Received: 1 February 2022 | Revised: 5 April 2022 | Accepted: 5 April 2022

DOI: 10.1002/lipd.12343

ORIGINAL ARTICLE

AOCS WILEY

Blue-green light is required for a maximized fatty acid unsaturation and pigment concentration in the microalga *Acutodesmus obliquus*

Mark Helamieh[1,2] | **Marco Reich**[1] | **Sophie Bory**[1] | **Philipp Rohne**[3] | **Ulf Riebesell**[4] | **Martin Kerner**[2] | **Klaus Kümmerer**[1]

[1]Institute of Sustainable Chemistry, Leuphana University of Lueneburg, Lueneburg, Germany

[2]Strategic Science Consult Ltd, Hamburg, Germany

[3]Institute of Pharmacy and Biochemistry, Therapeutical Life Sciences, Johannes Gutenberg-University Mainz, Mainz, Germany

[4]GEOMAR Helmholtz Centre for Ocean Research Kiel, Kiel, Germany

Correspondence
Mark Helamieh, Institute of Sustainable Chemistry, Leuphana University Lueneburg, Universitaetsallee 1, 21335 Lueneburg, Germany.
Email: mark.helamieh@stud.leuphana.de and markhelamieh@web.de

Funding information
Federal Institute for Research on Building, Urban Affairs and Spatial Development, Initiative Future Building, Germany, Grant/Award Number: SWD-10.08.18.7-17.02

Abstract

Blue-green light is known to maximize the degree of fatty acid (FA) unsaturation in microalgae. However, knowledge on the particular waveband responsible for this stimulation of FA desaturation and its impact on the pigment composition in microalgae remains limited. In this study, *Acutodesmus obliquus* was cultivated for 96 h at 15°C with different light spectra (380–700 nm, 470–700 nm, 520–700 nm, 600–700 nm, and dark controls). Growth was monitored daily, and qualitative characterization of the microalgal FA composition was achieved via gas chromatography coupled with electron impact ionization mass spectrometry (GC-EI/MS). Additionally, a quantitative analysis of microalgal pigments was performed using high-performance liquid chromatography with diode array detection (HPLC-DAD). Spectra that included wavelengths between 470 and 520 nm led to a significantly higher percentage of the polyunsaturated fatty acids (PUFA) 18:3 and 16:4, compared to all other light conditions. However, no significant differences between the red light cultivations and the heterotrophic dark controls were observed for the FA 18:3 and 16:4. These results indicate, that exclusively the blue-green light waveband between 470 and 520 nm is responsible for a maximized FA unsaturation in *A. obliquus*. Furthermore, the growth and production of pigments were impaired if blue-green light (380–520 nm) was absent in the light spectrum. This knowledge can contribute to achieving a suitable microalgal pigment and FA composition for industrial purposes and must be considered in spectrally selective microalgae cultivation systems.

KEYWORDS
blue-green light, fatty acid, lutein, microalgae, photosynthetic pigment

INTRODUCTION

Microalgae are considered a promising source of products for the food and fuel industry (Adarme-Vega et al., 2012; Ahmad et al., 2011; Rösch et al., 2019; Schenk et al., 2008; Singh et al., 2011; Tang et al., 2020). Compared to land-based crops, microalgae have several advantages, such as higher growth rates, a lower water demand, and no requirement for

Abbreviations: CDW, cell dry weight; DAD, diode array detector; EI, electron impact; FA, fatty acid; FAME, fatty acid methyl ester; GC, gas chromatography; HPLC, high-performance liquid chromatography; IS, internal standard; MS, mass spectrometry; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; rpm, rounds per minute; RT, retention time; SFA, saturated fatty acids; SIM, selected ion monitoring.

arable land (Ho et al., 2014; Miranda et al., 2018). Microalgal biomass can be used to produce green biofuels (Anto et al., 2020). Different components of the microalgal biomass are suitable for biogas production via anaerobic digestion, whereas lipids can be used to produce biodiesel (Chhandama et al., 2021; Zabed et al., 2020). For the production of biodiesel, a higher degree of fatty acid (FA) saturation is desirable (Piligaev et al., 2015). Additionally, microalgae might also serve as a production platform for valuable bioactive compounds in food and feed production (Goiris et al., 2012; Gong et al., 2011; Kusmayadi et al., 2021). For instance, carotenoids such as astaxanthin, lutein, and beta carotene are produced by microalgae and have a high value as supplements in the food, feed, and pharmaceutical industry (Gong et al., 2011; Vaz et al., 2016). Primarily lutein is frequently found in several microalgal species (Ho et al., 2014). Owing to its color and antioxidative properties, it has many different applications in the food, pharmaceutical, and cosmetic industry (Sun et al., 2014). Another group of essential components for the human diet, produced by microalgae, are specific FA (Behrens & Kyle, 1996). The optimal FA composition is a decisive factor for the nutritional value of food products. A high ratio of omega-3/omega-6 FA is required for healthy nutrition, whereas a lower omega-3/omega-6 ratio is supposedly associated with adverse health effects (Fabiani et al., 2021; Simopoulos, 2002). In western diets, high contents of omega-6 FA are prevailing, which might be related to several diseases of affluence, such as cardiovascular diseases, cancer, and autoimmune diseases (Shanab et al., 2018; Simopoulos, 2002). In contrast, higher chained omega-3 FA, such as eicosapentaenoic acid and docosahexaenoic acid, are important precursors of hormones and can be protective against neurodegenerative diseases such as Alzheimer's, Parkinson's, and multiple sclerosis disease (Shanab et al., 2018). These higher chained polyunsaturated fatty acids (PUFA) are biosynthesized from linoleic acid (18:2) and linolenic acid (18:3) precursors. A relatively high content of these FA can be found in fish oil (Shanab et al., 2018). However, several adverse environmental effects are related to the fishery industry (Blanco Gonzalez et al., 2017; Worm et al., 2006). Therefore, novel approaches for the production of PUFA are required.

The use of microalgae for PUFA production is viewed as a greener alternative in the food industry (Adarme-Vega et al., 2012). Furthermore, the metabolism of microalgae can be influenced by cultivation parameters (Aussant et al., 2018; El-Sheekh et al., 2012; Hultberg et al., 2014). This, in turn, may be used for the targeted production of specific FA and other valuable compounds (Abomohra et al., 2013; Abomohra & Almutairi, 2020; El-Sheekh et al., 2012). For example, phytohormones and the pretreatment of microalgae with low-dose atmospheric plasma can increase growth and FA production (Almarashi et al., 2020; Esakkimuthu et al., 2020). It is also well-known that the cultivation temperature greatly impacts the FA composition of microalgae, whereby lower cultivation temperatures are related to a higher PUFA concentration and a higher degree of FA unsaturation (Degraeve-Guilbault et al., 2021; Patterson, 1970). Another abiotic parameter that impacts the FA composition is the intensity and spectral composition of light (Hultberg et al., 2014; Nzayisenga et al., 2020; Shu et al., 2012). A recent study showed that different light intensities strongly impact the PUFA and pigment composition in the microalga *Chlorella sorokiniana* (Krimech et al., 2022). Moreover, blue and green light can influence FA composition and content in microalgae (Hultberg et al., 2014; Shu et al., 2012). This coincides with a higher observed content of photosynthetic pigments and xanthophylls upon blue light treatment (Fu et al., 2013; Ho et al., 2014; Sharmila et al., 2018). Furthermore, it was postulated that a maximum FA unsaturation in microalgae requires blue or blue-green light (Helamieh et al., 2021; Poliner et al., 2021). However, the detailed activating waveband was only roughly estimated between 450 and 550 nm (Helamieh et al., 2021). Although the underlying cause is unclear, it is assumed that the higher degree of FA desaturation upon blue-green light treatment might be caused by a rearrangement of the thylakoid system of the microalgal chloroplasts, which contain high percentages of highly unsaturated FA (Helamieh et al., 2021; Hugly & Somerville, 1992; Hultberg et al., 2014). If this is the case, the increase of highly unsaturated FA should be coupled with increased photosynthetic pigments. Besides the effects of blue light, the impact of red light on many metabolic processes and the FA composition in eukaryotic microalgae is not yet well understood (Helamieh et al., 2021; Poliner et al., 2021).

