ALGAE-DERIVED BIOFUELS: COMPARATIVE ALGAL YIELD OF AUTOTROPHIC, HETEROTROPHIC, AND MIXOTROPHIC GROWTH CONDITIONS

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Abstract

With limited reserves of fossil fuels, the energy crisis is driving exploration into alternative sources of energy such as biofuels. Biofuels derived from algae are practical because algae can be grown and harvested in mass and they produce significant lipids compared to other oil crops. In this experiment, the freshwater microalgae species *Chlorella vulgaris* was studied under autotrophic, heterotrophic, and mixotrophic growth conditions to compare the growth rate and lipid yield of each condition. The autotrophic and mixotrophic experiments were grown on a light-dark cycle of 18:6 hours. The heterotrophic experiments were grown in complete darkness to prevent the algae from photosynthesizing. The glucose concentration used in the experiments was 500 mg/L and the DCMU concentration was 4.7 mg/L.

Results of this study indicated that heterotrophic growth conditions produced the highest lipid yields of 6.7% DCW, 5.3% DCW, and 4.7% DCW and second-highest growth rates of 0.1473 days^{-1}, 0.0725 days^{-1}, and 0.1216 days^{-1} for the Light, DCMU, Glucose experiment, Dark, DCMU, Glucose experiment, and Dark, Glucose experiment, respectively. Additionally, the mixotrophic condition (Light, Glucose experiment) was shown to produce the highest growth rate overall of 0.2124 days^{-1}. The high probability of the DCMU compound providing an additional carbon source for the algae was also observed.

***Keywords: Chlorella vulgaris*, autotrophic conditions, heterotrophic conditions, mixotrophic conditions, lipid yield**

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1. Introduction

Fossil fuels are becoming scarcer as the world's energy demand increases. Attention has been directed to biofuels, because they are a renewable and sustainable source of energy that do not have detrimental environmental effects (Shen et al., 2009). Algae colonies are a plausible source of biofuel because it is relatively easy to grow and harvest algae in bulk. In 2007, researcher Yusuf Chisti reported a study proving that algae produce more oil per U.S. cropping area than many other oil crops such as corn, soy, and jatropa. Algae produce fatty acids called unsaturated lipids, which are extracted and processed to form biofuel. When algae are stressed, they produce copious amount of lipids. These lipids do not need extensive pretreatment before being formed into biodiesel (Woertz, 2007).

While considering financial and environmental energy constraints, research has been initiated in the field of light-deprived algae. When the photosynthetic process of algae is removed, algae gains energy from alternative organic processes that convert sugar into lipids (Perez-Garcia et al., 2010). In the light, autotrophic algae gain energy through a light supply using photosynthesis. In the dark, heterotrophic algae gain energy by consuming dissolved organic matter as opposed to photosynthesizing. Mixotrophic algae use both photosynthesis and the consumption of organic nutrients (Crane et al., 2010).

Growing autotrophic algae presents several disadvantages. The autotrophic algae reactor must have an extremely large surface area and shallow depth to allow for algae to gain light exposure by being close to the surface light source. Maintenance of both outdoor and indoor reactors to allow for sufficient light penetration is tedious and expensive. The demanding space requirements and constant need for light make autotrophic algae cultivation an expensive process. Growing algae heterotrophically presents economic advantages. The algae can be grown significantly denser, allowing for greater yield, because light does not need to penetrate the algae. Also, the cost of cultivation will decline because the space and maintenance requirements are not as demanding (Perez-Garcia et al., 2010).

Using glucose as an alternative source of energy is significantly less expensive than providing algae with light. Glucose is a complex carbon substrate that produces microalgal biomass and biochemical components of the algae such as lipids (Kong et al., 2012). If glucose is used as a source of carbon, cell growth rate and productivity can improve, which makes algae-derived biofuels more efficient (Kong et al., 2012).

The species of algae used in this study was *Chlorella vulgaris*, which is a resilient and versatile species of algae found around the world. It has a lipid content range of 14-22% by dry cell weight (Becker, 1994). *C. vulgaris* can be grown under a wide variety of environmental conditions, including heterotrophic growth environments (Perez-Garcia et al., 2010). An image of *Chlorella vulgaris* is shown below in Figure 1.

