Received: 20 July 2023 | Revised: 4 October 2023 | Accepted: 4 October 2023

DOI: 10.1111/php.13873

RESEARCH ARTICLE

Impact of green and blue-green light on the growth, pigment concentration, and fatty acid unsaturation in the microalga *Monoraphidium braunii*

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Funding information
Bundesinstitut für Bau- Stadt- und Raumforschung, Grant/Award Number: SWD-10.08.18.7-17.02

Abstract

The spectral composition of light is an important factor for the metabolism of photosynthetic organisms. Several blue light-regulated metabolic processes have already been identified in the industrially relevant microalga *Monoraphidium braunii*. However, little is known about the spectral impact on this species' growth, fatty acid (FA), and pigment composition. In this study, *M. braunii* was cultivated under different light spectra (white light: 400–700 nm, blue light: 400–550 nm, green light: 450–600 nm, and red light: 580–700 nm) at 25°C for 96 h. The growth was monitored daily. Additionally, the FA composition, and pigment concentration was analyzed after 96 h. The highest biomass production was observed upon white light and red light irradiation. However, green light also led to comparably high biomass production, fueling the scientific debate about the contribution of weakly absorbed light wavelengths to microalgal biomass production. All light spectra (white, blue, and green) that comprised blue-green light (450–550 nm) led to a higher degree of FA unsaturation and a greater concentration of all identified pigments than red light. These results further contribute to the growing understanding that blue-green light is an essential trigger for maximized pigment concentration and FA unsaturation in green microalgae.

KEYWORDS

algal metabolism, fatty acid desaturase, green microalgae, photosynthetic pigment, polyunsaturated fatty acid, spectral light composition, thylakoid membrane

Abbreviations: ALA, alpha linolenic acid; 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; LA, linoleic acid; 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.

INTRODUCTION

Light is a vital parameter for the metabolism of photosynthetic organisms such as microalgae. The light intensity and light regime are crucial for growth and photosynthetic activity.[1] Additionally, the spectral composition of light is highly relevant for microalgal metabolism. The impact of different light spectra on the growth of microalgae has been controversially discussed in the latest literature.[2–5] Recent studies have reported a high biomass production upon green and yellow light under high light conditions as well as high culture densities. This is inconsistent with the common opinion that weakly absorbed light spectra only make a minor contribution to the growth of microalgae.[3–7]

Besides, the importance for photosynthetic energy intake and biomass production, blue and green light are important environmental triggers for regulating many metabolic processes in higher plants and algae.[8–11] For instance, it is known that blue light is required for the cell division of the microalga *Chlamydomonas reinhardtii*.[12] In the green microalga *Monoraphidium braunii*, many well-known blue light-induced processes have been identified. Blue light is a positive switch signal for the nitrate, nitrite, chloride, and hydrogen carbonate uptake in *M. braunii*.[13–15] Moreover, it is also an activation signal for enzymes involved in the nitrogen metabolism of this organism.[16] Activation of the enzyme nitrate reductase by blue light has also been reported in other algae species and is presumably widespread among green microalgae.[9,17] Furthermore, blue and green light can influence the composition of photosynthetic pigments and fatty acids (FA). Recently, we have reported that blue-green light between 450 and 550 nm is required for a maximized FA unsaturation and a maximized pigment concentration in the green microalga *Acutodesmus obliquus*.[18] These blue-green light-triggered physiological reactions might be related to a light activation of FA desaturase enzymes and the rearrangement processes of thylakoid membranes of chloroplasts.[5,19–21] Many of such light-triggered processes in organisms are highly conserved in the evolution of organisms.[22,23] Therefore, investigating the impact of blue-green light on the FA and pigment composition in photosynthetic organisms is of great interest, both phylogenetically and biochemically.

