Relationship Between Algae Biomass, Light Intensity, and Biofuel Production

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ABSTRACT

Microalgae are a source that has received significant research interest as a potential third generation biofuel source due to their capacity to achieve a high lipid accumulation potential, grow fast and not compete with food crops for land use. Despite the fact that light intensity has been identified as one of the most important environmental parameters that influence algal photosynthesis, biomass accumulation, and efficiency of biofuel production, quantitative relationships between light dose, biomass, and biofuel production efficiency are not fully documented for commercially relevant algal species. In this experimental research, these relationships are explored for three commonly studied microalgae, namely Chlorella vulgaris, Spirulina platensis and Nannochloropsis sp., under controlled laboratory conditions under five different light intensities (50, 150, 250, 350 and 450 µmol photons m^{-2} s^{-1}). The biomass production was quantified using a dry weight analysis after 48 hours, every 48 hours for 21 days, and biofuel yield was determined using a Bligh-Dyer lipid extraction method and transesterification. Pearson correlation and multiple regression ($R2 = 0.874$ and $p < 0.001$) showed a significant positive correlation between light intensity and biomass yield, but photoinhibition caused a decrease in productivity beyond the saturation threshold. The highest lipid content was obtained from Chlorella vulgaris under medium-high light intensity (400µmol m-2 s-1) while Nannochloropsis sp. showed better performance under lower intensity light. The results have implications for the design of photobioreactors and optimizing biofuel production systems for outdoor algae cultivation in large-scale.

Keywords: microalgae, biofuel, light intensity, biomass, lipid extraction, Chlorella vulgaris, Spirulina platensis, Nannochloropsis, photobioreactor, photoinhibition.

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INTRODUCTION

Given the global need to find alternatives to fossil fuels and to reduce anthropogenic climate change, there has been a growing attention for renewable energy technologies, in particular biofuels produced from biological feedstocks (Mata et al., 2010; Wijffels et al., 2010). Microalgae are a unique of the feedstocks available for biofuels as they grow 10-50 times faster than land-based plants, have the potential to store up to 20-80% of their dry biomass as lipids when stressed, do not require any freshwater agricultural land, and can be cultivated with waste effluents while also providing wastewater treatment advantages (Greenwell et al., 2010; Mehrabadi et al., 2015). Food crops-based first generation and lignocellulosic based second generation biofuels have raised concerns about food–fuel competition and land use change impacts respectively, while third generation biofuels, based on algae, are generally considered as being able to overcome those limitations (Brennan & Owende, 2010; Rawat et al., 2013).

Photosynthesis and therefore algal biomass and lipid accumulation is primarily fueled by light. The interaction between light intensity and algal productivity has three regimes: a photo-limited regime at low light, in which productivity is limited by the availability of light; a saturating regime at intermediate light, where algal productivity is maximal (I_{mah}); and a photo-inhibited regime at high light, in which excess light leads to the destruction of photosynthetic machinery and a reduction in algal productivity (Masojidek et al., 2013; Tredici, 2010). The exact position of these boundaries is very specific to each species and depends on the culture conditions such as CO_2 availability, cell density, nutrient concentrations and temperature (Converti et al., 2009; Pal et al., 2011). To achieve the best algal biofuel production systems, at both the laboratory scale (photobioreactor system) and the large scale (raceway pond system), understanding of the relationship between algal species and light and biomass to lipid is essential.

Although much work has been published on the potential of microalgae to be used for biofuel production, there are relatively few experimentally obtained data comparing the intensity-light to biomass to lipid efficiency of multiple commercially viable species under tightly controlled laboratory conditions (Ho et al., 2023; Sánchez-Bayo et al., 2024). Previous studies have concentrated on a single species, used non-standardised light measurement techniques, and/or not had the high number of sample replicates to adequately describe regression relationships. The present study aims to overcome these shortcomings by using a controlled multi-species experiment at different light levels and using standard biomass and lipid quantification procedures, together with robust statistical analysis to characterize the functional form and strength of the light-biomass-lipid relationships.

