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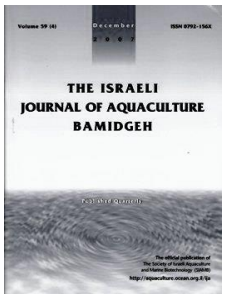

Growth and Biochemical Composition of *Navicula sp*. Cultivated at Two Light Intensities and Three Wavelengths

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Abstract

Many studies have reported that the exposure of microalgae cultures to red light increases the production of carbohydrates, while blue light promotes the production of protein. There are several studies about *Navicula*, however there are few, if any, studies of the combined effects of wavelength and light intensity on the biochemical composition of this genus In this study we evaluated the combined effect of three wavelengths: white (400-750nm), blue (430-480nm), and red (650-750nm), at two light intensities (50 and100 µmol/m^2/sec) on the growth and biochemical composition of *Navicula* sp. cultured on a laboratory scale. The experiment was carried out under controlled conditions utilizing a factorial design 2x3 (light intensity and wavelength) with white light as the control. The cell concentration was measured daily. Dry biomass of filtered cells was incinerated at 450ºC in a muffle oven. The biochemical content was measured using micro methods. The cell concentration was higher with white light at both intensities (291,875 and 90,938 cells/mL at 50 and100 µmol photon/m^2/sec, respectively). Microalgae grown under blue wavelengths at 100 µmol photon/m^2/sec had the highest dry biomass (1607 pg/cell). The highest percentage of protein was obtained under the blue light (22.83%), carbohydrates under the white light (4.13) and lipids under the red light (35.25%) all these results were observed under the low light intensity (50 µmol photon/m^2/sec). The highest cell concentration and growth rate was observed under the low light intensity the largest proportions of which were proteins produced under the blue light. Lipid composition was not affected by light intensity or wavelength.

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Introduction

Microalgae are organisms of great interest due to the great variety of polysaccharides that can be extracted under culture conditions (Laurienzo 2010). Several studies have reported the effect of exposure to different wavelengths and light intensity on microalgae cultures. It has been observed that red light increases the production of carbohydrates and blue light promotes the production of protein in *Chaetoceros* cultures (Ramos-Lemuz 2000; Ramírez-Trejo 2002; Korbee et al. 2005). White light is the combined effects of red and blue wavelengths (Korbee et al. 2005).

Benthic microalgae have high potential for production of polysaccharides (Leal et al. 2010) because they generate mucilage which is high in extracellular polymeric substances (EPS), including lipids, proteins, nucleic acids, and carbohydrates (Staats et al. 1999; Leal et al. 2012). These compounds have a wide range of applications in biotechnological industries, such as gels production, cosmetics, antioxidants, antibacterials, antivirals, and others (Staats et al. 1999; Lee et al. 2006; Melo-Ruiz y Cuamatz, 2007; Laurienzo 2010; Amaro et al. 2011; Raposo et al. 2013).

Although there have been studies on the genus *Navicula* these have not investigated the combined effects of wavelength and light intensity on the biochemical composition of species of the genus. In this study we evaluated the effect of three wavelengths: white (400-750nm), red (650-750nm), and blue (430-480nm), at two light intensities (50 and100 µmol photon/m^2/sec) on the growth, biomass, and lipid, protein and carbohydrate content of *Navicula* sp.

Materials and Methods

Selection of microalgae strain. In this study we evaluated the benthic microalgae *Navicula* sp., using a strain from the collection of Department of Scientific and Technological Research of the University of Sonora (DICTUS).

Experimental design. The study was carried out under indoor controlled conditions. A factorial 3X2 (wavelengths X intensities) experimental design with 4 replicates per treatment was performed. Treatments consisted of three wavelengths: (control) white (400-750nm), red (650-750nm), and blue (430-480nm), and two light intensities (50 and 100 µmol photon/m2/sec). The experimental units consisted of transparent plastic containers with 10 L of F media (Guillard and Ryther 1962). The stocking density of microalgae was 35,000 cells/mL. Light was supplied by Light Emitting Diode lamps (LED) electronically controlled to the desired intensity. The irradiance was measured using a quantic spherical sensor Li-Cor 193SA to obtain the desired wavelength.