Besides this foundational knowledge, investigating these spectrally triggered metabolic processes in microalgae may have practical applications. The detailed data can be harnessed for the targeted production of valuable compounds, such as FA, for the food, feed, and biofuel industries. In particular, cultivation parameters can be used to influence microalgal metabolisms and enable the targeted industrial production of valuable compounds in microalgae.[24–28] Pigments are used as colorants and antioxidants in the food industry.[29–31] In particular, the pigment lutein is found in many microalgae and is highly valued in the pharmaceutical, cosmetic, and food industries.[32–34] Polyunsaturated fatty acids (PUFA), such as alpha-linolenic acid (ALA, 18:3n-3), are produced by different microalgae species.[35,36] The value of ALA for human nutrition is very high, due to its key function as a precursor for higher chained PUFA, which are in turn important for different physiological reactions in the human body.[37] Therefore, the food industry aims to produce products enriched in ALA.[38] But the n-3 /n-6 FA ratio is also vital for the nutritional value of food products. In particular, the ratio of the n-3 FA ALA toward the n-6 FA linoleic acid (LA, 18:2n-6) can have a strong impact on human health. A high ALA/LA ratio can even provide protection against several neurodegenerative diseases.[39–41] However, the supply of these PUFA from traditional sources, such as aquaculture and the fishery industry, is insufficient to satisfy global demand.[42] Moreover, these sources are associated with adverse environmental effects.[43,44] In contrast, microalgae are a renewable production platform for crop production and other agricultural demands.[45] Additionally, they have several advantages over land plants, such as no requirement for arable land, a lower water quality requirement, and higher growth rates.[46,47]

In this study, a combined investigation of the impact of different broad light spectra on the growth, FA, and pigment composition of *M. braunii* was conducted. It provides new insights into the light-related metabolic processes of green microalgae. The species was chosen due to the many already known blue light-triggered metabolic processes and its high industrial relevance.[5,48,49] Moreover, it has a close phylogenetic relation to *A. obliquus*, for which the impact of blue-green light on the FA composition and pigment concentration has already been described in detail Ref. [5,50].

MATERIALS AND METHODS

Microalgae preparation

The green microalga strain, *Monoraphidium braunii* SAG-202-7b (*M. braunii*) from the Culture Collection of Algae (SAG) of the University of Göttingen, Germany, was used for this study. Strain preservation was conducted in flasks on an orbital shaker (IKA KS 501 digital; 100–110 rpm), continuously illuminated by a fluorescent lamp with $50 \pm 10\,\mu mol\,photons\,m^{-2}\,s^{-1}$ (Osram Lumilux, cool white, 18W), resulting in a temperature of $25 \pm 1°C$. The flasks contained sterilized Flory Basis Fertilizer 1 (Euflor, Germany) and KNO_3 (Fisher Scientific, Germany) as described in Ref. [5,48]. The pre-cultivation and

determination of cell dry weight (CDW) were performed according to Ref. [5,18].

Cultivation device and test conditions

Only a brief description of the cultivation experiments and methodological differences is provided here since they have already been described in detail in Helamieh et al.[5].

Microalgae cultivation was carried out in column reactors as a batch culture for 96 h, with the cultivation temperature set at 25 ± 0.5°C to promote high growth and a high FA unsaturation. Two Philips MSR HR CT, 575 W metal halide lamps with a sun-like light spectrum provided the light. The metal halide lamps generated the white light spectrum, which was further referred to as "white light" (380–700 nm). The white light was additionally attenuated with a shading net (polyethylene, aperture size 2 × 10 mm, Hermann Meyer KG, Rellingen, Germany). Other light spectra were obtained using the optical filter foils "light red" "dark green," and "dark blue" (LEE-Filters, England) in combination with the metal halide lamps. These light spectra were named "red light" (580–700 nm), "green light" (450–600 nm), and "blue light" (400–550 nm). All microalgae experiments were conducted at a photon flux density of 210 µmol photons $m^{-2} s^{-1}$ for all tested light spectra (shown in the Appendix S1).