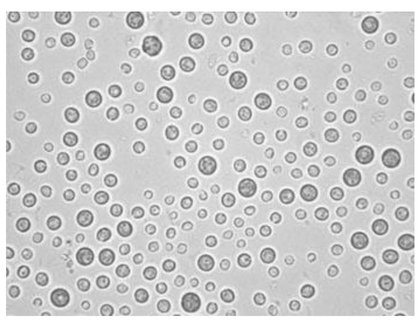

Figure 1: Photograph of *Chlorella vulgaris*

The objective of this experiment was to compare autotrophic, heterotrophic, and mixotrophic algal growth and lipid yield to determine the most effective growth condition.

2. Materials and Methods

A total of sixteen 1000mL beakers were used for this experiment. The ratio of Bold's Basal Medium to algae in each beaker system was 600:400mL. The algae species was purchased from the culture collection bank at the University of Texas (UTEX). Each beaker contained a concentration of glucose, a concentration of DCMU (photosynthesis blocker), or a combination of both compounds. A glucose concentration of 500 mg/L was used in the beakers containing glucose and a DCMU concentration of 4.7 mg/L was used in the beakers containing DCMU. The glucose concentration was determined using the fact that there is 0.039 percent CO_2 by volume in the atmosphere. The carbon concentration in air was replicated in the beakers. The DCMU concentration was determined based on a similar experiment where the conditions of algae were light-deprived (Melis, 2007).

Figure 2 displays the compounds present in each beaker. Each condition was conducted in duplicates to ensure accuracy and consistency.

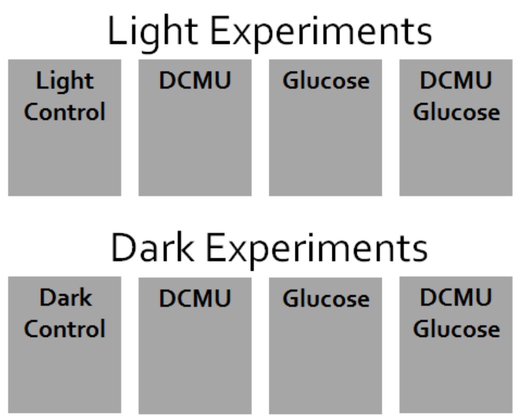

Figure 2: Beaker Contents for Autotrophic, Heterotrophic, and Mixotrophic Growth

Experiments were conducted using a modified jar test setup that continuously agitated the algae at 90 revolutions per minute. For the light, autotrophic and mixotrophic experiments, the light-dark cycle ratio was 18:6 hours at 400 ft-c. For the dark, heterotrophic experiments, the entire modified jar test set-up was placed in a sealed fume hood behind a black-out curtain. Each individual beaker was retrofitted with a cardboard box with a door cut-out for taking samples. These precautions were taken to ensure that no light entered the system.

Temperature, pH, and algal growth were monitored daily throughout the experiment. Carbon consumption in each of the sixteen beakers was measured four times over the course of the experiment with the use of HACH Test N Tube high range COD. Temperature and pH were measured using a Celsius-thermometer and a HACH pH meter. Algal growth was observed using optical density values measured by a HACH DR 4000 Spectrophotometer. Lipid extractions were conducted on day 14 of the experiment using the Bligh and Dyer method (1959).

3. Results and Discussion

The purpose of this experiment was to determine the most effective growth environment (heterotrophic, autotrophic, or mixotrophic) for lipid production and algal growth of *C. vulgaris*. By observing a heterotrophic growth condition, energy consumption and cost of algal cultivation will decrease, while feasibility of algal production will increase.

Figure 3 presents the growth curves for each of the eight growth conditions. The dashed lines represent growth conditions in the light and the solid lines represent growth conditions in the dark.