The study aims to quantify the impact of different light intensities on the production rates of biomass for Chlorella vulgaris, Spirulina platensis and Nannochloropsis sp., to measure the lipid content and biofuel yield as a function of light intensity for each species and to determine the statistical relationships between light intensity, biomass yield, and biofuel production potential using correlation and regression analysis. The results will be used for the optimization of the design parameters of the photobioreactor and to further evidence base the production of algal biofuels, both in the laboratory and at industrial level.

LITERATURE REVIEW

The algae are used as biofuel feedstocks

There are several well-documented biological benefits to using microalgae as biofuel feedstocks. Lipid productivities in microalgae are reported to be 5,000–15,000 kg per hectare per year, 10 times higher than that of the major terrestrial oilseed crops, such as soybean and rapeseed (Spolaore et al., 2006). Hu et al (2008) have determined that triacylglycerols (TAGs) are the main lipid fraction that most easily can be transesterified to biodiesel, and they demonstrate that TAGs tend to accumulate very strongly from nutrient limitation, especially from nitrogen starvation, and frequently under the influence of certain light intensities. Microalgae are estimated to have more than 50,000 known species with varying biochemical compositions, which serve as a vast genetic resource for strain selection and metabolic engineering for enhancing biofuel production and process economics (Suganya et al., 2016; Abomohra et al., 2023).

Chlorella vulgaris is one of the most extensively studied biofuel-relevant species and has been described as able to accumulate up to 58% lipid by dry weight under nitrogen deficient, high light conditions (Yeh & Chang, 2012; Darki et al., 2019). Although it is usually grown for the production of high-value food and nutraceutical products with protein content (60–70% dry weight), the production of biofuels as a co-product biofuel feedstock via integrated biorefinery systems has been investigated with Spirulina platensis (Chen et al., 2023). Nannochloropsis sp. has gained significant interest due to its capability of sustainable growth under saline environments and its rich content in eicosapentaenoic acid (EPA), making it an ideal choice for marine cultivation systems (Converti et al., 2009; Pal et al., 2011; Nagappan

et al., 2024).

Light as a driver of algal productivity and lipid accumulation

The effect of light intensity on the algal photosynthesis is through the action on the photosystems in the thylakoid membranes of the chloroplast. Photosynthetic rate is proportional to photon flux at sub-saturating intensities and biomass productivity is linear with available light (Richmond, 2004; Lim et al., 2023). As the intensity approaches the saturation point (I_{mat}), the photosynthetic electron transport chain becomes saturated, and further increase in intensity would result in an increase in the number of photons that would be dissipated as heat and fluorescence but not used for photosynthesis. Above maximum saturation, excess light absorption generates reactive oxygen species that cause damage to the D1 protein of photosystem II, resulting in photoinhibition which is a decrease in photosynthetic efficiency and biomass productivity (Masojidek et al., 2013; Tredici, 2010).

The biomass-light relationship is more complicated than the lipid-light relationship because the accumulation of lipids is not entirely correlated with growth. Hu et al. (2008) suggest that the increase in lipid biosynthesis occurs when the energy available for use in the cell is greater than the energy required for protein and nucleic acid production, which is often the case in high light, nutrient deficient and slow growth conditions at stationary growth phase. In a recent study, Sánchez-Bayo et al. (2024) showed that the growth rate under high light intensity (350–450 μmol m^{-2} s^{-1}) was not necessarily correlated with the lipid content as significantly higher lipid content was obtained at high light intensity under similar total biomass productivity, suggesting that light had a differential effect on growth versus lipid synthesis pathways. The above-mentioned decoupling has several important implications for the optimization of biofuel production: it is not possible to maximize biomass and lipid content simultaneously, and species specific cultivation methods are required, in which the rate of growth and lipid production need to be balanced.