The cultivation experiments started at an optical density of 0.5 and were measured with a UV/VIS spectrometer at 750 nm (Pharmacia LKB Ultrospec III).[5,51] The cell dry weight (CDW) was calculated using a linear correlation between optical density at 750 nm (OD_{750}) and CDW, previously determined in measurements:

$$CDW = 0.436 \times OD_{750} - 0.099$$

Samples were taken daily and subsequently stored at −80°C for the FA and pigment analysis. The CDW was determined using the linear correlation by measuring the OD_{750} immediately after daily sampling.

Fatty acid analysis

The analytical method was performed according to Helamieh et al.[5]

In contrast, a volume equivalent to 0.015 g CDW of the thawed samples was used for the FA extractions and heneicosanoic acid (Sigma Aldrich, Taufkirchen, Germany) 20 µmol dissolved in hexane was employed as an internal standard (IS) in this study. The extraction and transesterification of all FA-containing lipids into fatty acid methyl ester (FAME) were carried out according to Ref. [5,52–54]. The retention times (RT) in a GC–MS chromatogram with the identified FA can be found in the Appendix S1. The ratio of the identified FAME was set into relation with the respective area of the IS, and the relative percentage of the total FAME was calculated.

Pigment analysis

The pigment analysis was performed according to Helamieh et al.[18]

Data evaluation and statistical analyses

The cultivations were performed in triplicates for each tested condition in two independent experiments, and the means ± standard errors were calculated from the values obtained. All statistical tests were done according to Helamieh et al.[18]

RESULTS

Growth

In the growth experiments, *M. braunii* was cultivated for 96 h at 25°C, with equal photon flux densities under white, red, green, and blue light conditions. Under white light treatment, the CDW reached 1.94 ± 0.04 g L^{-1}. With red light, a maximum of 1.62 ± 0.04 g L^{-1} CDW was reached. Green light and blue light treatment led to a maximum CDW of 1.37 ± 0.04 and 1.06 ± 0.01 g L^{-1}, respectively (Figure 1). The differences in biomass production after 96 h cultivation are significant ($p \leq 0.05$) for all spectra, except for the green light cultivation compared to the red light cultivation (Figure 1).

Fatty acids

All identified FA of *M. braunii* are shown in Table 1, and a chromatogram of the GC-EI/MS analysis is shown in the supplementary data (see Appendix S1). The FA 18:1, 16:1, and 16:3 were observed in different, not specifically characterized isomeric forms. Under white light, the dominant PUFA were 18:2 (18.2% ± 0.6%) and 18:3 (15.7% ± 0.6%), with a total PUFA percentage of 57.2% ± 0.6%. The rare PUFA 16:4 and 18:4 are typical for microalgae and were also identified in *M. braunii* (Table 1).[55]

The light spectrum had a strong effect on the FA composition. Higher proportions of the FA 18:3 were found in the white, green, and blue light cultivation compared to

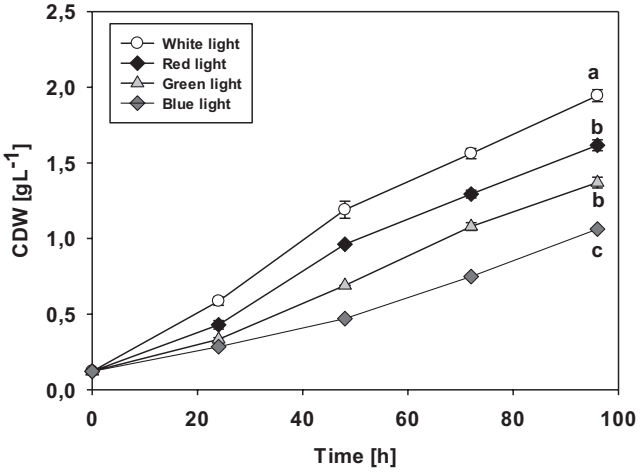

FIGURE 1 Biomass production of *Monoraphidium braunii* exposed to 210 µmol photons $m^{-2} s^{-1}$ white light (400–700 nm), red light (580–700 nm), green light (450–600 nm), and blue light (400–550 nm) at 25°C. The cell dry weight (CDW) was determined by a correlation with the optical density at 750 nm. Values represent means ± standard errors of two experiments. Different superscript letters (a–c) mean significant differences ($p \leq 0.05$) between the groups.