In addition to the fundamental scientific interest in a detailed understanding of the spectral impact on microalgal metabolism, this knowledge can be practically applied in photovoltaic-photosynthesis hybrid systems. These systems combine spectrally selective solar cells with photobioreactors to concomitantly harvest sunlight to produce microalgal biomass and electricity in one comprehensive system. For example, a recently developed, germanium-based semitransparent solar cell enables a high transmission of specific wavebands and can be spectrally adjusted, depending on the layer thickness (Osterthun, Neugebohrn, et al., 2021). Thus, detailed knowledge of the stimulating light waveband is required to adjust the transmission of the photovoltaic to the microalgal demands accordingly. Besides producing electricity and biomass in these systems, selected light wavelengths might modulate microalgal metabolism to produce a desirable FA and pigment composition for biofuel, food, and feed applications.

To close this knowledge gap, the impact of different light spectra on the growth, FA, and pigment composition of the microalga *Acutodesmus obliquus* was studied. The respective light spectra were chosen to identify the stimulating light wavebands that trigger a high degree of unsaturation and pigment production in microalgae. Biomass production of *A. obliquus* was assessed, and qualitative characterization of the FA profiles was analyzed by gas chromatography coupled with electron impact ionization mass spectrometry (GC-EI/MS). Additionally, the pigment composition was determined by high-performance liquid chromatography with diode array detection (HPLC-DAD).

MATERIAL AND METHODS

Microalgae preparation

The microalga strain *A. obliquus* (syn. *Scenedesmus obliquus; Tetradesmus obliquus*) (No. U169) from the Microalgae and Zygnematophyceae Collection Hamburg (MZCH, previously SVCK) of the University of Hamburg was used (von Schwartzenberg et al., 2013). The cultivation medium was composed of Flory Basis Fertilizer 1 (Euflor, Germany) and KNO_3 (Fisher Scientific, Germany). The pH was adjusted at 7.0 using HCl (Fisher Scientific, Germany) and NaOH (Fisher Scientific, Germany). To study heterotrophic growth, 2 g L^{-1} of glucose (Sigma-Aldrich, Taufkirchen, Germany) was added in the heterotrophic dark control. The determination of cell dry weight (CDW) was done gravimetrically. Microalgae inoculum was obtained in a pre-culture in 1 L Schott flasks at 25°C and a constant photon flux density of 150 μmol m^{-2} s^{-1} emitted by a Sylvania T9 circline 32 W fluorescent tube with a white light spectrum. A CO_2-enriched air (5% v/v) was used for the aeration of the pre-culture. A magnetic stirrer constantly homogenized the microalgae suspension at 200 rpm stirring speed. In order to obtain a factor to convert optical density into dry weight, a defined volume, depending on the optical density, of algae suspension was taken from each pre-culture and four dilution steps of the sample were prepared. The dilutions were filtered and subsequently dried at 80°C for 24 h. It was previously tested that a constant weight was reached after 3–8 h. In parallel, a subsample of each dilution was used to measure the optical density at 750 nm(OD_{750}). A conversion factor was calculated from the linear correlation between OD_{750} and dry weight.

Cultivation unit

Microalgae were cultivated as a batch in bubble column reactors. For that purpose, glass tubes of a length of 490 mm and a diameter of 40 mm each holding a volume of 350 ml, were used. Twelve glass tubes were submerged into an optical transparent acrylic glass water bath in black acrylic brackets. These brackets kept the tubes vertically and prevented ambient light from reaching the back and the sides of the tubes. All tubes were exclusively irradiated from the front side of the water bath with metal halide lamps (Philips MSR HR CT, 575 W), with a sun-like light spectrum (Figure 1a) (Helamieh et al., 2021; Osterthun, Helamieh, et al., 2021). The metal halide lamps generated the applied white light spectrum (380–700 nm). All wavelengths below 380 nm emitted by the metal halide lamps were absorbed by the acrylic glass water bath. For all other spectra, the optical filter foils "light red," "deep straw," and "medium yellow" (LEE-Filters, England) were used in combination with the metal halide lamps. These optical filters were fixed to the outer front side of the water bath. The resulting light spectra are further referred to as "red light" (600–700 nm), "orange light" (520–700 nm), and "yellow light" (470–700 nm) (Figure 1b–d). All spectra were measured and adjusted to the same photon flux density for each experiment (Table 1). Absolute photon fluxes were determined by a UV–Vis spectrometer (BLACK-Comet, StellarNET, Tampa, USA) within a range of 400–700 nm. Additionally, one section of the front side was shaded with aluminum foil for the dark control samples.

Test conditions

Pre-cultures were diluted to an OD_{750} of 0.5 for each experiment, measured with a UV/VIS spectrometer (Pharmacia LKB Ultrospec III). All cultivation experiments were started by using a volume of 350 ml of microalgae suspension. The growth was monitored via the OD_{750}, which was measured directly after sampling (Admirasari et al., 2022; Helamieh et al., 2021; Osterthun, Helamieh, et al., 2021; Reymann et al., 2020). The CDW was determined via the linear correlation between OD_{750} and CDW as established by the measurements.

$$CDW = 0.873 \times OD_{750} - 0.156$$

All experiments were performed as batch experiments for 96 h, with triplicates for each test condition in two experiments. The microalgae were irradiated constantly with a photon flux density of 195 ± 20 μmol m^{-2} s^{-1} in experiment 1 and 209 ± 24 μmol m^{-2} s^{-1} in experiment 2 for all light spectra, respectively (Table 1). The dark control was cultivated in the absence of light. In one dark control, 1 g L^{-1} of glucose was added at the start to compensate for the absence of light and enable a heterotrophic microalgal growth. In previous tests, under similar conditions, it was observed that glucose was depleted after 72 h of cultivation. Therefore, 1 g L^{-1}