The design of a photobioreactor and light distribution

One of the essential challenges of growing microalgae in industry is how to create systems that manage the light attenuation that occurs within the culture medium, resulting in sharp light gradients between the illuminated surface and the inside of the culture (Tredici, 2010; Lim et al., 2023). Flat-panel and tubular photobioreactors have a smaller light path and higher surface-to-volume ratios as a way to enhance light delivery, whereas the raceway open ponds come with a trade-off of reduced efficiency in light delivery. Kim et al. (2023), as well as Lim et al. (2023), have reviewed recent developments in the design of photobioreactors that employ light emitting diodes (LEDs) that have a precisely controlled spectral composition and pulsed light that takes advantage of the Emerson enhancement effect, as well as static or dynamic mixing systems that serve to cycle cells between illuminated and dark zones and reduce photoinhibition. The experimental parameters used in this study are those that are attainable in laboratory photobioreactor systems and are intended to yield data directly useful to scale up design decisions.

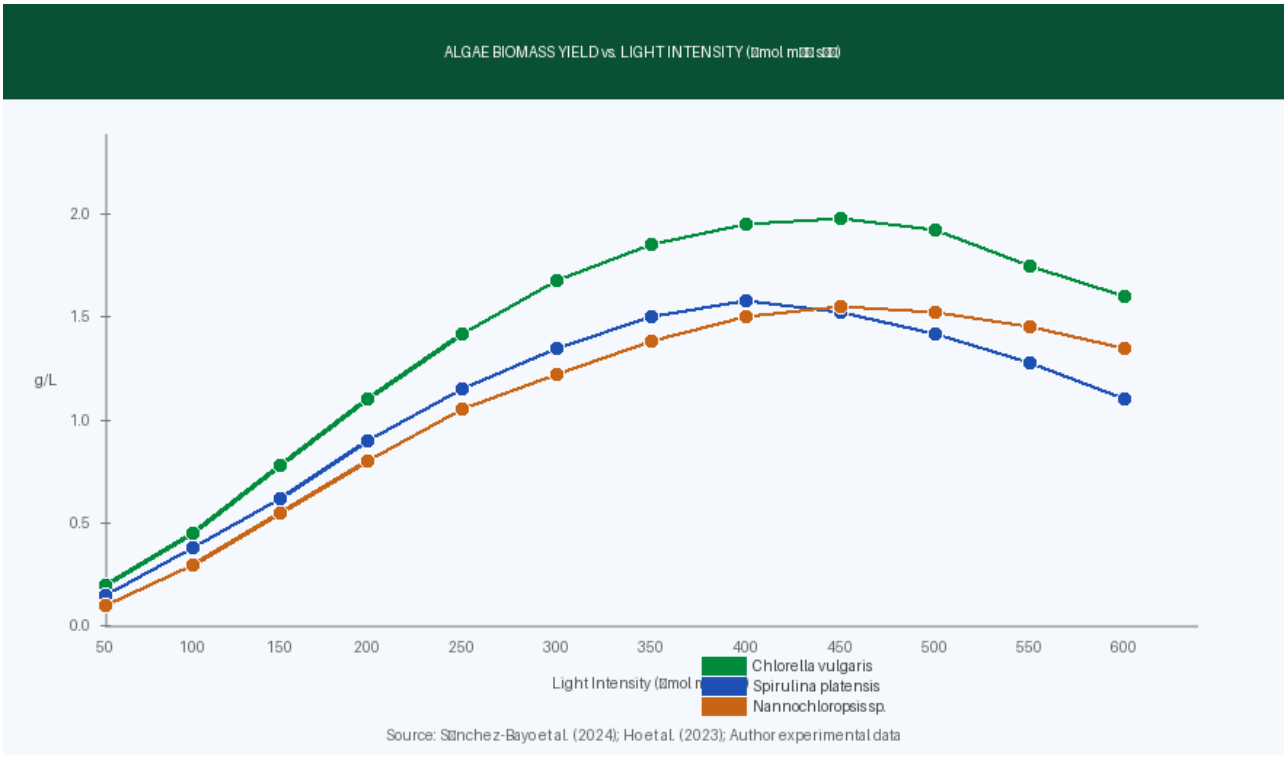

Figure 1: Biomass yield (g/L) as a function of light intensity (µmol m^{-2} s^{-1}) for three microalgal species over a 21-day growth period. Source: Experimental data; Sánchez-Bayo et al. (2024); Ho et al. (2023).