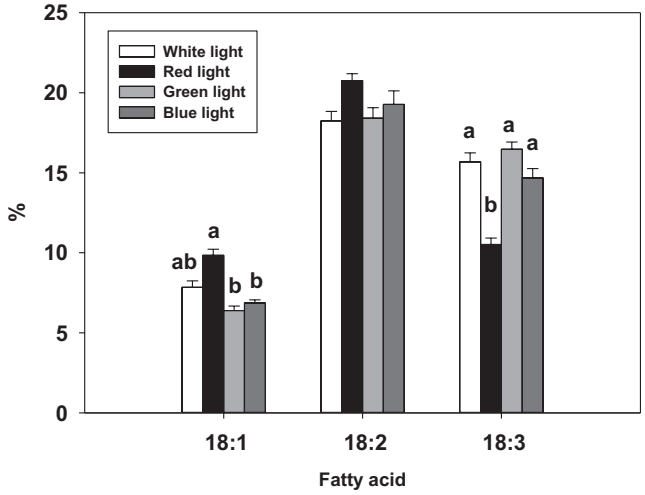

FIGURE 2 Relative proportions [%] of the fatty acids 18:1, 18:2; and 18:3 in *Monoraphidium braunii* exposed to 96 h with 210 µmol photons $m^{-2} s^{-1}$ white light (400–700 nm), red light (580–700 nm), green light (450–600 nm), and blue light (400–550 nm) at 25°C. The isomers of 18:1 were summed up. Values represent means ± standard errors of two experiments with triplicates of each light condition. Different superscript letters (a, b) mean significant differences ($p \leq 0.05$) between the groups.

TABLE 1 Fatty acid (FA) profile of *Monoraphidium braunii*.

FA	%	FA	%
14:0	≤1	18:1 isomer	≤1
15:0	≤1	$18:2^{\Delta 9,12}$	18.2 ± 0.6
16:0	30.7 ± 0.1	$18:3^{\Delta 9,12,15}$	15.7 ± 0.6
16:1 isomers	1.8	$18:4^{\Delta 6,9,12,15}$	2.2 ± 0.2
$16:2^{\Delta 7,10}$	7.5 ± 0.3	22:0	≤1
17:0	≤1	24:0	≤1
16:3 isomer	1.4 ± 0.1		
16:3 isomer	5.6 ± 0.2		
$16:4^{\Delta 4,7,10,13}$	6.4 ± 0.6	SFA	32.6 ± 0.1
18:0	≤1	MUFA	10.2 ± 0.6
18:1 isomer	7.8 ± 0.4	PUFA	57.2 ± 0.6

Note: The samples were taken after 96 h of cultivation with 210 µmol photons $m^{-2} s^{-1}$ white light at 25°C. Values represent means ± standard errors. The experiment was repeated twice in triplicates.

Abbreviations: MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; SFA, saturated fatty acids.

