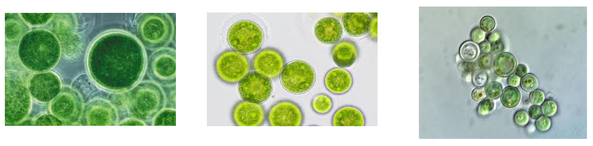
The Effect of the Type of Extraction Method on the Amount of Crude Algal Lipids Recovered for Economically Feasible Biofuel Production

Algae Biofuel Background

Problems

1. High Cost (~$20/gal)
2. Energy Intensive
3. Not Environmentally friendly
4. Low Lipid Yields
5. Need to Maximize Product

Chlorella Vulgaris



Pictures obtained from: http://www.healthyfellow.com, http://www.algaeindustrymagazine.com, http://botany.natur.cuni.cz

Purpose

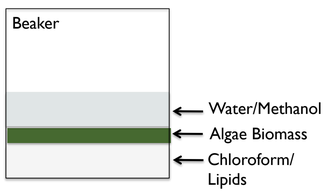
To determine which extraction method or pretreatment (Bligh and Dyer, Modified Bligh and Dyer, Electroporation, Osmotic Pressure, Microwave, Enzyme-Assisted Extraction, or Fungus-Assisted Extraction) recovers the greatest percentage of crude algal lipid, is the cheapest, uses the least amount of energy, and requires the least amount of time.

Hypothesis

If the extraction method affects the amount of crude lipids recovered for economically feasible biofuel production, then the Bligh and Dyer Method with no pretreatments will be the most economically feasible because it is the oldest and most reliable method for obtaining algal lipids without depreciating the lipid quality. It is cheap to do and does not require extensive time.

Methods

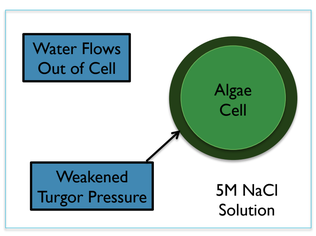
Baseline Solvent Extraction (#1-4)



How it Works

The hydrophobic solvent, chloroform, pulls the lipids out from the cell wall and dissolves them. The bottom layer is removed after centrifugation, and the chloroform is evaporated from the mixture. This process can also refine the oil simultaneously and is effective in quantifying and extracting algal lipids.

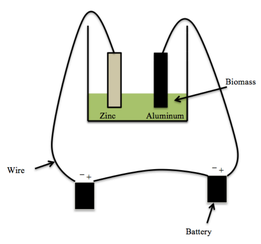
5M NaCl (Osmotic Pressure) (#5, 7)



How it Works

The high concentration of salt creates a hypertonic solution for the algae cell. The water from inside the cell will flow into the solution, causing loss of turgor pressure. This effectively weakens the cell wall, providing for easier solvent extraction.

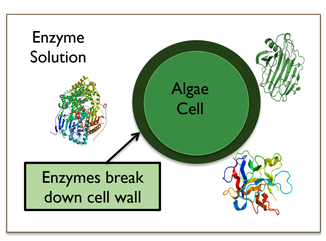
Electroporation (#6, 7)



How it Works

The electrical current will cause the algae solution to flocculate (clump together) and will weaken the cell wall without killing it. This provides for easier solvent extraction. It also damages the fungal cell wall, releasing the enzymes into the medium.

Fungal-Assisted Extraction (#10-12) (Designed)



How it Works

Enzymes from the fungus, such as cellulase, are released into the medium through electroporation and then they break down the cell wall. This allows for easier solvent extraction and nearly maximum lipid recovery, as proved by the data.

The Solution? Fungal- Assisted Extraction

Methods Tested

Baseline Method
1. Bligh and Dyer Chloroform: Methanol

Modified Baseline
2. Bligh and Dyer NaCl Water
3. Bligh and Dyer Chloroform: Ethanol
4. Bligh and Dyer Chloroform: Isopropanol

Pretreatments
5. 5M NaCl
6. Electroporation
7. Electroporation/5M NaCl
8. Microwave
9. Enzyme Mixture

Designed Pretreatments
10. Fungus Medium
11. Fungus Electroporation
12. Fungus Sonication

The numbers correspond to the same numbers on the graphs below

Benefits

1. Low Cost (Reduced by 50%)
2. No Additional Energy
3. Symbiotic Relationship
4. Maximum Lipid Yields
5. Potential to Grow on Wastewater