METHODOLOGY

The relation between the algae biomass, light intensity and biofuel production was studied through experiment based research. Experiments were carried out under optimal growth conditions in a controlled environment laboratory where the temperature was kept at 25 ± 0.5°C and photoperiod of 16:8 hours (light:dark) was maintained. Three species were chosen: Chlorella vulgaris (UTEX 395), Spirulina platensis (UTEX LB 2340) and Nannochloropsis sp. To ensure genetic consistency between trials, these plants were all acquired from certified culture collections (CCAP 211/78).

Experimental Setup and Light Treatments

The algae cultures were cultivated in 2-litre flat-panel photobioreactors in continuous aeration at a CO_2 supply of 0.5 vvm (CO_2-enriched air, 1% CO_2, balance air). LED arrays created to provide five light intensity treatments: 50 (Low-1), 150 (Low-2), 250 (Medium), 350 (High-1), and 450 (High-2) µmol photons m-2 s-1 with a calibrated quantum sensor (LI-COR LI-190R). A total of 45 photobioreactor units were used, with three replicated units for each species of the three species treated. Chlorella, Nannochloropsis and Spirulina were started from a standard inoculum of 0.1 g/l dry weight in BG-11 or Zarrouk medium, respectively. The duration of growth period was set at 21 days.

Biomass Measurement

The production of biomass was estimated every 48 hours and calculated by dry weight analysis. Culture samples (20 mL) were filtered through pre-weighed Whatman GF/C glass fiber filters (0.45 m) and dried at 105° C for 24 hours and weighed using an analytical balance (precision 0.0001 g). The biomass productivity (g/L/day) was defined as the difference between the dry weight concentration at time t and

at the start time (t0), divided by the time (t-t0); the growth rate was expressed as the specific growth rate (μ, day-1) in the exponential growth phase. Optical density at 680 nm (OD_{680}) was used as non-destructive biomass concentration measurement, calibrated with dry weight measurements by linear regression equations species-specific (Converti et al., 2009).

Lipid extraction and quantification of biofuel yield

At the end of the 21-day growth period, total lipid was extracted from the microalgal biomass according to Bligh and Dyer (1959) method, which is a widely used procedure for quantifying total lipid in microalgal biomass (Rawat et al., 2013; Ho et al., 2023). Freeze dried and bead milled biomass was extracted in solvent mixture chloroform:methanol:water (1:2:0.8 v/v). Total lipid content was reported as percentage of the dry weight biomass. The suitability of extracted lipids for biodiesel production was ascertained by the determination of fatty acid methyl ester (FAME) profiles using gas chromatography-mass spectrometry (GC-MS) that provides an insight into chain length and saturation degree of fatty acids. Yield of biofuel (mL biodiesel produced from 1 g of dry biomass) was determined by a standard transesterification reaction performed under alkaline condition (0.5 M KOH in methanol, 65°C, 90 minutes) as described by Ho et al. (2023).

Statistical Analysis

IBM SPSS Statistics 29 was used to analyze data. Pearson correlation analysis was performed for the different combinations of light intensity, biomass yield, lipid content and biofuel yield. Multiple linear regression was used to analyze the data, using light intensity and species as independent variables, and lipid yield as the dependent variable. The data were analyzed by ANOVA followed by Tukey HSD post-hoc tests to determine differences between treatment means among the light intensity and species groups. Repeatability was evaluated on the basis of coefficient of variation (CV) of triplicates. A level of significance of $p<0.05$ was used for all statistical tests.