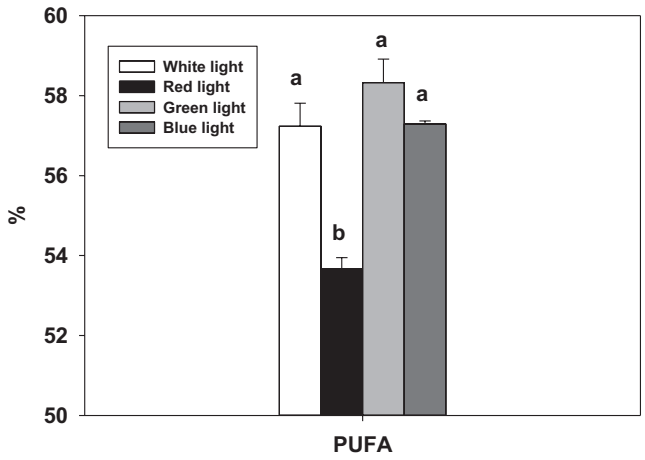

FIGURE 3 Relative proportions [%] of polyunsaturated fatty acids (PUFA) in *Monoraphidium braunii* exposed to 96 h with 210 µmol photons $m^{-2} s^{-1}$ white light (400–700 nm), red light (580–700 nm), green light (450–600 nm), and blue light (400–550 nm) at 25°C. Values represent means ± standard errors of two experiments with triplicates of each light condition. Different superscript letters (a, b) mean significant differences ($p \leq 0.05$) between the groups.

red light conditions (Figure 2). A similar pattern was also observed for the PUFA 16:4, even though these differences were not significant (see Figure S2).

Contrary to the aforementioned effect on the FA 18:3, a reverse tendency was observed for the lower desaturated n-C18 FA (18:1 and 18:2). Upon red light cultivation, a higher percentage of the 18:1 and 18:2 was detected, compared to the other spectral cultivations. Taken together, upon white, green, and blue light cultivation, a higher proportion of 18:3 toward 18:2 and 18:1 was identified, in comparison to red light cultivation (Figure 2). Additionally, a higher percentage of the summarized PUFA was also observed under white, green, and blue light, compared to red light cultivation (Figure 3). Therefore, the white, green, and blue light treatment led to a higher total degree of FA unsaturation than monochromatic red light.

Photosynthetic pigments

In the HPLC DAD analysis, six pigments were identified in *M. braunii* (Table 2). The following composition was found in the white light control after 96 h of cultivation. The primary photosynthetic pigments, chlorophyll a and b were found in concentrations of $9694 \pm 31\,\mu g\,g^{-1}$ CDW and $3370 \pm 45\,\mu g\,g^{-1}$ CDW, respectively. Lutein was found to be the major carotenoid in concentrations of $535 \pm 22\,\mu g\,g^{-1}$ CDW. Furthermore, the carotenoids and xanthophylls neoxanthin ($398\,\mu g\,g \pm 14\,\mu g\,g^{-1}$ CDW), violaxanthin ($228 \pm 8\,\mu g\,g^{-1}$ CDW), and alpha-carotene ($22 \pm 1\,\mu g\,g^{-1}$ CDW) were identified.

The light spectrum had an influence on the concentration of the identified photosynthetic pigments. Pigment concentrations for all identified pigments reached a higher value in the white, green, and blue light cultivations, compared to the red light cultivation (Table 2; Figure 4). For example, only 69.0% of lutein was found upon red light treatment compared to white light cultivation. Likewise, the values for the red light-treated samples reached 72.6% for neoxanthin, 78.5% for violaxanthin, 63.6% for alpha-carotene, and 80.0% and 87.9% for chlorophyll a and b, in comparison to white light. A similar relation of pigments can also be observed if red light treatment is compared to the green or blue light-cultivated samples (Table 2; Figure 4).

DISCUSSION

In the cultivation experiments, the biomass production of *M. braunii* was strongly affected by the light spectrum. This underlines the importance of the parameter light spectrum for the biomass production of microalgae. A comparably low growth upon blue light was also observed in recent studies on microalgae.[5,56] Even though the absorption spectrum of the primary photosynthetic pigment, chlorophyll *a*, has a high overlap with the blue light spectrum, this waveband is inefficiently used for the biomass production of *M. braunii* (Figure 1). Besides chlorophyll *a* and *b*, carotenoids and xanthophylls in *M. braunii* can also absorb blue light.[57] Xanthophylls and carotenoids mainly convert light energy into heat as a photoprotective mechanism, which might compromise biomass production.[58,59] This is one possible explanation for the low growth performance under blue light.

