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Lipid content and biomass analysis in autotrophic and heterotrophic algal species

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Abstract

Biofuels are a form of renewable energy derived from living matter, typically plants. The push for biofuels began in order to decrease the amount of carbon dioxide (CO_2) released into the atmosphere, as biofuels are essentially carbon neutral. The idea is the same amount of CO_2 the plants took in to perform photosynthesis will then be released in the burning of the biofuels. Algae is an excellent source of biofuels because it grows quickly and is versatile in terms of the type of fuel it can produce. The two most common mechanisms for algae growth are heterotrophic or photoautotrophic. Heterotrophically grown algae uses an exogenous energy source, such as glucose, and uses the energy stored in it to perform cellular functions. Glucose also serves as a source of carbon and hydrogen, which are the primary elements found in lipids. In addition heterotrophic algae requires other nutrients for survival, such as water, vitamins, and inorganic ions. Algae grown photoautotrophically uses pigments in cellular photoreceptors to convert energy from light into adenosine triphosphate (ATP), an energy source, and to produce glucose. It also requires water, vitamins, and inorganic ions like the heterotrophic algae does. Some algal species, such as *Chlorella zofingiensis*, can be grown both photoautotrophically and heterotrophically. This algae species will be the subject of our experiment.

Our experiment seeks to discover the most efficient way of growing algae to produce the highest amount of lipids. In addition to serving as a key component of cell and organelle membranes, lipids are a common form of high efficiency, long-term energy storage for living organisms, which is why lipids are extracted and processed to form biofuels. We propose growing one species of algae photoautotrophically by providing it with proper amounts of light but eliminating any glucose available. We will also grow the same species heterotrophically, with exogenous access to glucose, but eliminating all exposure to light sources. Finally, we will grow the same species mixotrophically with access to both glucose and light. Once the algae is grown, it will be harvested and analyzed for its lipid profile to determine which algae sample has the highest percent lipid content. We will also measure the percent biomass of each sample to determine which primary energy source leads to the greatest amount of total algal growth, percent organic material, and percent lipid content.

We predict the algae grown with access to both sunlight and exogenous glucose will produce both the highest lipid content and the highest percent of biomass.

Growth Conditions of *Chlorella zofingiensis*

Experiment Trials					
	Light	Glucose	Orbital Shaking	Kulh Media (Table 2)	Temper-ature
Heterotrophic	No	Yes	Yes	Yes	25°C
Photoautotrophic	Yes	No	Yes	Yes	25°C

Analysis Method

- Measure a sample of algae into a reaction vial.
  (Prepare three reaction vials of each sample to insure precise measurements.)
- Add 1% H_2SO_4 in Methanol to each reaction vial to catalyze the reaction.
- Place the reaction vials in a Thermomixer where they undergo a transesterification reaction.
  - Transesterification is the process of converting triglycerides and phospholipids to fatty acid mythel esters (FAME) and glycerol as is demonstrated in Figure 4.
- Extract the FAME from the esterified mixture using hexane.
- After centrifugation, the reaction vial will have a distinguishable hexane layer at the top of the sample. Pipette only this hexane layer into a gas chromotography (GC) vial which has been prepared with a C17 FAME solution; this is a standard which allows for interpretation of the results.
- Perform GC/MS analysis of the FAME in the GC vials using a 7890B/5977A Series Gas Chromatograph Mass Selective Detector, Figure 3.
- Measure the percent biomass of the algae sample by combusting it at 500°C inside a muffle furnace. The heat of the furnace consumes the organic mass, leaving behind the inorganic mass.

Kulh Medium		
Compound	**Density (mg/L)**	**Concentration (mols/L)**
KNO_3	1011.1	1×10^{-2}
$NaH_2PO_4 \bullet 1H_2O$	621	4.5×10^{-3}
$Na_2HPO_4 \bullet 2H_2O$	89	0.5×10^{-3}
$MgSO_4 \bullet 7H_2O$	246.5	1×10^{-3}
$CaCl_2 \bullet 2H_2O$	14.7	1×10^{-4}
$FeSO_4 \bullet 7H_2O$	6.95	2.5×10^{-5}
H_3BO_3	0.061	1×10^{-6}
$MnSO_4 \bullet 1H_2O$	0.169	1×10^{-6}
$ZnSO_4 \bullet 7H_2O$	0.287	1×10^{-6}
$CuSO_4 \bullet 5H_2O$	0.00249	1×10^{-8}
$(NH_4)_6Mo_7O_{24} \bullet 4H_2O$	0.01235	1×10^{-8}

Table 1. Composition of the medium the algal colonies were grown on (Kulh and Lorenzen, 1964) The depletion of these nutrients acts as a stressor on the algae, causing it to produce lipids for long-term energy storage, not just for cellular functions.

