Interaction of Light, Geothermal Water and Nutrients on Biomass Production of *Chlorella sorokiniana* Microalgae (CS-101)

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Abstract

Geothermal water is a source of water rich in nutrient content. In this study geothermal water was used for growing *chlorella sorokiniana* species of microalgae (CS-101), using different combination of lights (12:12, 16:8, and 24:0) and three different sources of nutrients as growth media (geothermal water, Bold's Basal Medium and ½ BBM & ½ geothermalwater). Based on factorial experiment with CRD arrangement, the result indicated 16:8 light cycle proved to be the best for algae biomass production with all three mediums ($P<0.05$). High significant differences were observed in biomass production when combination of ½ BBM & ½ geothermal water were used ($P<0.05$). The pH in BBM increased from 7 to 10.42 and was significantly higher from geothermal water (9.02) and ½ geothermal & ½ BBM (9.25).

***Key words**: Microalgae, Geothermal water, Biomass, Biofuel, Chlorella sorokiniana*

1 Introduction

The global economy runs on energy. Our modern society is faced with the dilemma of energy prices and environmental concerns, as well as potential shortages of energy and water supplies. The global appetite for energy is enormous, representing $6 trillion per year, or about 13% of global gross domestic product [1]. In addition to costs and availability of fossil fuels, one of the paramount importance concerns with using fossil fuels is the fact that they release numerous amount of CO_2 into atmosphere result in global warming which is effecting on food resource, water, ecosystems and environment [1]. Hence, scientist put concerted efforts to find an alternative for fossil fuels which is sustainable, renewable, and able to balance the CO_2 [2]. Microalgae have been found to be a great substitute aiming to lessen our dependence to fossil fuels. Algae strains are renewable and sustainable feedstock for the production of biofuels from nonfood sources which is a very vital advantage of using microalgae. Raw materials that are generally being commercially exploited to produce biofuels consist of edible fatty oils derived from rapeseed, soybean, palm, sunflower and other plants [3].The biofuel from edible oils is controversial due to the increase in global food prices, depletion of ecological resources and intensive agricultural practices in crop cultivation. Furthermore, microalgae produce more oil than oil crops. Some algae strains can produce up to 10 to 100 times of oil per unit area of land compared to oil crops [4]. Microalgae do not require arable land to grow and it can utilize wastelands; therefore it is not competing with land for growing crops [5]. Microalgae need massive amount of water to grow [6]. Many species of microalgae are able to effectively grow in sources other than fresh water such as geothermal water, because of their ability to utilize abundant organic carbon, inorganic nitrogen (N), and phosphorus (P) in wastewater [7]; thus, they do not deplete fresh water supplies which is limited especially in areas where algae productivity has great potential like arid and semi-arid regions with abundant amount of sunshine and alternative water resources. *Chlorella sorokiniana* strains are able to increase their biomass under harsh heat desert environment [8]. Studies and evidence have found that *Chlorella sorokiniana* has the adaptability and capability to grow in condition with high organic pollution such as the sewage stabilization pond and wastewater treatment [9]. Geothermal water is an excellent water source for growing algae since it is rich in HCO_3 and other nutrient. This research analyzed the growth of algae strain in different growing conditions of one microalga, CS -101 in order to determine the optimal lighting and media conditions lead to the increase of biomass yield. CS- 101 is *Chlorella sorokiniana* strain which was originally isolated from the freshwater sample collected from Inner Mongolia Province in China [9].

2 Material and Methods

2.1 Algae

One strain of *Chlorella Sorokiniana* was used, CS-01. It was cultivated from commercially available samples until six liters were obtained. In each increase in volume, samples were allowed to grow for three weeks then the volume was increased 500% with the addition of Bolds Basal Medium [10].