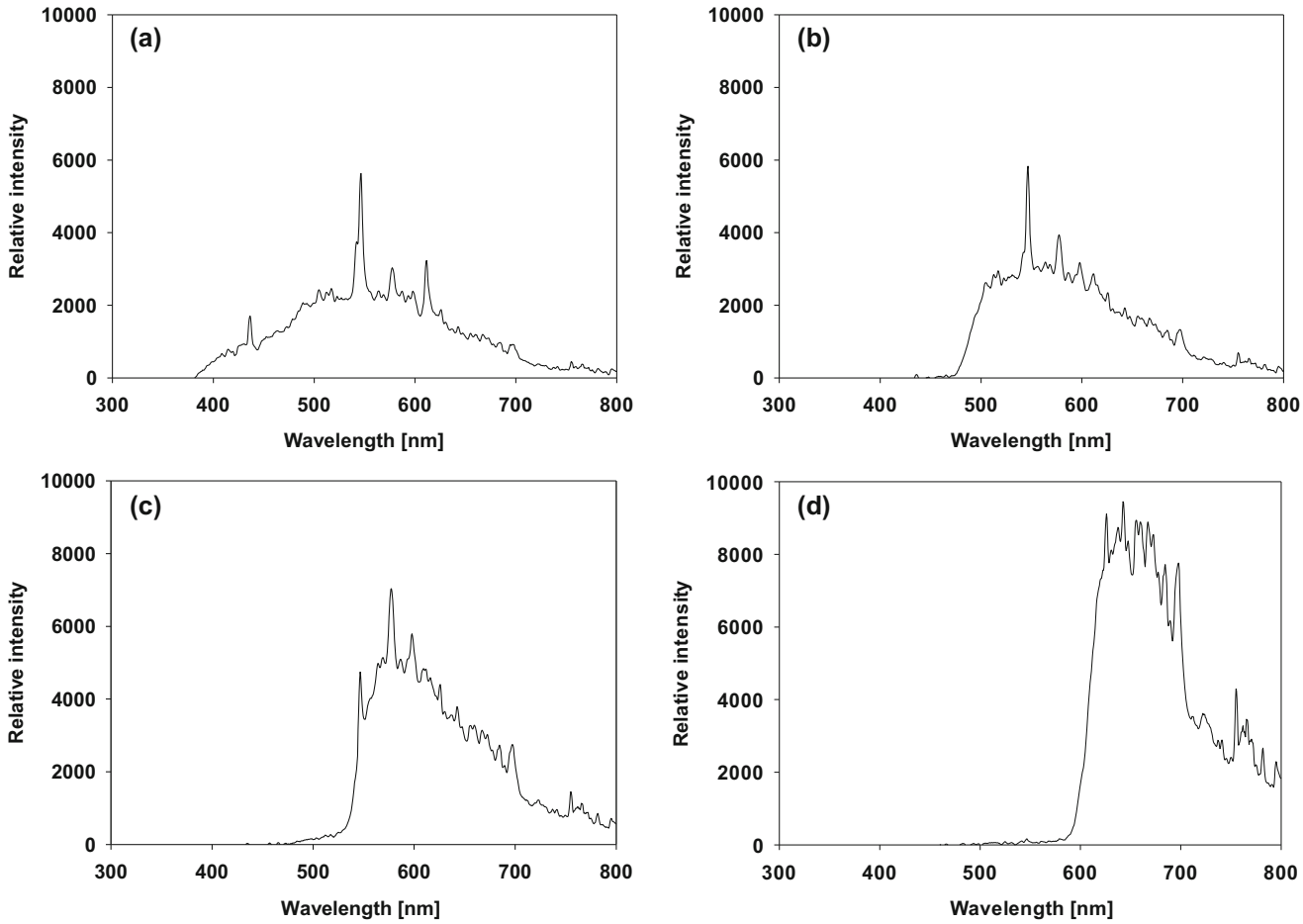

FIGURE 1 The light spectrum of the MSR 575 HR CT metal halide lamp. Unfiltered (white light) (a); in combination with the optical foils: medium yellow (yellow light) (b); deep straw (orange light) (c), and light red (red light) (d) (Lee filters, England). The relative emission spectra (Jλ,rel in counts) were determined by a UV–vis spectrometer (Black-comet, StellarNET, Tampa, USA) within a range of $\lambda = 300$–800 nm (integration time = 10 ms)

TABLE 1 Test conditions during the different experiments

Experiment	Light conditions	White light	Yellow light	Orange light	Red light	Repeated
1	Photon flux [μmol m^{-2} s^{-1}]	188 ± 11	195 ± 20	195 ± 10	184 ± 10	2×
Exp.	Light conditions	White light	Dark control	Dark + GLc	Red light	Repeated
2	Photon flux [μmol m^{-2} s^{-1}]	209 ± 24	0	0	210 ± 1	3×

Note: The temperatures and the estimated accuracies of measurement. Values for the photon fluxes represent the means ± standard deviation of triplicates.

of glucose was added at 72 h upon cultivation started. These dark controls were used to observe if red light impacts the FA and pigment composition. A detailed description of all conditions is summarized in Table 1. The bubble column reactors were aerated and mixed by humidified and CO_2-enriched air (5% v/v) at an airflow of 0.2 L min^{-1}. The temperature in the water bath was kept at 15° ± 0.5°C by a chiller (AD15R-30, VWR, Pennsylvania, USA) in all experiments to enable a maximum of unsaturated FA. Samples for the FA analysis were taken from the pre-culture. After the cultivation was started, 3–20 ml (depending on the biomass concentration) samples were taken after 24 h and 96 h of cultivation for FA and pigment analysis and subsequently stored at −80°C prior to analysis. The FA compositions of samples taken after 24 h and 96 h of cultivation were subsequently analyzed by GC-EI/MS. Additionally, the pigment composition of the samples taken after 96 h of cultivation was analyzed by HPLC-DAD.

Fatty acid analysis

Details of the analytical method can be found elsewhere (Helamieh et al., 2021).

FA extraction: The frozen samples were thawed and 0.0025 g CDW was used for the FA-extractions. 20 μmol heptadecanoic acid (Sigma Aldrich, Taufkirchen, Germany) dissolved in hexane was used as internal standard (IS). A modified Folch extraction was employed to extract the FA (Ichihara & Fukubayashi, 2010; Reich et al., 2012, 2013). The solvents of the extraction were evaporated under a constant and gentle stream of nitrogen and the transesterification was done according to (Helamieh et al., 2021).

Instrumental conditions (GC): The samples were analyzed according to (Helamieh et al., 2021).

GC/EI-MS was performed with a Thermo Scientific™ ISQ™ 7000 Single Quadrupole GC–MS system. The samples (1 μl) were injected with a sampler and the injector was operated in splitless mode and kept at 260°C. To separate the target compounds, a TRACE™ TR FAME fused silica capillary column (0.25 mm, 0.25 μm × 30 m) with helium as carrier gas was used with a constant pressure of 100 kPa, with a flow rate of 1.5 ml min^{-1}. The oven temperature was set to start at 60°C, plateau for 1 min, followed by a ramp rate of 6.5°C min^{-1} until the final temperature of 260°C was reached and then held for 8 min. The electron energy was 70 eV. The ion source was set to 270°C and the mass range of *m*/*z* 60–400 was recorded in the full scan mode. Fragment ions included *m*/*z* 74, *m*/*z* 79, *m*/*z* 81, and *m*/*z* 87 for the fatty acid methyl ester (FAME) detected during the measurement in the GC/EI-MS selected ion monitoring (SIM) mode.

Pigment analysis

The microalgal pigments were analyzed by HPLC according to (van Heukelem & Thomas, 2001). A HPLC Ultimate 3000 (Thermo Fisher) equipped with a DAD-3000 diode array detector, an FLD-3400RS fluorescence detector, a pre-column (Eclipse XDB-C8 Grd Crtds 4.6 × 12.5), and a separation column (Eclipse XDB-C8 3.5u 4.6 × 150) were used for the analysis of pigments. Spectra were recorded with a wavelength range of 380–800 nm. Separation was done isocratic, whereby solvent A (70:30 Methanol: 28 mM Tetrabutylammonium acetate, pH 6.5) and solvent B (100% Methanol) were added linearly with a flow-rate of 1.1 ml min^{-1}, from 5% to 95% within 22 min. The column oven temperature (TCC-3000SD) was set at 60°C.

Data evaluation and statistical analyses

The mass spectra and the RT were used for the qualitative analysis of the separated FAME. The data obtained from the SIM mode measurement were used to set the peak area ratios (sum of the four recorded *m*/*z* values) of the identified FAME into relation with the respective area of the IS. Heptadecanoic acid was used as IS. Finally, the proportion of each FA (in %) was calculated. All samples were taken in triplicates. The mean values ± standard error were calculated. Experiment 1 was repeated three times, and experiment 2 was repeated twice for the biomass production and FA analysis. The pigment analysis was repeated twice for experiment 1 (Table 4) and once for experiment 2 (Table 5). Statistical analyses were carried out using RStudio (version 1.2.5033), and the programming language R (version 3.6.2). Data import, visualization, and analysis were supported using the tidyverse, ggpubr, and rstatix packages. To examine whether the observed effects are statistically significant, a One-way ANOVA with Tukey post hoc test was performed.

RESULTS

Growth of *A. obliquus*

The produced biomass of *A. obliquus*, cultivated under all light conditions, was compared in two experiments. In experiment 1, the CDW reached a maximum value of 1.4 g L^{-1} for the white light treated samples after 96 h of cultivation. However, the maximum CDW of the red light treated samples reached a slightly but significantly lower value during the same cultivation time (Figure 2a). In the dark control, to which glucose was added to partially compensate for a decreased synthesis in the absence of light, a CDW of 1 g L^{-1} was reached after 96 h. No biomass increase was observed in the absence of light without glucose (Figure 2a). In experiment 2, the red light treated approaches reached a significantly lower maximum CDW, compared to all other tested spectral approaches, accordingly (Figure 2b).

Impact of the light conditions on the fatty acid composition

In this study, 15 FA were identified in *A. obliquus* (see supplementary). The main variations were observed in the FA 16:1, 16:2, 16:3, 16:4, 18:1, 18:2, and 18:3. Therefore, the focus was set on these FA.

It was found that the tested light conditions had a significant effect on the FA composition of *A. obliquus* (Figure 3, Tables 2, and 3). In experiment 1, the highest relative percentages of the FA 16:4 and 18:3 were observed upon cultivation with white light, compared to all other conditions. The dark controls and red light treated cultivations reached a significantly lower percentage of 16:4 and 18:3 at the same time (Figure 3a and b). In experiment 2, the highest relative percentages of the FA 16:4, and 18:3 were observed upon cultivation with white light and yellow light, compared to the red light and orange light treated approaches (Table 3, Figure 3a and b).