In contrast, the green light treatment led to higher biomass production than blue light (Figure 1). In classical plant physiology, the prevailing assumption of a low contribution of green light to photosynthetic production dominates.[60] However, a more complex understanding has emerged in very recent studies.[2–5,56] Due to the low absorptance, green light has a lower contribution to the photosynthetic production in plants and microalgae compared to red, and blue light at low photon flux densities. However, green light might efficiently contribute to biomass production under high and medium photon flux densities and high biomass concentrations. In this case, the low absorptance of green light is compensated by a more uniform absorption and more efficient use of light energy.[4] Green light can penetrate deeper layers, enabling better light dilution in the leaf tissue or microalgae solution. In contrast, red and blue light are strongly absorbed by the first layers of chloroplasts in leaf layers or microalgae solutions, whereas deeper layers remain unilluminated. This results in a lower photon quantum yield due to unequal illumination, a higher heat dissipation, and perhaps, even a higher degree of photoinhibition of the photosystems.[2,4,5,56] Very efficient use of red light and green light under medium photon flux densities was also observed in our study on *A. obliquus*.[5] Nevertheless, a combination of blue and red light was found to cause higher photosynthetic efficiencies compared to monochromatic red light in the same algae strain.[61] This was also observed in our recent study on the microalgae *A. obliquus* and might explain the high biomass production under white light, which contains red light and blue light (Figure 1).[18]

TABLE 2 Results of the HPLC-DAD pigment analysis in *Monoraphidium braunii*.

	Chl *a* ($\mu g\,g^{-1}$ CDW)	Chl *b* ($\mu g\,g^{-1}$ CDW)	Lutein ($\mu g\,g^{-1}$ CDW)	Neo ($\mu g\,g^{-1}$ CDW)	Vio ($\mu g\,g^{-1}$ CDW)	Alpha ($\mu g\,g^{-1}$ CDW)
W	9694 ± 31	3370 ± 45	535 ± 22	398 ± 14	228 ± 8	22 ± 1^{a}
R	7754 ± 923	2935 ± 405	369 ± 33	289 ± 31	179 ± 17	14 ± 1^{ab}
G	9881 ± 343	3234 ± 121	490 ± 10	360 ± 26	223 ± 5	17 ± 1^{ab}
B	9505 ± 808	3518 ± 420	475 ± 41	354 ± 1	222 ± 8	15 ± 1^{b}

Note: The analysis was repeated two times. Values represent the means ± standard errors. Different superscript letters (a, b) mean significant differences ($p \leq 0.05$) between the groups.

Abbreviations: Alpha, Alpha-Carotene; B, blue light; CDW, cell dry weight; Chl *a*, Chlorophyll *a*; Chl *b*, Chlorophyll *b*; G, green light; Neo, Neoxanthin; R, red light; Vio, Violaxanthin; W, white light.

