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Influence of Photoperiod, Light Intensity and Temperature for Optimizing Biomass and Pigment Productivities of *Chlorella salina*

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ABSTRACT

A study was conducted to investigate the effect of temperature (18°, 22°, 26°, 30°C) and four different light: dark cycles (24:0, 16:8; 12:12; 8:16) with three different light intensities (100, 200 and 400 µmol m-2 s-1) on maximum growth rate, maximum yield and pigment content (Lutein) of a marine chlorophyte Chlorella salina in batch culture to provide a more mechanistic understanding of the role of environmental factors. The results revealed that C. salina preferred growth under high light intensity of 200 µmol m-2 s-1, a maximum of 22°C temperature and 16:8 light/dark periods. The interaction effect of light intensity and photoperiod has resulted in the increase of total carotenoids and lutein content. The results indicate that varying the light regime; it is capable to manipulate the biochemical composition of the marine isolate C. salina, producing valuable antioxidant lutein under 22°C, with the light intensity of 200 µmol m-2 s-1 and a photoperiod regime of 16:8 light/dark cycle.

Keywords: Microalgae, Chlorella salina, Light intensity, Photoperiod, Lutein

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INTRODUCTION

Microalgae are a major natural source for a vast array of valuable compounds as lipids, proteins, carbohydrates, pigments among others. Despite many applications, only a few species of microalgae are cultured commercially because of poorly developed cultivation process. The cultivation of these photosynthetic microorganisms represents an attractive process for obtaining biochemical components with high potential applications in different industries [1, 2, 3, 4]. Microalgae combine properties typical of higher plants (efficient oxygenic photosynthesis and simplicity of nutritional requirements) with biotechnological attributes properties of microbial cells (fast growth in liquid culture and ability to accumulate or secret some metabolites). This particular combination represents the basis of microalgal biotechnology for the use of these microorganisms on high-valued metabolites production [2]. The productivity optimization of microalgae cultures is seen as a key factor for the process of cultivation aimed at the production of metabolites. Higher productivities are necessary to become the green feedstock economically viable and overcome the marks achieved by traditional cultures of terrestrial plants. The cell growth rate of microalgae are affected by a combination of environmental parameters like light intensity, photoperiod, temperature and nutrient composition of the culture medium [5]. With regard to light radiation, it may be limiting to the cultivation, particularly in dense cultures due to the shadowing effect of cells [6] inhibiting the growth, or when in excess due to the process known as photoinhibition which reduces the biomass production. Therefore, the identification and selection of tolerant species to light without productivity losses is intended [7].

In order to produce higher carotenoid content in microalgae, some physical and chemical factors which influence growth and formation of carotenoid were considered; including light, temperature and photoperiod. Higher amount of lutein is produced and accumulated rapidly under stress conditions such as bright light, high salinity and nutrient deprivation (e.g., nitrogen and phosphorous). Furthermore, light-dark cycles can be adjusted to optimize the cell growth and carotenoid accumulation. Concerning the effect of illumination cycles, the investigation by [8] showed that 24:0 photoperiods was more effective than 12:12 photoperiod for carotenoid formation under the same light intensity. The same study indicated that illumination duration is more crucial than light intensity in carotenoid biosynthesis.

Microalgal pigments change with algal variety, and therefore, the influences of different light qualities upon the physiological properties of different algae, such as growth, photosynthesis and cellular metabolism, are diverse. Microalgae from Chlorella species is considered to be one of the most robust species for cultivation in open ponds due to its capability of resisting contamination, and also a promising candidate for commercial lipid production due to its rapid growth [9]. For photoautotrophic algal culture, the light regime is a critical component in determining the biomass production of a culture [10, 11, 12, 13]. Numerous studies have examined how the growth rates of photoautotrophic algal culture are affected under different conditions of photoperiod and irradiance [14, 15, 16]. A change in the light regime can also influence the rate of nutrient uptake [17, 18]. A better understanding of the effects of light and temperature on growth kinetics will contribute to the improvement of biomass productivities and reduce the costs associated with the optimization of culture parameters. The objective of the present investigation is to evaluate the influence of different environmental factors i.e. temperature and illumination on the growth and pigments of *Chlorella salina* to optimize the culture condition for its large scale production.