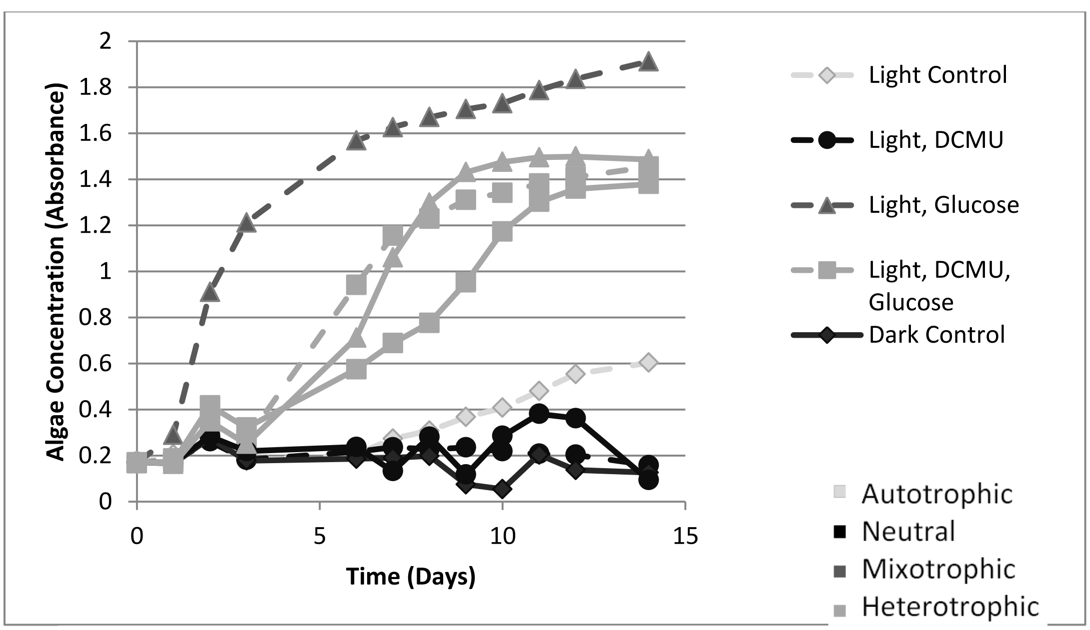

Figure 3: Growth Curves for Each Condition

It is apparent from Figure 3 that the mixotrophic growth condition, which supplied both light and glucose to the algae, resulted in the highest growth rate. The comparatively high growth rate of the mixotrophic condition was expected. The addition of an organic carbon source in combination with the carbon provided to the algae through light-driven photosynthesis represents the ideal growth condition, which is referred to as a luxury condition. The algae are grown in an environment with a surplus of nutrients, so the growth increases as the algae takes advantage of ideal conditions.

Following the mixotrophic growth condition, the heterotrophic condition had the second highest growth rate. The algae grown in this condition were removed from the autotrophic condition of the stock cultures. A heterotrophic environment was unfamiliar, but all heterotrophic experiments grew well with a glucose-based carbon source. The autotrophic growth condition grew the least. This result was not expected. Algae are grown in autotrophic conditions in the lab as well as in nature, so they are adapted to grow with light as a source of energy. The autotrophic growth condition was expected to have the second highest growth after the mixotrophic condition. The fact that all cases of heterotrophic growth were higher than autotrophic growth is encouraging, because heterotrophic conditions are more economical and feasible.

Figure 4 presents the carbon consumption curves for each growth condition. Again, the dashed lines represent growth conditions in the light and the solid lines represent growth conditions in the dark.

