level and the corresponding average photon flux at the center of the reactor is depicted in the table inset in Fig. 1. Average light intensity was determined as the mean value of the irradiance measured in five directions (N–S–E–W and upward) at the center of the base of empty reactor placed in light enclosure. Light measurements were performed using a light meter (model LI-250A, Li-Cor Biosciences, Lincoln, NE, USA) equipped with a quantum sensor (model LI-190SA, Li-Cor Biosciences, Lincoln, NE, USA). The average light intensities with four, eight, or 12 fluorescent tubes switched were calculated to be 91.2, 177.8, and 273.1 $\mu mol\ m^{-2}s^{-1}$, respectively.

The reactors for the experiments were placed on stir plates that were used to rotate the built-in paddle impellers. An air–CO_2 mixture was sparged through each reactor, and flow rates were monitored using gas flow meters. The entire reactor setup containing media and sparge system was autoclaved prior to inoculation. After start of the experiments, gas flow rates were manually adjusted using in-line valves to maintain circum-neutral pH. This method of pH adjustment has been successfully used in previous studies [26], and in our experiments, the overall gas flow changes required to maintain near-neutral pH were only 10% higher than the initial set point. Besides pH control, gas sparging supplemented inorganic carbon to the algal cultures and also provided a means for axial mixing in the reactor in addition to the radial fluid motion provided by the reactor paddles. Throughout the experiments, the cultures were exposed to a light–dark cycle of 12 h that was maintained using a timer connected to the light circuit.



Experimental Schemes (light intensities measured in µmoles/m^2/s)

		Scheme I	Scheme II
	Tubes A on both sides are on	Level 1= 91.2	
	Tubes A and B on both sides are on	Level 2= 177.8	Level 2 = 177.8
	Tubes A, B, and C on both sides are on	Level 3 = 273.1	Level 3 = 273.1

Fig. 1 Schematic diagram of the reactor and illustration of light levels

Experimental Design

Initial experiments were performed at six PFDs—70.8, 91.2, 130.4, 177.8, 220.0, and 273.1 µmol $m^{-2}s^{-1}$ to determine the effect of different light intensities on algal biomass accumulation under constant illumination. Thereafter, effects of sequential increase in incident light intensities on growth were studied using three light levels and two illumination schemes. All the three light levels were used sequentially in scheme I, whereas in scheme II, only levels 2 and 3 were employed in sequential order. In illumination scheme I, growth was started at a light intensity equal to 91.2 µmol $m^{-2}s^{-1}$ (scheme I, level 1) and sequentially increased to 177.8 µmol $m^{-2}s^{-1}$ (scheme I, level 2) and finally up to 273.1 µmol $m^{-2}s^{-1}$ (scheme I, level 3). In the illumination scheme II, light intensities of 177.8 (scheme II, level 2) and 273.1 µmol $m^{-2}s^{-1}$ (scheme II, level 3) were sequentially employed. During both illumination schemes, changes to higher light levels were made when growth was observed to stop at the lower intensity.

Analytical Methods

During growth studies, 15 mL of sample was periodically withdrawn from each reactor and analyzed for optical density, total suspended solids (TSS), and nitrate. For all samples, optical density was measured as absorbance at 685 nm using a Spectronic Genesys 5 spectrophotometer (Thermo Fisher Scientific Inc., Waltham, MA, USA). At higher culture densities, TSS measurements were also made using procedures outlined in the Standard Methods recommended by the American Public Health Association (method 2540 D) [27]. Due to poor method sensitivity at low culture densities, TSS was measured only when at least partial growth was achieved. Correlations between optical density and TSS were developed and TSS concentrations in dilute cultures were determined from the calibration based on absorbance data. Nitrate (NO_3–N) was measured by the Cadmium reduction (method 4500 –NO_3^-I) [28] using Hach nitrate analysis kits (Hach Company, Loveland, CO, USA).