MATERIAL AND METHODS

Microalgae culture

The microalgae strain used in this study was *C. salina* from CIBA, TamilNadu, India. The volume of inocula added corresponded to 10% of the volume of the sterile medium. The organism was kept in 1L Erlenmeyer flask containing 900ml of Conway medium [19].

Experimental setup

C. salina was cultivated for 21 days. To determine the suitable temperature, light source and photoperiod for the growth; 24:0, 12:12, 16:8, 8:16 light dark photoperiods were chosen under different light intensities, 100, 200 and 400 µmol m^{-2} s^{-1} with different temperature ranges 18°, 22°, 26°, 30°C. The initial cell concentration of *C. salina* was counted as $7.07x10^3$ cells/ml for 24:0 light dark cycles and $9.46x10^3$ cells/ml for

12:12 light dark cycle. Each growth condition was set up at least in triplicate. Cell growth was monitored by recording the value obtained for light scatter at 750nm in OD units, with the time interval of 2 hr between measurements during the culture period.

Specific growth rate (μ) and doubling time (T_d) of all cultures were calculated according to the values of OD_{750nm} recorded. The Specific growth rate (μ) and doubling time (T_d) were calculated according to:

$$\mu = \ln (x_2/x_1)/t_2-t_1$$
$$T_d = \ln 2/\mu$$

Where x_2 and x_1 refer to OD at time (h) and time zero respectively

Analytical Methods

Biomass concentration was determined by dry weight measurements. For this, 10 mL aliquots of the cell suspension were centrifuged, washed with distilled water, and the algae were dried in an oven at 80°C for 24 h. The physiological status of the cells was measured by quantifying the fluorescence of chlorophylls.

Pigments were extracted from the harvested biomass using 80% (v/v) acetone. The absorbance of the acetone extract without cell debris was measured at wavelength of 480 nm for total carotenoids. The content of total carotenoids was calculated according to Strickland & Parsons [20]:

$$\text{Total Carotenoids } (\mu g\ ml^{-1}) = 4.0 \times Abs480_{nm}$$

where $Abs480_{nm}$ is the absorbance of 80% acetone extract measured at 480 nm.

Chlorophyll a, b and total Chlorophyll were evaluated by measuring the absorbance of acetone extract at 664 nm and 647 nm and calculated according to Porra et al. [21]:

$$\text{Chl a } (\mu g\ ml^{-1}) = (12.25 \times Abs664\ nm) - (2.25 \times Abs647\ nm);$$
$$\text{Chl b } (\mu g\ ml^{-1}) = (20.31 \times Abs647\ nm) - (4.91 \times Abs664\ nm);$$
$$\text{Total Chl } (\mu g\ ml^{-1}) = \text{Chl a } (\mu g\ ml^{-1}) + \text{Chl b } (\mu g\ ml^{-1})$$

where Abs_{664nm} and $Abs_{647\ nm}$ refer to the absorbance of 80% acetone extract measured at 664 nm and 647 nm

RESULTS

Biomass productivity at different temperature, light intensity and photoperiod regimes

Biomass productivity of *C. salina* was evaluated at various temperatures and light intensities for a period of 21 days. Growth analysis of cultures grown at different temperatures showed significant difference in growth pattern. Maximum biomass concentration (as dry weight) i.e. 0.76 $g.L^{-1}$ was observed at temperature 22°C and least i.e. 0.27 $g.L^{-1}$ was found at temperature 18°C (Fig. 1). The maximum growth rate i.e. 0.098 doubling day^{-1} was observed at 22°C, but with further increase in temperature reduction in growth rate was observed. At 18°C culture showed 0.032 doubling day^{-1} which is almost half as compared to growth rate at 22°C (Fig. 2).

