Biodiesel Production in *Chlorella vulgaris* and *Synechococcus*

Sarah Mertson, Elizabeth Klosko, Alex Wilkinson, Daniel Bergman, Colden Grossman, Theodore Hovling, Annabel Rupp, Kjersti Mygland, Nicole Gretzinger, and Dr. Barnabas Gikonyo
Chemistry Department, SUNY Geneseo, Geneseo, NY 14454

Introduction

Fossil fuels are the largest contributors to global climate change, accounting for nearly 75% of total greenhouse gas emissions. A green energy solution can be found in autotrophs, which both sequester carbon in their growth and can be made into biodiesel. *Chlorella vulgaris* has been studied for efficient lipid growth and biodiesel production. *Synechococcus,* a genus of cyanobacteria that grows prolifically in Conesus Lake, may be an even better source of fuel than *C. vulgaris*. Growth of *Synechococcus* was observed in a variety of media and it was determined that BG-11 fosters the most prolific growth. *Synechococcus* phospholipids were extracted and converted into biodiesel using transesterification. These results from *Synechococcus* were compared to previous studies on *C. vulgaris* to determine which organism is the better source of biodiesel.

Growth Methods

1. Testing Various Media for *Synechococcus* Growth

To determine which media supports the most prolific growth, *Synechococcus* was grown for 28 days at 27 ºC in six different medias [BG-11, BBM, BG-11 + glycerol, BBM+Glycerol, granulated BG-11, and water]. A standard curve to determine cell concentrations from the absorbance of ultraviolet light at 685 nm was created. Then, we collected absorbance values from each flask over 28 days.

Figure 1. *Synechococcus* experimental growth setup

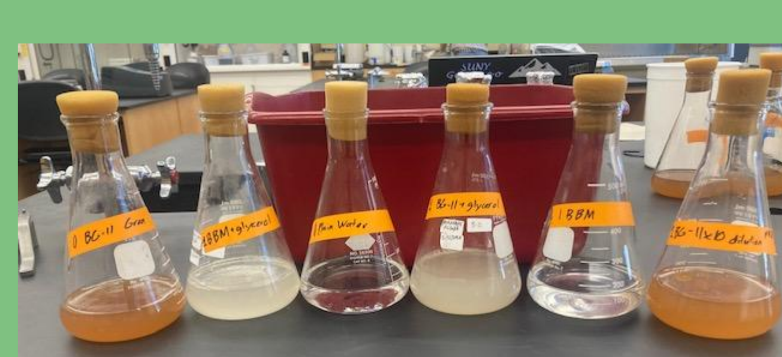

Figure 2. *Synechococcus* growth variation, Cloudiness indicates non-synechococcus growth

Biodiesel Methods

2. Lipid Extraction/Transesterification Of *C. vulgaris* and *Synechococcus*

The algae was shaken in a 2:1 chloroform-methanol solution. The resulting mixture was centrifuged and added to a separatory funnel with a 0.72% NaCl solution. The organic was collected, and excess solvent removed via a rotary evaporator. The transesterification reaction was run for 30 minutes at 65ºC. A final IR analysis was carried out to determine if the synthesis was successful.

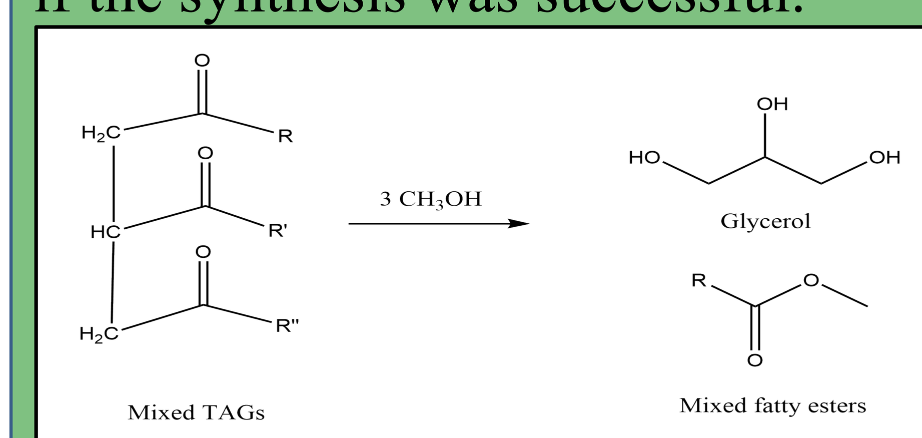

Figure 3. Biodiesel transesterification reaction schematic.