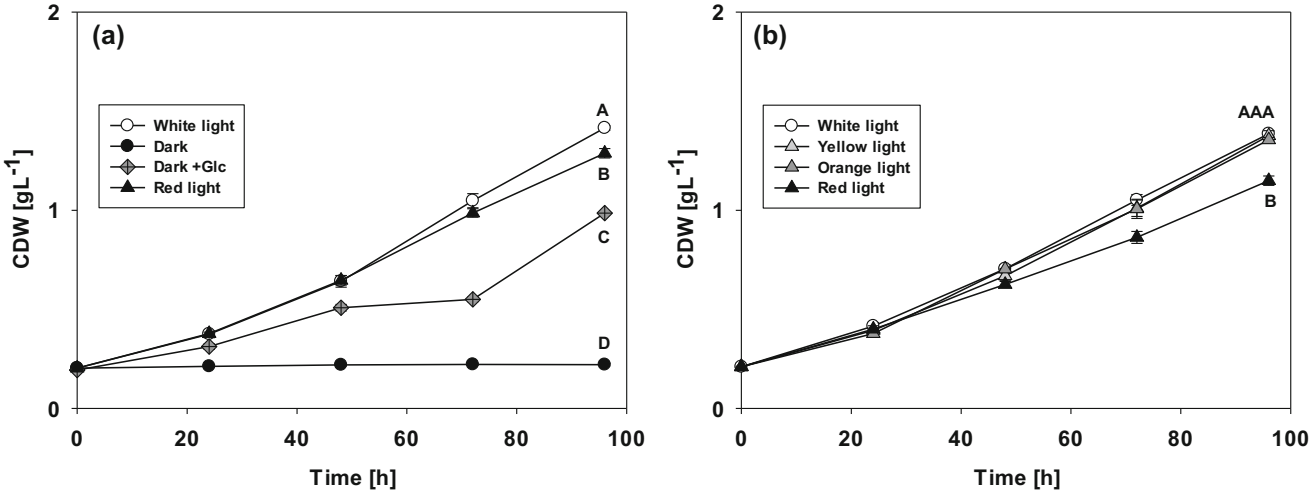

FIGURE 2 Biomass production of *A. obliquus* exposed to (a) white light (380–700 nm), red light (600–700 nm), dark controls with, and without 2 gL^{-1} glucose (experiment 1), and (b) white light (380–700 nm), yellow light (470–700 nm), orange light (520–700 nm), and red light (600–700 nm) (experiment 2). Both experiments were done at 210 μmol m^{-2} s^{-1} (if light was applied) and 15°C temperature. The cell dry weight (CDW) was determined by a correlation with the optical density at 750 nm. Values represent means and standard errors. Experiment 1 was repeated three times and experiment 2 was repeated two times. Different superscript letters (A–D) mean significant differences between groups ($p < 0.05$)

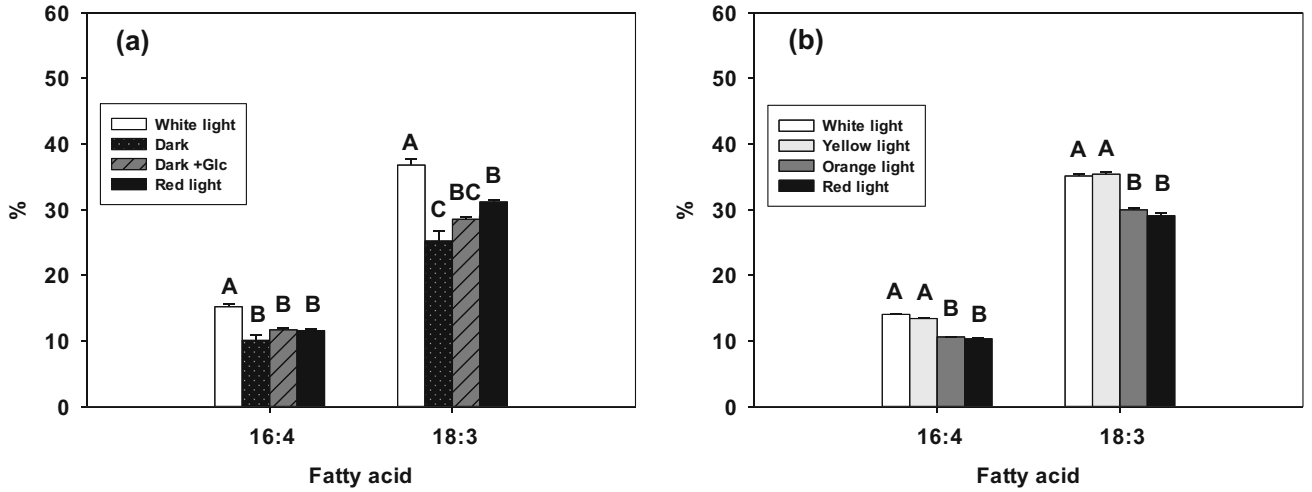

FIGURE 3 Relative percentage of the fatty acids 16:4 and 18:3 in *A. obliquus* exposed to (a) white light (380–700 nm), red light (600–700 nm), dark controls with, and without 2 gL^{-1} glucose (experiment 1), and (b) white light (380–700 nm), yellow light (470–700 nm), orange light (520–700 nm), and red light (600–700 nm) (experiment 2). Both experiments were done at 210 μmol m^{-2} s^{-1} (if light was applied) and 15°C temperature. The measurement of fatty acid were carried using GS-MS. all values shown are the means ± standard errors of two (experiment 1) and three independent experiments (experiment 2). Data were analyzed by using one-way ANOVA, followed by Tukey's multiple comparison test. Different superscript letters (A–C) mean significant differences between groups ($p < 0.05$)

Notably, no significant differences could be observed between the white light and yellow light treated cultivations, as well as between the red and orange light treated groups (Figure 3a and b). Furthermore, no significant differences were observed between the red light treated samples and the heterotrophic dark control samples (Figure 3a). No significant impact of the light conditions on the relative percentage of 18:4 in *A. obliquus* was observed (data not shown). Additionally, the percentage of 16:1 increased considerably after adding glucose in the absence of light (Table 2).

Impact of the light conditions on the pigment concentration

In the pigment analysis, two photosynthetic pigments (chlorophyll a and chlorophyll b) and four xanthophylls (lutein, violaxanthin, neoxanthin, and beta carotene)

TABLE 2 Fatty acids. Results of the fatty acid analysis of experiment 1. The analysis was repeated three

	16:1 [%]	16:2 [%]	16:3 [%]	16:4 [%]	18:1 [%]
W	1.4 ± 0.1^{bc}	0.1 ± 0.1^{c}	2.3 ± 0.1^{c}	15.2 ± 0.4^{a}	2.3 ± 0.1^{b}
D	2.0 ± 0.2^{b}	0.4 ± 0.1^{b}	4.8 ± 0.3^{a}	10.1 ± 0.8^{b}	4.8 ± 0.2^{a}
D + Glc	4.8 ± 0.1^{a}	0.7 ± 0.1^{a}	3.7 ± 0.1^{b}	11.7 ± 0.2^{b}	4.8 ± 0.1^{a}
R	1.3 ± 0.1^{c}	0.3 ± 0.1^{b}	4.5 ± 0.1^{ab}	11.6 ± 0.2^{b}	4.8 ± 0.3^{a}
	18:2 [%]	**18:3 [%]**	**SFA [%]**	**MUFA [%]**	**PUFA [%]**
W	1.7 ± 0.1^{c}	36.8 ± 0.9^{a}	37.7 ± 0.1	3.7 ± 0.1^{c}	58.6 ± 0.9^{a}
D	7.4 ± 0.4^{a}	25.2 ± 1.5^{c}	43.0 ± 1.0	6.8 ± 0.2^{b}	50.2 ± 1.0^{b}
D + Glc	5.7 ± 0.1^{b}	28.67 ± 0.3^{bc}	37.8 ± 0.3	9.5 ± 0.1^{a}	52.6 ± 0.1^{ab}
R	5.5 ± 0.1^{b}	31.2 ± 0.3^{b}	38.0 ± 0.1	6.1 ± 0.2^{b}	55.9 ± 0.3^{ab}

Note: Values represent the means ± standard errors. W, white light; D, dark control; D + Glc, dark with additional glucose. The isomers (c/t) of 16:1 were summed up. Different superscript letters (a–c) mean significant differences between groups ($P < 0.05$).

