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Short Communication

Effect of wavelengths on the growth and chemical-proximal composition of diatoms *Amphora* aff. *amoena* and *Chaetoceros muelleri*

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ABSTRACT. *Amphora* aff. *amoena* and *Chaetoceros muelleri* are potential diatoms with good proximal composition and the ability to grow rapidly. Like other microalgae, this composition can be improved by adjusting their culture conditions, such as exposure to different light conditions. However, there is still a need to explore these culture conditions further and their effect on these diatoms. This study aimed to evaluate the chemical-proximal composition of the diatoms *A.* aff. *amoena* and *C. muelleri* under three wavelengths: white (WL) range (400-750 nm), blue (BL) range (430-480 nm), and red (RL) range (595-660 nm), at an irradiance of 200 µmol m^{-2} s^{-1} using LED lights. In both species, *A.* aff. *amoena* and *C. muelleri*, the highest cell concentration under WL was: 216,250 and 1,198,125 cells mL^{-1}, respectively. The carbohydrate content was 5.80% for *A.* aff. *amoena* and 2.21% for *C. muelleri*, while protein content was 13.41% for *A.* aff *amoena* and 12.31% for *C. muelleri*. Lipids were found to be most abundant in *A.* aff. *amoena* when exposed to RL (37.19%) and *C. muelleri* (60.48%) when exposed to BL. No significant differences were found in *A.* aff. *amoena* under different light conditions.

Keywords: *Chaetoceros muelleri*; *Amphora* aff. *amoena*; wavelengths; chemical-proximal composition; growth, diatoms

Microalgae are photosynthetic organisms that inhabit various aquatic habitats, from oceans to freshwater environments (Blackburn & Volkman 2012). They also participate in biogeochemical cycles of important macroelements and carbon dioxide fixation (Farrelly et al. 2013). In addition to the high efficiency of converting solar energy into chemical energy, they have fast growth and do not compete for water and land in agricultural areas, being an advantageous characteristic of their culture (El Gamal 2010). Recently, studies have been oriented toward pharmaceuticals, nutrition, and biodiesel due to their rich biochemical composition, which molecules could have potential bioactivity (Miao & Wu 2006, Hosikian et al. 2010, Simas-Rodrigues et al. 2015). Microalgae naturally produce different bioactive compounds, which allow them to resist various environmental conditions (Hosikian et al. 2010). One of the most diverse and ubiquitous groups are diatoms (around 200,000 species of diatoms globally) (Mann & Vanormelingen 2013), which have been used for decades in traditional industry, although they have been more widely used in the aquaculture sector; they have been becoming candidates for other industrial applications as high added value products (De Tommasi 2016, Hamed 2016).

Diatoms are abundant in various extracellular polymeric substances, including lipids, proteins, carbohydrates, carotenoids, sterols, and isoprenoids. These compounds are valuable for their uses in dietary supplements, aquaculture, cosmetics, biomedical and pharmaceutical applications (such as antiproliferative, antioxidant, antibacterial, antiviral), and bioremediation,

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among other uses (Lee et al. 1989, Arad & Yaron 1992, Brown 2002, Mendes et al. 2003, Spolaore et al. 2006, Zammit 2016, Chew et al. 2017). Two potential candidates for utilization due to their rich metabolites are the benthic diatom *Amphora* aff. *amoena* and the planktonic diatom *Chaetoceros muelleri*.

It has been observed that manipulating their cultivation conditions favors a biomass rich in bioactive compounds. Factors such as limitation of nutrients, changes in pH, temperature, salinity, and light modifications are most important in its development (Markou et al. 2012). Intensity and wavelength are key in the development of microalgae. Various studies have shown that, by modifying the lighting conditions, it is possible to obtain a better production of biomass and bioactive compounds in various species of microalgae (Schulze et al. 2014, He et al. 2015, Helena et al. 2016, Jaubert et al. 2017), including diatoms (Nilawati et al. 1997)

Light is a factor that influences the development of microalgae. Several studies have shown that exposure to various light conditions can stimulate the production of biomolecules, directly influencing the proximal composition (Markou et al. 2012, Fimbres-Olivarría et al. 2018).

