Investigating the Effects of Growth Medium and Light Sources on the Growth of *Botryococcus Braunii*

Ayse Busra Sengul, abalbayrak@hotmail.com
Muhammad Mustafizur Rahman, muhammad.rahman@wichita.edu
Eylem Asmatulu, e.asmatulu@wichita.edu

Department of Mechanical Engineering
Wichita State University
1845 Fairmount St., Wichita, KS 67260, USA

ABSTRACT

In the present study, the effect of medium and light source on the growth of *B. braunii* was investigated for large-scale and economical production of biomass and hydrocarbons. Three types of media and light sources were used with incubation temperature of 23±2°C under different light source with a 12h-light/12h-dark cycle to observe different growth rates. The growth rate of *B. braunii* was measured via ultraviolet visible (UV-Vis) spectrophotometry and biomass dry weight. The results of this study showed the maximum growth rate was reached after approximately three weeks of incubation and then was followed by the stationary phase. Among the different media and lights tested, medium consist of sodium nitrate, sodium phosphate and iron chloride with red/blue light was found to be the best combination for the growth of the algae.

Keywords: algae, temperature, light, growth medium, biomass.

1 INTRODUCTION

Economic developments and civilization are the driving forces to produce more fuel from different resources. The world’s demand for liquid, gas and solid fuels has been drastically increasing while the supply of these fuels are considerably decreasing. Many countries lacking safe access to energy will be having economic, political and militarily disadvantages [1]. Considering the national energy security, it is important to develop long term and domestic fuel sources. Because of these reasons, the United States imports about 60 percent of its liquid fuel worldwide. For the economic security, every year about $400 billion is going abroad in exchange for the fuel that the US consumed. This money could be gained back to the US economy with domestically produced renewable fuels, which will allow the country to overcome major economic problems [1].

Renewable energies are the most effective sources to fight against the climate change [2]. Greenhouse gas emission can be reduced by developing renewable and sustainable energy sources which will also protect the natural resources, such as water, soil and air. Liquid transportation fuels reason the one third of the fossil-fuel carbon emissions footprint [1]. Biofuel can be a key solution for all these challenges. Biodiesel is an alternative fuel which can be produced from domestic renewable energy resources. It can also be used in diesel engines with small or no modification. Biodiesel is nontoxic and free of sulfur, and can be blended at any level with petroleum diesel to produce a biodiesel mixture [3].

Algae are photosynthetic and heterotrophic organisms and has huge potential as a biofuel source. They have the high productivities of algal biomass that can be grown in many locations on the earth. Microalgae can produce wide range of commercially fascinating byproducts such as oils, fats, sugars, and functional bioactive compounds under the difficult agro-climatic conditions (e.g., temperature, water availability, etc.) [4]. According to the recent research studies, algae could be 10 or even 100 times more productive than traditional bioenergy feedstocks. Once algae is harvested, it can be easily processed into the raw material to make fuel for cars, trucks, trains, and even aircraft [5,6].

Botryococcus braunii (B. braunii) is the algae of special interest because of its performance of accumulating extracellular hydrocarbons. Three forms of these algae colony have developed diverse metabolic tactics to produce the alage oil [7,8]. About 75-86% of the dry weight of B. braunii might be long-chain hydrocarbons [9]. These species are remarkable for their capacity to produce high amounts of hydrocarbons, particularly oils in the structure of Triterpenes, that are usually around 30–40% of their dry weight [7].

The great majority of these hydrocarbons are botryocuccus oils: botryococcenes, alkadienes and alkatrienes. Since the Botryococcus oil is fatty acid triglycerides and it is not a vegatable oil, transesterification method cannot be used for making biodiesel out of it. Botryococcus oil is inedible and has very different chemical structure, so it doesn’t have free oxygen atom for the transesterification process. However, Botryococcus oils can be consumed as feedstock for hydrocracking in an oil refinery to produce kerosene, diesel, as well as octane (gasoline, a.k.a. petrol). Considering the hydrocraking process, Botryococcenes are chosen over alkadienes and alkatrienes because Botryococcenes can be converted into a higher octane fuel [10]. Based on all these information, oil content is important for algae to get biodiesel out of it. Algae can grow in hard environmental conditions while the conditions like air, water, temperature, and growth medium have significant effects on algae growth and its oil/lipid content [11,12]. This paper describes effects of growth media and light sources on algae growth, biomass yield, and their possible applications in the future.

2 EXPERIMENT

2.1 Microalgae Strain and Medium

B. braunii was selected as a microalgae strain for this study. The *B. braunii* was purchased from "algabiotics.com". The microalgae strains were grown in 1000 mL of three types of media. The first type was the starter mix consisting of sodium carbonate, potassium nitrate, sea salt, ammonium phosphate, strong green tea, and iron sulfate. The starter mix was prepared by dissolving 1 tablespoon starter mix and ½ squeeze metal solution in 1 L distilled water. The second type was the Miracle-Gro prepared by dissolving ½ tablespoon of commercial Miracle-Gro in 1 L distilled water and adding KOH to adjust pH to 7. The third type was the commercial synthetic fertilizer prepared by dissolving ½ tablespoon of it in 1 L distilled water.