Cell count, biomass and proximate composition. The cell concentration was measured using a Neubauer chamber and an optical microscope (Carl Zeiss Axiostar plus) with the following equation:

$$\#\frac{\text{cells}}{\text{ml}} = (\text{Average number of cells in eight large squares})(10^4)$$

Samples for biomass evaluation were taken by filtering 250 mL culture water in Whatman GFC paper filters (diameter 47 mm) previously calibrated. The filters were dried for 8 h at 75ºC in a conventional oven, then incinerated for 12 h at 450ºC in a muffle oven, and finally weighed with a digital balance. Biomass was determined by difference in weight. The microalgae protein content was measured following the methodology described by Lowry and modified by López-Elías et al. (1995); the carbohydrate concentration was estimated by the "phenol-sulfuric acid" method reported by Dubois et al. (1956), and the lipid content was calculated by a colorimetric method described by Pande et al. (1963).

Statistical analysis. The cell concentration data was analyzed with descriptive statistics (average and standard deviation), in addition to an analysis of two-way ANOVA and a posteriori Tukey test to determine differences between treatments in terms of cell concentration, biomass, and biochemical composition of microalgae (Zar, 1999). For statistical analysis STATISTICA for Windows (StatSoft, 1995) was used.

Results

Cell concentration. Growth (measured as cell concentration) of control cultures (white light) and cultures treated with red light increased up to second day at light intensity of 50 µmol photon/m^2 /sec, while in cultures under blue light, they increased up to the third day at the same intensity. No significant differences in final cell concentration between blue light and the control (Figure 1a) were found (F=34.77, p>0.05). Growth of cultures in the control (white light) treatment, exposed to 100 µmol photon/m^2/sec was significantly higher on the first day, compared to the other wavelengths (F=34.77, p <0.05) (Figure 1b).

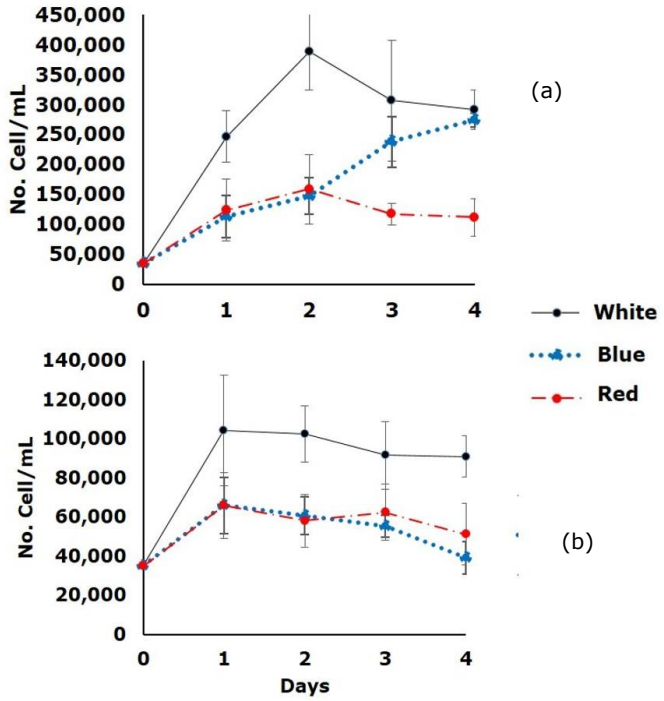

Fig. 1. Growth curves of the microalgae *Navicula* sp. cultivated at two light intensities:
(a) 50 µmol/m^2/sec and
(b) 100 µmol/ m^2/sec
at three wavelengths (white: 400-750nm, blue: 430-480nm and red: 650-750nm).

Cell concentration levels of microalgae at the end of the experiment were higher in white light at both intensities (291,875 cells/mL at 50 µmol photon/m^2/sec and 90,938 cells/mL at 100 µmol photon/m^2/sec), with significant differences ($F_{illumination}$= 350.1, p≤ 0.01; $F_{wavelength}$= 53.58, p≤ 0.01) between them (Table 1).

Table 1. Cell concentration and growth rates at the end of the culture of *Navicula* sp. cultivated at two light intensities (50 µmol photon/m^2/sec and 100 µmol photon/m^2 /sec) and three wavelengths.