Water has a high absorption of red light (RL, 720-740 nm). Therefore, it is the first to be absorbed. In deep waters, blue light (BL, 430-480 nm) and green light (500-570 nm) are predominant (Levine & MacNichol 1982). The type of preferential length of each species depends on the physiological adaptations of the microalgae. For example, benthic algae, naturally distributed in the seabed or aquatic substrate, tend to perform higher when exposed to BL. On the other hand, planktonic algae tend to prefer the RL, as they are distributed on the surface and in the water column (Guo & Fang 2020).

RL, particularly the far-red, promotes high growth rates; however, cell size is smaller in many microalgae (De Tommasi 2016). In RL, the spectrum of chlorophyll absorption is wider, and there is more carbon accumulation, favoring the production of lipids and carbohydrates; likewise, it reinforces photosystem II (De Tommasi 2016).

On the other hand, BL has been reported to promote the activation of enzyme cascades (ferredoxin), increase levels of alanine, aspartate, glutamate, glutamine, and malate, promote protein synthesis, and strengthen photosystem I (Schulze et al. 2014, De Tommasi 2016).

Due to microalgae's great importance and usefulness in various industrial fields, it is essential to generate information about its proximal composition under exposure to various wavelengths. This will allow us to delve further into the potential use of these biomolecules in multiple areas, such as nutrition, aquaculture, cosmetics, and bioremediation.

The benthic diatoms *A.* aff. *amoena* and *C. muelleri* were obtained from the strain collection of the Department of Scientific and Technological Research of the University of Sonora (DICTUS, by its Spanish acronym). They were cultivated in 1 L Erlenmeyer flasks with 700 mL of medium F, salinity of 35, and average temperature of 24.5 ± 1.3°C. The medium was prepared with previously filtered and sterilized seawater. The microalgae were exposed to three wavelengths: BL (430-480 nm), white light (WL, 400-750 nm), and RL (595-660 nm), at an irradiance of 200 µmol m^{-2} s^{-1} during 24 h using light-emitting diodes (LED). Phillips®, Amsterdam. The cultures were carried out in three biological replicates (each of three technical replicates). Irradiance was measured with an immersion photometer brand LI-COR Model LI-250 Light Meter (Arredondo-Vega & Voltolina 2007).

For the cell count, 3 mL of each treatment were taken and fixed with Lugol (solution of I_2 at 1% and KI at 2% in distilled water) (Andersen 2005). Cell counts were performed daily and were carried out in a Neubauer chamber (0.1 mm depth) and observed in an optical microscope (Carl Zeiss Axiostar Plus). Considering an initial concentration of 50,000 cells mL^{-1} for *A.* aff *amoena* and 100,000 cells mL^{-1} for *C. muelleri*. The number of cells was obtained using the following equation (Arredondo-Vega & Voltolina 2007).

$$\text{Number (cells mL}^{-1}) = \left(\frac{\text{\# total cells}}{\text{\# plots counted}}\right)(10)^4$$

Biomass was determined gravimetrically by weight difference between the GFC glass fiber filter (GFC 45 mm diameter) with and without a microalgae sample, with a known concentration for a duplicate of each culture. The filters were previously washed with distilled water and dried for 8 h in a conventional oven (THELCO® Laboratory Oven, Precision Science, Model 130). The biomass and filters were washed with 3% ammonium formate to remove salt. The filters were placed in aluminum foil in the oven for about 12 h. The total dry weight (mg^{-1} L) was obtained by calculating the weight difference between the weight of the dry filter without the sample and the weight of the filtered sample, divided by the volume of the filtered culture.

The filter with the dry and weighed biomass was incinerated in a muffle (Felisa®, Model 3 60D) at 490°C for 7 h. The filters were weighed; by the weight difference between the filter with dry biomass and the filter with ash, the inorganic weight (ashes) was obtained. Organic matter was obtained by weight difference with dry sample and ash filter (Sorokin 1973, Arredondo-Vega & Voltolina 2007).

For the chemical analysis, a known volume of each flask was filtered through the Whatman GFC of 25-45 mm diameter filters. Proteins were determined following the methodology described by Lowry et al. (1951) and modified by López-Elías et al. (1995); and the methodology of DuBois et al. (1956) for the determination of carbohydrates and Pande et al. (1963) to estimate lipid content.