Experimental Fungal-Assisted Extraction

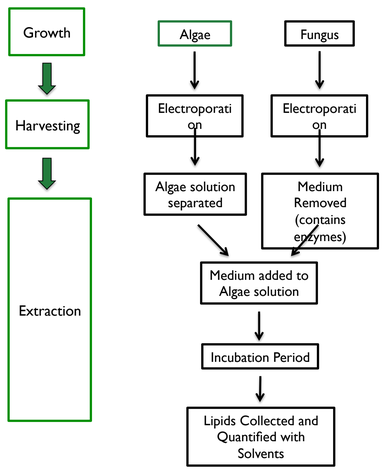
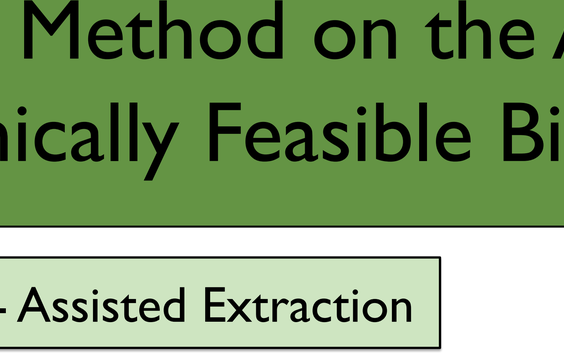


Figure 1: Percent of Total Algal Mass Lipid Yield

Figure 2: Total Cost of Growth, Harvesting, and Extraction



*Note for Above and Below Charts: Methods 8, 9, and 12 were removed due to extremely high costs (>$100/gal)

Figure 3: Lipid Yield Improvements from Baseline Method

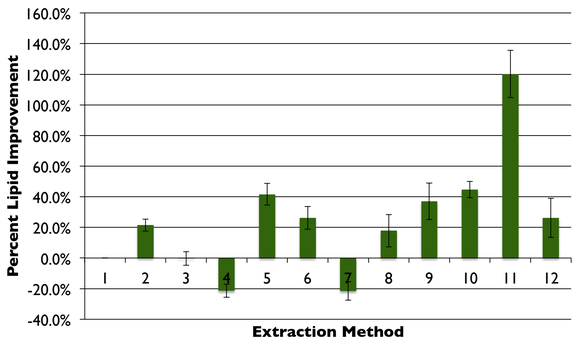
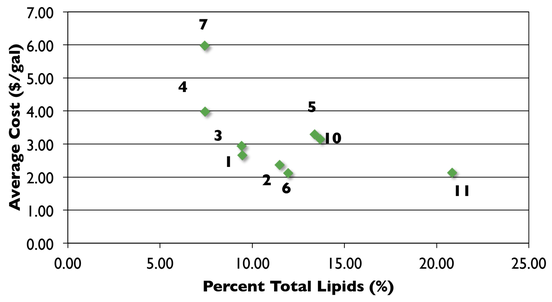


Figure 4: Extraction Cost vs. Percent Lipid of Total Mass



Statistical Analysis

Analysis of Variance (One-Way)

Summary

Groups	*Sample size*	*Sum*	*Mean*	*Variance*
B&D Chloroform:Methanol	3	28.38	9.46	0.246
B&D NaCl Water	3	34.47	11.49	0.214
B&D Chloroform: Ethanol	3	28.29	9.43	0.239
B&D Chloroform: Isopropanol	3	22.31	7.44	0.234
5M NaCl	3	40.19	13.40	0.385
Electroporation	3	35.82	11.94	0.405
Electroporation/5 M NaCl	3	22.28	7.43	0.333
Microwave	3	33.44	11.15	0.575
Enzyme Mixture	3	38.88	12.96	0.649
Fungus Super Medium Without Algae	3	41.07	13.69	0.570
Fungus Electroporation Without Algae	3	62.48	20.83	0.839
Fungus Sonication Without Algae	3	35.82	11.94	0.697

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*p-level*	*F crit*
Between Groups	416.25	11.00	37.841	52.10	<.00001	2.22
Within Groups	17.44	24.00	0.727			
Total	433.69	35.00				