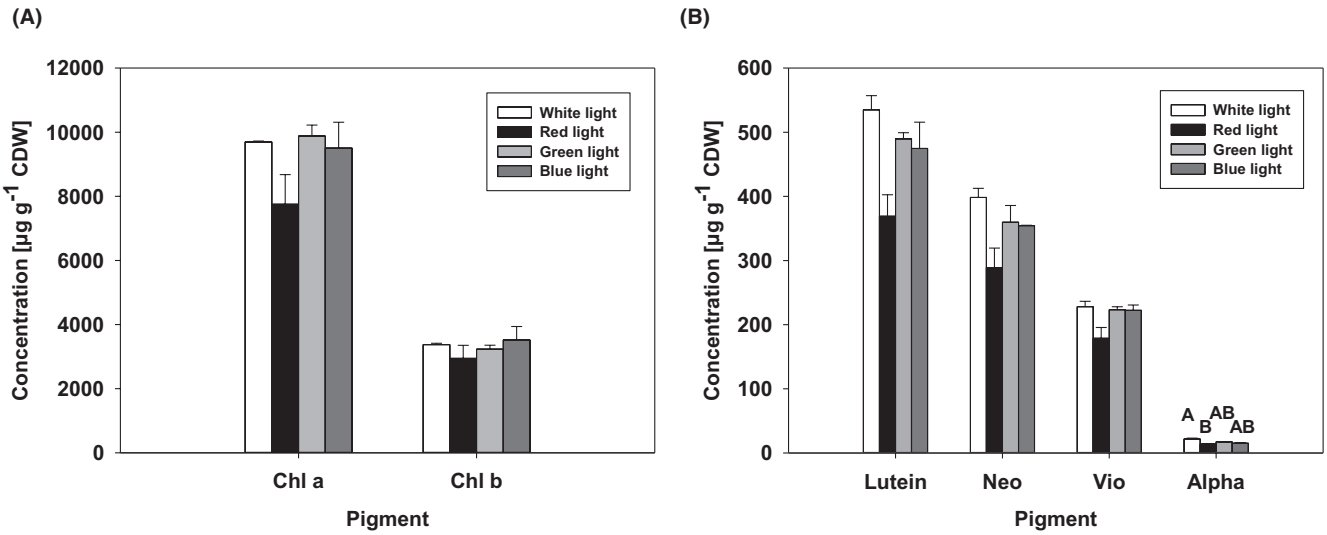

FIGURE 4 Results of the HPLC-DAD analysis of *Monoraphidium braunii*. Chl *a*: Chlorophyll *a*; Chl *b*: Chlorophyll *b* (A). Alpha, Alpha-Carotene; Neo, Neoxanthin; Vio, Violaxanthin (B). CDW, cell dry weight. The analysis was repeated two times. Values represent means ± standard error. Different superscript letters (A, B) mean significant differences ($p \leq 0.05$) between the groups.

The FA composition of *M. braunii* has a high content of PUFA, and therein a comparably high percentage of the FA 18:3 and 18:2 (Table 1). These are essential FA for human nutrition.[62] The FA composition is in accordance with other studies on the genus *Monoraphidium*.[49,55] A strong impact of the light spectrum on the FA composition of *M. braunii* was observed. The degree of FA unsaturation was higher upon white, green, and blue light treatment, compared to red light conditions. In our recent study on the green microalga *A. obliquus*, we observed a similar spectral effect. We hypothesized that blue-green light between 450 and 550 nm is required for a maximum FA unsaturation in *A. obliquus*.[5] The waveband between 450 and 550 nm was contained in all tested light spectra except red light. Therefore, the aforementioned FA desaturation effect is apparently also present in *M. braunii* and might explain the higher degree of unsaturation upon white, blue, and green light compared to red light in this study (Figures 2 and 3). This spectral waveband might be required for the maximum activity of FA-desaturase enzymes in green microalgae. Some of these FA-desaturases in photosynthetic organisms are regulated via different environmental cues, such as temperature and light.[63,64] However, it is not yet known which part of the light spectrum is required for the light regulation of FA-desaturases.

The changes in the ratio of the n-C18 FA indicate that the different light spectra impact the FA desaturation process (Figure 2). In particular, the desaturation of the FA 18:2, which is the last biochemical step in the biosynthesis of 18:3, seems to require blue-green light in *M. braunii*. It is well-known that the FA are desaturated step by step via FA-desaturases that are specific for each reaction.[21,64,65] Moreover, the activation of desaturases by light is already recognized as widespread in different phylogenetically distanced organisms.[21,63,64] Therefore, we assume that blue-green light (450–550 nm) is also required for a maximized degree of unsaturation in *M. braunii*, presumably by activating FA-desaturases.