Calculations

Total moles of incident photons during algal growth were calculated based on incident light flux as

$$N_{SL} = A_{eff}(I_{in}\Delta t) \tag{1}$$

where N_{SL} represents the total incident photons during growth at Lth level under Sth illumination scheme of the experiment. I_{in} is the average incident light flux or PFD (μmol $m^{-2}s^{-1}$) at that level, and Δt represents the total duration during which light irradiance was available to the algal culture at that light level in the given scheme of experiment. A_{eff} is the effective surface area of the bioreactor (m^2) that is calculated as the projected area of liquid culture exposed normal to the incident light and given by

$$A_{eff} = \pi DH \tag{2}$$

where H is the liquid depth in the reactor (=7 in.), and D is the internal diameter of the reactor (= 7 in.).

For example, total incident photons during growth at level 2 in scheme II of the experiment can be calculated using I_{in}=177.8 μmol $m^{-2}s^{-1}$, Δt=13 d at 12 hr/d=561,600 s, and A_{eff}=$\pi\times7\times7$ in.^{2}=154 in.^{2}=0.1 m^2.

$$\text{Hence, } N_{22} = 9.98 \times 10^6\ \mu\text{mol}$$

Rates of growth $\Delta X/\Delta t$ were calculated as the slope of the linear portion of the growth curve, whereas biomass yields per gram of NO_3–N consumption, $Y_{X/N}$, were calculated as the ratio of biomass accumulated during a given time period in an illumination scheme and NO_3–N consumed in that duration.

Results and Discussion

Photosaturation Studies with *N. oleoabundans*

In photobioreactors, available irradiance depends on geometry of the reactor in addition to incident light intensity [29]. Therefore initial studies were performed to determine the incident light flux that would result in favorable algae growth in our reactor set-up. These

tests were performed using constant irradiance in the range 70–273.1 μmol $m^{-2}s^{-1}$. Final biomass concentrations obtained at the end of these experiments were measured.

The results from these tests are shown in Fig. 2. Biomass concentrations were observed to first increase with incident PFD but after a certain level of illumination, a decrease in the final amount of biomass produced was observed, suggesting a behavior consistent with photosynthetic saturation. At low light intensity, the photosynthetic processing capabilities are not adequately utilized resulting in low bioproductivity. Higher illumination results in photosynthetic saturation where all the photons are fully utilized by the phototrophic organism, and at still higher intensities, the available photons are not fully utilized and instead get dissipated [30]. Eventually, as irradiance level increases, photosynthesis is inhibited [31]. Most commonly, photosynthesis saturation curves are reported in terms of microalgal growth rates [12, 21, 32]. However, since biomass concentrations in photobioreactors, such as stirred vessels, are also proportional to incident light intensities, yield based data can also describe the photosaturation effects [33]. In such systems, under conditions of moderate mixing, phototrophic growth rates have been reported to be proportional to biomass concentrations [33].

Based on Fig. 2, it appears that photosaturation occurs at approximately 180 μmol $m^{-2}s^{-1}$ and is consistent with other observations that report flux tolerances in the range 200–400 μmol $m^{-2}s^{-1}$ for microalgal cultures grown in photobioreactors [14]. To avoid inhibition of low density starting cultures, initial light intensities for subsequent tests were chosen to be 91.2 and 177.8 μmol $m^{-2}s^{-1}$.

Algal Growth Studies with Sequential Increase in Light Intensities

Growth data for the two sequential illumination tests are shown in Fig. 3. Nitrate concentrations monitored in these experiments to obtain nutrient-specific stoichiometric yield coefficients are also included. These data show the progressive accumulation of biomass over time and the corresponding nutrient consumption. It can be observed that the nitrogen consumption over the entire experimental duration was less than 50% of what was initially added. Also, less than half the phosphorus initially added was consumed (data not shown), suggesting that the growth was not nutrient limited. However, at each illumination level, growth stopped after a certain biomass concentration was achieved. Increase in light intensity led to a resumption of growth but the cultures reached stationary phase again without complete depletion of nutrients. This phenomenon is likely due to inadequate availability of light to sustain growth at higher cell concentrations due to the balance

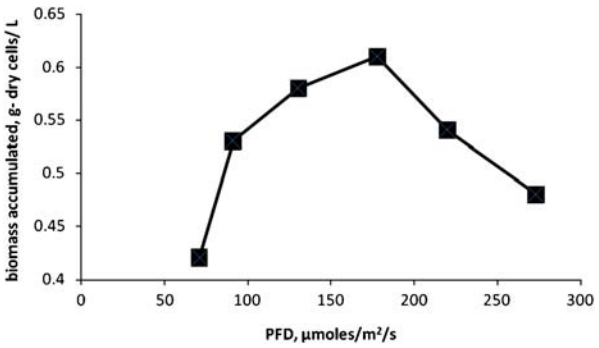


Fig. 2 Biomass accumulation at different incident light intensities with constant-light intensity throughout the growth