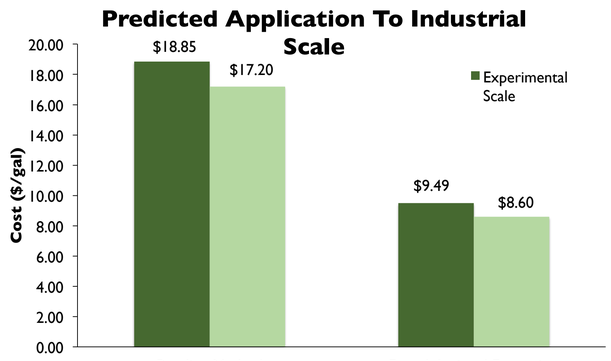
Discussion

- The goal of the project was to determine the most economically feasible method of lipid extraction in the algae strain *Chlorella vulgaris* in a wet growth medium.
- The enzyme method of extraction is one of the most promising methods for enhanced extraction because of its high yields and low energy requirements. However, the problem is that it is too expensive.
- It is so costly because it requires an extensive process to extract and purify the enzymes. The extraction process is very similar to extracting the lipids from algae: if the two methods are combined, then the cost of the enzymes is almost entirely eliminated, like in the fungus method.
  - *Aspergillus niger,* was found to contain a wide variety of enzymes that break down the algal cell wall.
  - To release the enzymes from the fungus, electroporation proved to be an effective method. These enzymes assisted in breaking down the cell wall, providing for easier solvent extraction and maximum lipid recovery.
- First, the lipid yields of all the methods were graphed in **Figure 1**. The lipid yield is the percent of the total mass of the algae sample that was retrieved as lipids in each method.
- The highest lipid yield was Fungus Electroporation (Method #11) with 20.83% and the second was Osmotic Pressure (Method #5) with 13.40%.
- Next, the percent lipid yields were compared to the baseline method: the Bligh and Dyer Chloroform:Methanol (Method #1) in **Figure 3** as lipid yield improvement.
  - The Fungus Electroporation Method had the highest percent yield improvement at 120%.
- Looking at economic analysis in **Figure 4**, average cost and algal lipid yield were compared on a scatterplot, looking for the method that had the lowest cost and highest yield.
  - The results showed that the fungus electroporation method (critical point #11) was one of the cheapest and had the highest yield, costing $2.13 per gallon of oil and having a 20.83% yield.
  - It is interesting to note the cheapest method is Electroporation (critical point #6) however its percent yield is not nearly as high, therefore the overall cost is much higher ($14.95 per gallon)
  - When looking at time, the Fungus electroporation method (critical point #11) had the greatest time (11,530 seconds). This is due to the two-hour incubation period for the enzymes to break down the cell wall.
- When amplifying the cost of each method to include the Growth, Harvesting, and Extraction steps combined, the cost of algae biofuels is significantly reduced with the Fungus Electroporation Method. (**Figure 2**)
  - When more lipids are able to be retrieved from the algae, less algae has to be grown and harvested, significantly decreasing the cost.
  - The cost of the Fungus Electroporation method is $9.49 per gallon including the growth, harvesting, and extraction step. The cost of the baseline is $18.85 per gallon, thus reducing the cost by 49.7%.
- One error resides in the fact that the experiment was done on a small scale.
  - This results in the cost being much higher per gallon of oil created than if the oil were produced in a large factory. The proportions of costs are still comparable due to the ANOVA test results. However, they may not represent the equivalent costs of the project if it were done in large scale.
- One limitation of the data is the type of lipids was not analyzed, therefore further research into the Fungal-Assisted Method should be done to measure its potential for success on industrial scale. This was a preliminary study and more research is needed before the method is feasible.

Applications and Conclusions

- According to the Department of Energy, the price per gallon of algae oil is around $20.00 per gallon, whereas the cost of gasoline per gallon in the United States is anywhere from $2.00 to $4.00.
- With this years project, the cost is further reduced with the new designed method of fungus electroporation.
- The cost does not take into account the potential of combining the harvesting and pretreatment method (electroporation), growing the algae with the fungus, or the additional lipid content the fungus provides.
- Future research could be focused on creating optimal conditions for growing the fungus and algae together and combining the harvesting and extraction pretreatment of electroporation.
  - Fungus and algae form the symbiotic relationship known as lichens and are extremely hardy, being able to survive almost any conditions. This will allow for further stressing on both the organisms and less requirements for growth—increasing the yield of lipids and lowering the costs.
- Other future research can focus on how this method can benefit the water industry and water purification.
  - It is already proven that the algae can clean up wastewater, however it is not perfect, so what if the combination of *Aspergillus niger* and *Chlorella vulgaris* will aid in removing toxins from water?
  - The speed, amounts of water, and recycling processes can all be researched, providing the most efficient and environmentally friendly device for growing, harvesting, and extracting the algae all at once.

Predicted Application To Industrial Scale



Grow on Wastewater

Lichens

Pictures obtained from: http://www.water-technology.net, https://en.wikipedia.org/wiki/Fruticose_lichen

References

"Biodiesel from Algae Oil - Oilgae - Information, News, Links for Algal Fuel, Algs Bio-diesel, Biofuels, Algae Biofuel, Energy - oilgae.com." *Biodiesel from Algae Oil - Oilgae - Information, News, Links for Algal Fuel, Algs Bio-diesel, Biofuels, Algae Biofuel, Energy - oilgae.com.* N.p., n.d. Web. 16 Sept. 2012. <http://www.oilgae.com>.