Growth kinetics, cell concentration, dry biomass, ash, organic matter, and chemical analysis (carbohydrates and proteins) were analyzed by one-way ANOVA with a significance level of $P \leq 0.05$. The Tukey test separated the means for those parameters in which statistically significant differences were observed. Statistical analyses were performed with the JMP 11 statistical software.

In this study, different wavelengths of light impacted cell growth and physiochemical determinations on the diatoms *A.* aff. *amoena* and *C. muelleri*. Light is one of the most important factors for microalgae, and it is essential to initiate photosynthesis (Schulze et al. 2014). The quality of light includes the different wavelengths that directly affect their development (Lepetit et al. 2017). The visible spectrum represents a small portion of the electromagnetic radiation spectrum, spanning from UV (380 nm) to RL (780 nm) (Sliney 2016).

Sunlight is the primary source of the microalgae in their natural habitat or cultivated outdoors. However, in laboratory settings, using LEDs emitting specific wavelengths has proven to be a very efficient alternative (Li et al. 2020).

BL and RL subserve the development of microalgae (Lepetit et al. 2013, Schulze et al. 2014, Fimbres-Olivarría et al. 2018, Iwasaki et al. 2021), WL has the entire visible spectrum, it contains BL and RL in a balanced way, that can favor a greater accumulation of carbon. Therefore, this light provides the illumination condition to which microalgae are exposed in their environment (Lehmuskero et al. 2018). The WL influenced growth kinetics on the microalgae *A.* aff. *amoena*, reaching an average of 216,250 ± 4,389 cells mL^{-1}, followed by BL (187,083 ± 1,512 cells mL^{-1}), meanwhile the lower cell concentration was observed when exposed to the RL (130,312 ± 5,340 cells mL^{-1}) (Fig. 1). The same tendency occurred on *C. muelleri*, the lower concentration was obtained when exposed to the RL, 242,500 ± 13,248 cells mL^{-1}, on BL 599,375 ± 18,561 cells mL^{-1}, and the highest at the WL exposition (1,198,125 ± 29,4506 cells mL^{-1}) (Fig. 2). Other authors reported a decrease in the number of cells in the genus *Chaetoceros* when exposed to RL (Li et al. 2020, Iwasaki et al. 2021). In *Chlorella vulgaris* and *Dunaliella tertiolecta*, the RL causes low cell production (Tang et al. 2011, Yan et al. 2013).

The different wavelengths did not affect the biomass and ash of *A.* aff. *amoena*. Nevertheless, in the diatom *C. muelleri*, a greater accumulation of organic matter and ash was observed in the cells exposed to RL (84.50 ± 0.09 g L^{-1} of dry weight (DW) and 77.00 ± 0.05 (%DW), respectively, and in equal proportion under BL and WL (Table 1). Concerning the ashes (%DW), BL and WL exhibited the highest concentrations (33.00 ± 0.05 and 32.38 ± 0.04).

The accumulation of carbohydrates in the diatoms *A.* aff. *amoena* and *C. muelleri* were favored with the WL (5.80 ± 0.02 and 2.21 ± 0.07%, respectively), followed by BL (0.44 ± 0.02 and 1.58 ± 0.16%, respectively). Further, a low concentration was recorded in the microalgae *C. muelleri*, though no carbohydrates were detected in *A.* aff. *amoena* exposed to RL. Previous studies have reported a higher content of carbohydrates for other benthic species, such as *Navicula* sp. (Fimbres-Olivarría et al. 2015). In the present study, *A.* aff. *amoena* was exposed to high irradiance (200 µmoL m^{-2} s^{-1}), considering its benthic nature, this could cause photoinhibition, which leads to cell damage, therefore affecting the synthesis of primary metabolites (Lehmuskero et al. 2018).

Regarding proteins, both species reached the major content under WL (13.41 ± 0.001 and 12.31 ± 0.01%, for *A.* aff. *amoena* and *C. muelleri*, respectively). However, RL generated the lowest amount in the diatoms *A.* aff. *amoena* and *C. muelleri* (3.29 ± 0.001 and 3.30 ± 0.01%, respectively) (Table 1). In other studies, a consortium of *Chlorella variabilis* and *Scenedesmus obliquus* showed a higher amount of protein, 56% (Gatamaneni-Loganathan et al. 2020). Previously, it has been reported that BL plays a large role in protein production due to its participation in photosynthesis, which promotes a large accumulation of nitrogen and favors protein production. In this study, WL increases the accumulation of this biomolecule (Miao et al. 2016, Lehmuskero et al. 2018, Guo & Fang 2020) since it contains the entire visible spectrum. It has been observed that RL can affect photosynthesis,

