THE EFFECTS OF LIGHT INTENSITY ON THE GROWTH RATE OF CYANOTHECE

By

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Abstract

This research was directed towards investigating the effect of light intensity on the growth rate of algae in an aqueous environment. An exopolysaccharide producing cyanobacterium, cyanothece, was grown in a controlled photo bioreactor environment while the specific growth rate, and cell density were monitored at different light intensities. The light intensities varied between 40 – 300 μmol photon m·2 s·1, actual values being: 44, 58, 93, 96, 289 and 306 $\frac{\mu mol\ photon}{m^2\ s}$. The unique characteristics of the cyanothece provided great properties facilitating algae growth in varying conditions. To ensure optimum harvesting, the salinity, pH and temperature of the reactor medium were controlled, as the light intensity varied. After two months of cultivating, the highest cell and exopolysaccharide produced were recorded, measuring 3.0 g/L and 2.6 g/L, respectively. The experimental results showed that the specific growth rate and algae optical density increased as the light intensity increased. In an effort to relate the microbial growth rates in the photo bioreactor environment to the concentration of a limiting nutrient, the Monod equation was used.

Introduction

As energy demands continues to increase, while traditional energy sources (such as fossil fuels, coal, oil and natural gas) decreases, more renewable resources are being explored. One of the most versatile energy sources is biofuel, based on its ability to be produced from so many renewable energy sources. Additionally, there are several advantages to producing biofuel over fossil fuel. Some of these advantages are that biofuels: produce less greenhouse gases overall than fossil fuels when they are burned, creates a greater fuel security for countries without oil reserves, and plants are preserved in the production of biofuel. However, a complete transition from traditionally derived fuels to biofuel is impossible due to production capacity restraints and cost.

Though biofuels do not compete with traditional energy resources (Singh *et at.*, 2010), algae, as the third generation to produce biofuels, has several advantages to reduce the cost compared to the first and second generations (Zhang, 2014). A few advantages are that it does not compete with food resources, does not use arable land, has higher growth rate, and can be cultivated in saline water or wastewater (Noraini et al., 2014; Chen et al., 2014). Due to the variety of algae species

and the applications, the development of methods for high throughput cultivation and efficient harvesting of microalgae has, over the past decades, constituted an active field of research (Hassan, 2013).

Cyanobacteria has high photosynthetic efficiencies and diverse metabolic capabilities that provides the ability to convert solar energy into a variety of biofuel. These unique characteristics of the unicellular cyanobacterium made it attractive for this research. Cyanothece, a cyanobacterium isolated from a shallow lake in the Florida Keys. Cyanothece can fix nitrogen, tolerate high salinity and secrete an extracellular biomaterial which contains proteins and carbohydrates (Phlips *et at.*, 1989; Moreira, 2014). Having the ability to fix nitrogen at near maximum rates, in mediums lacking nitrogen, helps to minimize cost of cultivation, because no nitrogen is required. (Phlips et al., 1989; Zhang, 2014) Additionally, due to the tolerances for high salinity, fresh water resources can be conserved and the risk of contamination from other microorganisms can be minimized (Zhang, 2014).

There are many variables that could contribute to algal growth, some of these includes the concentration of carbon dioxide/available air, the concentration of phosphorous, temperature and light intensity. However, the effect of light intensities on specific growth rate (μ) will be discussed in this paper.

Method and Material

There are two primary means of cultivating algae, open systems and closed systems. For this specific investigation, the closed system (photo bioreactors) cultivation method were used. In order to maintain a controlled environment, six 500 mL serum bottles were used as PBRs. In the cultivation of the algae, Allen medium without nitrogen (A-Na medium) was developed by creating a series of stock solutions, this provided a controlled environment. The medium comprised of ethylenediaminetetraacetic acid disodium salt, dihydrate, potassium chloride, calcium chloride dihydrate, dipotassium phosphate, magnesium sulfate heptahydrate, iron, vitamins, (molybdenum), sodium chloride and sodium bicarbonate as the buffer.