Ben, Browne. "Oil Extraction from Microalgae." *Algae Oil Extraction Capstone* (2009). Print.

Chehri, Javad. "Factors Affecting The Growth of Biomass of Aspergillus Niger." *Medical Sciences and Public Health* 1.1 (2013). Print.

Edwards, Mark R., *Green algae strategy: end oil imports and engineer sustainable food and fuel*. S.l.: CreateSpace], 2008. Print.

Grayburn, W. S., et al. "Harvesting, Oil Extraction, And Conversion Of Local Filamentous Algae Growing In Wastewater Into Biodiesel." *International Journal Of Energy & Environment* 4.2 (2013): 185-190. *Academic Search Premier*. Web. 9 Dec. 2013.

Hartman, Evana . "A Promising Oil Alternative: Algae Energy." *The Washington Post* 6 Jan. 2008: All. *The Washington Post*. Web. 16 Sept. 2012.

Hnain, Antoine K., Lisa M. Cockburn, and Daniel D. Lefebvre. "Microbiological

Hnain, Antoine K., Lisa M. Cockburn, and Daniel D. Lefebvre. "Microbiological processes for waste conversion to bioenergy products: Approaches and directions." *Environmental Reviews* 19.NA (2011): 214-237. Print.

Hou, Li Xia, Xiao Lei Shang, Xiaoxia Wang, and Jing Liu. "Application of Enzyme in Aqueous Extraction of Sesame Oil." *Eur Food Res Technol European Food Research and Technology* (2013): 1027-030. Print.

Hunds, K., and B.V. Reddy. "Algal Biodiesel Production From Power Plant Exhaust And Its Potential To Replace Petrodiesel And Reduce Greenhouse Gas Emissions." *International Journal Of Low Carbon Technologies* 6.4 (2011): 294-298. *Academic Search Premier*. Web. 9 Dec. 2013.

Irving, Tyler E., and D. Grant Allen. "Species and material considerations in the formation and development of microalgal biofilms." *Applied Microbiology and Biotechnology* 92.2 (2011): 283-294. Print.

Kilpp, Edda, Bodil Nordlander, Roland Krüger, Peter Gennemark, and Stefan Hohmann. "Integrative Model of the Response of Yeast to Osmotic Shock." *Nat Biotechnol Nature Biotechnology* 23.8 (2005): 975-82. Print.

Kumar, Ramanathan Ranjith, Polur Hanumantha Rao, and Muthu Arumugam. "Lipid Extraction Methods from Microalgae: A Comprehensive Review." *Frontiers in Energy Research Front. Energy Res.* 2 (2015): 1-9. Print.

Pienkos, Philip, Lieve Laurens, and Andy Aden. "Making Biofuel from Microalgae." *American Scientist* 99 (2011): 474-81. Print.

Susilick, Kenneth S., William B. McNamara, and Yuri Didenko. "Conditions During Multi-Bubble Cavitation." *American Institute of Physics* (2000): 463-66. Print.

Complete references can be found in the research journal.

Pictures

"Xanthoria Parietina." Wikipedia. Wikimedia Foundation, n.d. Web. 02 May 2016.

"Best of Chlorella and Spirulina." *Healthy Fellow RSS*. N.p., n.d. Web. 02 May 2016.

"World Bank Approves Funds for Wastewater Treatment Regulations in Vietnam." *Water Technology*. N.p., n.d. Web. 02 May 2016.

"Key Ingredients." *MDidacs*. N.p., n.d. Web. 02 May 2016.

"Frankfort Municipal Utilities." *Frankfort Utility*. N.p., n.d. Web. 02 May 2016.

All pictures below were taken by the research scientist.

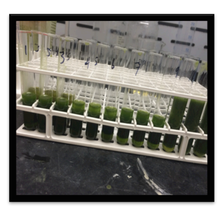


Image 1: This is a picture of the same glass tubes in Image 2, except this is after five minutes. The separate layers can now be clearly seen.

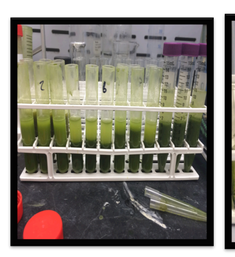


Image 2: This is a picture of the different treatments sitting for five minutes after all solvents had been added.

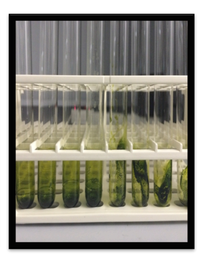


Image 3: This is the resulting lipid mixture after the chloroform had been evaporated.