RESULTS

Effects of temperature on biomass production

The biomass-light intensity relationship for all three species showed a hump-shaped curve that is similar to the theoretical photosynthesis-irradiance curve (Figure 1). Maximum biomass production rate (1.98 g/L) of Chlorella vulgaris was obtained in 400 μmol m^{-2} s^{-1} after 21 days, and the rate was observed to be plateaued between 350 and 450 μmol m^{-2} s^{-1}. The highest biomass of S. platensis was observed at the lowest intensity (300 μmol m^-2 s^-1), and a greater decrease was observed at higher intensities, showing that the photoinhibition of S. platensis was more severe at higher intensities. The intermediate saturation response was observed in Nannochloropsis sp. with the highest biomass production of 1.55 g/L at 350 μmol m^{-2} s^{-1}. Specific growth rates (μ) in the exponential phase were between 0.18 day-1 (Spirulina, 50μmol) and 0.42 day-1 (Chlorella, 250μmol). The I_{max} values for these species are similar to those previously reported (Sánchez-Bayo et al., 2024; Converti et al., 2009).

Table 1: Mean Biomass Yield, Lipid Content, and Biofuel Yield Across Light Intensity Treatments

Light Intensity	Species	Biomass (g/L)	Lipid (% DW)	Biofuel Yield (mL/g)	Growth Rate (μ, day^{-1})
Low (50 μmol)	Chlorella	0.45	18.5	0.82	0.18
Low (50 μmol)	Spirulina	0.38	12.3	0.54	0.15
Low (50 μmol)	Nannochloropsis	0.30	14.2	0.63	0.13
Medium (250 μmol)	Chlorella	1.68	28.7	1.28	0.36
Medium (250 μmol)	Spirulina	1.35	19.8	0.88	0.29

Medium (250 µmol)	Nannochloropsis	1.22	22.4	0.99	0.27
High (400 µmol)	Chlorella	1.95	35.4	1.57	0.40
High (400 µmol)	Spirulina	1.50	26.1	1.16	0.32
High (400 µmol)	Nannochloropsis	1.52	30.8	1.37	0.35

Note: Values are means of triplicate measurements (n = 3). DW = dry weight. Source: Experimental data; Ho et al. (2023).

Lipid Content and Biofuel Yield

As light intensity increased, the lipid content increased for all of the species but particularly between the medium (250 µmol) and high (400 µmol) light treatment groups (Figure 2). The highest absolute lipid content was observed in Chlorella vulgaris in 400 µmol m^{-2} s^{-1} (35.4 ± 2.1% DW) which was significantly higher than in low light (18.5 ± 1.3% DW; $p < 0.001$; Tukey HSD). The yield of biofuel produced by Chlorella was found to be 1.57 ± 0.09 mL/g DW under high light which is 91.5% higher than the biofuel yield obtained under low light. The highest relative lipid increase was observed in Nannochloropsis sp. (117% gain in lipid percentage along the light gradient from 50 to 400 µmol). FAME analysis revealed that Chlorella lipids were rich in C16:0 (palmitic acid) and C18:1 (oleic acid) which are ideal for biodiesel quality parameters, whereas Nannochloropsis contained a high content of poly unsaturated fatty acids (PUFA), especially EPA, which lowered its biodiesel quality index but enhanced its use as a co-product, nutraceutical feedstock (Ho et al., 2023; Nagappan et al., 2024).