Figure 4. Separating lipids after extraction

Growth Results

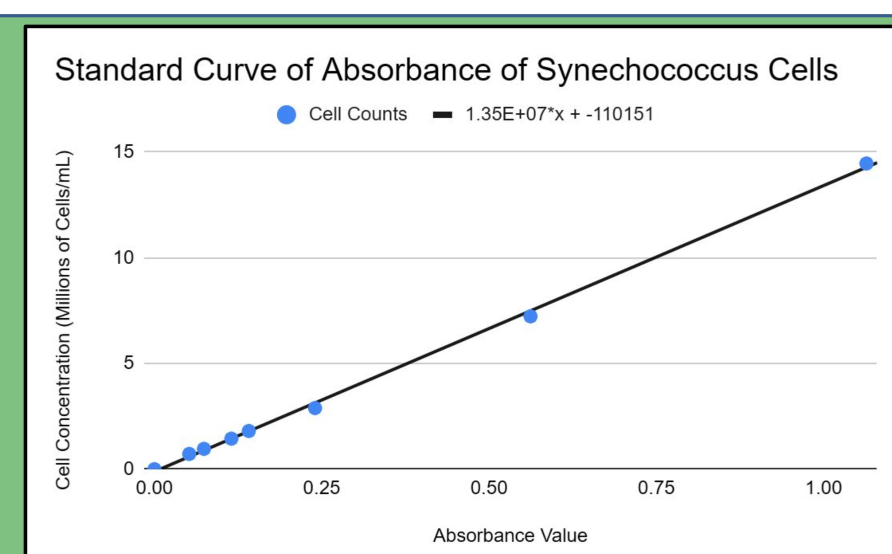

Figure 5. Standard Curve of absorbance values of *Synechococcus* cell numbers ($y=1.35*10^7x - 110151$) There are an estimated 14,460,160 cells/mL of solution.

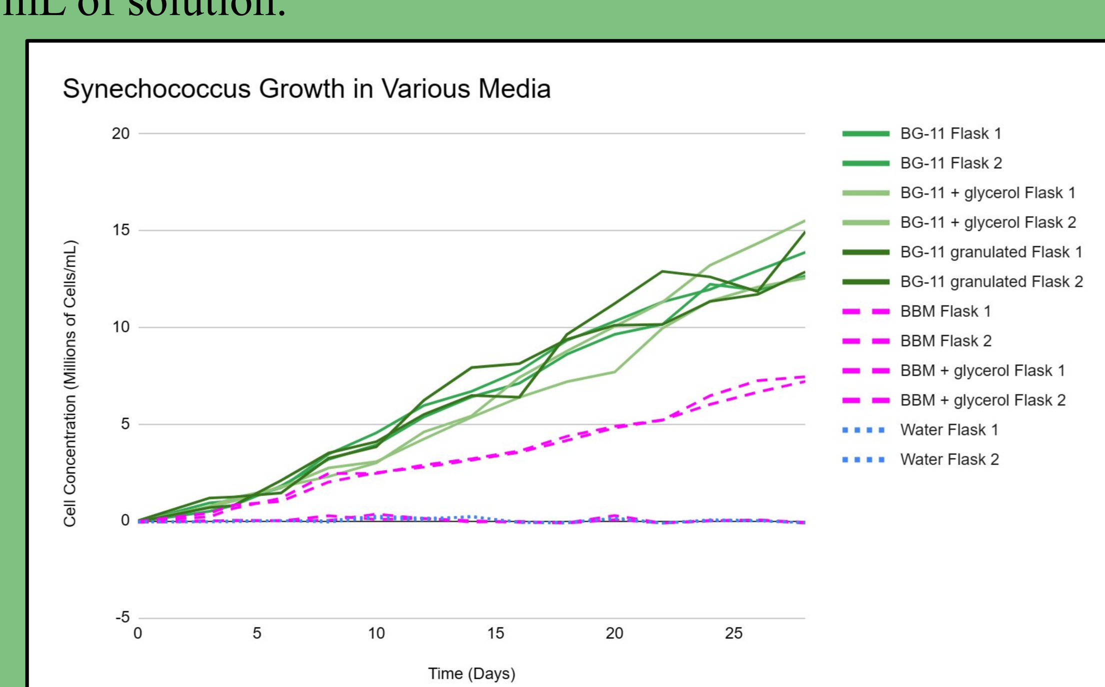

Figure 6. *Synechococcus* growth was most successful in BG-11 media variations. Maximum cell concentration was 15.5 million cells/mL