To reduce potential contamination, the PBRs were autoclaved at 121 °C for 30 minutes before used. 250 ml of the medium was then added to each PBR, in the presence of 1 mL of algae. The system was them monitor and controlled to maintain conditions. The pH of the solutions was maintained at 7.5±0.25. Instead of a stirring rod, air was pumped into each PBR, through a plastic tube and a filter stone, at 2 $L \cdot min^{-1}$. To mitigate contamination, alcohol was used to clean the air tubes and stones before being placed into solution environment.

The PBRs were then placed in an algae cultivation chamber. A modified refrigeration, containing LED lights, was used as the cultivation chamber. The PBR systems were exposed to 13 hours of light and 11 hours of darkness per day, during which the temperatures were controlled at 30±2 °C during light cycle and 22±2 °C during dark cycle. The light intensities varied within the chamber, this allowed different cultures to operate under specific light intensities. The light intensities ranged between 40 – 300 μmol photon $\cdot m^{-2} \cdot s^{-1}$.

Sample testing and analysis was divided into two periods. The first period was one week long, while the other was for the duration of the cultivation period. For the first period, 1 mL samples for analysis were taken daily for one week. After the first week, samples were then taken every two days and analyzed throughout the cultivation period. The optical density, salinity and dry weights were measured and analyzed from the samples; the pH of these samples was measured to maintain at 7.5±0.25.

For the analysis of the optical density, 1mL samples were taken and centrifuged at 10000 rpm for 15 minutes. Afterwards, the supernatant was separated from the biomass pellets. The biomass pellet was then resuspended by adding 1 mL of distilled water, after which the optical density was measured. In the event that the optical density of the biomass was greater than 0.6, the sample was then diluted in effort to increase accuracy.

The salinity was maintained at approximately 35 psu. A deviation in the salinity based on evaporation and other factors, resulted in the adding of autoclaved distilled water. The amount of water to be added for salinity deviation was calculated using equation (1) below:

$$\text{water volume added} = \frac{(\text{salinity read} - 35) \times \text{liquid volume in the reactor}}{35} \quad (1)$$

For the dry weight measurement of cells and exopolysaccharide (EPS), 15 mL algae culture was centrifuged at 10,000 rpm for 20 mintures. Then the supernatant was removed and 15 mL DI water was added to resupend the cell pellet. Then the resupsension was centrigued again until the salinity was lower than 2 psu. Then 10 mL of supernatant and cell was withdrawn, and placed on weighed aluminum dishes. The dishes were then placed into an oven, where the samples were dried at 105 °C until constant weight. Then the samples were burnt at 550 °C, the difference of the two weights was the volatile solid (VS) dry weight of the cells and the EPS. Because of an extra loss of the culture medium, the VS of the EPS should subtract the loss of the medium to get the EPS dry weight. This mass was then measured, and observed throughout the algae cultivation.

Light intensities were set for specific reactors throughout the course of the experiment. The light intensities ranging from low to medium, were used as the specific algae growth was observed. A table outline the light intensity associated with specific PBRs is illustrated below.

Table 1. Illustration of light intensities associated with specific PBR

Light Level	**High**		**Medium**		**Low**	
Light Intensity ($\frac{\mu mol\ photon}{m^2\ s}$)	289	306	96	93	58	44
Reactor #	R1	R2	R3	R4	R5	R6

Results and Discussion

Algae was cultivated in PBR over a two month period. Periodically, the biomass was recorded for analysis. Over the course of the 60 day period, optimal biomass dry weight was recorded which correlated with the reactor with the highest light intensity. Figure 1. Illustrates a graph of the measured data.