The growth of microalgal populations depends on various abiotic factors viz. available light, temperature, level of nutrients such as nitrogen and phosphorus. Among these factors, the light that directly influences photosynthesis mechanism is an important factor in defining optimal growth conditions for the culture. Different species of microalgae respond in a different way to light intensity and photoperiod [22]. Considering light as the most important energy source for the photoautotrophic algae, many studies have focused on the effect of light intensity. Both photoperiod and light intensity had a significant effect on the final cell number of *C. salina* at the end of 21 day experiment. The specific growth rate and cell concentration increased linearly with light intensity. The cell density of cultures under light intensity of 200 μmol m^{-2} s^{-1} was significantly higher than the other two light intensities. Over this limit, the increase of light intensity did not result in any enhancement of the growth rate suggesting that the saturation point of photosynthesis was

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reached. Fig: 3 illustrates the effects of light intensity and photoperiod on DCW and biomass productivity of *C. salina*. The cell density was found to be increased with the increase in light intensities. Results of the present study show a direct relationship between light intensity, growth rate, and biomass productivity. In a similar kind of study, higher light intensity produced higher biomass in less time as compared to lower light intensity in *Tetraselmis chui* [22]. According to the literature, the amount of light received and stored by the cells has a direct relationship with the carbon-fixation capacity consequently determining the biomass productivity and cell growth rate. Cells grown under saturated light conditions accumulate carbohydrate and triacyglycerals as storage materials, resulting in high content of biomass. However, at very high irradiance, photoinhibition may lead to cell damage thus inhibiting the growth rate and finally causes cell death [23]. Hence, an adequate supply of light energy is most critical factor in biomass and secondary metabolite productivity.