In the pigment analysis, six photosynthetic pigments were analyzed (Figure 4a,b; Table 2). The pigment composition is in accordance with previous studies on this species.[66] Furthermore, a spectral influence on the pigment composition of *M. braunii* was observed in this study. It is already known that blue light triggers the production of xanthophylls and photosynthetic pigments.[34,67,68] This was also observed in this study for *M. braunii*. Similar to the higher FA unsaturation, the concentrations of all pigments were also higher upon white, green, and blue light treatment compared to the red light cultivation (Figure 4a,b; Table 2). Both, the different pigment concentrations and the different degrees of FA saturation, might be related to rearrangement processes of thylakoid membranes of chloroplasts.[5,19,20] These membrane systems are the locations of the photosynthetic complexes that contain the carotenoids and chlorophyll a and b.[8,69] Furthermore, the thylakoid membrane systems have high proportions of PUFA.[19–21] Therefore, the application of blue-green light might trigger an elevation of the thylakoid membrane system in *M. braunii*. This potentially explains the higher values of photosynthetic pigments and PUFA upon blue-green light-containing spectra.

Many light-regulated processes are highly conserved in the evolution of organisms.[22,23] Therefore, further investigation of the impact of blue-green light on the PUFA and pigments in different phylogenetically

distant organisms is required. This investigation might reveal the extend to which the light wavelength dependent effect on the FA and pigment composition is spread among photosynthetic organisms. To validate the hypothesized mechanism of thylakoid membrane rearrangements, additional research is necessary. For example, electron microscopic images of microalgae samples cultivated under specific light spectra might visualize blue-green light-triggered rearrangement processes in the chloroplasts of microalgae. To uncover the potential role of FA-desaturases, a detailed investigation of these enzymes is required. A transcriptome analysis of genes encoding FA-desaturases or enzyme assays involving FA-desaturases can provide insights into the role of these enzymes in blue-green light-triggered FA unsaturation. A detailed analysis can contribute to a deeper understanding of the light-regulated mechanisms in microalgae and plants. This knowledge can be leveraged for targeted industrial production of valuable compounds in microalgae.[24–28] Further practical applications of this knowledge are photovoltaic-photosynthesis hybrid systems with comprehensive spectral use of the sunlight for the production of electricity and biomass in one system.[61]

CONCLUSION AND IMPLICATIONS

Monochromatic red light and green light led to higher biomass production than monochromatic blue light. This underlines the high relevance of red and green light for microalgal growth. However, the highest biomass production was observed upon white light treatment. In addition, the strong impact of blue-green light on the FA and pigment composition of *M. braunii* was observed. Light spectra that contained blue-green light (450–550 nm) led to a maximized FA unsaturation and concentration of photosynthetic pigments. These results contribute to a growing understanding that blue-green light is a crucial environmental trigger for the lipid metabolism of photosynthetic organisms. The knowledge generated in this study may potentially be used to influence the metabolism of microalgae for industrial applications. It might pave the way for a targeted production of specific FA by light wavelength management for the biofuel and food industry. A lower degree of FA unsaturation is suitable for the production of biofuels, whereas a high content of specific PUFA is associated with beneficial nutritional effects. The degree of FA saturation in microalgae can be influenced by light wavelength management during microalgae cultivation, particularly by choosing a light spectrum that includes or excludes the waveband between 450 and 550 nm.

AUTHOR CONTRIBUTIONS

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

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 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 also thank Stina Krings for her contribution to microalgae cultivation and sampling. Open Access funding enabled and organized by Projekt DEAL.

CONFLICT OF INTEREST STATEMENT

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this article.

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How to cite this article: Helamieh M, Reich M, Rohne P, Riebesell U, Kerner M, Kümmerer K. Impact of green and blue-green light on the growth, pigment concentration, and fatty acid unsaturation in the microalga *Monoraphidium braunii*. *Photochem Photobiol*. 2024;100:587-595. doi:10.1111/php.13873