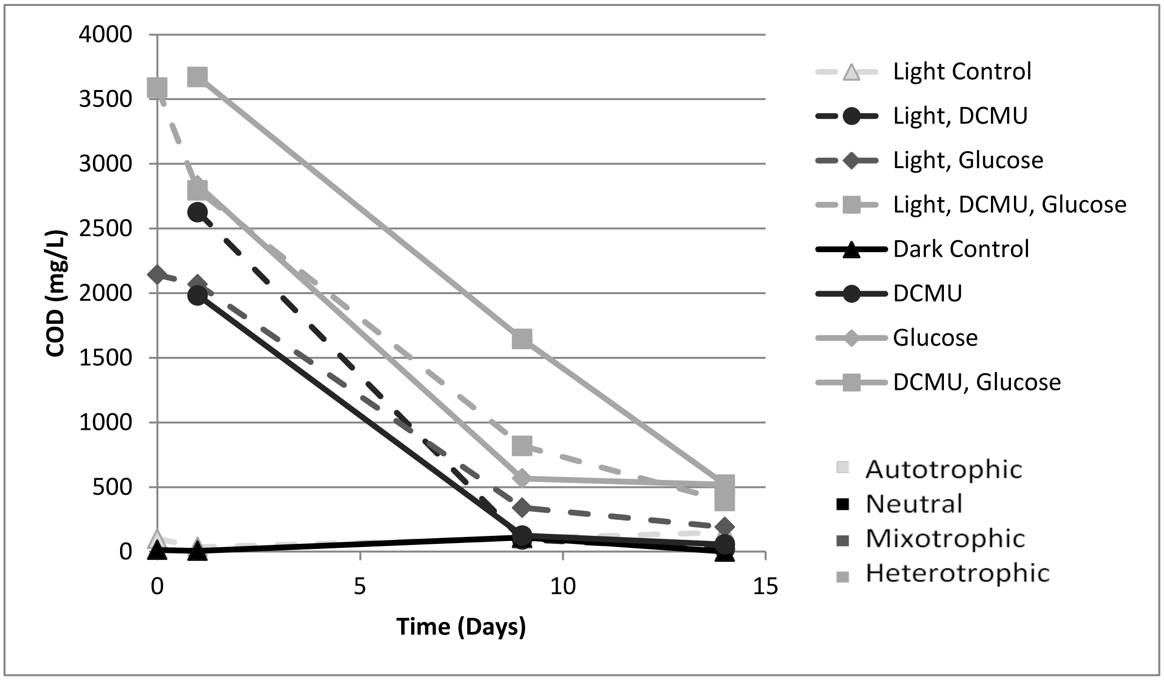

Figure 4: Carbon Consumption in Different Growth Conditions

The heterotrophic algae, as expected, had the most organic carbon consumption. The heterotrophic growth conditions had organic carbon directly imputed into the system as a source of energy for the algae, so it is clear that the algae were using the glucose as a source of energy. The carbon concentration of the heterotrophic conditions nearly reached zero at the conclusion of the 14-day experiment. Organic carbon was also introduced to the mixotrophic condition, which is why the mixotrophic condition had the second highest carbon consumption. The algae were gaining energy from both light and the glucose. The autotrophic condition was grown in the light without any addition of glucose, so there cannot be any organic carbon consumption, as is indicated in Figure 5.

The DCMU compound was added to the cultures grown in the dark in order to determine whether or not the algae would use DCMU as an additional carbon source. Based on Figure 4, it is apparent that the DCMU compound was contributing to the algae's carbon consumption. The two growth conditions with the most drastic carbon consumption were the heterotrophic dark condition containing DCMU in addition to glucose and the forced heterotrophic light condition also containing DCMU in addition to glucose. The results of the experiment indicate that the DCMU may not be performing its desired function, but rather it is being used as an additional energy source for the algal cultures.

In the case of the heterotrophic dark environment with the addition of DCMU, the algae did not have access to a light source and was forced to convert the organic carbon that was initially provided into the energy it needed to survive. Similarly, the forced heterotrophic light environment took away the algae's ability to photosynthesize, so it also was forced to use the glucose provided to survive.

Figure 5 displays a detailed analysis of lipid yield based on growth conditions. According to the data, the most optimal growth condition for lipid yield was the heterotrophic condition. This supports the fact that algae produce profuse amounts of lipids when grown in stressful conditions (Woertz, 2007).

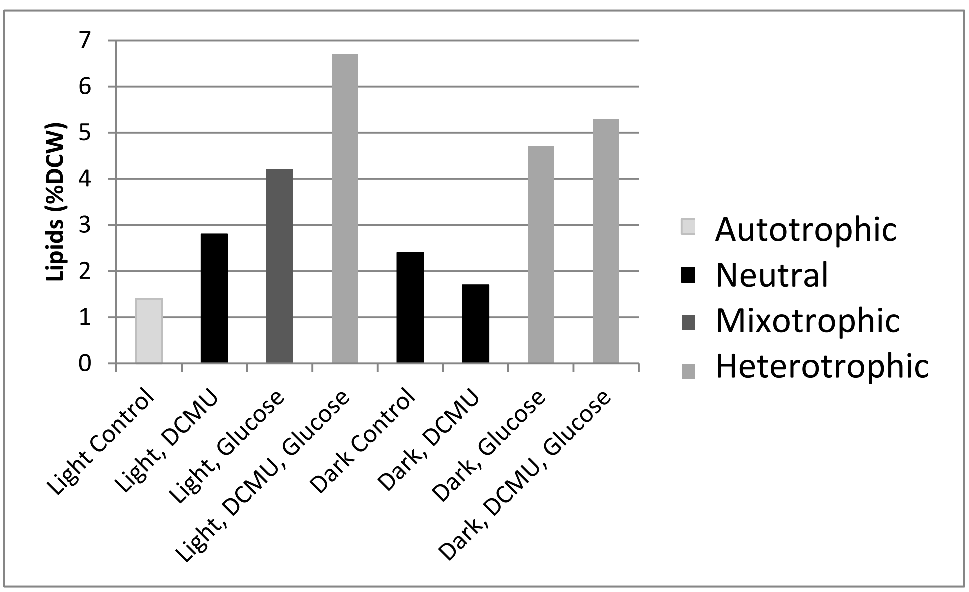

Figure 5: Lipid Analysis of Autotrophic, Heterotrophic, and Mixotrophic Conditions