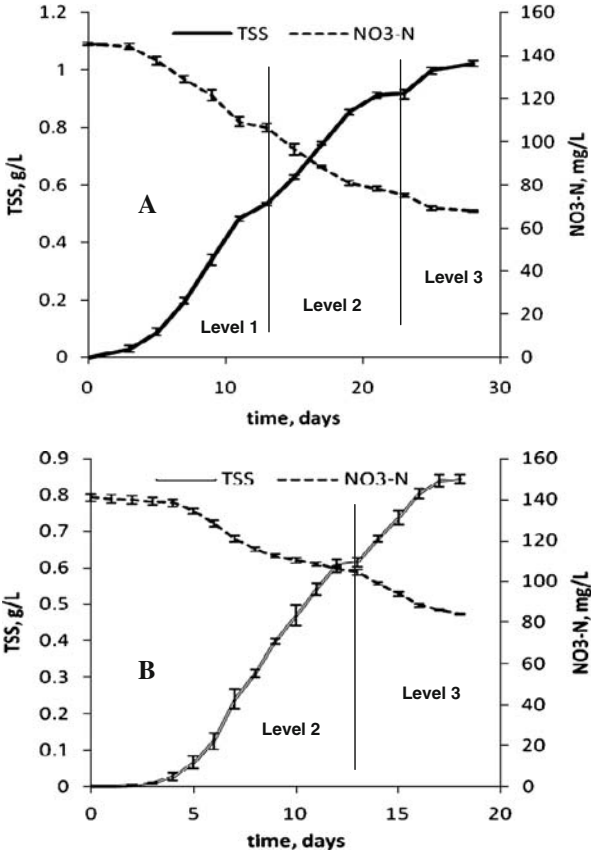


Fig. 3 Algal growth and NO_3–N uptake during studies under **a** illumination scheme I (sequentially illuminated with light intensities of 91.2, 177.8, and 273.1 μmol $m^{-2}s^{-1}$) and **b** illumination scheme II (sequentially illuminated with light intensities of 177.8 and 273.1 μmol $m^{-2}s^{-1}$)

between energy absorbed by the photosynthetic mechanism and that dissipated by respiration as hypothesized by previous research [34–36].

Overall biomass accumulation was enhanced when light intensities were sequentially increased under both illumination schemes (Fig. 3). When light flux closer to the photosynthetic saturation limit was initially used (illumination scheme II with initial PFD of 177.8 μmol $m^{-2}s^{-1}$), the growth rates and yields were higher, as expected (Table 1). Also, when suboptimal photon flux was used initially (illumination scheme I with initial PFD of 91.2 μmol $m^{-2}s^{-1}$), biomass yield based on nitrate consumption ($Y_{X/N}$) was lower. These results are corroborated by other previous research, which shows that algal cells show higher specific uptake of nitrogen when grown under low light and slower growth conditions in comparison to growth under higher light intensities that facilitate faster growth [37].

Figures 4 and 5 show the comparison between algal growth under illumination schemes I and II at lower (scheme I level 1 and scheme II level 2) and higher (scheme I level 2 and scheme II level 3) light levels, respectively. It can be seen that when the light intensity was increased to a higher level under both illumination schemes, growth rate trends were similar to those observed during lower level tests. Although illumination scheme II used light concentrations that were observed to be photoinhibitory during single light intensity experiments (Fig. 2), it appears that the inhibitory effect was mitigated due to exposure of the dense growing culture to this light intensity. Since the penetration of light is less in

Table 1 Yields and NO_3–N consumption at the end of different levels of growth in schemes I and II.