Light intensities	*Wavelength (nm)*	*Final cell concentration (Cell/mL)*	*Growth rate (µmax)*	*Average growth rate*	*Accumulated growth rate*
50	400-750	291,875± 33,064^{d}	2.80±0.26^{b}	1.26±0.98^{b}	9.02±5.32^{a}
50	430-480	274,583±12,140^{d}	1.64±0.42^{a}	0.87±0.58^{ab}	5.07±3.23^{b}
50	650-750	112,500±31,225^{c}	1.72±0.71^{a}	0.75±0.68^{ab}	4.16±2.51^{ab}
100	400-750	90,938±10,625^{bc}	1.54±0.37^{a}	0.62±0.56^{a}	4.25±2.53^{ab}
100	430-480	39,063±8,189^{a}	0.88±0.31^{a}	0.31±0.36^{a}	1.91±1.41^{a}
100	650-750	51,250±15,579^{ab}	0.88±0.36^{a}	0.33±0.35^{a}	2.23±1.29^{ab}

Different letters in the same column indicate significant differences at P<0.01.

Final cell concentrations were higher in cultures grown under blue and red wavelengths at low intensities of 50 µmol photon/m^2/sec compared with the concentrations reached in cell cultures exposed to the higher light intensity of 100 µmol photon/m^2/sec at the same wavelengths. All cultures had the lowest concentration of cells at the highest light intensity during the experiment (Table 1).

Maximum growth rate was obtained at 50 µmol photon/m^2/sec light intensity under white light (2.80 divisions/day), significantly higher than the other treatments ($F_{illumination}$= 20.75, p≤ 0.01; $F_{Wavelenght}$= 4.77, p≤ 0.01) (Table 1). There were no significant differences (F=0.81, p>0.05) between maximum growth rates in cultures exposed to 100 µmol photon/m2/sec of light intensity at the three wavelengths.

Biomass production and biochemical composition. The highest production of dry biomass per cell was found in cultures exposed to light intensity of 100 µmol photon/m^2/sec at the three wavelengths, those grown at blue wavelengths being the greatest (Table 2), while the cultures exposed to 50 µmol photon/m^2/sec had the lowest dry biomass production, being greater in the cultures exposed to the red wavelength ($F_{illumination}$ = 180.90, p≤ 0.01; $F_{wavelenght}$ = 24.33, p≤ 0.01) than in the other wavelengths (Table 2).

Table 2. Biomass in pg/cell (picograms per cell) and final biochemical composition of the microalgae *Navicula* sp. cultivated at two light intensities (50 µmol photon/m^2/sec and 100 µmol photon/m^2/sec) and three wavelengths: white (400-750nm), blue (430-480nm), and red (650-750nm).

Light intensities	*Wavelength (nm)*	*Dry biomass (pg/cell)*	*Organic matter (pg/cell)*	*Protein %*	*Carbohydrate %*	*Lipid %*
50	400-750	199.14±11.35^{a}	99.7±3.40^{a}	16.54±2.13^{a}	4.13±0.70^{a}	25.40±2.60^{a}
50	430-480	178.22±68.16^{a}	132.6±14.09^{a}	22.83±2.42^{b}	3.06±0.86^{a}	25.32±5.32^{a}
50	650-750	423.56±25.17^{ab}	213.3±12.02^{ab}	15.12±4.79^{a}	3.33±0.78^{a}	35.25±4.54^{e}
100	400-750	592.16±105.98^{b}	320±59.96^{b}	15.02±2.12^{a}	3.49±0.85^{a}	30.34±6.20^{d}
100	430-480	1607.66±243.49^{c}	947.2±143.40^{d}	14.36±1.67^{a}	3.46±1.13^{a}	19.15±1.82^{ac}
100	650-750	1468.29±536.30^{c}	743.4±131.91^{c}	12.83±2.84^{a}	3.70±0.52^{a}	14.87±3.93^{b}

Different letters in the same column means significant differences at P<0.01.

As shown in table 2, a significant increase of dry biomass and organic matter production (pg/cell) was observed as the light intensity increased, with the greatest values for the microalgae cultivated at 100 µmol photon/m^2/sec.

The highest protein level was observed in cultures exposed at light intensities of 50 µmol photon/m^2/sec in blue light (23%) (Table 2). This was significantly different than in the other treatments ($F_{illumination}$= 8.76, p≤ 0.01; $F_{wavelength}$= 5.72, p≤ 0.01).

The total carbohydrate content observed in cultures of *Navicula* sp., was higher at intensities of 50 µmol photon/m^2/sec in white light; however, no significant difference between the two intensities of light and the three wavelengths (p≤ 0.01) were found (Table 2).

In this study, the lipid content was higher in cultures exposed to light intensities of 50 µmol photon/m^2/sec; the greatest lipid percentage was observed in growth under red light (Table 2).

