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Biodiesel Production from Algae

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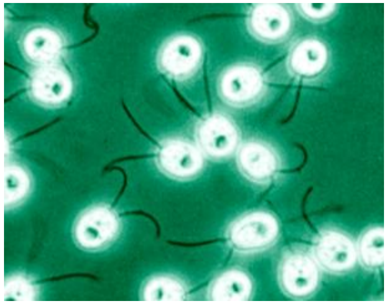
Introduction

- Algae fuel is a good alternative to fossil fuels in a time of high oil prices, increasing energy needs, and competition between food demands and biofuel sources
- Harvest cycle of 1 to 10 days
- Does not require freshwater – can be produced using ocean or wastewater
- Biodegradable and relatively harmless
- Can produce 300 times more oil per acre than conventional crops, such as rapeseed, palm, soybean, and jatropha

Background of Algal Strains

- *Chlamydomonas reinhardtii*:
  - Most widely used laboratory species. Single celled chlorophyta. Grows in freshwater and soil, and has a reported lipid content of about 21%.
- *Haematococcus droebakensis*
  - Single celled chlorophyta. Grows in freshwater, and has a reported lipid content of nearly 33%.
- *Platymonas*
  - Single celled chlorophyta. Grows in freshwater, and has a reported lipid content of 15-22%.

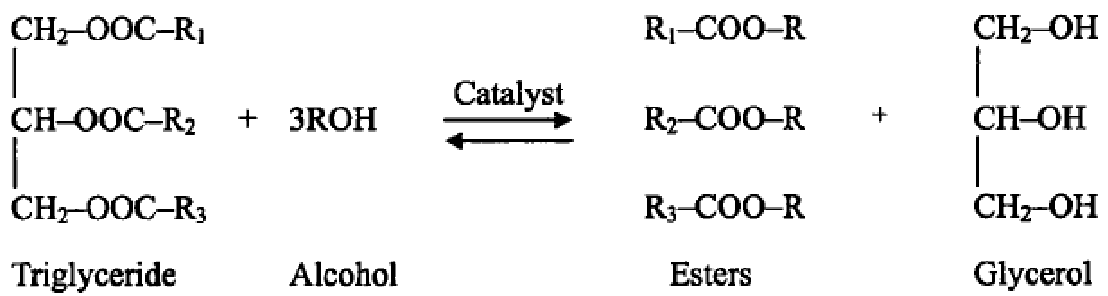


Chlamydomonas reinhardtii

Haematoccocus droebakensis

Platymonas

Trans-esterification of Triglycerides



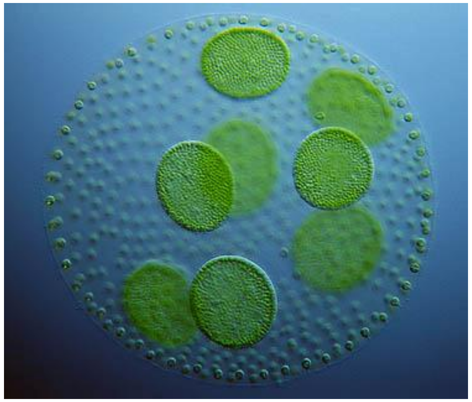
Trans-esterification is a chemical reaction between triglyceride and alcohol in the presence of a catalyst. It consists of a sequence of three reactions where triglycerides or triacylglycerides [TAGs] are converted at first to diglycerides and then to monoglycerides, eventually followed by the conversion of monoglycerides to glycerol (MacDougal et al., 2011). The esters corresponding to the chains R_1, R_2 and R_3 are formed. Three moles of alcohol are stoichiometrically required for each mole of triglyceride, but a higher molar ratio of five to six is often employed for maximum ester production depending on process conditions (Sharma et al., 2008). Some of the fatty acid methyl esters (FAMEs) may be transformed to diesel hydrocarbons without oxygen species.

MacDougall, K.M., McNichol, J., McGinn, P.J., O'Leary , S.J. B., and Melanson, J.E. (2011). Triacylglycerol profiling of microalgae strains for biofuels feedstock by liquid chromatography–high-resolution mass spectrometry. Analytical and Bioanalytical Chemistry, 401, 2609–2616.

Sharma, Y.C., Singh, B., and Upadhyaya, S.N. (2008). Advancements in development and characterization of biodiesel: A review. Fuel, 87, 2355-2373.