TABLE 3 Fatty acids. Results of the fatty acid analysis of experiment 2

	16:1 [%]	16:2 [%]	16:3 [%]	16:4 [%]	18:1 [%]
W	1.3 ± 0.1	0.1 ± 0.1^{c}	2.4 ± 0.1^{c}	14.1 ± 0.1^{a}	2.4 ± 0.1^{b}
Y	1.1 ± 0.1	0.2 ± 0.1^{b}	3.1 ± 0.2^{b}	13.4 ± 0.1^{a}	2.8 ± 0.1^{b}
O	1.2 ± 0.1	0.4 ± 0.1^{a}	4.7 ± 0.1^{a}	10.7 ± 0.1^{b}	4.8 ± 0.3^{a}
R	1.3 ± 0.1	0.4 ± 0.1^{a}	4.9 ± 0.1^{a}	10.4 ± 0.2^{b}	5.2 ± 0.2^{a}
	18:2 [%]	**18:3 [%]**	**SFA [%]**	**MUFA [%]**	**PUFA [%]**
W	1.8 ± 0.1^{d}	35.1 ± 0.3^{a}	40.5 ± 0.2	3.7 ± 0.1^{b}	55.8 ± 0.2
Y	2.9 ± 0.1^{c}	35.4 ± 0.4^{a}	38.4 ± 0.5	4.0 ± 0.1^{b}	57.7 ± 0.4
O	5.7 ± 0.1^{b}	30.1 ± 0.2^{b}	39.8 ± 0.4	6.0 ± 0.2^{a}	54.2 ± 0.2
R	6.4 ± 0.1^{a}	29.1 ± 0.5^{b}	39.6 ± 0.6	6.5 ± 0.1^{a}	53.9 ± 0.4

Note: The analysis was repeated two times. Values represent the means ± standard errors. W, white light; R, red light; O, orange light; Y, yellow light. The isomers (c/t) of 16:1 were summed up. Different superscript letters (a–d) mean significant differences between groups ($P < 0.05$).

TABLE 4 Results of the pigment analysis of experiment 1

	Chl a μg g^{-1} CDW	Chl b μg g^{-1} CDW	Lutein μg g^{-1} CDW	Vio μg g^{-1} CDW	Neo μg g^{-1} CDW	Beta μg g^{-1} CDW
W	18,003 ± 2099	5345 ± 579	1594 ± 250	300 ± 45	857 ± 119	855 ± 141
D	14,049 ± 404	4345 ± 164	975 ± 135	219 ± 32	769 ± 73	372 ± 83
D + Glc	6720 ± 679	2249 ± 249	449 ± 35	85 ± 11	390 ± 36	132 ± 2
R	15,646 ± 5408	4549 ± 1620	1188 ± 356	250 ± 80	805 ± 260	651 ± 87

Note: The analysis was performed once. Values represent means of triplicates ± standard deviation. Chl a, Chlorophyll-a; Chl b, Chlorophyll-b; Vio, Violaxanthin; neo, Neoxanthin, beta, Beta carotene; W, white light; D, dark control; D + Glc, dark with additional glucose; R, red light; O, orange light; Y, yellow light.

were identified in *A. obliquus* (Tables 4 and 5). The pigment composition in *A. obliquus* is explained in detail for experiment 2. Under white light treatment in experiment 2 the photosynthetic pigment, chlorophyll-a, was identified in concentrations of 14,939 ± 332 μg g^{-1} CDW. The second porphyrin pigment, chlorophyll b, was identified in concentrations of 5794 ± 309 μg g^{-1} CDW. Lutein was found in concentrations of 1529 ± 58 μg g^{-1} CDW. It is known that *A. obliquus* is a lutein-rich microalga (Ho et al., 2014). Furthermore, the xanthophylls violaxanthin, neoxanthin, and beta carotene were identified in amounts of 269 ± 31 μg g^{-1} CDW, 907 ± 78 μg g^{-1} CDW, and 428 ± 18 μg g^{-1} CDW, respectively (Table 5). A similar pigment composition was also observed in experiment 1 (Table 4).

Similar to the results received for the FA, a strong impact of the light spectrum on the pigment concentration was observed. The concentration decreased successively with the decreasing share of blue light in the yellow, orange, and red light spectra, respectively (Table 5, Figure 1). Under red light conditions, only 47.1% of the lutein concentration was found, compared to white light conditions (Table 5). The same tendency was also observed for the pigments chlorophyll a, violaxanthin, neoxanthin, and beta carotene (Table 4). A similar effect was also observed in the pigment data

TABLE 5 Results of the pigment analysis of experiment 2

	Chl a µg g^{-1} CDW	Chl b µg g^{-1} CDW	Lutein µg g^{-1} CDW	Vio µg g^{-1} CDW	Neo µg g^{-1} CDW	Beta µg g^{-1} CDW
W	14,939 ± 332	5794 ± 309	1529 ± 58	269 ± 31	907 ± 78	428 ± 18
Y	10,490 ± 2085	4265 ± 520	1034 ± 216	188 ± 46	603 ± 170	326 ± 100
O	7910 ± 1835	3435 ± 251	747 ± 157	132 ± 28	464 ± 119	207 ± 64
R	8355 ± 2572	5090 ± 1082	721 ± 189	133 ± 33	541 ± 161	163 ± 44

Note: The analysis was repeated two times for the samples of experiment 2. Values represent the means ± standard error in the experiment. Chl a, Chlorophyll-a; Chl b, Chlorophyll-b; Vio, Violaxanthin; neo, Neoxanthin, beta, Beta carotene; W, white light; D, dark control; D + Glc, dark with additional glucose; R, red light; O, orange light; Y, yellow light, CDW, cell dry weight.

of experiment 1. The highest concentrations were observed for the white light cultivation, compared to the red light and dark treatments (Table 4).

DISCUSSION

In this study, *A. obliquus* was cultivated at 15°C under different light conditions. The low temperature was chosen to maximize the FA-unsaturation. Microalgae raise the PUFA content with lowered environmental temperatures to maintain the fluidity of cell membranes (Degraeve-Guilbault et al., 2021; Patterson, 1970). However, the used cultivation temperature is lower than the optimum temperature of this species, which is around 30°C (Hindersin et al., 2012). Therefore, the biomass production was lower than in previous studies with the same strain, which were performed around the optimum temperature of this species (Admirasari et al., 2022; Helamieh et al., 2021; Reymann et al., 2020). In addition, the growth under monochromatic red light was significantly lower compared to all other tested light spectra (Figure 2). This finding is also supported by a recent study on the same algae strain. It was shown that wavelengths below 500 nm combined with red light (600–700 nm) lead to higher photosynthetic efficiency and growth in *A. obliquus*, compared to monochromatic red light (600–700 nm) (Osterthun, Helamieh, et al., 2021).

The FA composition is in accordance with several published data on the same microalgal strain (Abomohra et al., 2013; Helamieh et al., 2021). However, due to the lower cultivation temperature, a higher relative percentage of PUFA, such as 18:3 and 16:4 was observed in this study. The relative percentage of the PUFA 18:3 and 16:4 reached up to 36.8%, and 15.2% of the relative FA percentage under white light conditions (Table 2, Figure 3).

This study observed a significant impact on the FA composition (Figure 3, Tables 2 and 3). We have already postulated that light wavelengths between 450 and 550 nm are required for maximum FA unsaturation in *A. obliquus* (Helamieh et al., 2021). This was confirmed in the present study. Moreover, this study's results can contribute to localizing the required waveband with a higher spectral resolution. The highest percentages of the PUFA 16:4 and 18:3 were observed upon white light (380–700 nm) and yellow light (470–700 nm) treatments, whereas red light (600–700 nm), orange light (520–700 nm), and dark treated samples reached significantly lower values in all experiments (Figure 3a and b). The highest percentage of 16:4 and 18:3 were found upon white and yellow light treatment. Both light spectra share the wavelength range between 470 and 520 nm. Based on these data, it can be concluded that this 50 nm broad waveband is relevant for the FA unsaturation in *A. obliquus*.

Accordingly, the red light and orange light treated group, as well as the dark controls, showed the lowest percentage of 16:4 and 18:3. No significant differences could be observed between the red and orange light treated cultivations (Figure 3a and b). Additionally, no significant differences were observed between the red light treated samples and the heterotrophic dark control samples (Figure 3a). Therefore, no impact of the wavelengths between 520 and 700 nm (contained in orange and red light) on the FA desaturation was found.