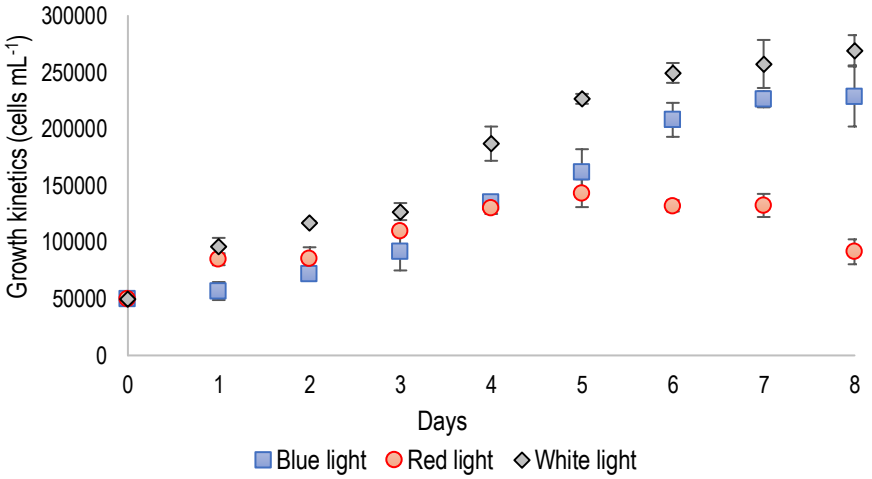

Figure 1. Growth kinetics of *Amphora* aff. *amoena* cultivated at different wavelengths.

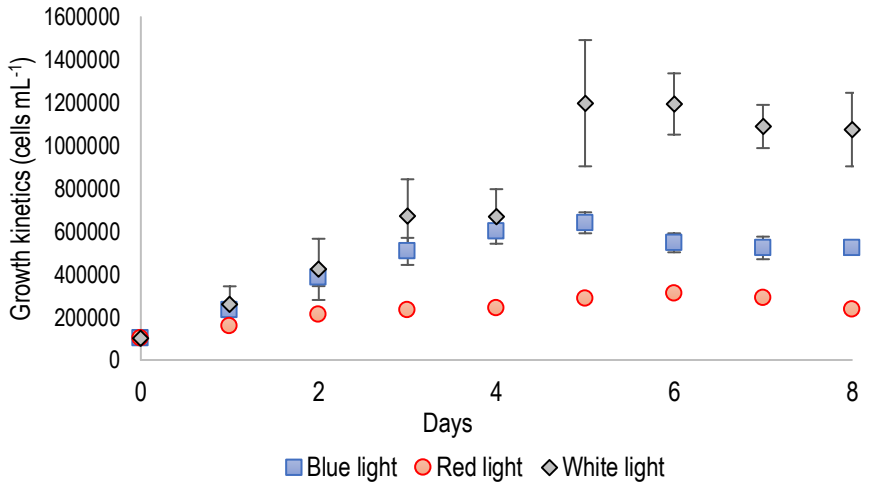

Figure 2. Growth kinetics of *Chaetoceros muelleri* cultivated at different wavelengths.

causing an inhibition of protein production, as Pei et al. (2022) reported in the microalgae *Isochrysis zhanjiangensis*.

Lipids were the most abundant biomolecule in the two diatoms. In *A.* aff. *amoena*, the effect of light exposure was also demonstrated; the highest percentage was in the RL (37.19 ± 0.57%) and the lowest in the BL (17.27 ± 0.29%). In lipids, the major accumulation was recorded in the RL, which aligns with the findings by Fimbres-Olivarría et al. (2015) for the benthic diatom *Navicula* sp. (35.24 ± 4.54%) at an irradiance of 50 µmol m^{-2} s^{-1}; this light has a longer wavelength and generates a greater accumulation of carbon, which can promote the lipids synthesis. Also, *Navicula* sp. had the highest lipid content with the exposition to this light (Fimbres-Olivarría et al. 2015). Yang & Weathers (2015) reported that brief exposure to RL increased lipid production in the microalgae *Ettlia oleoabundans*. The increase in lipid production with exposure to RL could be attributed to a reduction in photosynthetic activity caused by the isolation of exposure to a single wavelength and light intensity. Light stress conditions have been reported to favor the accumulation of lipids in the cell (Severes et al. 2017).