Research Objectives

- Explore a cleaner alternative to petroleum diesel by extracting oil from algae and converting it into biodiesel
- Increase the efficiency and practicality of algae production by optimization of important growth variables
- Compare the growth rate and lipid content of different algal species under the same growth conditions
- Determine the chemical composition of the algae biodiesel through gas chromatography



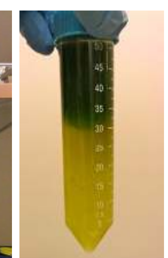
Experimental Techniques

- Bioreactor Studies: Three high-yielding algal species were tested for producing biodiesel by photosynthesis --- *Chlamydomonas reinhardtii*, *Haematococcus droebakensis,* and *Platymonas. The growth rates were determined by gravimetric methods under varying conditions.*
- Lipids Extraction Studies: The energy-rich lipids were extracted from the algae by ultrasonication for the disruption of algal cells by cavitation at high frequencies (greater than 20,000Hz) using 1,2-dichloroemethane and methanol (2:1 vol.) according to modified Folch's technique (Folch et al., 1957).
- Esterification: Chemical conversion by trans-esterification of algal oil from lipids to fatty acid methyl esters (FAMEs) and to biodiesel hydrocarbons.

*Folch, J., Lees, M., and Stanley, G.J. S. (1957). A simple method for isolation of total lipids from animal tissues. Journal of Biological Chemistry, 226 (1), 497-509.

Photosynthesis bioreactors

Algal biomass



Ultrasonication for lipids disruption and extraction by phase separation

Experimental Techniques (continued)

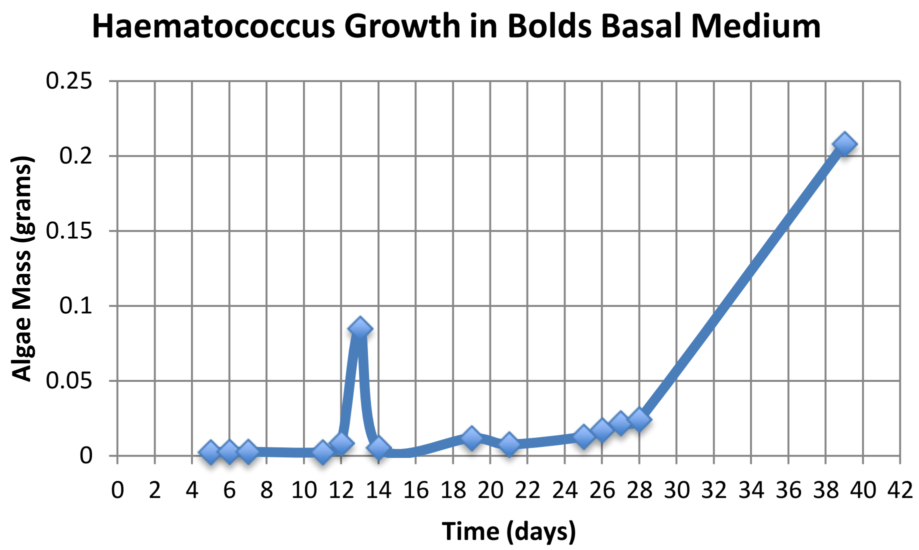
- Analysis of important biodiesel products by gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS)
- Gas chromatography employing a Varian 3900 instrument equipped with a fame ionization detector, and a DB-5 capillary column (30 m x 0.32 mm x 0.25 µm), with temperature programming.
- Gas chromatography-mass spectrometry using a Bruker 400 GC in combination with a Bruker Daltonics MS-300 detector, equipped with a DB-5 capillary column for separation and identification of products.

Gas chromatography

Gas chromatography-mass spectrometry

Results

- The graph shows *Haematococcus* growth in Bold's Basal Medium for 39 days. Overall growth between additions of nutrients predominantly showed exponential and declining growth periods. Note that more Bold's Basal Medium was added on days 18 and 32.
- *Haematococcus droebakensis*, was the best algal species among the species tested based on the results of the FAME (fatty acid methyl esters) analyses using gas chromatography and mass spectrometry techniques.
- Adding more nutrients before the stabilization of death phases attained more algae for future oil extraction (Day 32 was in growing phase).
- After ultrasonication, the lipids were extracted by the Folchs method. The lipid content was 8.6% by weight.