Illumination scheme	Level 1			Level 2			Level 3		
	ΔX_1, g cells/L avg. (standard deviation)	$\Delta X/\Delta t$, g cells L^{-1} day^{-1} avg. (standard deviation)	$Y_{X/N}$, g cells/g N avg. (standard deviation)	ΔX_2, g cells/L avg. (standard deviation)	$\Delta X/\Delta t$, g cells L^{-1} day^{-1} avg. (standard deviation)	$Y_{X/-N}$, g cells/g N avg. (standard deviation)	ΔX_3, g cells/L avg. (standard deviation)	$\Delta X/\Delta t$, g cells L^{-1} day^{-1} avg. (standard deviation)	$Y_{X/N}$, g cells/g N avg. (standard deviation)
I	0.53 (0.006)	0.050 (0.0005)	13.57 (0.98)	0.38 (0.010)	0.047 (0.002)	12.74 (1.04)	0.11 (0.015)	0.055 (0.003)	11.5 (0.71)
II				0.61 (0.013)	0.075 (0.001)	16.68 (1.23)	0.23 (0.011)	0.056 (0.002)	12.40 (0.92)

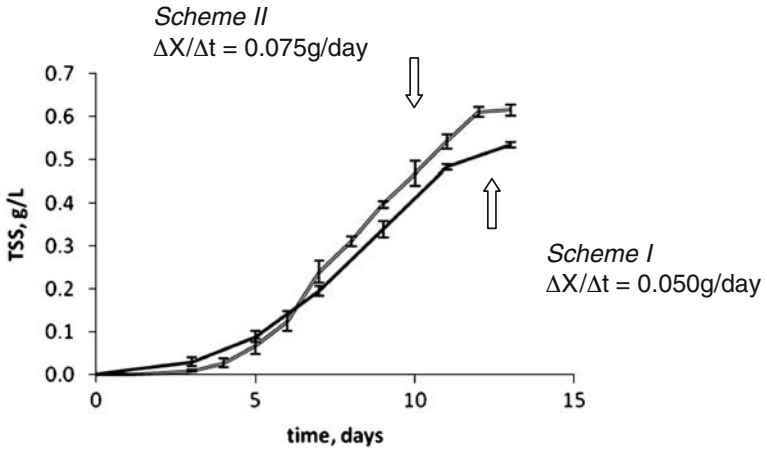


Fig. 4 Comparison between lower (first) light level growths under illumination schemes I and II. Photon flux densities during this stage of growth were 91.2 and 177.8 μmol $m^{-2}s^{-1}$, respectively, for the two illumination schemes

dense cultures, cells were likely exposed to the higher intensities for smaller periods as they moved in and out of the illuminated region due to mixing. This limited exposure likely prevented the inhibitory effects that would otherwise be observed if cultures were exposed to high light intensities initially when fewer cells are present in the liquid medium [14]. Although cultures grew faster under illumination scheme II that had overall higher light intensities, the final biomass concentrations (after exposure to light level 3) were higher during growth under illumination scheme I. It can be observed from Figs. 4 and 5 that under illumination scheme I, growth was prolonged, although slower, such that final culture densities were higher. One possible explanation for this behavior could be that under illumination scheme I, light intensities doubled between lower (level 1) and higher (level 2)

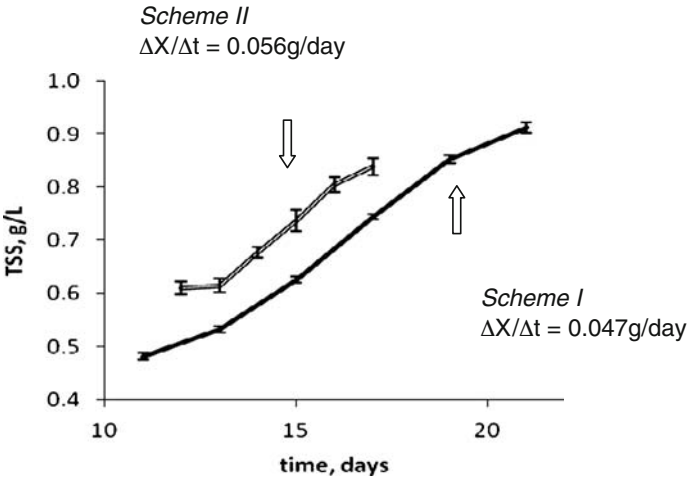


Fig. 5 Comparison between higher (second) light level growths under illumination schemes I and II. Photon flux densities during this stage of growth were 177.8 and 273.1 μmol $m^{-2}s^{-1}$, respectively, for the two illumination schemes

levels, whereas they increased by a factor of only 1.5 under scheme II. The bigger jump in light intensity in scheme I possibly allowed better penetration of light through the algal culture, which resulted in phototrophic activity for a longer duration.