2.2 Experimental Apparatus

Regular mouth, one pint, Kerr brand mason jars were used for the experiment. Each had a ¼ inch hole in the center of the lid to allow for the air tube. Each light cycle used of four GE, F40PL/AQ-ECO, wide spectrum, 40W florescent tubes with a 3100K color temperature producing 1900 limens. The average distance from bulb to experiment was 25cm. All weights were measured using an Acculab AL-204 scale with an accuracy of +/- 0.0001g. An Eppendorf 5804 centrifuge was used to separate biomass from medium. Drying of centrifuged biomass was done in a Fisher oven. Air supply was room air which was pumped and regulated using standard aquatic pumps, ¼ inch tubing, and gang valves. The pH was obtained using an Accumet AB15/15+ pH meter. Eppendorf 1-10ml Research plus pipette was used for inoculation and transferring of algae. Medium volumes were measured using volumetric flasks.

2.3 Test Procedures

In this study geothermal water from the Aggie Mountain site of New Mexico State University was used for growing *Chlorella sorokiniana* species of microalgae (CS-101). BBM was prepared using the standard BBM protocol. Where applicable, all equipment was washed, rinsed with distilled water, and autoclaved. Each strain of algae was divided into three light cycles, [24:0], [16:8], and [12:12]. Within each light cycle three mediums were tested (BBM, geothermal well water, and ½ BBM & ½ geothermal well water). Each light cycle/medium set consisted of triplicate samples for a total sample set of 27/algae strain. The glass jars were filled with 200ml medium. The pH was measured and found to be 7.03 for the BBM medium, 6.79 for the geothermal water medium, and 6.90 for the ½ BBM / ½ geothermal water medium. The inoculating algae were stirred until homogenous. Each sample was then inoculated with 50ml of algae. For each strain of algae, nine jars (3 of each medium) were randomly placed in each light cycle environment. Air hoses were inserted into the jars and the air flow was adjusted to 5ml/s. The experiment ran for 134 hours, starting with the off light cycle. Total number of hours of lights on-vs.-lights off was 134-0 for the [24:0] light cycle, 86-48 for the [16:8] light cycle, and 74-72 for the [12:12] light cycle. Ambient temperature was measured throughout the experiment and found to be 32.2°C ± 2.0°C. The biomass of each strain of inoculating algae was determined by taking six 10ml samples. The samples were centrifuged at 10,000 rpm (14,857 rcf) for one minute. The liquid was poured off and the samples dried for 60 hours at 40° C. The resultant inoculating biomass/sample was found to be 0.0305g for CS-101. At the end of the experiment (134 hours) the pH of each sample was taken. 10ml from each sample was then centrifuged at 10,000 rpm (14,857 rcf) for one minute. The liquid poured off and samples dried for 24 hours at 80°C. The dry biomass was weighed and biomass increase calculated. Data were analyzed through GLM procedure. Assumptions were checked using SAS 9.2[11]. Date were analyzed and means were compared using Tukey's Test ($p<0.05$).

3 Result and discussion

The result indicated 16:8 light cycle proved to be the best for algae production with all three mediums ($P<0.05$) (Figure1).

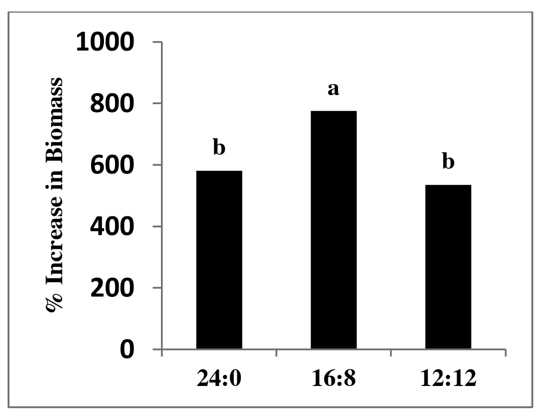

Figure1: Effect of Light cycle on % increase in Biomass ($P<0.05$).