The elevation of the FA16:1 in the heterotrophic dark treated samples is remarkable, even though no immediate explanation for it can be found in the literature. In addition, different from (Helamieh et al., 2021), no impact of the light conditions on the relative percentage of 18:4 in *A. obliquus* was observed (data not shown).

The photosynthetic pigments were identified in proportions and concentrations that are typical for *A. obliquus* (Admirasari et al., 2022; Ho et al., 2014; Wiltshire et al., 2000). Previous work showed that exposure to specific light spectra is decisive for the production of pigments in microalgae (Chainapong et al., 2012; Ho et al., 2014). Our results confirm that the light conditions strongly impact the pigment composition. A stable tendency was observed for the carotenoid, lutein. The highest concentration was detected under white light treatment. A higher concentration of pigments in microalgae upon white light exposition was already observed (Ho et al., 2014). We assume, that similar to the FA data, the blue light component in the white light spectrum is decisive for the maximum pigment production (Figure 1). Blue light is associated with the synthesis of chlorophyll-a and is additionally involved

in rearrangements of chloroplasts (Sánchez-Saavedra & Voltolina, 2002). The carotenoids and xanthophylls have their absorption maximum in the waveband between 400 and 500 nm (Peterman et al., 1997). The impact of the applied spectra on the pigment concentrations shows similar patterns to the described blue-green light effect on the FA unsaturation. Though different from the FA composition, the concentrations of the pigments decrease successively, rather than abruptly, with the decreasing blue light content in the spectra (Tables 2–5, Figure 1, Figure 3).

Blue-green light effects on the metabolism of *A. obliquus*

The results show that blue-green light has an important function in the metabolism of *A. obliquus*: The growth under red light was reduced compared to all other tested light spectra. It was reported that supplementation of blue light could substantially improve the growth of *A. obliquus* compared to monochromatic red light (Osterthun, Helamieh, et al., 2021). Although the physiological reason for this finding is not apparent, we assume that blue light has vital functions in the metabolism required for high biomass production. However, several functions of blue light in organisms are already known in literature and can give some insights into putative mechanisms behind the finding. For instance, it is already known that the cell division of microalgae is regulated via blue light (Münzner & Voigt, 1992). Furthermore, blue light is essential for the entrainment of the circadian clock in many organisms (Hajdu et al., 2018; Li et al., 2016).

If the spectrum contained blue light, not only the CDW but also the concentration of the carotenoids and chlorophyll a in *A. obliquus* reached higher values compared to the treatment with monochromatic red light (Tables 4 and 5). Furthermore, also the FA metabolism is impacted by blue-green light. Only if light with wavelengths between 470 and 520 nm was applied, a significantly higher percentage of the FA 16:4 and 18:3 was observed compared to all other light treatments (Figure 3). Moreover, the higher concentration of pigments upon blue-green appears to be paralleling with the increase of FA unsaturation. Both effects are putatively related to rearrangement processes in the chloroplasts (Ho et al., 2014; Sánchez-Saavedra & Voltolina, 2002). Higher unsaturated FA can be found in thylakoid membranes of chloroplasts in high percentages (Hugly & Somerville, 1992; Hultberg et al., 2014; Poliner et al., 2021). Therefore, this part of the light spectrum might increase the thylakoid membrane systems, with a concomitant elevation of pigments and specific PUFA in *A. obliquus*.

Blue light is also involved in posttranscriptional activations of different enzymes. For example, in the green microalga *Monoraphidium braunii*, the import of nitrate and the nitrate reductase is activated by blue light (Aparicio et al., 1976; Aparicio & Quiñones, 1991; Quiñones & Aparicio, 1990). Recently, a blue light-driven decarboxylation of FA to alkanes in *Chlorella variabilis* was reported (Huijbers et al., 2018). It was suggested that this spectrally selective photoactivation of the decarboxylase could be harnessed for the enzymatic production of biodiesel. Another group of light-dependent enzymes are FA desaturases. It is well known that these enzymes in plants and microorganisms are activated by environmental cues, such as light (Kis et al., 1998). In *A. obliquus* and the stramenopile microalga *Nannochloropsis oceanica*, this activation of FA desaturases is assumed to be triggered by blue-green light exclusively (Helamieh et al., 2021; Poliner et al., 2021). This study on *A. obliquus* also supports this assumption. Moreover, the responsible part of the light spectrum was further localized and narrowed down to a 50 nm broad waveband between 470 nm and 520 nm.

SUMMARY AND OUTLOOK

The variety of blue light effects on the metabolism of *A. obliquus* and the important function for FA desaturation shows the need for further research. Many light perceiving processes in organisms are highly conserved in the evolution of life. Therefore, it might be interesting to elucidate if the requirement of blue light for FA unsaturation is a widespread effect in plants and microalgae. Besides the basic scientific research, this knowledge can be used for industrial applications. For example, the selective illumination with supplementation of blue-green light can be harnessed for a maximized production of PUFA and bioactive compounds, such as lutein in microalgae. This is especially desirable for microalgal feed and food products. Vice versa, the relative percentage of PUFA can be reduced for the production of biofuels from microalgal FA, applying a spectrally selective illumination that lacks the waveband between 470 and 520 nm. One technical application of this knowledge is possible in photovoltaic-photosynthesis hybrid systems.

This study evaluated the impact of different light spectra on the growth, FA composition, and pigment concentration of *A. obliquus*. We were able to show that blue-green light (380–520 nm) is crucial for a maximized production of pigments and a high percentage of the PUFA 16:4 and 18:3. Especially, the narrow light waveband between 470 and 520 nm is important for a high degree of FA unsaturation. Therefore, the importance of blue-green light should be taken into account in spectrally selective illumination microalgae cultivation systems. This knowledge might be the base for a light stimulated production of microalgal pigments and specific FA for the food and biofuel production.

ACKNOWLEDGMENTS

This study was funded by the research initiative Zukunft Bau of the Federal Institute for Research on Building, Urban Affairs, and Spatial Development (No. SWD-10.08.18.7-17.02). A special thanks goes to Christoph Stegen of the technical support team of the Leuphana University Lüneburg for his excellent technical support and expertise to optimize the experimental setup for microalgae cultivation. We thank Kerstin Nachtigall of the GEOMAR Helmholtz Centre for Ocean Research Kiel, for performing the HPLC-DAD analysis. Finally, we want to thank Professor Dr. Dieter Hanelt, PD Dr. Klaus von Schwartzenberg, and the staff of the Microalgae and Zygnematophyceae Collection Hamburg (MZCH, previously SVCK) microalgae collection of the University of Hamburg for giving us the chance to work with one of their microalgae strains.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

CREDIT AUTHORSHIP CONTRIBUTION STATEMENT

All authors have made a scientific contribution and approved the final draft of the manuscript. **Mark Helamieh**: Conceived and designed the study. Carried out the research analysis and interpretation of data. Wrote the first draft of the manuscript. **Marco Reich**: Supervision and support in the methodology part. Analysis and interpretation of data. Contribution to the writing process. Editing and review of manuscript. **Sophie Bory**: Carried out the research. Analysis of data. **Philipp Rohne**: Analysis of data and made the statistical work. Contribution to the writing process. **Ulf Riebesell**: Carried out the research. Analysis of data. **Martin Kerner**: Analysis of data. Contribution to the writing process. Supervision of the study. **Klaus Kümmerer**: Supervision and support in the methodology part. Contribution to the writing process. Analysis and interpretation of data. Editing and review of the manuscript.

ETHICS STATEMENT

No human or animal subjects were used in this study.