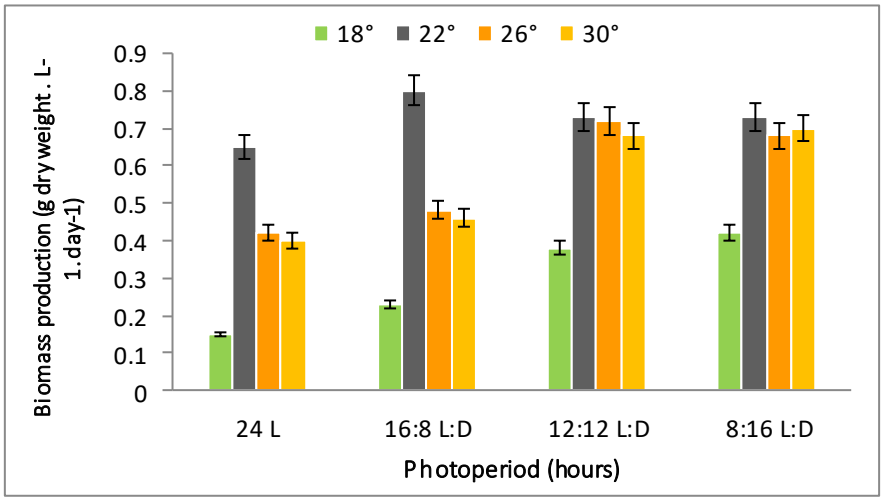

Fig: 1 Dry weight of *C. salina* under different temperature and photoperiods

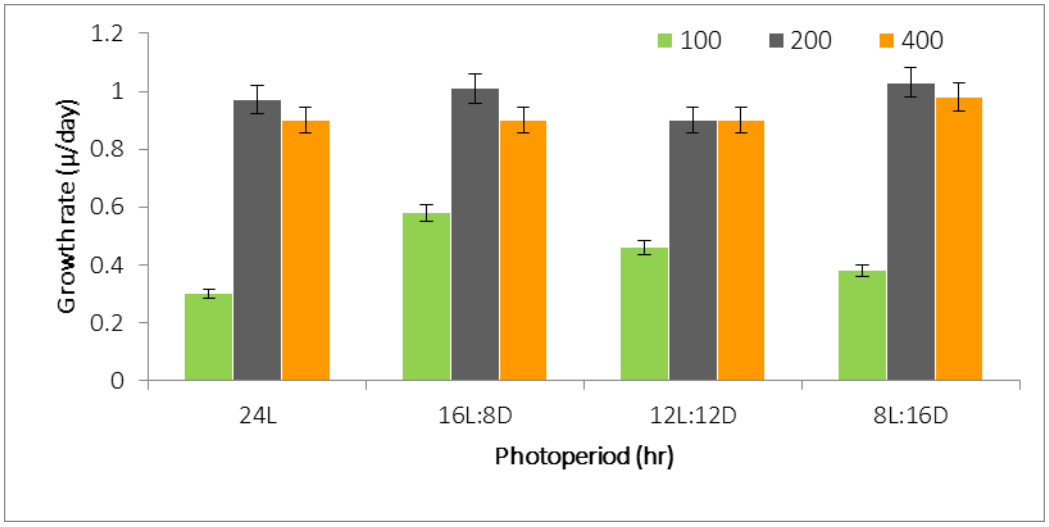

Fig: 2 Growth rate of C. Salina under different light intensities and photoperiods

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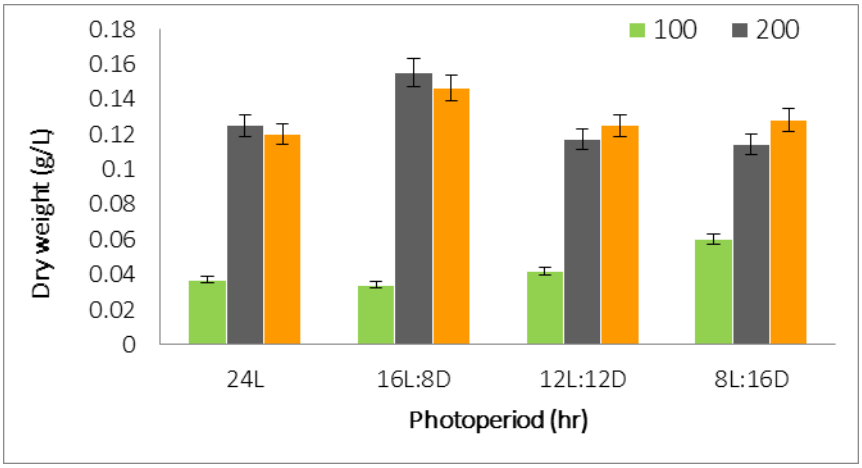

Fig: 3 Dry weight of *C. salina* under different light intensities and photoperiods

Table 1: Maximum cell density and specific growth rate of *C. salina* at 100, 200 and 400 µmol m^{-2} s^{-1} light intensities and different photoperiod cycles.

Light intensity (µmol m^{-2} s^{-1})	Photoperiod (L:D cycle) h	Max. Cell density (x 10^7 cell/ml)	Specific Growth rate µ (day $^{-1}$)
100	24:0	1.3	0.09
	16:8	2.8	0.209
	12:12	2.2	0.372
	8:16	2.0	0.399
200	24:0	3.2	0.287
	16:8	4.5	0.473
	12:12	3.6	0.429
	8:16	3.42	0.406
400	24:0	3.6	0.323
	16:8	4.28	0.489
	12:12	4.02	0.478
	8:16	3.8	0.479

There was no significant difference in DCW and biomass productivity in cells grown under 12 or 16 hr light (153.10 and 168.7 mg/L DCW, respectively). However, continuous light and 16 hr dark grown cultures yielded lower DCW resulting in lower biomass productivity. Similar result was also reported by [24] in *Nannochloropsis sp*. in which higher growth rate and biomass productivity was found in 12 or 18 hr light grown culture. As described in literatures, biomass production in many microalgae increased under high light conditions which generally cause an increase in reproduction until the saturation point intensity [25]. This similar trend was observed in present study. This effect is caused by the photooxidation reaction inside the cell as the excess light cannot be absorbed into the photosynthetic apparatus [26]. An optimal growth rate was observed in cultures grown under 16:8 light dark periods (0.16 µ/day).

