Research in Marine Sciences
Volume 3, Issue 3, 2018
Pages 371 -376

Effect of light and temperature factors in optimizing the growth of Parachlorella microalgae

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Received: 2018-04-16 *Accepted: 2018-07-29*

Abstract

Determining the optimal culture conditions for algae growth is one way of increasing its products, such as carbohydrates, which may assume an important role in increasing the amount of algae-yielded ethanol. The present study was conducted to determine the effects of light-dark photoperiod (12-12h, 16-8h, 20-4h) and temperature (25 ºC, 29 ºC, and 33 ºC) on growth rate and sugar content of *Parachlorella kessleri* Microalgae. In this study, microalgae were cultured in nine different treatments (each with three replications) of specified photoperiod and temperature conditions. Data analysis showed that temperature has no significant effects on the growth factor ($p \geq 0.05$); however significant effect of photoperiod was observed on the growth factor ($p \leq 0.05$). In addition, the effect of interaction of photoperiod and temperature on this factor was not significant ($p \geq 0.05$). In conclusion, the optimum culture conditions obtained in the photoperiod of 12-12h (light-dark) and temperature of 25 º C.

Keywords: Culture optimization; Parachlorella Algae; Light; Temperature; Growth.

1. Introduction

At present shortage of oil resources and environmental pollution are two major challenges that must be considered. Since limited resources are being leveled out and their amount is declining rapidly, it has led to an increase in the global price of fossil fuels and a recession in the global economy. Also, scientific research has shown that excessive use of fossil fuels has caused environmental pollution and, consequently, global warming and severe climate change. In order to solve such problems, humans seek to use renewable

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energy alternative, which is one of the types of renewable bio-fuels (Wang, 2013).
At the moment, we are faced with some environmental issues, including the pollution caused by excessive use of fossil fuels, that human creativity can play a significant role in achieving sustainable solutions for life on the planet and the availability of alternative fuels. The use of microalgae was started in some countries since the previous years due to their high potential in producing raw materials for bio-fuels, as well as their genetic manipulation to achieve the best algae for bio-fuel production, and also brought valuable results to along. With this view, the effect of temperature and light period factors on the growth rate of *Parachlorella sp.,* as the native species of the Persian Gulf have been considered in this study.

2. Materials and methods

Two-parameter factorial experiment was carried out in a completely randomized design with three replications. The factors were studied in three levels of light-dark period (12-12, 16-8, 20-4) and three temperature values (25, 29, and 33 ºC). Normality of data was analyzed using Kolmogorov-Smirnov test. After verifying the normality of the data, Tukey test was used at 5% level to compare the average values. Data analysis was performed using SPSS software (v. 23).

2.1. Data

The *parachlorella* algae sample was prepared from an algae collection at the Persian Gulf Science and Technology Park. According to the molecular supplementary studies and the pattern of the alien tree, which was carried out by Masumi and colleagues on the algae, the mentioned strain was identified as *parachlorella kessleri* (Masoumi *et al.,* 2014).
In this study, light intensity of 2000Lux was used for all treatments. The light period is one of the important conditions in microalgae experiments, which has a great impact on the growth and proportion of the food composition. Nine treatments with three replications including three treatments of light period and three temperature treatments were investigated. The light period treatments consisted of three light-dark periods (12-12, 16-8, and 20-4), and as the second variable three temperature treatments (25 ºC, 29 ºC, and 33 ºC) were considered. For this purpose, a warming-cooling system was used to reach the required temperatures. Also, the optimum pH value and the salinity were considered in 7.4 and 25 ppt.

2.2. Method in Growth Rate

In order to determine the microalgae growth rate, the samples were taken every 24 hours under the hood and by sterilized samplers. Using spectrophotometer, the absorbance of the samples was read at 490 and 650 nm wavelengths until the end of the twentieth day. Distilled water was used as Blanc.

3. Results

The daily growth rates of algae in different treatments from day 9 to 20 are shown in Figure 1. Based on this, it was found that the growth rate of algae has been increasing steadily through the end of the testing period.
Data analysis showed that during the 20 days of the growth period, the most growth was observed in days 16 to 20, and there was no

