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Extraction and Analysis of Triacylglyceride from Marine Mircoalgae for Biodiesel Production

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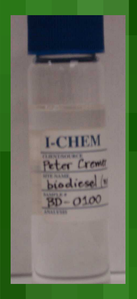
ABSTRACT

Production of biomass derived fuels is important in reducing the national dependence on imported petroleum. Certain diatoms, unicellular eukaryotic algae, have shown to produce up to 60% cellular mass, as a triacylglyceride (TAG), used for the production of biodiesel. Difficulties in efficient biodiesel production from microalgae lie in TAG production and conversions. Thus, we are continuing to investigate and develop efficient techniques for extracting TAGs from *Thalassiosira Weissflogii* and *Thalassiosira Pseudonana*. Parameters in the experiments include: extraction solvent, agitation process, and extraction temperature. We discovered that hexane extractions yield 5 – 10% TAGs based on the dry weight of the algal biomass. Analysis of extracted TAGs included: Thin-Layer Chromatography as a TAG screening process, and Gas Chromatography Mass Spectroscopy techniques to identify the TAG chemical components.

Bio-diesel

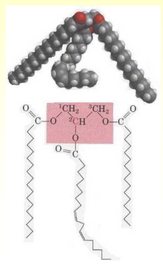


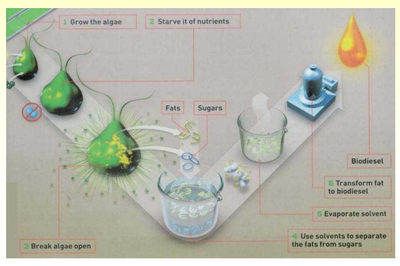
Project Goal

To investigate and develop efficient techniques for extracting TAGs

- We are using 2 species with different agitation methods
- We are looking to find initial efficiencies in these methods

A Tri Acyl Glycerol or TAG is a molecule which is extracted from a diatom and consists of 1 to 3 lipid chains, acyls which are the atoms that replace the hydrogen in the lipid chains, and a glycerol molecule.

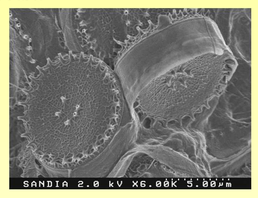
* 

** 

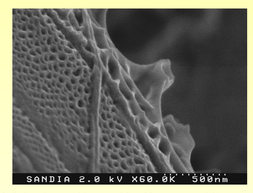
Background

If it costs more energy and money to create the fuel than what it is actually worth than it is not worth producing. Therefore, we are studying whether or not we can ultimately make a bio-fuel efficiently.

- A bio-fuel is a classification of oils (i.e. corn can be made into bio-fuel)
- From bio-fuel we can create bio-diesel, which is a fuel made from lipids
- Can obtain bio-diesel from biomass, which is the starting material
- We used micro-algae which are made from diatoms
- Diatoms are a group of unicellular brown algae
- There are as many as 100,000 diatom species



T. Weissflogii Feedstock



T. Weissflogii Centrifuged

Why we chose to work with micro-algae?

- It can be found in water and they grow rapidly
- We worked with microalgae from ocean and thus saved freshwater resources
- Diatoms replicate themselves by mitosis and also sexually at roughly three replications a day
- They are also readily available and inexpensive

The diatoms that we worked with are:

Thalassiosira Weissflogii and Thalassiosira *Pseudonana*

Why we chose to work with these specific diatoms?

- They have been studied more and are more well known
- The complete genomic sequence for *Thalassiosira Pseudonana* has been completed

Methods

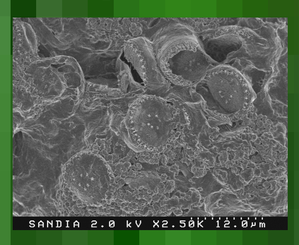
Extractions

- Concentrated the biomass by methods of centrifuging, lyophilizing, or filtration
- Some parameters considered before the extraction process…
  - ○ Extraction solvents (i.e. hexane, chloroform, methanol, etc.)
    - We have been mainly using Hexane to compare with past data.
  - ○ Agitation processes: sonication and/or stirring
  - ○ Extraction temperature.
    - Measure out a certain amount of the biomass into a vial
- Added solvent
- Sonicated and/or stirred vial for a variable amount of time at temperatures below the solvent's boiling point
- Removed the Supernatant, which is the TAGs and the liquid solvent, from the residual biomass and filtered
- Evaporated the solvent using compressed nitrogen air
  - ○ All that remained are TAG molecules.