Bio pigment accumulation at different temperature, light intensity and photoperiod regimes

Temperature is an environmental variable that affects algal growth, once it influences chemical reactions rates, diffusion rates in water, diffusion and transport through membranes, respiratory and photosynthetic electron transport, enzyme activities and, as a consequence, the rate of development of algal populations [27]. After every 5^{th} day, known amount of cell mass was harvested and analyzed for its pigment contents. At different experimental temperatures up to 26°C pigment content gradually increased but with further increase in temperature reduction in pigments was observed. Cultures grown at 22°C showed the highest chlorophyll a and carotenoid accumulation i.e. 1.47 % and 0.25% (of dry weight) respectively (Fig: 4). Maximum volumetric lutein content in the exponential phase and lutein productivity increased when temperature was raised, reached a maximum at 26°C and remained constant at even higher temperature.

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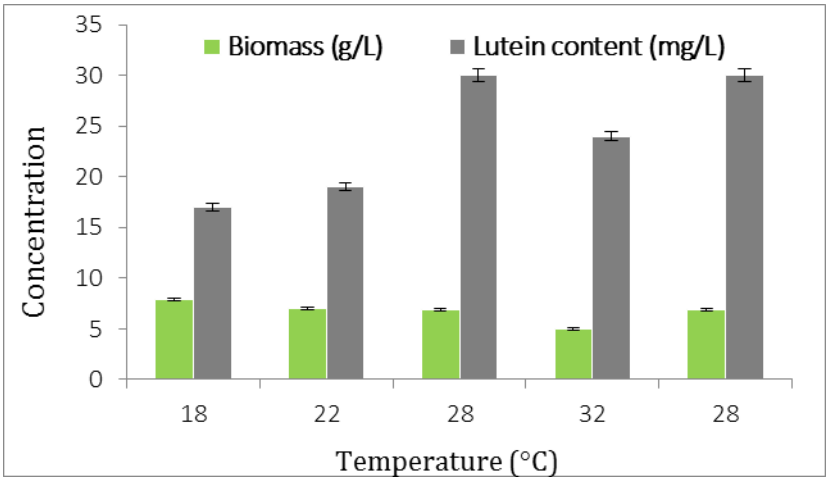

Fig: 4 Dry weight of *C. salina* under different light intensities and photoperiods

The light intensity is an important factor on the microalgae cultivation. Some microalgae species require greater light energy to conduct the photosynthesis process; however, excessive luminosity can cause photo inhibition and cell death, affecting directly the global cell growth [1, 28]. Microalgae cultures performed under stress conditions, such as excessive luminosity, tend to stimulate the synthesis and lipid accumulation [29, 28]. By considering the analysis of pigment concentration, cells exposed to different light intensities showed a decrease of Chl a content per cell and a relative increase in carotenoids content. Thus, *C. salina*, similarly to other photosynthetic organisms, showed an acclimation response by decreasing the Chl a content to reduce light harvesting ability and accumulating carotenoids which have an anti-oxidant activity. Among various light irradiances tested, 200 µmol m^{-2} s^{-1} light intensity was found optimum for pigment accumulation. The yield of chlorophyll a was maximum at 2,000 µmol m^{-2} s^{-1} light intensity. But carotenoid/chlorophyll value (0.181) was found maximum at 400 µmol m^{-2} s^{-1}. There was an improvement in total carotenoid by increasing light irradiance level. The maximum lutein content at the exponential phase increased with irradiance, exhibiting an optimum of 200 µmol m^{-2} s^{-1} (116 mg l^{-1} day^{-1}) and decreasing slightly (98.7 mg l^{-1} day^{-1}) at higher irradiance. The changes in pigments are considered to be an adaptation mechanism to high light [30]. Low light intensity synthesis of more photosynthetic units to aid light harvesting, while at high light, algae synthesize more photosynthetic units to prevent photo-damage [31, 26, 32].

Table: 2: Maximum Pigment production rate of C. salina at 100, 200 and 400 µmol m-2 s-1 light intensities and different photoperiod cycles.