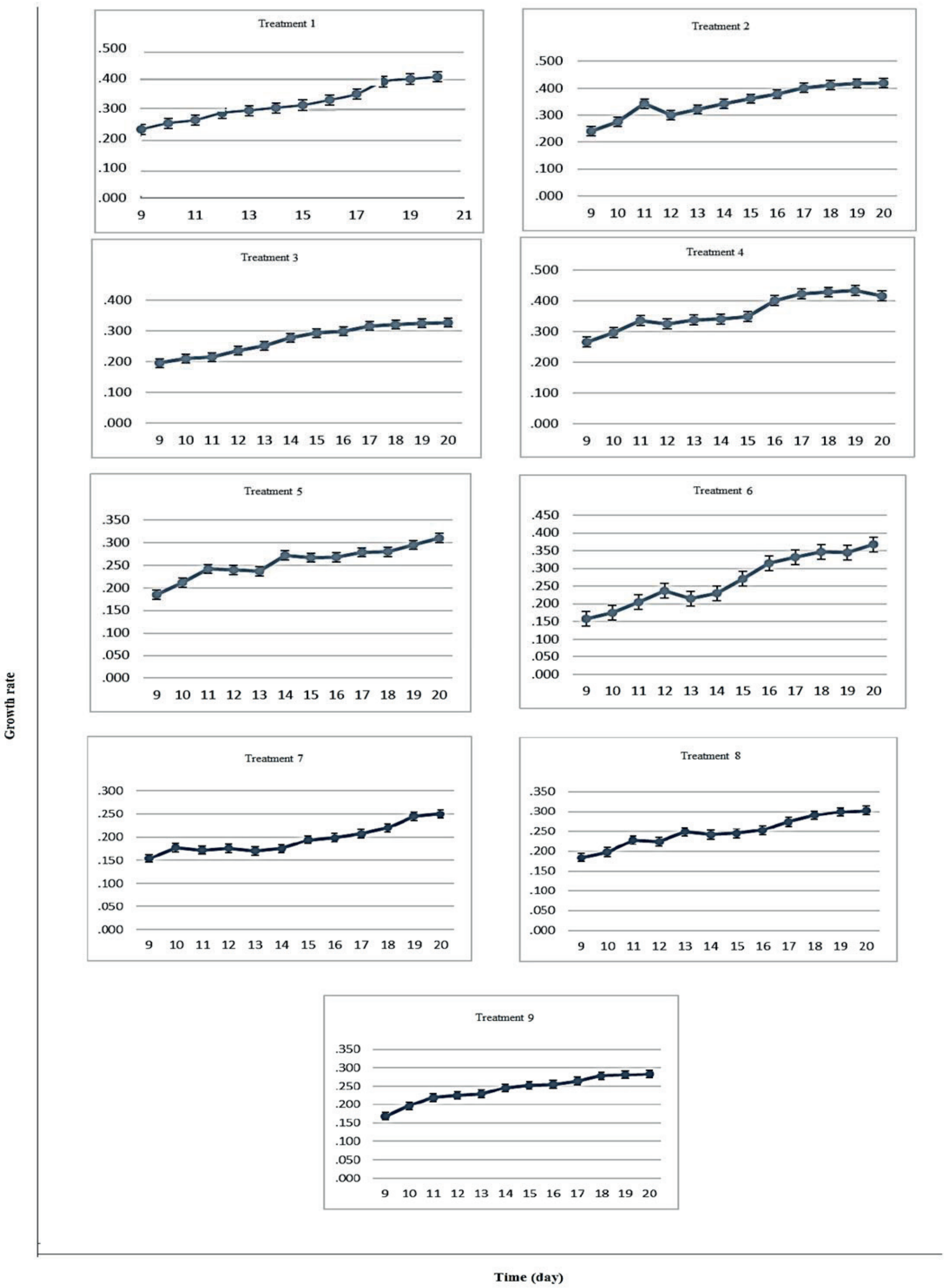

Figure 1. *Parachlorella* algae growth rate: The vertical axis is absorption at 650 nm and the horizontal axis is time (day)

significant difference between the growth rates of algae in different days, however, the growth rate of algae was higher in the 19th and 20th.

3.1. Effect of light and temperature period on the growth rate of parachlorella algae on the twentieth day

Data analysis showed that temperature had no significant effect on growth rate ($P \geq 0.05$), but the light period had a significant effect on algae growth ($P \leq 0.05$). Also, the interaction between temperature and light period was not significant ($P \geq 0.05$) .The highest growth rate in the light-dark period was 12-12h and 16-8h, and the two treatments did not differ significantly. But both were different with the light-dark period of 20-4h. In total, the highest growth rate was observed in treatment 1.