High significant differences were observed in biomass production when combination of ½ BBM & ½ geothermal water were used ($P<0.05$) (Figure2).

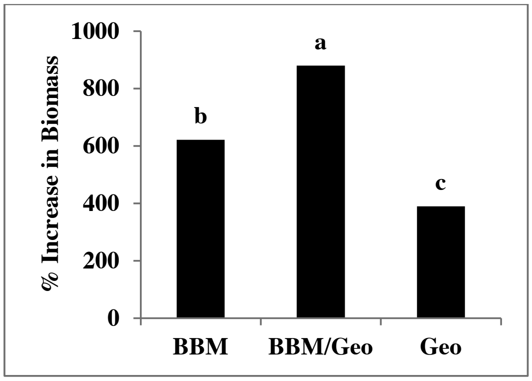

Figure2: Effect of Medium on % increase in Biomass ($P<0.05$).

Since the interaction is also significant, an interaction plot needs to be provided before drawing any conclusions. The interaction plot is shown below in figure 3.

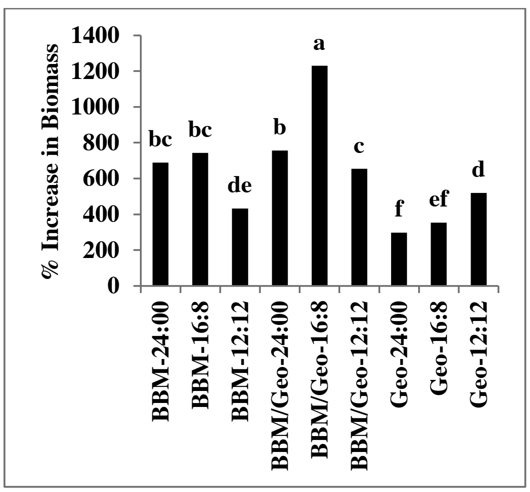

Figure 3: Interaction between Light cycle and Medium with Biomass increase ($P<0.05$).

From figure 3, it can be concluded that if 50% BBM and 50% geothermal with 16:8 light cycle is used the biomass increase will be maximized.

As it is shown in figure4, PH in BBM increased from 7 to 10.42 and was significantly higher from both geothermal water (9.02) and ½ geothermal & ½ BBM (9.25). There were no significant differences between geothermal water and ½ geothermal & ½ BBM. High pH in all BBM slowed growth compared to ½ geothermal & ½ BBM.

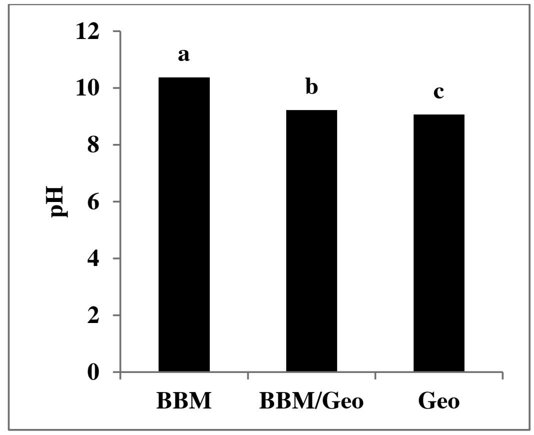

Figure4: Effect of Medium on pH ($P<0.05$).

Besides, there were no significant differences in pH in different light cycles.

Low growth in all geothermal indicates geothermal lacking in some nutrient(s). Geothermal water contributed and worked as pH buffer, this can be the result of lower PH in geothermal compared to others.

4 Conclusions

One way to lower the cost of algae production is to lower the cost of inputs such as nutrients and CO_2. Geothermal water is a good water source for growing algae since it is rich in HCO_3 and other nutrient and it will lower the cost of product. Fresh water is mostly gathered as potable water and limited by human activities [12]. It is necessary to find alternative water resources for biofuel production. Geothermal water is a good candidate to be used as a new source for growing microalgae.

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