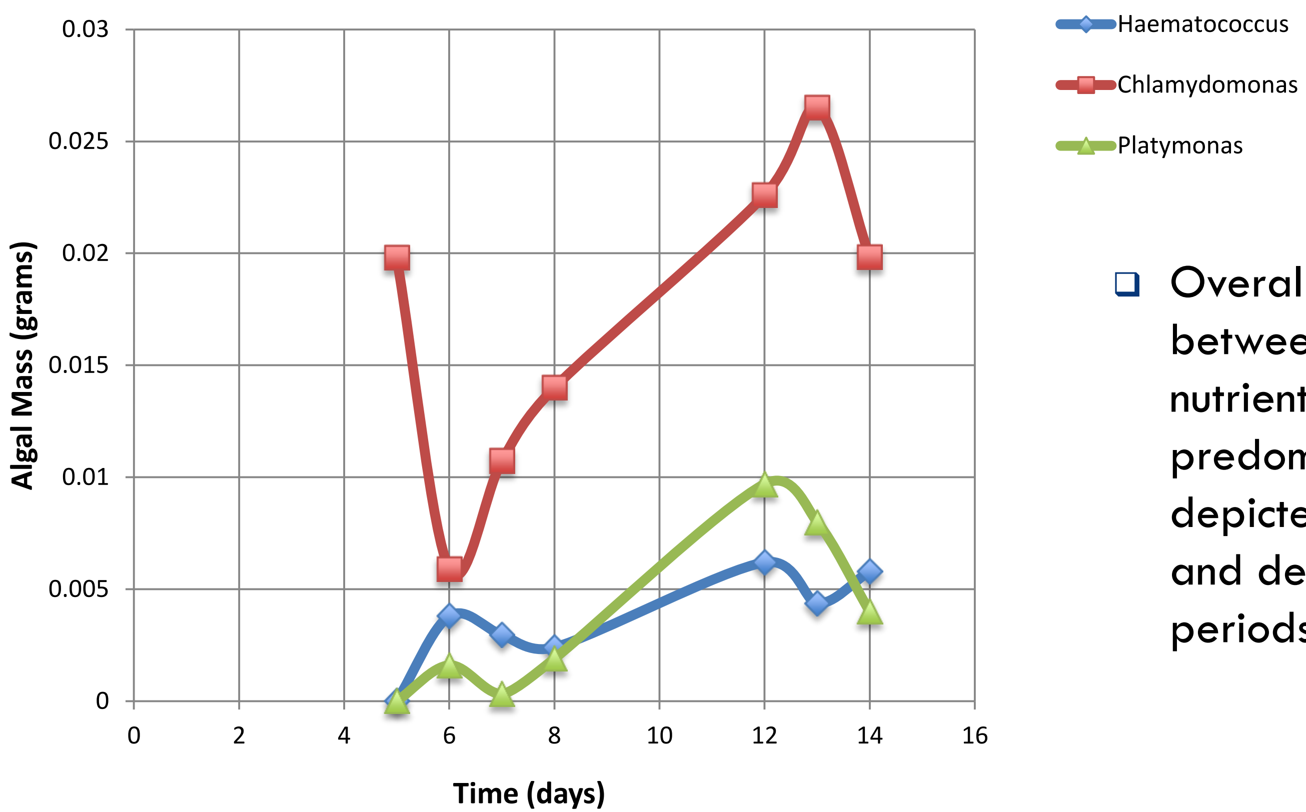


Haematococcus droebakensis growth in Bold's Basal Medium

*Day 13 results most likely error

Green residue containing lipids

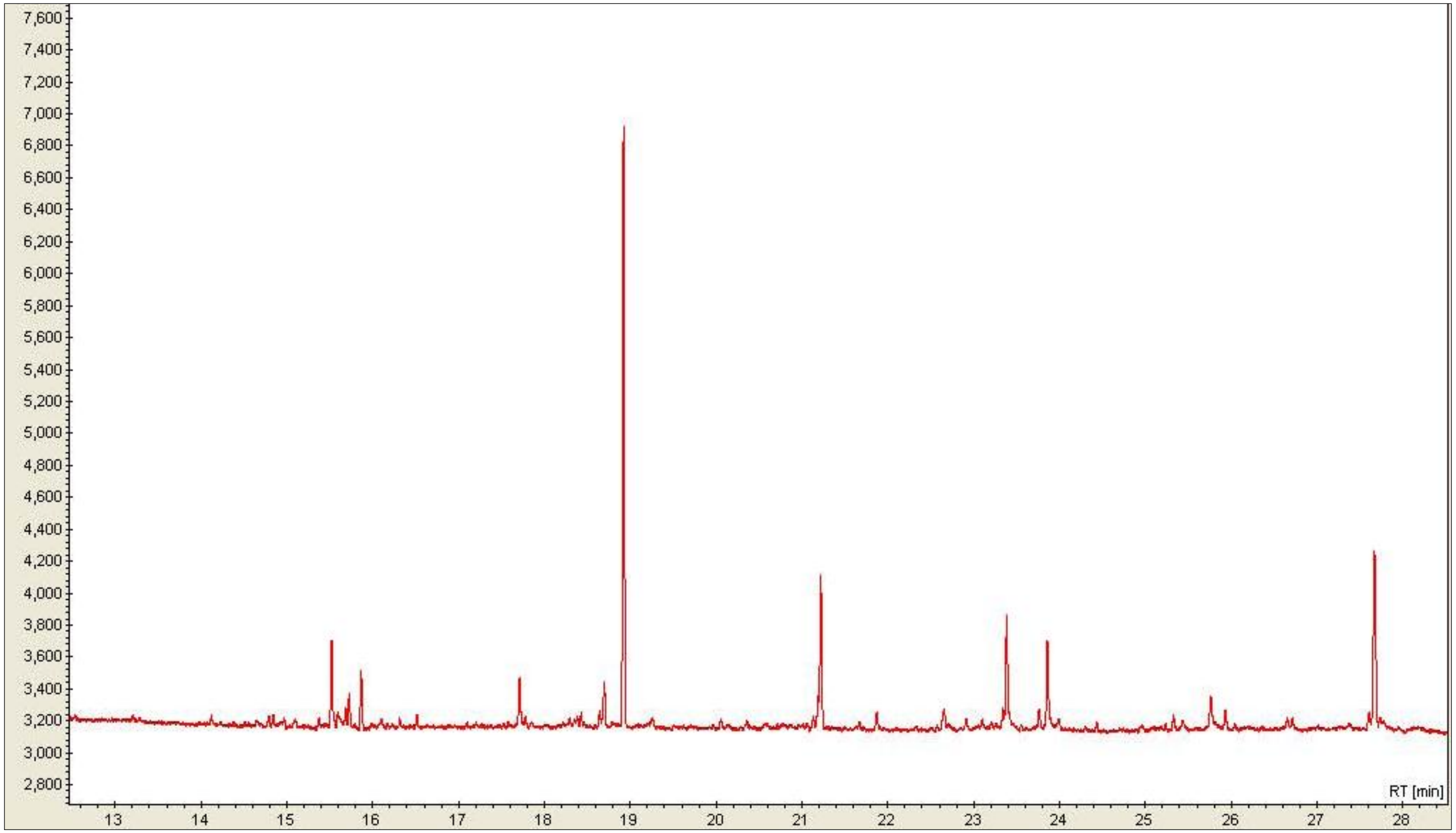
Results



- Overall growth between additions of nutrients predominantly depicted exponential and declining growth periods.

Growth patterns of *Haematococcus droebakensis, Chlamydomonas reinhardtii*, and *Platymonas* in Bold's Basal Medium for 15 days.