The autotrophic condition of growth is the least stressful to the algae. Algae are photosynthetic organisms, so the natural growth environment is autotrophic using light from the sun. The algae stock solutions of Rowan University are maintained and cultivated in an autotrophic state. Because the algae are in their naturally occurring, ordinary growth environment, the algae is not stressed in the autotrophic condition.

The mixotrophic state is observed to have the second-best lipids achieved after a 14-day growth period. This can be attributed to the mix of a familiar growth environment and an unfamiliar environment created with the introduction of glucose. With both light and glucose available to the algae, it had an abundance of nutrients and nutrient sources. This may have slightly stressed the algae out because the algae are not acclimated to a medium with alternative sources of carbon. The excess nutrients could account for the algal stress.

As expected, the heterotrophic growth condition of the algae was most effective for lipid production. The algae were removed from a light environment specifically engineered to cater to photosynthetic organisms. Instead, they were introduced to a completely dark environment with glucose as the only source of carbon. Although the algae are able to acclimate to the glucose-saturated water, the algae are not accustomed to forcing a different chemical process, and it is a large source of stress. The results prove that high lipid yields are, in fact, a true advantage of heterotrophic growth conditions.

Table 1 presents an overview of the final results for each growth environment. The last column is a product of the growth rate and lipid yield, which provides a value necessary to determine the most effective growth environment overall. The most effective combination of algae, glucose, light, and DCMU was light, DCMU, and glucose. It was expected that the Light, DCMU, Glucose experiment would closely parallel the Dark, Glucose experiment, because

DCMU is an inhibitor of photosynthesis, meaning the algae was essentially being grown in the dark wherever the compound was added. When looking at growth rates, the two conditions are very similar. But when looking at lipid yields, the Light, DCMU, Glucose experiment had a relatively large lipid yield compared to the Dark, Glucose experiment. This alludes to the idea that DCMU is actually providing an alternative source of carbon for the algae to consume in the system.

Also as was expected, the Dark Control and the Dark, DCMU experiments had negative growth rates, indicating that both cultures were dying off without access to either light or glucose. However, the carbon consumption for the Dark, DCMU experiment was very high, again indicating that the DCMU compound was providing the algae with an additional carbon source.

Table 1: Summary of Experimental Results

Growth Condition	Growth Rate (Day^{-1})	R^2 (unitless)	Carbon Consumption (%)	Lipid Yield (% DCW)	Lipid Normalized Production Weight ($Days^{-1}$)
Light Control	0.0578	0.955	-	1.4	0.0809
Light, DCMU	0.0468	0.829	99.2	2.8	0.1310
Light, Glucose	0.2124	0.897	90.8	4.2	0.89208
Light, DCMU, Glucose	0.1473	0.925	86.0	6.7	0.9869
Dark Control	-0.0070	1.000	-	2.4	0.0168
Dark, DCMU	-0.0005	1.000	97.2	1.7	0.0008
Dark, Glucose	0.1216	0.885	81.7	4.7	0.5715
Dark, DCMU, Glucose	0.0725	0.9092	85.9	5.3	0.3842

4. Conclusions

C. vulgaris cultivated in the heterotrophic conditions (Light, DCMU, Glucose experiment, Dark, DCMU, Glucose experiment, and Dark, Glucose experiment) showed the highest lipid yields of 6.7% DCW, 5.3% DCW, and 4.7% DCW, respectively. *C. vulgaris* grown in a mixotrophic condition (Light, Glucose experiment) showed the highest growth rate of 0.2124 day^{-1}. Conclusions drawn from this experiment include the correlation between stresses placed on the algae and high lipid yield, and the relationship between the mixotrophic “luxury” growth condition and highest growth rate. In addition, the results supported the idea of DCMU being utilized as an additional carbon source for algal consumption. Based on the results of this experiment, heterotrophic algal growth conditions were shown to provide an effective environment for lipid production and growth of *C. vulgaris*. These results are promising, because heterotrophic algae is more economical and feasible than autotrophic or mixotrophic conditions.

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