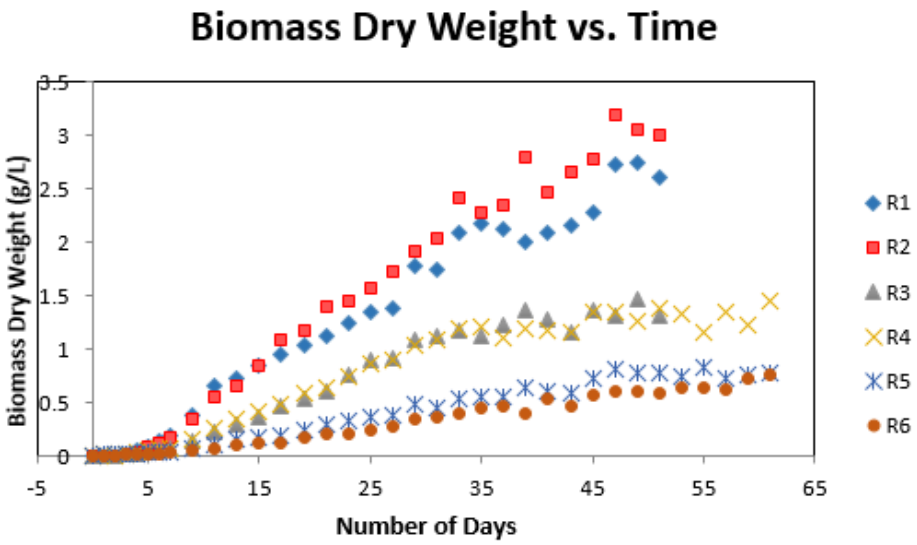

Figure 1. Cell growth overtime

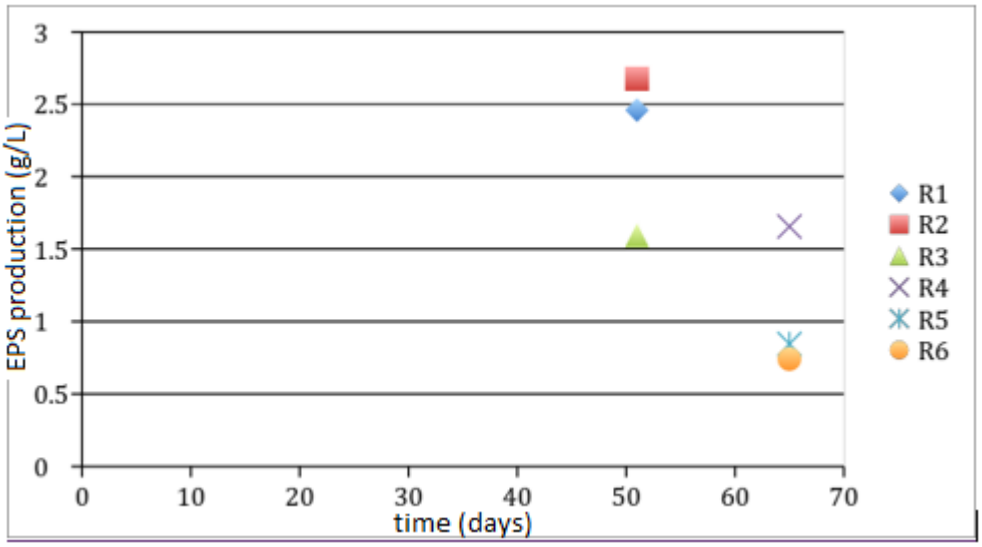

Figure 2. Exopolysaccharide production overtime

For the first five days of cultivation, there were minimal differences in the dry mass weight. However, over the later days, the biomass dry weight increased as the light intensity increased. The cell and EPS dry weight recorded as high as approximately 3.0 g/L and 2.6 g/L, respectively (Figure 1 and 2). Based on the performance displayed by the data obtained, one can conclude that increasing light intensity increased the cell and EPS dry weight. Having a high EPS dry weight is critical for processing. The high carbon and hydrogen content makes lipids an energy

dense molecules. This feature makes it a favorable target for processing and usage as an alternate energy source to traditional fuels.

Data from figure 1 was used to calculate the specific growth rate at the specified intensity. The equation used to calculate the specific growth rate is seen below:

$$X = X_0 e^{(\mu(t - t_{lag}))}$$

X - OD measured at time t (day)
X_0 - represents OD measured at t_o.
t_{lag} -lag time to reach exponential phase

Using the above equation, the specific growth rates was calculated for the resistive light intensities. A table with the calculated values can be seen below in Table 2.