Results

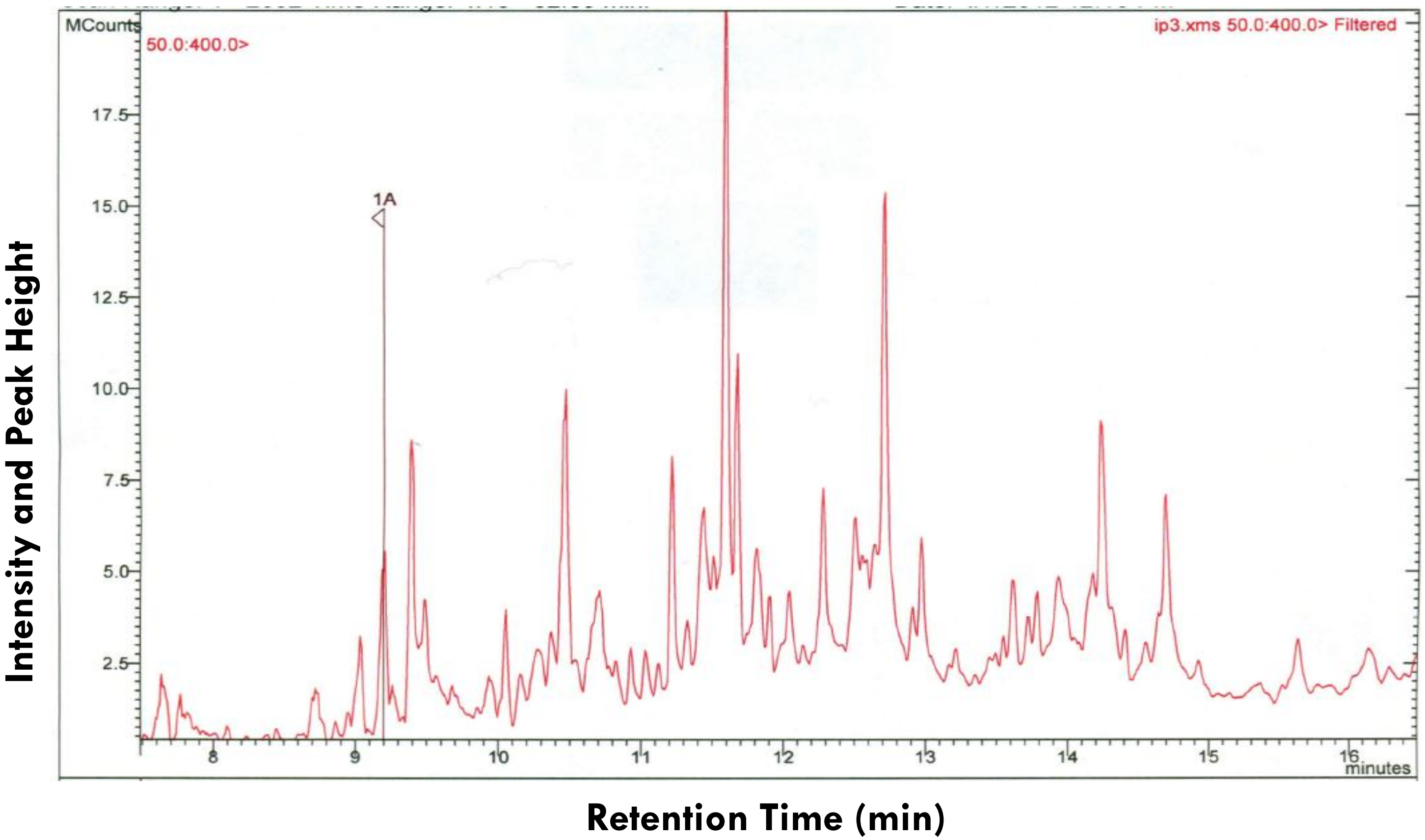


Intensity and Peak Height

Retention Time (min)

Gas chromatography spectrum showing major organic compounds typically found in algal biodiesel produced by the species *Platymonas*