2.2 Algal Cultivation

The cultures were incubated at room temperature (23-25ºC) under different light source with a 12h-light/12h-dark cycle. Three different light sources providing yellow, white, and pink/blue illumination were evaluated. The light sources were obtained from a local store with the following emission parameters: yellow (incandescent), 380-2500 nm wavelength; white, 360-950 nm wavelength; pink/blue, 400-700 nm wavelength. Note that the exact wavelengths of the light sources are not specified on the products. Air was aerated into the jar through an air sparger at the bottom of the jar. The strains were checked for 25 days growth period.

2.3 Evaluation of Algae Growth

The growth rate of B. braunii was measured via ultraviolet visible spectrophotometry (Hitachi U-2900) and biomass dry weight. The cell concentration of the culture was determined regularly by measuring optical density (OD680) at wavelength 680 nm. Before taking the individual reading, the vial was agitated continuously for 30 seconds before the measurements were taken daily. The cultures were determined gravimetrically and growth was expressed in terms of dry weight (mg/L). The cultures were harvested by centrifugation at 5000G for 10 min and the cells were washed with distilled water. Then, the pellet was freeze dried. The dry weight of algal biomass was determined gravimetrically and the growth was expressed in terms of dry weight (g/L).

3 RESULTS AND DISCUSSION

3.1 Effects of Light on Algae Growth

The purpose of this step was to determine the most suitable light sources for the growing of B. braunii. Optical density (OD680) measurements were conducted for algal medium under yellow, white, and pink/blue light source exposures. Among nine experiments, the highest optical density value was obtained for the culture which was grown under pink/blue light source and the lowest value was obtained for the culture which was grown under white light source. For starter mix medium (M1), the highest and the lowest optical density values, which were obtained from the pink/blue light source, were found to be 0.192 and 1.853, respectively. As expected, OD680 value of the culture increased day-by-day and the maximum OD680 value was obtained at the end of 23th day. For Miracle-Gro medium (M2), optical density values were read 0.177 as minimum and 0.675 as maximum under the pink/blue light source. For commercial synthetic fertilizer medium (M3), the optical density values varied between 0.361 and 1.610 under the pink/blue light source.

The dry weight values were found as 0.81g/L under yellow light source, 0.40g/L under white light source and 1.66g/L under pink/blue light source for the starter mix medium (M1). For Miracle-Gro medium (M2), dry weight values were found as 0.80g/L under yellow light source, 0.44g/L under white light source and 1.62g/L under pink/blue light source. For commercial synthetic fertilizer medium (M3), dry weight values were found as 1.28g/L under yellow light source, 1.54g/L under white light source and 1.34g/L under pink/blue light source. Variation of dry weight values shows similar characteristics to optical density values as expected. Specifically, the highest dry weight value was obtained for culture which was grown under pink/blue light source and the lowest value was obtained for the culture which was grown under white light source.

3.2 Effects of Medium on Algae Growth

In this study, three different culture media with varying chemical compositions were evaluated to enhance the growth rate of B. braunii. Among the three types of culture media used, starter mix medium (M1) had higher values of optical density throughout the tests. The optical density of B. braunii in starter mix medium (M1) showed progressive increment from the second day onwards until the 23th day under pink/blue light source, and then a slight decrease was observed. Commercial synthetic fertilizer medium (M3) showed the positive progression of the growth from the 4th day of culture until the 24th day and a stationary phase was observed afterwards under pink/blue light source. In Miracle-Gro medium (M2), the growth started within 16 days and did not show proper growth afterwards. The maximum values of the optical density in starter mix medium is 1.853, while in synthetic fertilizer medium and Miracle-Gro medium are 1.610 and 0.675, respectively. B. braunii maximum dry biomass of 1.66 g/L was obtained on the starter mix medium (M1).

4 CONCLUSIONS

B. braunii is a great source for environmental remediation and biofuel production. In order to improve the growth rate of the algae and biomass yield, three different growth media (starter mix, Miracle-Gro, synthetic fertilizer) and three light sources (white LED, pink/blue and yellow) were selected. The results showed that the B. braunii grows better in starter mix medium at room temperature (23-25ºC) under pink/blue light source with a 12h-light/12h-dark cycle. The maximum value of the optical density is 1.853 and dry biomass is 1.66 g/L in starter mix medium. Further research is needed to accomplish algal lipid extraction which will give an understanding of the yield lipid content of the dry biomass, as well as lipid characterizations.

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