Table 2: Light intensity and specific growth rate per reactor

Reactor #	**R1**	**R2**	**R3**	**R4**	**R5**	**R6**
Light Intensity ($\frac{\mu mol\ photon}{m^2\ s}$)	288.9	305.7	95.95	93.2	57.6	44.3
μ_{run1}(day^{-1})	0.57	0.6	0.3	0.31	0.21	0.19

In an effort to relate the microbial growth rates in the photo bioreactor environment to the concentration of a limiting nutrient, the Monod was used. The fundamental Equations governing this principle can be seen below.

$$\mu = \mu_{max} \frac{L}{K_L + L}$$

μ_{max} - Maximum specific growth rate (day^{-1})
L - Light intensity used for algal growth (μmol photon ·m^{-2}·s^{-1})
K_L - Value of L when $\mu/\ \mu_{max}$=0.5

The Monod equation was then used to compare modeled values to the experimental results. Figure 3. below illustrates how well the model fits the experimental data. Therefore, the model holds true, an increase in light intensity towards algae results in an increase in the specific growth rates (based on Monod's equation)

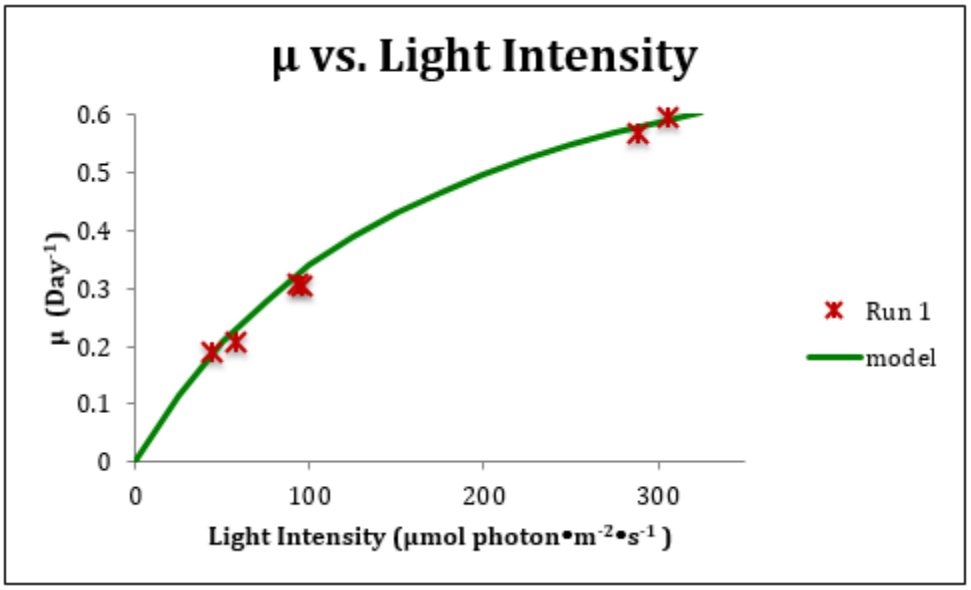

Figure 3. Relationship between specific growth rates for algae vs. light intensity

Conclusion

Though biofuel processes are usually more expensive than fossil the advantages of the third generation biofuel method, algae, makes it a favorable candidate. A few of these advantages includes algae: not competing with food resources, does not use arable land, has higher growth rate, and can be cultivated in saline water or wastewater. These advantages along with the unique characteristics of the cyanothece, provides the necessary properties to facilitate algae growth in varying conditions. To mitigate disturbances throughout the testing, bioreactors were used. Algae was harvested in a controlled environment to isolate the variable of interest (light intensity) and ensure optimum harvesting.

By operating within controlled parameters, the results showed that the specific growth rate and algae density increased as the light intensity increased. After two months of cultivating, the highest cell and EPS biomaterial produced were approximately 3.0 g/L and 2.6 g/L, respectively. The experimental data was then fitted to Monod's equation where a high correlational fit was observed. Therefore, the model holds true, an increase in light intensity towards algae results in an increase in the specific growth rates based on Monod's equation.

Reference

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