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EFFECT OF LIGHT WAVELENGTH, INTENSITY AND QUALITY PROMOTES EFFICIENT GROWTH OF *CHLORELLA MARINA*

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ABSTRACT

The growth of green algae Chlorella marina under the influence of three kinds of fluorescent lights was investigated in batch culture conditions. Five light intensities (20, 30, 40, 50, 60 and 80 µmol ·m $^{-2}$ ·s $^{-1}$) of the three lights, viz., blue, green and red were evaluated. The effect of light intensity and its quality explicated the specific growth rate, spectrum absorption coefficient and saturated light intensity of fluorescent lights. The procured results showed that the growth rates of Chlorella marina increase with the enhanced light intensity. However, there was inhibition of Chlorella marina growth was observed beyond the saturation light intensity. Further, in comparison with red and green light, the growth rate of Chlorella marina under blue light was found to be higher within saturated light intensity. In addition, the saturated light intensity of florescent lights was lower under blue light. Whereas it was found to be higher under green light.

KEY WORDS

Microalgae, saturated light intensity, Chlorella marina and growth rate.

INTRODUCTION

Use of microalgae for biotechnological applications covers many aspects, such as food additives in nutraceutics and animal feeding, cosmetics, pharmaceutics and energetics [3, 5, 8, 10]. Microalgae have many advantages compared to other photosynthetic organisms, such as their high growth rate and the requirement of little space for biomass production. Indeed, [4] reported a productivity of 10 g m-2 day-1 for microalgae compared to 1–2 g m for higher plants. Light intensity is attenuated exponentially with depth, and quality changes due to different extinction coefficients for various wavelengths. Both intensity and quality are affected by various factors, such as paniculate matter and dissolved substances. Planktonic algae are transported vertically by physical and or biological mechanisms exposing them to various light climates.

The marine green algae *Chlorella marina* has been considered as one of the dominant species in the coastal areas, and it distributes widely in the waters around the world. Many physical factors play important roles in the growth of this harmful alga. Among these factors, light is an important environmental factor which can affect microalgae growth, and it is one of the key environmental factors of the red tides happened. For a certain pH value, temperature, and nutrition conditions, the light intensity and the duration of illumination which decides the algae photosynthesis efficiency also play crucial roles on the growth rate of algae.

Many studies on the effects of light intensity, temperature and salinity have been reported. However, in terms of light intensity, most studies focused on the effects of fluorescent or the sun-light on the growth of the species. And the optimal light intensity for growth of *Chlorella marina* increases gradually to a maximum of 121.6 W m $^{-2}$ with temperature up to 25°C. Under this

temperature, the optimal irradiance was 7000 lx for *Chlorella marina* reported by [13].

In this work, we use fluorescent light source and study the effects of three monochromatic lights on the growth of *Chlorella marina* Based on the study on the effects of different wavelength of monochromatic light and different light intensities on the growth of *Chlorella marina*, we obtain the saturated light intensities of different monochromatic lights, and further provide the basis of analysis of the mechanism of the bloom occurrence.

2. MATERIALS AND METHODS

2.1. Sample collection and Microscopic observation

The phytoplankton sample was collected from vellar estuvary, Parangipettai, Tamilnadu, India. The samples were collected by towing the plankton net (mesh size 20 µm) on the surface of the water column. Soon after, the collected samples were transported to the laboratory and subjected for lively examination for dominant species identification and isolation under the light microscope (Magnus MLX-DX, Olympus (India). Sample were shacked well and analyzed under light microscope. The specimens were prepared as temporary slides and observed under microscope to determine the genera of phytoplankton community identify upto species levels with reference to the standard manuals [1, 2, 12].

2.2. Preparation of Culture medium

Unialgal in-vitro cultures were urbanized and maintained in F/2 medium [6]. Seawater used in this work is sterilized seawater that is filtered through Glass fiber (GF -3 Ø 47 mm) filter before used for culture medium preparation. During media preparation, the nutrient solutions were stored at 6°C.

2.3. Isolation and *in-vitro* culture of *C. marina*

All the nutrients were added to the seawater and sterilized [7]. Diatom species from the collected sample were isolated using the physical separations such as single cell picking method and inoculated in a test tube containing the medium. It was serially diluted to obtain a specific strain and pure bacterial free cultures were obtained by using antibiotics Ampicillin and Tetracycline (120mg/L). Axenic cultures were transferred to 2L Erlenmeyer flasks containing the culture medium, an enrichment of seawater. The flasks were kept for 10 days in artificial light under 12:12 light: dark photoperiod. The selected dominant microalgal sp.(*C. marina*) cells were transferred on to the freshly prepared media The lights used for the culture are provided by monochromatic fluorescent light with the center wavelength of 456 nm, 512 nm and 656 nm, respectively. The light intensity can be adjusted from 20µmol $m^{2}{\cdot}s^{-1}$ to 80µmol $m^{-2}{\cdot}s^{-1}$ by (PPFD-Photosynthetic Photon Flux Density) changing the distance between the light source and vessel. The light sources are measured by using Lux Meter through converting PPFD.