Biodiesel IR Results

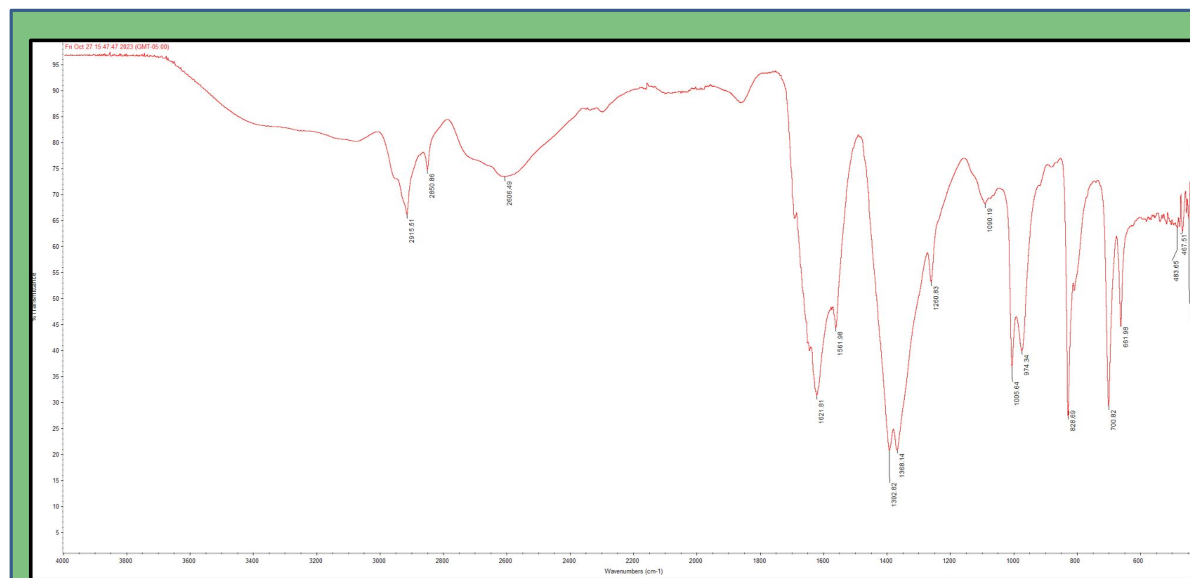

Figure 7. Biodiesel produced from *C. Vulgaris* lipids. The sharp peak at 1621.81 cm^{-1} indicates the ester carbonyl bond of the biodiesel. The peaks at 2915.51 cm^{-1} and 2850.86 cm^{-1} indicate methyl groups.

Figure 8. Biodiesel Standard from *Synechococcus* lipids. The peak at 1621.10 cm^{-1} demonstrates the the carbonyl group of the methyl ester biodiesel. The 2917.27 cm^{-1} and 2850.67 cm^{-1} peaks indicate methyl groups.

Conclusion

BG-11 produced the most prolific *Synechococcus* growth, reaching cell counts up to 68 times higher than found in filtered lake samples taken by group members. Adding glycerol to the media as an extra sugar source did not contribute to *Synechococcus* growth but rather another organism in the samples flourished. For *Synechococcus,* lipids yield was 2.26% by mass while in *C. vulgaris* the lipid yield was 25.65% by mass. IR spectra of the biodiesel produced from *Synechococcus* suggests a successful lipid extraction and transesterification, albeit little yield. Future directions include identifying this organism and altering our extraction- transesterification process to an *in situ* procedure.

Acknowledgements and References

[1] Mathimani, T., Uma, L, and Prabaharan, D. (2015) Homogeneous acid catalyzed transesterification of marine microalga Chlorella sp. BDUG 91771 lipid- An efficient yield and its characterization. Renew. Energy 81, 523-533.

[2] Milano, J., Ong, H., Masjuki, H., Chong, W., Lam, M., Loh, P., and Velleyan, V. (2016) Microalgae biofuels as an alternative to fossil fuel for power generation. Renew.Sustain.EnergyRev.58,180-197.

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