Total Lipid Content of Photoautotrophic and Heterotrophic Algae Cells (% of Total Fatty Acid)		
Fatty Acid	Photoautotrophic	Heterotrophic
C16:0	26.6±1.1%	22.2±1.0%
C16:1	2.2±0.1%	1.7±0.1%
C16:2	7.7±0.2%	8.3±0.4%
C16:3	6.6±0.3%	2.1±0.1%
C16:4	1.1±0.1%	0.2±0.0%
C18:0	3.7±0.1%	1.2±0.1%
C18:1	17.9±0.9%	35.2±1.2%
C18:2	20.8±1.2%	20.2±1.1%
C18:3 (n-6)	1.4±0.1%	0.5±0.0%
C18:3 (n-3)	10.8±0.4%	7.8±0.5%
C18:4	1.1±0.1%	0.4±0.00%

Table 2. (Liu et al., 2010)

C16:0 represents a fatty acid chain. The first number (e.g. in C16:0, 16) tells how many carbon atoms are in the chain, while the second number (e.g. in C16:0, 0) tells how many double bonds are in the chain. The fatty acids are what we test for in order to determine the most efficient cell type for biofuel use. Note that the only fatty acid with a significant different is C18:1. This indicates this fatty acid is part of the biochemical mechanism that differentiates between heterotrophic and photoautotrophic. C18:1, oleic acid, is a key fatty acid in biofuels. The more a cell produces, the more efficient it is at being converted to biofuels.

Figure 1. Reaction vials after transesterification
(personal photograph by Thomas Walker, March 12, 2016)

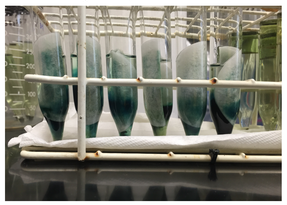

Figure 2. GC Vials (www.agilent.com)

Figure 3. GC/MS Agilent 6890 Series (www.agilent.com)

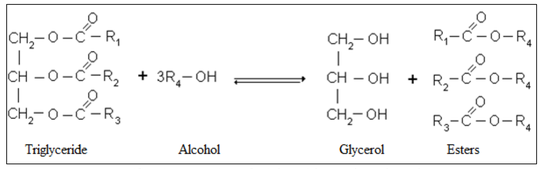

Figure 4. Transesterification Reaction of Triglycerides and Methanol to Form Fatty Acid Methyl Esters and Glycerol

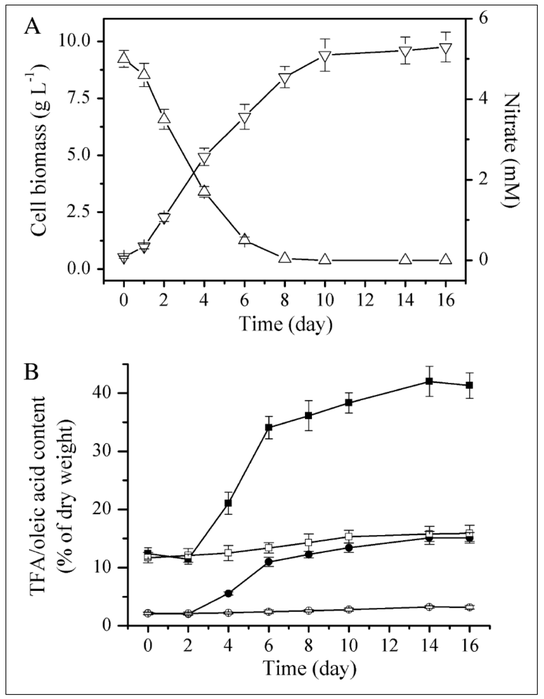

Figure 5. Cell growth (▽) and nitrate consumption (△) by heterotrophic *C. zofingiensis* (A) and accumulation of total fatty acid (TFA) (•/▫) and oleic acid (○/●) in autotrophic (▫/○) and heterotrophic (•/●) algal cells (B). (Liu et al., 2010)

Figure 5A shows the growth of the heterotrophic algae cells versus the nitrate consumption. The cells consume the nitrate rapidly (which was found in the medium, see Table 1), then when they deplete that resource, the cells undergo stress causing the increased production of lipids.

Figure 5B shows the fatty acid accumulation in both the heterotrophic and photoautotrophic algae cells. Heterotrophic cells accumulate more total fatty acid and more oleic acid in the same time period as the photoautotrophic cells.

Results

- At the end of the experiment, the heterotrophic cells accumulated more than double the total fatty acid and oleic acid as compared to photoautotrophic cells in the same amount of time.
- **The study clearly shows that heterotrophic algal cells have a higher yield of lipids, and the lipids they produce are more useful for biodiesel production.**

Conclusions

- In our theoretical experiment, we plan to perform an additional trial of algae growth and lipid analysis by growing it with access to both sunlight and exogenous glucose.

Theoretical Experiment Trials					
	Light	Glucose	Orbital Shaking	Kulh Media (Table 2)	Temper-ature
Heterotrophic	No	Yes	Yes	Yes	25°C
Photoautotrophic	Yes	No	Yes	Yes	25°C
Mixotrophic	Yes	Yes	Yes	Yes	25°C

- Based on the results recorded by Liu et al., 2010, we expect algae with access to both energy sources for lipid production will produce the highest concentration of lipids.
- Therefore, we expect that algae grown both photoautotrophically and heterotrophically (i.e. mixotrophically) will be the best growth option for the generation of biofuels.
- **Determining the growth mechanism which allows for the most efficient lipid production will have widespread effects in the fuel industry by maximizing the amount of fuel that can be extracted from the algae.**

Future Work

1. We suggest repeating this experiment with other species of algae that can also be grown mixotrophically, such as *C. vulgaris* and *C. protothecoides*. Other species may exhibit a preference toward one energy source over the other.
2. We suggest repeating this experiment with varying amounts of exogenous glucose and light instead of simply regulating their presence. The optimal conditions for lipid production in algae may be determined by varying the ratios of the nutrient sources.
3. We suggest repeating this experiment with changes to environmental factors, such as temperature and acidity to determine the most effective environment in which to grow algae.

References & Acknowledgments

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