Light intensity (µmol m^{-2} s^{-1})	Photoperiod (L:D cycle) h	Biomass (g/L)	Clorophyll a (mg/L)	Carotenoids (mg/L)	Lutein (mg/L)
100	24:0	0.10	0.106	0.0060	-
	16:8	0.18	0.119	0.0062	-
	12:12	0.22	0.135	0.0058	21
	8:16	0.24	0.364	0.0056	21
200	24:0	0.339	0.653	0.20	108
	16:8	0.553	0.725	0.28	116
	12:12	0.526	0.702	0.232	114
	8:16	0.428	0.716	0.20	111
400	24:0	0.502	0.711	0.19	104
	16:8	0.507	0.686	0.20	98.78
	12:12	0.528	0.314	0.23	98.7
	8:16	0.528	0.280	0.23	98.73

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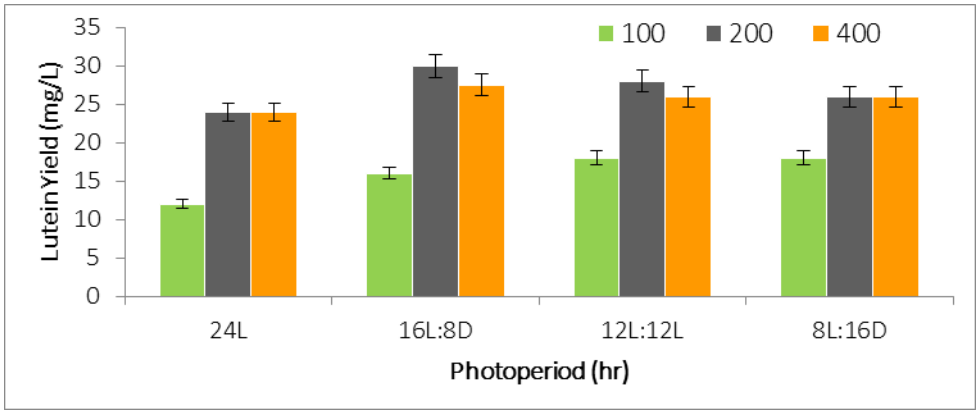

Fig: 5 Lutein yield of *C. Salina* under different light intensities and photoperiods

Pigment concentrations varied with respect to irradiances and photoperiod. Maximum photosynthetic efficiencies can be achieved when the light/dark cycle period approaches the photosynthetic unit turnover time. The change in photoperiod has shown different effects on photosynthetic activity of *C. salina*. Under 12 or 16 hr light regime, highest amount of Chl-a (10.19 µg/ml) was observed; whereas, in completely dark grown culture, lowest Chl- a (1.28 µg/ml) content was observed (Table: 2). Moreover, the carotenoid content was found highest in 16:8 light regime (4.11 µg/ml) and lowest in total dark condition (0.25 µg/ml). There was no correspondence between growth rates and pigment contents indicating that the most favourable conditions for growth were generally not coincident with those with highest pigment contents. On the last day of the investigation, the increase in pigment amount continued and studies showed that light regime had an effect on pigment amount of *C. salina*. And this investigation shows that 16:8 h is the best period of pigment content. Changes in light illuminance, quality and photoperiod bring about varies in their biomass and chemical composition of algae, therefore, showing various adaptations to different environmental conditions [33- 42]. These changes of the light have been shown to bring about differences in biochemical structure and pigment of microalgae [37, 38, 43].

CONCLUSION

With optimization experiments, it was found that factors light, photoperiod and temperature interfered in both growth rate and lutein productivity. The results revealed that *C. salina* grows best at light intensity of 200 µmol m^{-2} s^{-1}1 with the combination of 18:06 light: dark cycle with maximum cell density, specific growth rate and lutein yield. In contrast, *C. salina* showed a gradual decrease in cell density and specific growth rate when the photoperiod cycle was extended to 24:0 h light exposure at the same light intensity. Results indicated improved specific growth rates are accompanied by improved pigment yields. Low temperature and light intensity restrained algal growth. During the investigation, it is determined that pigment content increased at the different period so it is said that different light periods cause differences in pigment production.

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