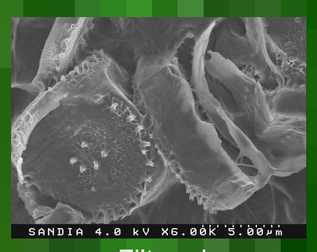
Supernatant of various samples

Thalassiosira Weissflogii

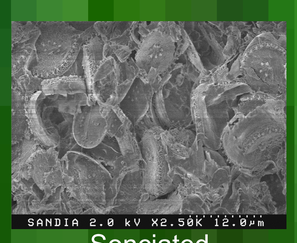
Centrifuged



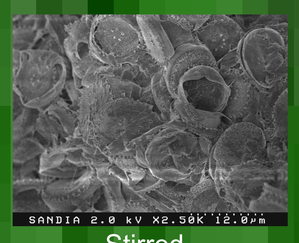
Lyophilized



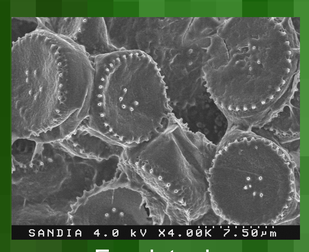
Filtered



Sonciated



Stirred

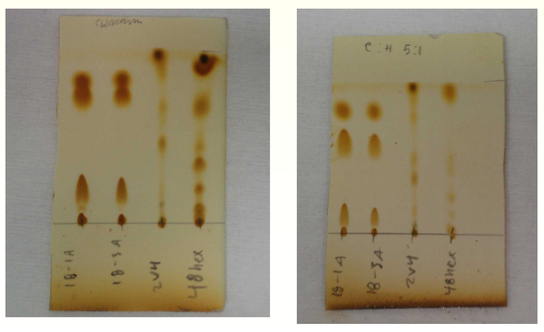


Feedstock

Thin-Layer Chromatography (TLC)

- We used TLCs to confirm that TAGs were actually extracted from the diatom samples
- TLC is an inexpensive, reliable, fast, and easy method to distinguish different compounds from each other.
- This method shows the presence of a compound but not how much of it
  - ○ Used UV florescent silica gel coated glass plates
  - ○ Placed a sample of the TAGs at one end of the plate along with a standard sample
    - ▪ Standard sample is known to contain TAG molecules
  - ○ Plate was dipped into a solvent (i.e. chloroform, methanol, hexane, water, ethyl acetate, etc.)
  - ○ The solvent moves up the plate by capillary action and the samples are dragged along
  - ○ To see the drag effect, we stained plates with iodine
  - ○ Samples should have similar drag effects to standards to confirm presence of TAGs

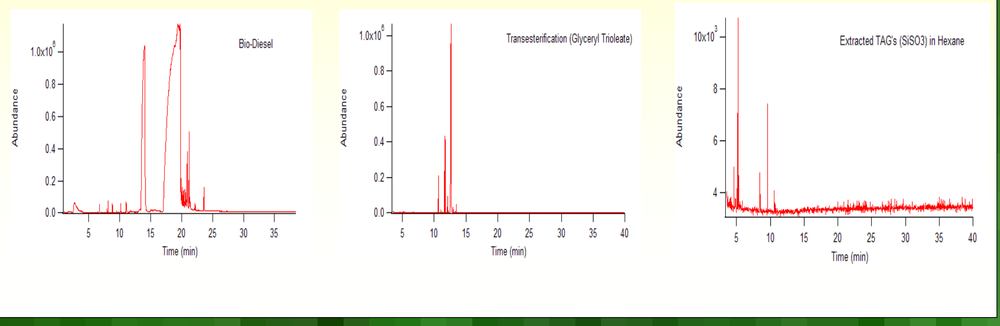
TLC plates showing presence of TAG compounds



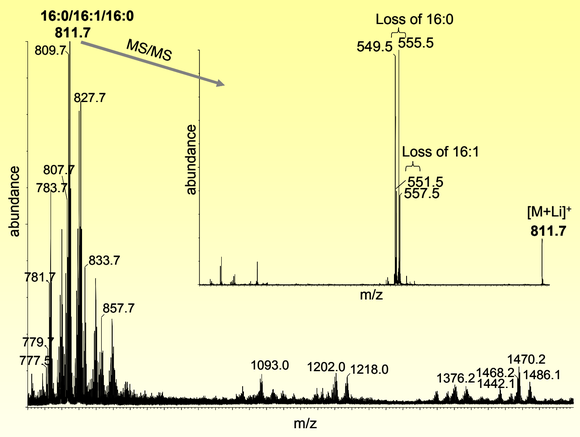
Gas Chromatography Mass Spectroscopy

Method for confirming TAGS and determining the compounds that make up the TAGs

- A sample of TAGs is pushed through a coated column with nitrogen gas and then vaporizes into a gas
- The particles are collided to break them apart into charged particles
- Only selected charged particles that successfully pass through a magnetic field are measured for mass and used for analysis



ESI MS/MS



ESI MS of TAGs extracted from *T. pseudonana.* Inset shows MS/MS of lithiated TAG 16:0/16:1/16:0 at m/z 811.7. The major product ions arise from loss of an intact carbon chain, allowing the length of each chain to be determined

Conclusions

- As of today, we have not come to any conclusions as to which method is best for extracting TAGs
- We are continuing to investigate and develop efficient methods

The knowledge gained from this work will ultimately aid in the manipulation of microalgal metabolic pathways to enhance biodiesel precursor production and possibly lead to the advent of efficient mechanisms for the production of biodiesel.

Illustrations:

*Triacylglycerol molecule, Ch 11 Lipids

** Growing to extraction to biodiesel process Popular Science, *How To Make Fuel from Algae*, Time4 Media, Inc., 2007, pg. 79.