4. Discussion

4.1. Effect of light period and temperature on the growth of parachlorella algae

Microalgae culture requires the full control of all growth factors including nutrients, pH, O_2 and CO_2 concentrations, temperature, and light (MORRIS, 1981). Therefore, researchers in micro-algae cultivation technology are seeking to optimize the performance of the main factors. In this regard, it seems necessary to obtain a complete understanding of the behavior of algae species that are under different environmental conditions. The study of the interaction between these factors and growth parameters allows us to find optimum conditions for selected large scale production. Generally, the growth of the microalgae population is dependent on various biological and non-biological factors, which the most important non-biological factors are light, temperature and nutrient content such as nitrogen, phosphorus, and silicate (for diatoms) (Falkowski *et al.,* 1985). Among these factors, it has a direct impact on the photosynthesis mechanism and is one of the important factors in determining optimum conditions for cultivation. In the absence of algae food restriction, the main control of microalgae is through light and temperature factors. In addition to the temperature and light intensity which are the main and effective factors in the production of microalgae biomass, the length of day and the duration of light are also determinative in the development of their cultivation and growth. In fact, daytime or night-time rhythms are effective in photosynthesis, respiration, cell division, and growth rates. They also affect enzyme activity and macromolecular synthesis (Kirk, 1994).

In this research, data analysis showed that temperature did not have a significant effect on growth rate. But the light period had a significant effect on the growth rate of algae. Also, the interaction between temperature and light period did not affect on the growth rate. The largest rate occurred in the light-dark periods of 12-12h and 16-8h, and these two treatments did not differ significantly, but they were different for the light-dark period of 20-4h. In overall, the highest growth rate was observed in treatment 1 with light-dark period of 12-12h, and 25˚C temperature.

In the same way, a research carried out by Srirangan *et al.* (2015) on *Dunaliella viridis* microalgae. They evaluated the number of cells to evaluate the effect of light period and temperature on the growth rate of microalgae, and they found that the algae under continuous lighting conditions (24-0h) their cell growth increased by 4-5 times, while in the light conditions (12-12h) showed 3-times enhancement. Also, by examining the effect of temperature on cell growth, according to the data obtained in each period, it was found that the temperature change from 25 °C to 35 °C significantly does not affect the rate of cell division (Falkowski *et al.,* 1985).

The effect of light on growth in a constant radiation has shown that maximum growth occurs in continuous lighting conditions. Accordingly, microalgal growth is dependent on the amount of energy received by the cultivation environment. But the problem with continuous light conditions is that the prolonged exposure to light reduces cell division. The accumulation of light irradiation is a factor that can limit the cell division in the micro-organisms under test. However, with less than 15 hours of light exposure, a change in the growth rate will be observed. In a method with a light-dark period of 15-9h, it seems that the cells have enough energy to divide the cell at a specific time. Therefore, it seems that 9 hours of darkness is enough to accomplish all the phenomena that should occur in the absence of light. Therefore, in most cases it is generally recommended not to use the continuous lighting period. In fact, the light period of darkness also increases the final concentration and reduces the cost of production. Generally, the growth of algae depends on the light period (Bouterfas *et al.,* 2006).

The necessity for a dark period is due to the fact that the photosynthesis process takes place in two phases: the first step is a photochemical and light-dependent stage, and the other is a biochemical, independent to the light (Seliger and McElroy, 2013). The compounds in the first stage, light dependency, include high-energy molecules of ATP and NADPH, whose energy is used in the dark period and light-free phase to synthesize the essential metabolic molecules for the growth. In addition, Laval and Mazliak (1995) stated that some of the enzymes in the pentose cycle at the dark stage, the CO_2 fixation phase, were deactivated during the light period. The dark stage is necessary at least to restore the production of NAD + and NADP + cofactors that are necessary for the first phase of photosynthesis, which indicates the importance of the dark phase.

Another point that can be mentioned in the importance of the dark stage is that, if cells that divide mitosis are exposed under dark-light conditions, cell division occurs after stopping a lightning stage (Lee *et al.,* 2015). Therefore, it is better to use a light-dark period of 15-9 h in order to balance the anabolic and catabolic reactions during the light period. In addition, for industrial applications and applicable planning, to reduce energy costs and increase biomass production, a light period of 12 to 15 hours is generally suggested as an optimum condition for the algae growth. The results can be verified according to the explanations given based on the best light period between 9-15h and the most suitable treatment obtained in the present study on the microorganism's growth with a light period of 12-12h.

Conclusions

In this study, the effect of temperature and light period on growth in *Parachlorella kessleri* algae was investigated. The results indicated that temperature did not have a significant effect on growth rate. But the light period had a significant effect on the growth rate of algae. Also, the interaction between temperature and light period did not affect on the growth rate. The largest growth rate occurred in the light-dark periods of 12-12h and 16-8h, and the treatments did not differ significantly. In overall, the highest growth rate was observed in treatment 1 with light-dark period of 12-12h, and 25°C temperature.

Acknowledgements

Authors are grateful to contributors from Bushehr Science and Technology Park, especially Mr. Javid Esfandiari, to support this project and provide laboratory facilities.

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