During growth studies under illumination scheme I, a third level of light intensity (273.1 μmol $m^{-2}s^{-1}$) was applied when growth stopped after level 2. The cultures appeared to continue to grow as a result of this switching for first 2 days but showed no enhancement in cell concentrations after the third day. During this period (2 days), about 0.11 g/L of biomass was accumulated, yielding a growth rate of 0.055 g cells $L^{-1}day^{-1}$—the highest observed among all the levels in illumination scheme I. These unexpected high growth rates could be attributed to "flashing effect" where cells were likely exposed to very high intensities for short periods due to the small penetration depth of light in these dense cultures. It has been reported in previous studies that flashing or intermittent exposure of high intensity light increases the efficiency of photosynthesis by algal cells [14, 38], and it is possible that the fortuitous combination of mixing speeds, light intensity, and culture density resulted in this phenomenon in our tests.

Comparison of Culture Performance Between Illumination Conditions Tested

The concentrations of biomass accumulated at the end of every level of growth during both illumination sequences are shown in Table 1. We can consider level 1 tests in each illumination sequence experiment as the constant-light control since uniform illumination was supplied during this portion of growth. On comparing biomass accumulated at the end of first level with biomass obtained from the entire growth study under the individual illumination sequence (including all levels), we can get the net effect of sequential change in light intensity on overall algal biomass accumulation. Through simple calculations, we can see that during scheme I, the overall biomass accumulation ($\Delta X_1 + \Delta X_2 + \Delta X_3 =$ 1.02 g cells/L) increases by 1.92-fold as compared to level 1 growth (ΔX_1=0.53 gcells/L). Similarly, a 1.38-fold increase is observed during scheme II experiments ($\Delta X_2 + \Delta X_3 =$ 0.84 compared to ΔX_2=0.61). Another appraisal of the system performance is through measure of productivity under the two illumination schemes tested; 1.02 g cells/L were obtained over 22 days (neglecting the 2 days of no growth) when illumination scheme I was employed such that the productivity under this scheme was 0.046 gcells $L^{-1}day^{-1}$. Productivity with illumination scheme II can similarly be calculated to be 0.0525 gcells L^{-1} day^{-1} showing that although final biomass concentrations were lower during growth on higher intensity lights, overall productivities stayed higher.

One final measure of system performance is through evaluation of efficiency of photon energy use during the two illumination schemes. Table 2 shows total incident photons on the liquid culture during different levels of algal growth. Using these data, it can be calculated that total moles of photons supplied under illumination scheme I ($N_{11}+ N_{12} + N_{13} = 10.55$ mol) is only 0.66 times of photons supplied under illumination scheme II ($N_{22} + N_{23} = 15.88$ mol). However, the final biomass accumulation at the end of scheme I ($\Delta X_1 + \Delta X_2 + \Delta X_3 = 1.02$ g cells/L) is 1.21 times of biomass accumulated at the end of

Table 2 Total moles of incident photons, N_{SL}, supplied to the culture at different stages of the experiment.

Illumination scheme	Level 1	Level 2	Level 3
I	$N_{11} = 5.12 \times 10^6$ μmol	$N_{12} = 3.07 \times 10^6$ μmol	$N_{13} = 2.36 \times 10^6$ μmol
II		$N_{22} = 9.98 \times 10^6$ μmol	$N_{23} = 5.90 \times 10^6$ μmol

scheme II ($\Delta X_2 + \Delta X_3 = 0.84$). This suggests that it is more energy efficient to start culture growth with lower intensity light to maximize utilization of incident radiation.

Conclusions

The sequential change in light intensity or PFD during algal growth studied in this research has shown to improve algal growth up to 2-fold. There are multiple factors that affect the growth rate and final cell concentration in this process, which include starting incident light intensity, the difference in light intensity levels or jump in light levels, and the number of light levels used over the favorable range of illumination for a specific algal strain. This study also showed that the utilization of supplied light not only depends on intensity of incident light but also on the culture density. Our study has also shown that simple periodic increase in light intensity can effectively increase the overall performance of photobioreactors.

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