Discussion

Light is one of the main factors involved in the development of microalgae; quality and quantity of light affects both, growth rate and biomass composition (Markou et al., 2012). In this study relatively low cell concentrations were obtained at 50 µmol photon/m^2/sec and 100 µmol photon/m^2/sec, respectively, cultivated under white light. Leal et al. (2013) reported higher cell concentrations (around 300,000 cells/mL) cultivating the microalgae *Navicula* at light intensities (between 120 and 130 µmol photon/m^2/sec) in white light. This difference is probably due to the different culture medium they used.

During our experiment all cultures of *Navicula* exposed to high light intensities (100 µmol photon/m^2/sec) produced the lowest cell concentrations. This intensity may have produced photo inhibition, causing a decrease in microalgae growth (Markou et al., 2012). Under natural conditions the benthic microalgae reach their maximum photosynthetic rate for a short period during the peak hours of light saturation, and after that go down to the sediment or produce cell aggregates to avoid photoinhibition (Blanchard et al., 2004; Cartaxana et al., 2013).

Biomass content in cultures exposed to 100 µmol photon/m^2/sec at white light, was similar to that found by Leal et al. (2013) who reported values of 410 pg/cell of dry biomass and 270 pg/cell of organic matter from the microalgae *Navicula germanolpolonica* cultivated at salinity of 35 at light intensities between 120 and 130 µmol/m^2/sec. Although the cell concentration was lower in the cultures exposed to higher intensities, the dry biomass was higher compared with those cultures exposed to lower intensity showing an increase as light intensity increased. There are no reports that explain the biomass enhancement in respect to light intensity stress. However this has been documented for other stressors. There was a reported increase of dry biomass in *Dunaliella* sp. when grown in a culture limited in nitrogen (Fimbres Olivarría et al., 2010). Morphological changes and increase in cell size in *Chaetoceros wighamii* and *Dunaliella parva*, respectively were observed when grown in a culture limited in phosphorus (de Castro Araújo and Garcia, 2005); Said, 2009).

Cell size of algae tends to increase as the salinity of the culture medium increases (García et al., 2012). This could indicate that cells such as microalgae, exposed to stressors like nutrient limitations, inadequate salinity, and even high light intensities, tend to increase in size in order to survive in stressful environments.

The values we obtained for biochemical composition of *Navicula* sp., are similar to those reported by other authors for marine microalgae. Brown et al. (1997) reported values between 6-34% protein, 7-23% lipids, and 5-23% carbohydrates, all of which are within the range of those found in the present study. Several studies have reported that the exposure of microalgae cultures to red light increases the production of carbohydrates, while blue light promotes the production of protein.

In this study, the protein content was higher in cultures exposed to blue light; the effect of this light on the photosynthetic cells has been studied mostly in unicellular green algae, and higher plants, showing that exposure to this wavelength promotes the synthesis of proteins, enzyme activation, and accumulation of nitrogenous compounds such as photoresist pigments (Ramos-Lemuz 2000; Ramírez-Trejo 2002; Korbee et al. 2005; Marchetti et al. 2013).

The total carbohydrate content of cultures of *Navicula* sp. was equal between all treatments, which suggests that exposure to these conditions does not influence the production of carbohydrates in this particular species. An increase in the content of total carbohydrates in cultures of microalgae exposed to red wavelengths, has been attributed to to the accumulation of carbon in the media (Ramos-Lemuz (2000); Ramírez-Trejo (2002); Korbee et al., (2005).

Benthic diatoms have been poorly investigated (Leal et al. 2010) even though there is increasing interest in analyzing their potential as a new source of polysaccharides with high added value (Melo et al. 2007).

The major component of the biochemical composition of *Navicula* sp. in this study was lipids. One of the main nutritional characteristics of benthic diatoms is the high content of these biomolecules (Leal et al., 2010). In our study the highest concentration of lipids (32.25%) was seen in cultures exposed to 50 µmol photon/m^2/sec under red light; these values are higher than those reported for *N. germanopolonica* cultivated between 120 and 130 µmol photon/m^2/sec under white light (Leal et al., (2013) and are also higher than those reported for other species of benthic diatoms (Lee et al. 2009); however, the values found in this study correspond with those obtained by Chen (2012) who evaluated biomass and total lipid content in 12 species of marine diatoms reporting values between 30 and 45%.

Conclusion

These results will facilitate studies related to the production of biomass from *Navicula,* rich in bioactive compounds like carbohydrates which despite being the minor components in this species, have great potential in obtaining high value added polysaccharides with antiviral, antioxidant, antibacterial, and others properties.

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