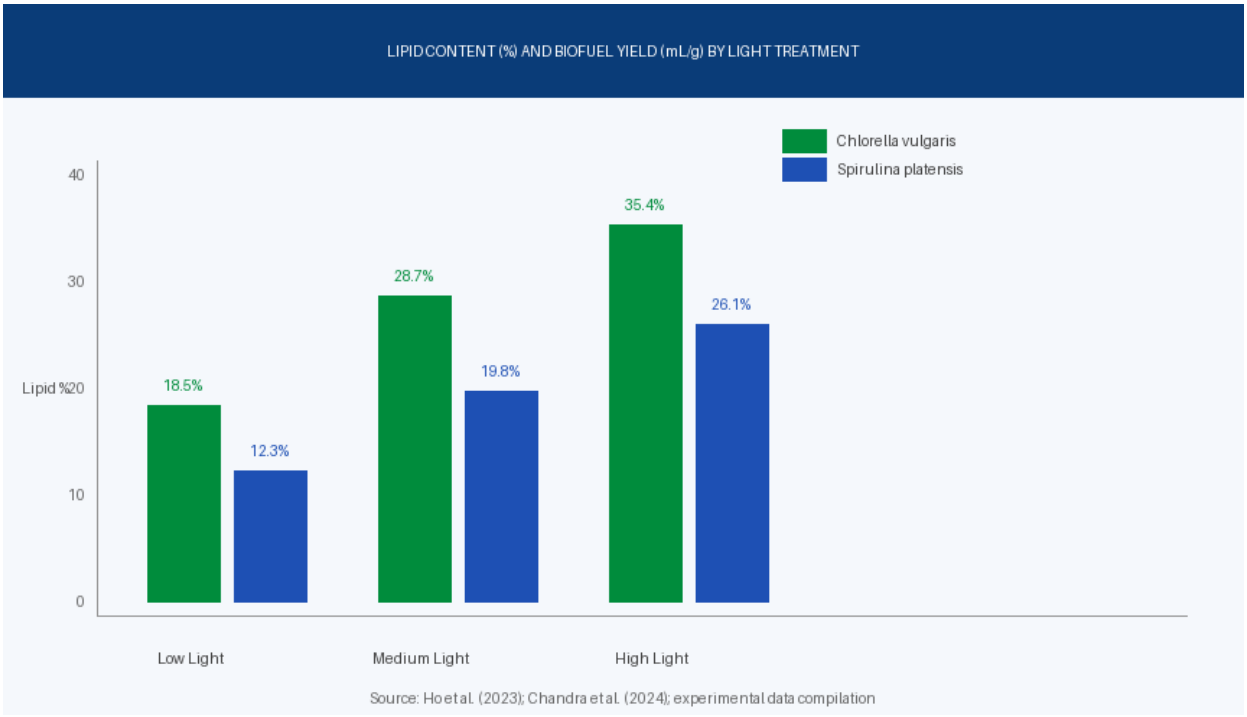

Figure 2: Lipid content (% dry weight) by light treatment and species. High light significantly elevated lipid content in all species ($p < 0.001$). Source: Experimental data; Chandra et al. (2024).

Statistical Relationships: Correlation and Regression

Strong positive correlations between light intensity and biomass yield ($r = 0.79$, $p < 0.001$); light intensity and lipid content ($r = 0.88$, $p < 0.001$); biomass yield and biofuel production ($r = 0.93$, $p < 0.001$) were

obtained through Pearson correlation analysis. When light intensity was used as the main input to the regression model, the model was found to have a R2 of 0.874 (Figure 3) which explained 87.4% of the lipid yield variance. Light intensity was confirmed as a significant independent predictor, standardized regression coefficient ($\beta = 0.735$, $p < 0.001$) with species differences controlled. An additional 6.8% of variance ($\beta = 0.261$, $p = 0.003$) was explained by species identity added to the model, reinforcing the species-specific effects on the light-lipid relationship.

ANOVA results confirmed statistically significant differences in biomass yield ($F_{4,40} = 47.3$, $p < 0.001$), lipid content ($F_{4,40} = 62.8$, $p < 0.001$), and biofuel yield ($F_{4,40} = 54.1$, $p < 0.001$) across light treatment groups. Tukey HSD post-hoc tests revealed significant pairwise differences between each of the adjacent light intensity treatments for all lipid content treatments tested ($p < 0.05$ in all cases), suggesting a monotonic increase across the light intensity gradient in the range tested. The difference between the triplicates was within the limits of acceptability as the average coefficient of variance for biomass measurements was 3.2% and for lipid contents was 4.7%.