Results

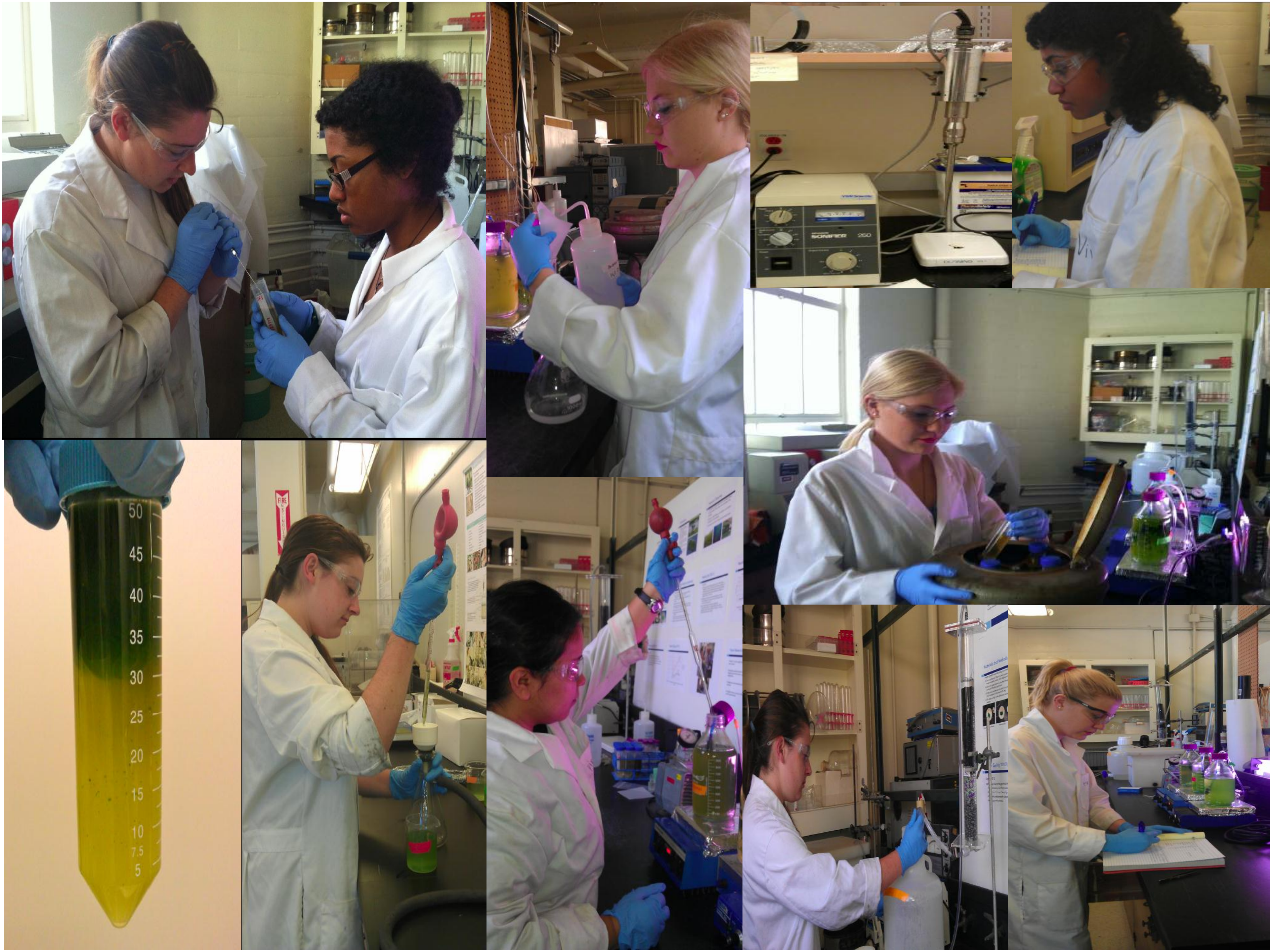


Gas chromatography-mass spectrometry spectrum for identifying the major organic compounds typically found in algal biodiesel produced by the species *Platymonas*

Results

Identification of major components of biodiesel produced by the species *Platymonas* from gas chromatography-mass spectrometry studies

Retention time (min)	Compound	CAS Number	Formula	Molecular weight (Daltons)
9.19	Eicosane	112-95-8	$C_{20}H_{42}$	282
10.64	Docosane	629-97-0	$C_{22}H_{46}$	310
11.22	Tetracosane	646-31-1	$C_{24}H_{50}$	338
11.60	Hexacosane	630-01-3	$C_{26}H_{54}$	366
12.72	Octacosane	630-02-4	$C_{28}H_{58}$	394
14.26	Triacontane	638-68-6	$C_{30}H_{62}$	422
14.69	Hentriacontane	630-04-6	$C_{31}H_{64}$	436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Summary and Conclusions

- *Chlamydomonas reinhardtii* manifested the fastest growth rate among various algal strains.
- *Platymonas* yielded the most amount of diesel hydrocarbons per unit of biomass. Hence it requires more investigations to arrive at accurate yields to chose the best algal strain.
- The oil or hydrocarbon content of the *Platymonas* species is about 35-45% of the dry weight based on preliminary estimates. The other species tested have lower yields at less than 15-20% of dry weight.
- The diesel hydrocarbons identified by gas chromatography-mass spectrometry were higher molecular weight alkanes (predominantly unbranched) in the C20 to C31 range. No esters were present in sizable amounts as they were probably transformed into pure hydrocarbons.
- More research is required to draw firm conclusions.

Future Research and Recommendations

- Test algal strains in greater detail to compare growth rate, lipid content, and hydrocarbon yields.
- Investigate carbon dioxide injection to observe kinetics of biomass growth and relation to possible carbon sequestration applicability.
- Study biomass growth at different temperatures and nutrient concentrations.
- Improve ultrasonication process to increase lipid extraction efficiency.
- Evaluate the use of metallic oxides (barium and zirconium oxides) and enzymes (lipase) to imrpove esterification and hydrocarbon yields.

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