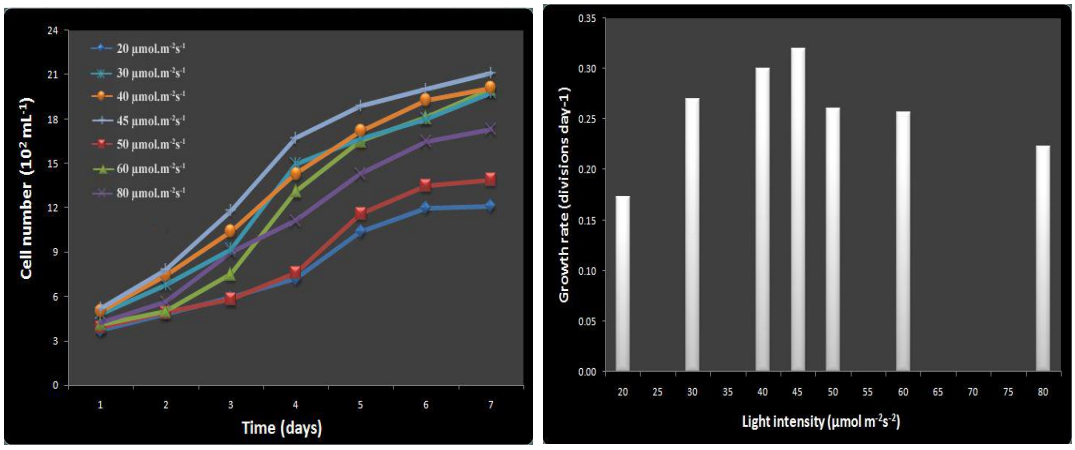
2. Method of Measurement

For researching the growth condition of *Chlorella marina* a 10 µL algal sample is fetched from each flask every day and then supported by a glass slide. The numbers of algal cell are counted under optical microscope and cell number counting's repeated for at least three times. The specific growth rate μ was calculated during the exponential growth period by [9, 11].

3. RESULTS AND DISCUSSION

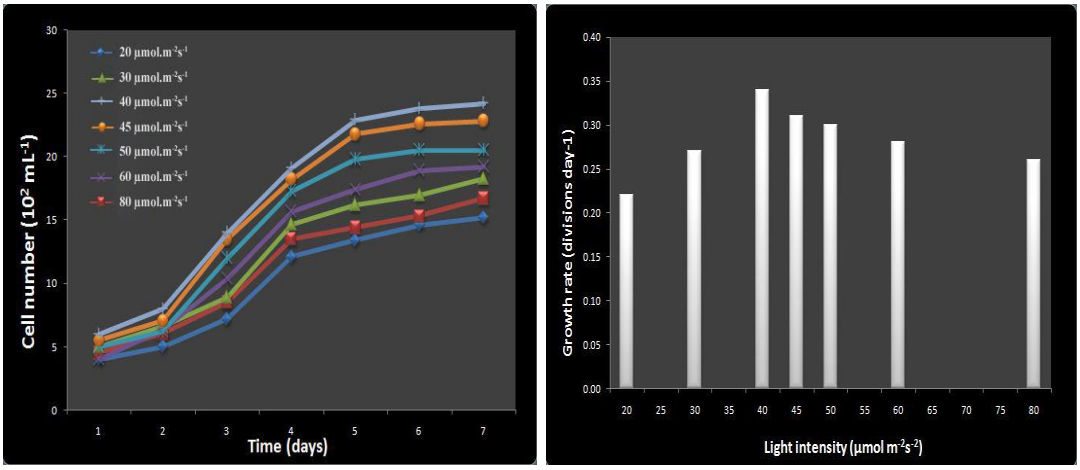
3.1. Effects of Light Intensity and Light Quality on the Growth of *Chlorella marina.*

Firstly, we study the effect of blue light on the growth rate of *Chlorella marina*. *Chlorella marina*, strain culture is maintained in conditions as mentioned above until the end of the exponential phase. The typical light intensity used are 20, 30, 40, 50, 60 and 80 µmol $\cdot m^{-2} \cdot s^{-1}$ light intensities, the *Chlorella marina,* has an exponential growth phase in seven days, as is shown in **Figure 1** (Considering that the cell cycle of *Chlorella marina* is about five day, we pay our attentions to the data in the first seven days), for different light intensities, the growth of *Chlorella marina* is significantly different, especially after two days. Based on the cell number, we can calculate the growth rates under different light intensities.