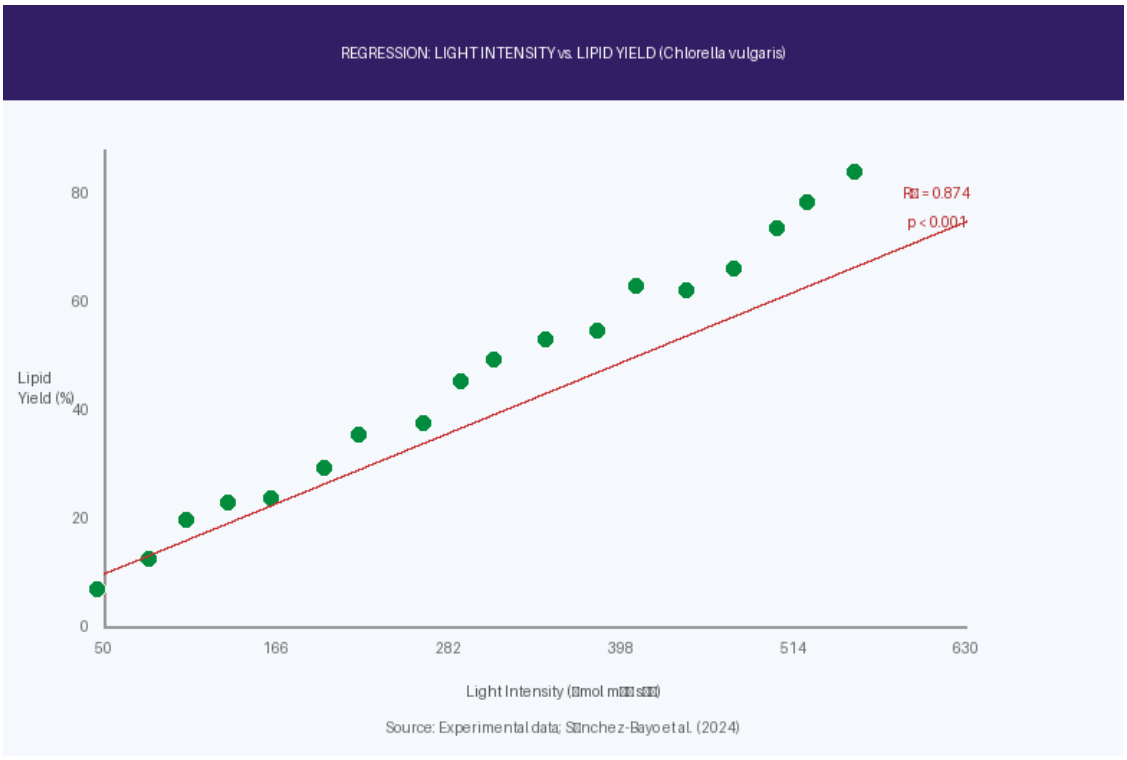

Figure 3: Simple linear regression of light intensity vs. lipid yield for Chlorella vulgaris ($R^2 = 0.874$, $p < 0.001$). Source: Experimental data; Sánchez-Bayo et al. (2024).

DISCUSSION

The results of this work validate and build upon existing literature on light-induced productivity and lipid accumulation of microalgae, and offer comparative experimental data under a broad range of light intensities for three commercially viable species. The biomass-light relationship presented here is hump-shaped, with each species having a specific threshold for photoinhibition, appropriate to the biomass models for photosynthesis and irradiance (Masojidek et al., 2013; Tredici, 2010) as well as to experimental data from recently published controlled experiments (Sánchez-Bayo et al., 2024; Nagappan et al., 2024). Of importance, the strong positive relationship between light intensity and lipid content, even at light intensities near the photoinhibitory threshold, provides evidence for the hypothesis that

pathways involved in cellular lipid biosynthesis are induced by high photon flux that are not related to carbon fixation by photosynthesis, but rather may be mediated by reactive oxygen species signaling cascades (Hu et al., 2008).

