Chlorella protothecoides Biodiesel

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Introduction

Biodiesel is a potential renewable, clean, and green energy. Using Chlorella protothecoides can help pave the path for this potential fuel. C. protothecoides has the ability to be grown both heterotrophically and autotrophically, and when grown heterotrophically growth restriction that burden many other strains are reduced. Optimization of growing C. protothecoides heterotrophically can greatly help us understand this species and the procedures to maximize lipid production genetically, greatly increase cell growth and density, and reduce lipid loss in the extraction process.

Gene Alternation

Figure 1: C. protothecoides that underwent UV exposure and was diluted 1:100,000 solution. These were then grown up in a flask and retested for lipid content.

Lipid content was optimized by mutating C. protothecoides DNA by UV radiation. Cells surviving UV radiation were then treated with Nile Red stain and the lipid content observed under a fluorescent microscope. Sample were then regrown through to create a serial enrichment of lipid content

Growth Optimization

Growth was optimized considering two main factors; ammonia and glucose. This was achieved by varying the volume of stock A, B, C and D, fully detailed in **Figure 2,** in the growing media. Samples were grown for 48 hours after which absorbances were collected. Results are shown in figure 3 with the greatest absorbance from stock B.

Group A 10x Stock		
98.57	grams/liter	MSG monohydrate
6.86	grams/liter	NaCl
3.18	grams/liter	CaCl2 monohydrate
10.95	grams/liter	KCl
54.76	grams/liter	MgSO4 septahydrate
4.05	grams/liter	Ammonium sulfate
65.71	grams/liter	Yeast extract
5.70	grams/liter	KH2PO4
1.10	grams/liter	Dow 1520US antifoam
Group B 10x stock		
52.667	grams/liter	FeSO4 septahydrate
94.249	grams/liter	citric acid
15.844	grams/liter	MnCl2 tetrahydrate
15.844	grams/liter	ZnSO4 septahydrate
0.204	grams/liter	CoCl2 hexahydrate
0.204	grams/liter	NaMoO4 dihydrate
10.580	grams/liter	CuSO4 pentahydrate
10.580	grams/liter	NiSO4 hexahydrate
Group C 10x stock		
11.2125	grams/liter	thiamine-HCl
0.184	grams/liter	vitamin B12
3.835	grams/liter	pantothenic acid hemi-calcium salt
Group D 10x Stock		
300	grams/liter	glucose