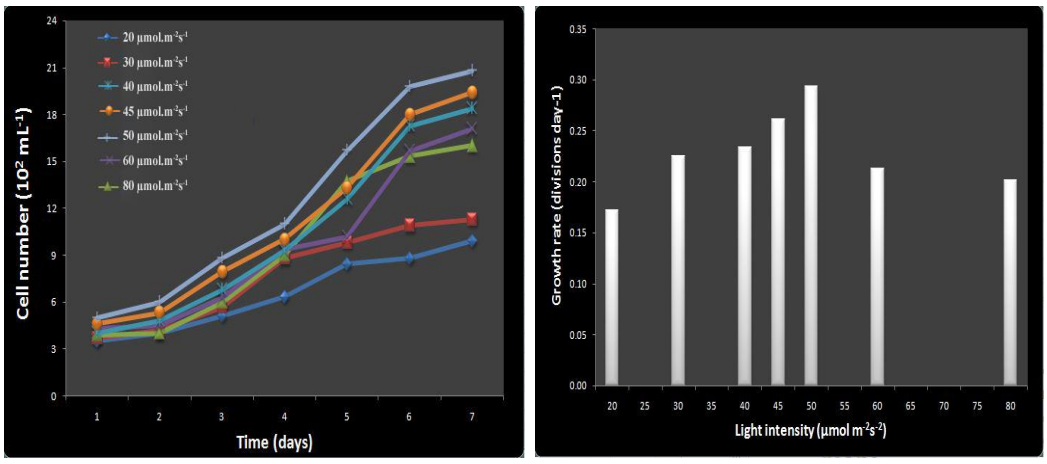
(a) **(b)**

Figure 1. (a) The growth curve of Chlorella marina under blue light; (b) Growth rate of Chlorella marina under blue light.



a) **(b)**

Figure 2. (a) The growth curve of Chlorella marina under green light; (b) Growth rate of Chlorella marina under green light



(a) **(b)**

Figure 3. (a) The growth curve of Chlorella marina under red light; (b) Growth rate of Chlorella marina under red light.

As is shown in **Figure 1 (b)**, the growth rates increase with the increasing light intensity from 20 to 80 µmol m^{-2} s^{-1}, the maximum growth rate is observed at 40 µmol m^{-2} s^{-1} **Figure 1(b)**. When the light level exceeds 40 µmol m^{-2} s^{-1}, the growth rates of *Chlorella marina* gradually decrease, which means the saturated light intensity of *Chlorella marina* under blue light is about 40 µmol m^{-2} s^{-1}. When the *Chlorella marina* is cultured under green light, the effect of the wavelength on the growth of the microalgae is not significant in the first two days **Figure 2(a)**, and then it goes significantly different after two days. Growth response curves as a function of intensity for *Chlorella marina* are shown in **Figure 2(b)**. The growth rates of *Chlorella marina* increase when light intensity increases from 20 to 50 µmol m^{-2} s^{-1}, and then decreases beyond 50 µmol m^{-2} s^{-1}. The highest growth rate of *Chlorella marina* is 0.293 at 50µmol·m^{-2}·s^{-1}. By the same method, we study the cell numbers and growth rates of *Chlorella marina* under red light, as is shown in **Figures 3 (a)** and **(b)**. The growth rates of *Chlorella marina* increase with the increasing light intensity from 20 to 45 µmol m^{-2} s^{-1}. When exceeds this light intensity range, the growth rates gradually decrease. The maximum and minimum growth rates of *Chlorella marina* are 0.173 at 20 µmol m^{-2} s^{-1} and 0.320 at 45 µmol m^{-2} s^{-1}, respectively. The saturated light intensity of *Chlorella marina* under red light is about 45µmol m^{-2} s^{-1}.

CONCLUSION

In conclusion, effects of light intensity and quality of three kinds of fluorescent lights light (blue, green, and red) on the growth of *Chlorella marine* are investigated. Results above show that when cells are exposed to blue, green or red light the growth conditions of *Chlorella marina* are greatly influence by different light wavelength. For three kinds of fluorescent light, the growth rates of *Chlorella marina* are significantly different. The growth rate under blue light is larger than under green and red light until it reaches saturation light intensity. In blue light condition, the saturation light intensity is about 40 µmol m^{-2} s^{-1}, which below the value of saturation light intensity of the green (50 µmol m^{-2} s^{-1}) and red light (45 µmol m^{-2} s^{-1}).

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