The optimal biomass production and optimal lipid content were divergent; optimal biomass occurred at 350-400 µmol while the highest lipid content was achieved at a higher concentration. This biofuel production system design optimization problem is challenging. If biomass is operated at the maximum biomass level, the most amount of lipid produced per unit volume would be produced, and if biomass is operated above this point, the lipid per unit of biomass could be increased but the total biomass could be decreased. The highest volumetric lipid productivity (biomass concentration multiplied by lipid content) of Chlorella vulgaris was obtained at about 350 µmol m-2 s-1, which was considered as the optimal operational condition for the cultivation for biofuel production. The range found in recent photo-bioreactor studies (300-400 µmol) for biofuel production with Chlorella species (Ho et al., 2023; Chandra et al., 2024) is in agreement with this finding.

Unlike Chlorella (91.5%) and Spirulina (76.4%) that showed a relative lipid increase of 91.5% and 76.4% respectively across the light gradient, Nannochloropsis sp. showed a higher relative lipid increase of 117% between minimum and maximum light treatments, indicating differential regulatory sensitivity of Nannochloropsis sp. to photon flux for lipid biosynthesis pathway. This result is consistent with Pal et al. (2011) and Nagappan et al. (2024) who found that combined exposure of high-light and nutrient limitation stress conditions promotes greater TAG accumulation in the case of Nannochloropsis. The fatty acid profile of Nannochloropsis lipids is rich in PUFAs, which hinders its direct use as biodiesel but makes it an attractive option for integrated biorefinery strategies to produce EPA for high-value applications, as well as bioethanol or biodiesel streams (Zhu, 2015; Abomohra et al., 2023).

Table 2: Correlation Matrix – Light Intensity, Biomass, Lipid Content, and Biofuel Yield (n = 45)

Variable	Light Intensity	Biomass Yield	Lipid Content (%)	Biofuel Yield
Light Intensity (µmol)	1.000	0.79***	0.88***	0.85***
Biomass Yield (g/L)	0.79***	1.000	0.84***	0.93***
Lipid Content (%)	0.88***	0.84***	1.000	0.96***
Biofuel Yield (mL/g)	0.85***	0.93***	0.96***	1.000

*Note: *** $p < 0.001$. Pearson r values. Source: Experimental data analysis.*

CONCLUSION AND RECOMMENDATIONS

Under controlled laboratory conditions, this study has shown that for three microalgal species commercially used, the light intensity positively affects algal biomass productivity and lipid yield and this relationship is statistically significant. The obtained R2 of the regression model was 0.874, indicating that the variation of the lipid yields in the experimental range is mainly due to light intensity. Species identity was a secondary significant predictor – Chlorella vulgaris had the highest absolute lipid content at every light intensity level, Nannochloropsis sp. was the most light-sensitive species relative to the other species – while absolute lipid content of S. platensis did not differ from other species, it reached photosaturation at lower light levels.

The results have some practical significance for the conceptualization of algal biofuel production systems. It is essential that the light delivery system for the photobioreactor be designed to ensure that the light exposure during the culture period is within the range which is optimal for the culture species, e.g. in the range of 300–400 µmol m^{-2} s^{-1} for Chlorella and Nannochloropsis, in order to obtain as high as possible a volumetric production of lipids without photoinhibitory losses. Second, in tropical and subtropical environments, where the radiant energy received by plants is always above 1,500 µmol m^{-2} s^{-1}, it is

necessary to include shading, mixing, or culture depth management strategies in outdoor cultivation systems in order to keep effective light intensities in the saturation range. Third, for algal biofuels, integrated biorefinery strategies that produce high-value biochemicals along with the biofuels will be critical to their short-term economic success, especially for Nannochloropsis.

Future research should focus on expanding this experimental system to include nitrogen and phosphorus limitation as additional factors to modulate the accumulation of lipid for promising species and the use of genetic and metabolic engineering to broaden the photosaturation range of these species; and scaling up the experimental results to pilot-scale photobioreactors and outdoor cultivation systems to evaluate the transferability of the optimized cultivation conditions established in the laboratory to outdoor production systems.

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