REFERENCES

Abomohra AEF, Almutairi AW. A close-loop integrated approach for microalgae cultivation and efficient utilization of agar-free seaweed residues for enhanced biofuel recovery. Bioresour Technol. 2020; 317:124027. https://doi.org/10.1016/J.BIORTECH.2020.124027

Abomohra AEF, Wagner M, El-Sheekh M, Hanelt D. Lipid and total fatty acid productivity in photoautotrophic fresh water microalgae: screening studies towards biodiesel production. J Appl Phycol. 2013;25(4):931–6. https://doi.org/10.1007/s10811-012-9917-y

Adarme-Vega TC, Lim DKY, Timmins M, Vernen F, Li Y, Schenk PM. Microalgal biofactories: a promising approach towards sustainable omega-3 fatty acid production. Microb Cell Fact. 2012;11(1): 1–10. https://doi.org/10.1186/1475-2859-11-96

Admirasari R, Hindersin S, von Schwartzenberg K, Hanelt D. Nutritive capability of anaerobically digested black water increases productivity of *Tetradesmus obliquus*: domestic wastewater as an alternative nutrient resource. Bioresource Technol Rep. 2022; 17:100905. https://doi.org/10.1016/J.BITEB.2021.100905

Ahmad AL, Yasin NHM, Derek CJC, Lim JK. Microalgae as a sustainable energy source for biodiesel production: a review. Renew Sustain Energy Rev. 2011;15(1):584–93. https://doi.org/10.1016/J.RSER.2010.09.018

Almarashi JQM, El-Zohary SE, Ellabban MA, Abomohra AEF. Enhancement of lipid production and energy recovery from the green microalga Chlorella vulgaris by inoculum pretreatment with low-dose cold atmospheric pressure plasma (CAPP). Energ Conver Manage. 2020;204:112314. https://doi.org/10.1016/J.ENCONMAN.2019.112314

Anto S, Mukherjee SS, Muthappa R, Mathimani T, Deviram G, Kumar SS, et al. Algae as green energy reserve: technological outlook on biofuel production. Chemosphere. 2020;242:125079. https://doi.org/10.1016/J.CHEMOSPHERE.2019.125079

Aparicio PJ, Quiñones MA. Blue light, a positive switch signal for nitrate and nitrite uptake by the green alga *Monoraphidium braunii*. Plant Physiol. 1991;95(2):374–8. https://doi.org/10.1104/PP.95.2.374

Aparicio PJ, Roldán JM, Calero F. Blue light photoreactivation of nitrate reductase from green algae and higher plants. Biochem Biophys Res Commun. 1976;70(4):1071–7. https://doi.org/10.1016/0006-291X(76)91011-1

Aussant J, Guihéneuf F, Stengel DB. Impact of temperature on fatty acid composition and nutritional value in eight species of microalgae. Appl Microbiol Biotechnol. 2018;102(12):5279–97. doi:10.1007/S00253-018-9001-X

Behrens PW, Kyle DJ. Microalgae as a source of fatty acids. J Food Lipids. 1996;3(4):259–72. https://doi.org/10.1111/J.1745-4522.1996.TB00073.X

Blanco Gonzalez E, De Boer F. The development of the Norwegian wrasse fishery and the use of wrasses as cleaner fish in the salmon aquaculture industry. Fish Sci. 2017;83:661–70. https://doi.org/10.1007/s12562-017-1110-4

Chainapong T, Traichaiyaporn S, Richard L. Ceridão_Nascimento_ALICEpdf, 2012; 8(5): 1593–1604.

Chhandama MVL, Satyan KB, Changmai B, Vanlalveni C, Rokhum SL. Microalgae as a feedstock for the production of biodiesel: a review. Bioresource Technol Rep. 2021;15:100771. https://doi.org/10.1016/J.BITEB.2021.100771

Degraeve-Guilbault C, Pankasem N, Gueirrero M, Lemoigne C, Domergue F, Kotajima T, et al. Temperature acclimation of the picoalga *Ostreococcus tauri* triggers early fatty-acid variations and involves a plastidial ω3-desaturase. Front Plant Sci. 2021; 12:639330. https://doi.org/10.3389/FPLS.2021.639330

El-Sheekh M, Abomohra AE-F, Hanelt D. Optimization of biomass and fatty acid productivity of *Scenedesmus obliquus* as a promising microalga for biodiesel production. World J Microbiol Biotechnol. 2012;29(5):915–22. https://doi.org/10.1007/S11274-012-1248-2

Esakkimuthu S, Krishnamurthy V, Wang S, Hu X, Abomohra AEF. Application of p-coumaric acid for extraordinary lipid production in *Tetradesmus obliquus*: a sustainable approach towards enhanced biodiesel production. Renew Energy. 2020;157: 368–76. https://doi.org/10.1016/J.RENENE.2020.05.005

Fabiani H, Mudjihartini N, Lestari W. Dietary omega-6 to omega-3 fatty acids ratio is correlated with high molecular weight adiponectin level in Indonesian office workers. Int J Nutr Pharmacol Neurol Dis. 2021;11(1):64. https://doi.org/10.4103/IJNPND.IJNPND_89_20

Fu W, Guomundsson Ó, Paglia G, Herjólfsson G, Andrésson ÓS, Palsson BO, et al. Enhancement of carotenoid biosynthesis in the green microalga *Dunaliella salina* with light-emitting diodes and adaptive laboratory evolution. Appl Microbiol Biotechnol. 2013; 97(6):2395–403. https://doi.org/10.1007/s00253-012-4502-5

Goiris K, Muylaert K, Fraeye I, Foubert I, de Brabanter J, de Cooman L. Antioxidant potential of microalgae in relation to their phenolic and carotenoid content. J Appl Phycol. 2012;24(6):1477–86. https://doi.org/10.1007/s10811-012-9804-6

Gong Y, Hu H, Gao Y, Xu X, Gao H. Microalgae as platforms for production of recombinant proteins and valuable compounds: Progress and prospects. J Ind Microbiol Biotechnol. 2011;38(12):1879–90. https://doi.org/10.1007/s10295-011-1032-6

Hajdu A, Dobos O, Domijan M, Bálint B, Nagy I, Nagy F, et al. ELONGATED HYPOCOTYL 5 mediates blue light signalling to the *Arabidopsis circadian* clock. Plant J. 2018;96(6):1242–54. https://doi.org/10.1111/TPJ.14106

Helamieh M, Gebhardt A, Reich M, Kuhn F, Kerner M, Kümmerer K. Growth and fatty acid composition of *Acutodesmus obliquus* under different light spectra and temperatures. Lipids. 2021;56:485–98. https://doi.org/10.1002/lipd.12316

Hindersin S, Leupold M, Kerner M, Hanelt D. Irradiance optimization of outdoor microalgal cultures using solar tracked photobioreactors. Bioprocess Biosyst Eng. 2012;36(3):345–55. https://doi.org/10.1007/S00449-012-0790-5

Ho SH, Chan MC, Liu CC, Chen CY, Lee WL, Lee DJ, et al. Enhancing lutein productivity of an indigenous microalga *Scenedesmus obliquus* FSP-3 using light-related strategies. Bioresour Technol. 2014;152:275–82. https://doi.org/10.1016/j.biortech.2013.11.031

Hugly S, Somerville C. A role for membrane lipid polyunsaturation in chloroplast biogenesis at low temperature. Plant Physiol. 1992;99(1):197–202. https://doi.org/10.1104/PP.99.1.197

Huijbers MME, Zhang W, Tonin F, Hollmann F. Light-driven enzymatic decarboxylation of fatty acids. Angew Chem Int Ed. 2018;57(41):13648–51. https://doi.org/10.1002/ANIE.201807119

Hultberg M, Jönsson HL, Bergstrand KJ, Carlsson AS. Impact of light quality on biomass production and fatty acid content in the microalga *Chlorella vulgaris*. Bioresour Technol. 2014;159:465–7. https://doi.org/10.1016/J.BIORTECH.2014.03.092

Ichihara K, Fukubayashi Y. Preparation of fatty acid methyl esters for gas-liquid chromatography. J Lipid Res. 2010;51(3):635–40. https://doi.org/10.1194/JLR.D001065/ATTACHMENT/55D3C28E-DB2E-47F2-A45B-FB118C1DF3A0/MMC1.PDF

Kis M, Zsiros O, Farkas T, Wada H, Nagy F, Gombos Z. Light-induced expression of fatty acid desaturase genes. Proc Natl Acad Sci USA. 1998;95(8):4209–14. https://doi.org/10.1073/PNAS.95.8.4209

Krimech A, Helamieh M, Wulf M, Krohn I, Riebesell U, Cherifi O, et al. Differences in adaptation to light and temperature extremes of *Chlorella sorokiniana* strains isolated from a wastewater lagoon. Bioresour Technol. 2022;350:126931. https://doi.org/10.1016/J.BIORTECH.2022.126931

Kusmayadi A, Leong YK, Yen HW, Huang CY, Chang JS. Microalgae as sustainable food and feed sources for animals and humans – biotechnological and environmental aspects. Chemosphere. 2021;271:129800. https://doi.org/10.1016/J.CHEMOSPHERE.2021.129800

Li X, Ma D, Lu SX, Hu X, Huang R, Liang T, et al. Blue light- and low temperature-regulated COR27 and COR28 play roles in the Arabidopsis circadian clock. Plant Cell. 2016;28(11):2755–69. https://doi.org/10.1105/TPC.16.00354

Miranda MT, Sepúlveda FJ, Arranz JI, Montero I, Rojas C. Physical-energy characterization of microalgae Scenedesmus and experimental pellets. Fuel. 2018;226:121–6. https://doi.org/10.1016/J.FUEL.2018.03.097

Münzner P, Voigt J. Blue light regulation of cell division in Chlamydomonas reinhardtii. Plant Physiol. 1992;99(4):1370–5. https://doi.org/10.1104/PP.99.4.1370

Nzayisenga JC, Farge X, Groll SL, Sellstedt A. Effects of light intensity on growth and lipid production in microalgae grown in wastewater. Biotechnol Biofuels. 2020;13(1):1–8. https://doi.org/10.1186/s13068-019-1646-x

Osterthun N, Helamieh M, Berends D, Neugebohrn N, Vehse M, Kerner M, Agert C. Influence of spectrally selective solar cells on microalgae growth in photo-bioreactors. 2021:70001.