Figure 2: Stock media recipes used in varying amount

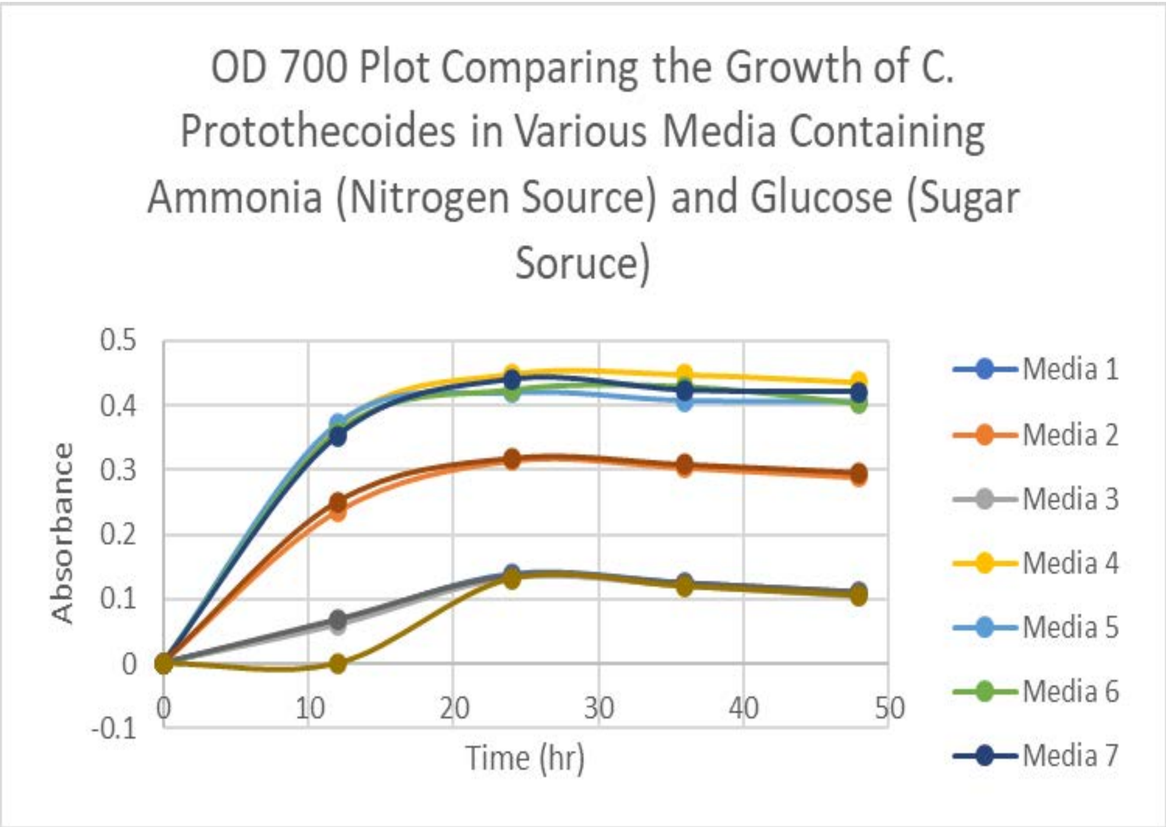

Figure 3: Absorbance of varying samples over time.

Extraction

Extraction optimization was achieved by varying salt, pH, and milling time. Samples were collected every five minutes for absorbance testing using propidium iodide and spectrophotometry. Ten minutes with no added salt and original pH resulted in the best extraction.