On the other hand, *C. muelleri* exhibited a higher concentration of lipids under BL (60.48 ± 2.4%). BL promotes the synthesis of RuBisCo and carbonic anhydrase, which favors lipid accumulation (Lehmuskero et al. 2018). Some studies have indicated that BL increases lipids in microalgae (Atta et al. 2013, Schulze et al. 2014).

Table 1. Biomass and proximal chemical composition of the microalgae *Amphora* aff. *amoena* and *Chaetoceros muelleri* cultivated at different wavelengths. One-way ANOVA and Tukey *a posteriori* $P > 0.05$. F: statistical value used to compare variances and determine significant differences between groups. Treatment values are means ± standard deviation of three biological replicates and three technical replicates. Different letters in the same row indicate significant differences. DW: dry weight.

Amphora aff. *amoena*				
	Wavelength			
	430-480 nm	650-750 nm	400-750 nm	$P > F$
Biomass (g L^{-1} DW)	$67.44^{b} \pm 12.65$	$37.94^{c} \pm 0.01$	$103.38^{a} \pm 10.27$	0.0001
Organic matter (%DW)	62.62 ± 0.02	67.50 ± 0.02	64.78 ± 0.02	0.1119
Ashes (%DW)	37.38 ± 0.02	32.50 ± 0.07	35.22 ± 0.03	0.2036
Total carbohydrates (%DW)	$0.44^{b} \pm 0.02$	$0.00^{c} \pm 0.00$	$5.80^{a} \pm 0.02$	0.0001
Total lipids (%DW)	$17.27^{c} \pm 0.29$	$37.19^{a} \pm 0.57$	$22.43^{b} \pm 0.23$	0.0001
Total proteins (%DW)	$12.46^{b} \pm 0.01$	$3.29^{c} \pm 0.01$	$13.41^{a} \pm 0.01$	0.0001
Chaetoceros muelleri				
	Wavelength			
	430-480 nm	650-750 nm	400-750 nm	$P > F$
Biomass (g L^{-1} DW)	$61.38^{b} \pm 0.08$	$84.50^{a} \pm 0.09$	$52.00^{b} \pm 0.01$	0.0006
Organic matter (%DW)	$67.00^{b} \pm 0.05$	$77.00^{a} \pm 0.05$	$67.62^{b} \pm 0.40$	0.0011
Ashes (%DW)	$33.00^{a} \pm 0.05$	$23.00^{b} \pm 0.04$	$32.38^{a} \pm 0.04$	0.0004
Total carbohydrates (%DW)	$1.58^{b} \pm 0.16$	$1.46^{c} \pm 0.01$	$2.21^{a} \pm 0.07$	0.0079
Total lipids (%DW)	$60.48^{a} \pm 2.4$	$17.03^{c} \pm 0.07$	$23.29^{b} \pm 1.01$	0.0002
Total proteins (%DW)	$8.46^{b} \pm 0.011$	$3.30^{c} \pm 0.01$	$12.31^{a} \pm 0.01$	0.0027

It is evident that the WL, containing the entire visible spectrum, supported the accumulation and production of biomass and biomolecules. Lipids were the most abundant biomolecule, and both BL and RL favored this accumulation in both species. RL increased the lipid accumulation in the diatom *A.* aff. *amoena*, whereas in *C. muelleri*, BL was found to enhance this increase. Based on the knowledge gained about the effects of different wavelengths on these microalgae, further studies should be conducted to investigate the presence of different compounds that can promote the bioactivities of these species under light stress. Additionally, their applications can be explored to improve various aspects of food production, including their potential use in aquaculture and other related industries globally.

Credit author contribution

M.L. Juárez-Gómez: conceptualization, validation, methodology, formal analysis, visualization, writing-original draft; C. Hayano-Kanashiro: funding acquisition, project administration, supervision, review, and editing; D. Fimbres-Olivarría: conceptualization, methodology, validation, visualization, supervision, review, and editing, data curation, formal analysis. All authors have read and accepted the published version of the manuscript.

Conflict of interest

The authors declare no potential conflict of interest in this manuscript.

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