Osterthun N, Neugebohrn N, Gehrke K, Vehse M, Agert C. Spectral engineering of ultrathin germanium solar cells for combined photovoltaic and photosynthesis. Opt Express. 2021;29(2):938. https://doi.org/10.1364/oe.412101

Patterson GW. Effect of culture temperature on fatty acid composition of *Chlorella sorokiniana*. Lipids. 1970;5(7):597–600. https://doi.org/10.1007/BF02531336

Peterman EJG, Gradinaru CC, Calkoen F, Borst JC, van Grondelle R, van Amerongen H. Xanthophylls in light-harvesting complex II of higher plants: light harvesting and triplet quenching†. Biochemistry. 1997;36(40):12208–15. https://doi.org/10.1021/BI9711689

Piligaev AV, Sorokina KN, Bryanskaya AV, Peltek SE, Kolchanov NA, Parmon VN. Isolation of prospective microalgal strains with high saturated fatty acid content for biofuel production. Algal Res. 2015;12:368–76. https://doi.org/10.1016/J.ALGAL.2015.08.026

Poliner E, Busch AWU, Newton L, Kim YU, Clark R, Gonzalez-Martinez SC, et al. Aureochromes are necessary for maintaining polyunsaturated fatty acid content in *Nannochloropsis oceanica*. BioRxiv. 2021;2021(01):428447. https://doi.org/10.1101/2021.01.27.428447

Quiñones MA, Aparicio PJ. Blue light activation of nitrate reductase and blue light promotion of the biosynthesis of nitrite reductase in *Monoraphidium braunii*. In: Ullrich WR, Rigano C, Fuggi A, Aparicio PJ, (eds), *Inorganic Nitrogen in Plants and Microorganisms*. Berlin, Heidelberg: Springer; 1990;171–7. https://doi.org/10.1007/978-3-642-75812-6_26

Reich M, Hannig C, Al-Ahmad A, Bolek R, Kümmerer K. A comprehensive method for determination of fatty acids in the initial oral biofilm (pellicle). J Lipid Res. 2012;53(10):2226–30. https://doi.org/10.1194/JLR.D026260

Reich M, Kümmerer K, Al-Ahmad A, Hannig C. Fatty acid profile of the initial oral biofilm (pellicle): an in-situ study. Lipids. 2013;48(9):929–37. https://doi.org/10.1007/S11745-013-3822-2

Reymann T, Kerner M, Kümmerer K. Assessment of the biotic and abiotic elimination processes of five micropollutants during cultivation of the green microalgae *Acutodesmus obliquus*. Bioresource Technol Rep. 2020;11:100512. https://doi.org/10.1016/J.BITEB.2020.100512

Rösch C, Roßmann M, Weickert S. Microalgae for integrated food and fuel production. GCB Bioenergy. 2019;11(1):326–34. https://doi.org/10.1111/GCBB.12579

Sánchez-Saavedra MP, Voltolina D. Efecto de las tasas de flujo de fotones de luz blanca y azul-verde en la eficiencia del crecimiento y contenido de pigmentos de tres especies de diatomeas en cultivos terminales. Cienc Mar. 2002;28(3):273–9. https://doi.org/10.7773/cm.v28i3.225

Schenk PM, Thomas-Hall SR, Stephens E, Marx UC, Mussgnug JH, Posten C, et al. Second generation biofuels: high-efficiency microalgae for biodiesel production. Bioenergy Res. 2008;1(1):20–43. https://doi.org/10.1007/s12155-008-9008-8

Shanab SMM, Hafez RM, Fouad AS. A review on algae and plants as potential source of arachidonic acid. J Adv Res. 2018;11:3–13. https://doi.org/10.1016/J.JARE.2018.03.004

Sharmila D, Suresh A, Indhumathi J, Gowtham K, Velmurugan N. Impact of various color filtered LED lights on microalgae growth, pigments and lipid production. Eur J Biotechnol Biosci. 2018;6(6):1–7. www.biosciencejournals.com

Shu C-H, Tsai C-C, Liao W-H, Chen K-Y, Huang H-C. Effects of light quality on the accumulation of oil in a mixed culture of Chlorella sp. and *Saccharomyces cerevisiae*. Chem Technol Biotechnol. 2012;87:601–7. https://doi.org/10.1002/jctb.2750

Simopoulos AP. The importance of the ratio of omega-6/omega-3 essential fatty acids. Biomed Pharmacother. 2002;56(8):365–79. https://doi.org/10.1016/S0753-3322(02)00253-6

Singh A, Nigam PS, Murphy JD. Renewable fuels from algae: an answer to debatable land based fuels. Bioresour Technol. 2011; 102(1):10–6. https://doi.org/10.1016/J.BIORTECH.2010.06.032

Sun Z, Zhi-gang Z, Jiang Y. Microalgae as a source of lutein: chemistry, biosynthesis, and Carotenogenesis. Adv Biochem Eng Biotechnol. 2014;123(July):127–41. https://doi.org/10.1007/10

Tang DYY, Yew GY, Koyande AK, Chew KW, Vo DVN, Show PL. Green technology for the industrial production of biofuels and bioproducts from microalgae: a review. Environ Chem Lett. 2020;18(6):1967–85. https://doi.org/10.1007/s10311-020-01052-3

van Heukelem L, Thomas CS. Computer-assisted high-performance liquid chromatography method development with applications to the isolation and analysis of phytoplankton pigments. J Chromatogr A. 2001;910(1):31–49. https://doi.org/10.1016/S0378-4347(00)00603-4

Vaz B d S, Moreira JB, Morais MG d, Costa JAV. Microalgae as a new source of bioactive compounds in food supplements. Curr Opin Food Sci. 2016;7:73–7. https://doi.org/10.1016/J.COFS.2015.12.006

von Schwartzenberg K, Bornfleth S, Lindner A-C, Hanelt D. The microalgae and Zygnematophyceae collection Hamburg (MZCH) – living cultures for research on rare streptophytic algae. Algolog Stud. 2013;142:77–107. https://doi.org/10.1127/1864-1318/2013/0131

Wiltshire KH, Boersma M, Möller A, Buhtz H. Extraction of pigments and fatty acids from the green alga *Scenedesmus obliquus* (Chlorophyceae). Aquat Ecol. 2000;34(2):119–26. https://doi.org/10.1023/A:1009911418606

Worm B, Barbier EB, Beaumont N, Duffy JE, Folke C, Halpern BS, et al. Impacts of biodiversity loss on ocean ecosystem services. Science. 2006;314(5800):787–90. https://doi.org/10.1126/science.1132294

Zabed HM, Akter S, Yun J, Zhang G, Zhang Y, Qi X. Biogas from microalgae: technologies, challenges and opportunities. Renew Sustain Energy Rev. 2020;117:109503. https://doi.org/10.1016/J.RSER.2019.109503

SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

How to cite this article: Helamieh M, Reich M, Bory S, Rohne P, Riebesell U, Kerner M, et al. Blue-green light is required for a maximized fatty acid unsaturation and pigment concentration in the microalga *Acutodesmus obliquus*. Lipids. 2022;57(4-5):221–32. https://doi.org/10.1002/lipd.12343