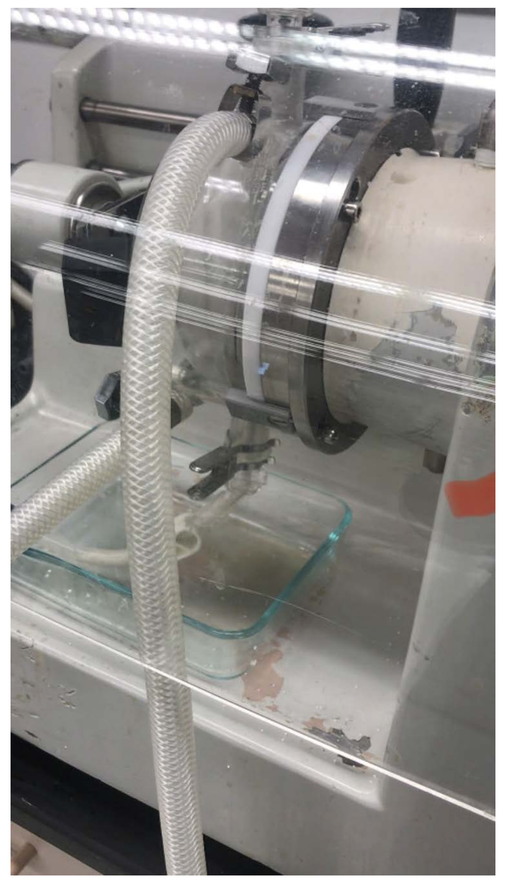

Figure 4: 300 mL glass bead mill

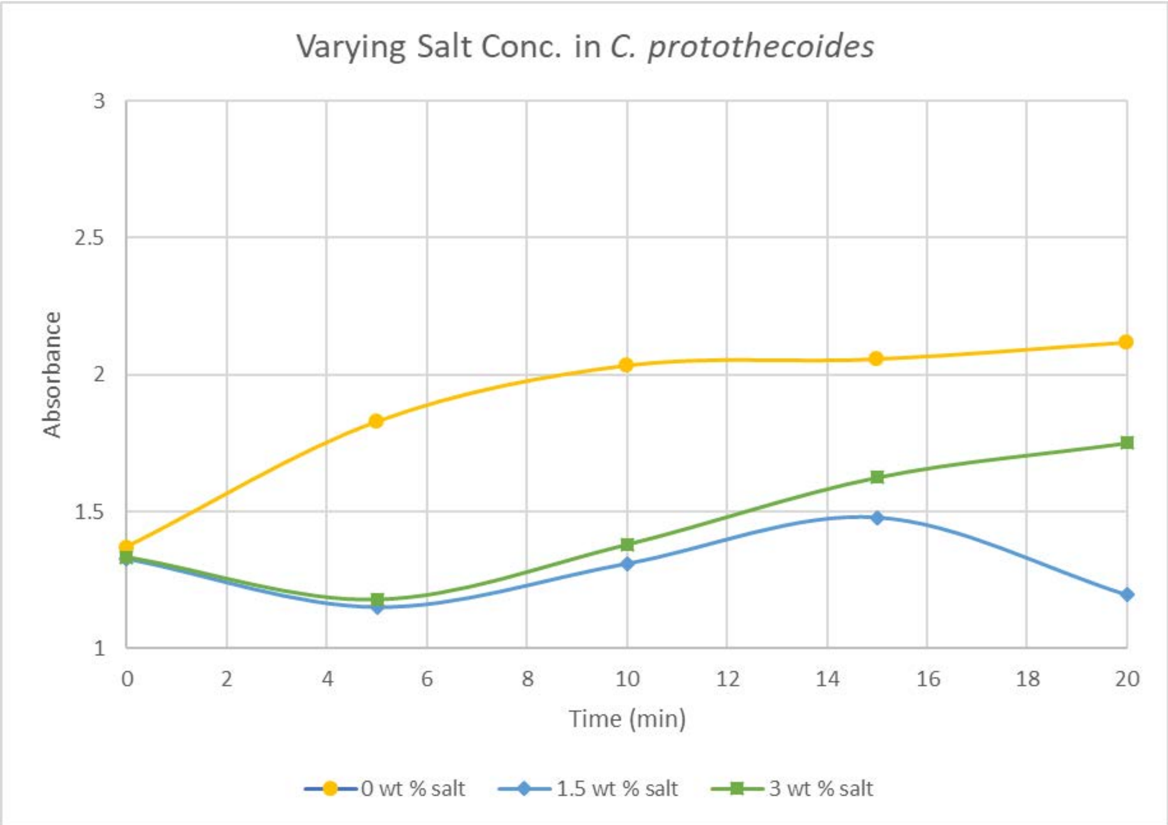

Figure 5: Absorbance of varying samples over time in the bead mill with wt% salt adjusted using sodium chloride.

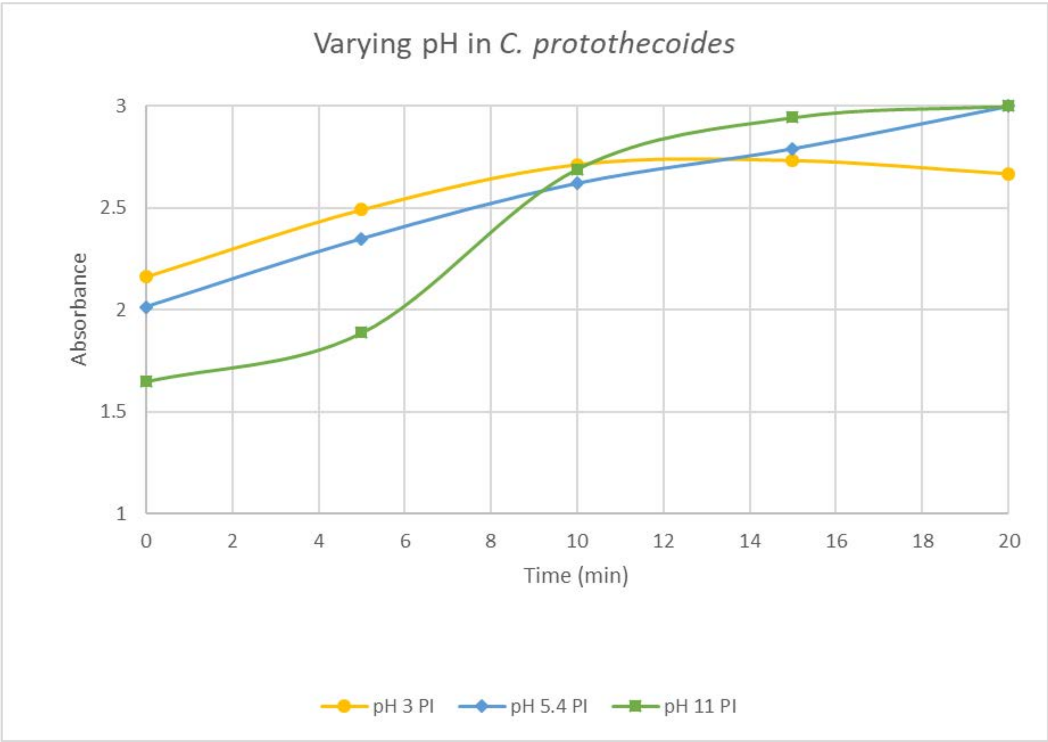

Figure 6: Absorbance of varying samples over time in the bead mill with pH adjusted using sulfuric acid and sodium hydroxide.

Conclusions

The team has explored several stages of optimizing Chlorella protothecoides for the production of biodiesel. The current data results suggest using a growing media in comparison to stock B for the most dense growth of Chlorella protothecoides followed by ten minutes in the bead mill using 0.8-1 μm beads. No adjustment to cell culture are needed for the best milling results. Future studies will provide for better optimization and reduced material input.

Future Work

The following are suggestions to further improve biodiesel from C. protothecoides.

- Kill samples need to be carried onward to test for lipid percentage.
- Media variants such as glycerol-ammonia, glucose-urea, and glycerol-urea experiments
- Will provide carbon and nitrogen sources for the algae while utilizing biofuel biproducts.
- Factors such as temperature and enzyme cocktails should considered in the milling process.

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