Algae Biofuel Background

<table>
<thead>
<tr>
<th>Problems</th>
<th>Benefits</th>
</tr>
</thead>
<tbody>
<tr>
<td>High Cost (~$20/gal)</td>
<td>Low Cost (Reduced by 50%)</td>
</tr>
<tr>
<td>Energy Intensive</td>
<td>No Additional Energy</td>
</tr>
<tr>
<td>Low Yields</td>
<td>Maximum Lipid Yields</td>
</tr>
<tr>
<td>Not Environmentally Friendly</td>
<td>Potential to Grow on Wastewater</td>
</tr>
</tbody>
</table>

**Chlorella vulgaris**

**Perilla Frutescens**

**Aspergillus oryzae**

**Extraction Methods**

<table>
<thead>
<tr>
<th>Baseline Solvent Extraction (1-14)</th>
<th>Fungal-Assisted Extraction (10-12, Designed)</th>
</tr>
</thead>
<tbody>
<tr>
<td>How It Works</td>
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</tr>
<tr>
<td>The hydrophobic solvent chloform, pulls the lipids out from the cell wall and dissolves them. The bottom layer is removed after centrifugation, and the chloform is evaporated from the mixture. This process can also refine the oil simultaneously and is effective in quantifying, and extracting algal lipids.</td>
<td>The electrical current will cause the algae solution to flocculate (clump together) and will weaken the cell wall without killing it. This process is also referred to as lipid extraction or the fungus method.</td>
</tr>
<tr>
<td>5M NaCl (Osmotic Pressure) (5, 7)</td>
<td>Enzymes from the fungus, such as cellulases, are released into the medium through electroporation and then they break down the cell wall. This allows for easier solvent extraction and near maximum lipid recovery, as proved by the data.</td>
</tr>
<tr>
<td>Electroporation (6, 7)</td>
<td>Fungal-Assisted Extraction (10-12, Designed)</td>
</tr>
</tbody>
</table>

**Hypothesis**

To determine which extraction method or pretreatment (Bligh and Dyer, Modified Bligh and Dyer, Electroporation, Osmotic Pressure, Microbes, Enzyme-Assisted Extraction, or Fungi-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.

**Purpose**

If the extraction method affects the amount of crude lipids recovered for economically feasible biodiesel 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 degrading the lipid quality. It is cheap to do and does not require extensive time.

**Methods**

**Baseline Solvent Extraction (1-14)**

- **How It Works**
  - The hydrophobic solvent chloform, pulls the lipids out from the cell wall and dissolves them. The bottom layer is removed after centrifugation, and the chloform 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 solubilizes the cell membrane, pulls the lipids out from the cell wall and dissolves them. The bottom layer is removed after centrifugation, and the chloform is evaporated from the mixture. This process can also refine the oil simultaneously and is effective in quantifying, and extracting algal lipids.

**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 process is also referred to as lipid extraction or the fungus method.

**Fungal-Assisted Extraction (10-12, Designed)**

- **How It Works**
  - Enzymes from the fungus, such as cellulases, are released into the medium through electroporation and then they break down the cell wall. This allows for easier solvent extraction and near maximum lipid recovery, as proved by the data.

**Results**

- **The goal of the project was to determine the most economically feasible method of lipid extraction in the algal 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 simple, except for 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 provides a non-invasive method.** These enzymes assist 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 recovered as lipids in each method.
- **The highest lipid yield was Fungi Electroporation (Method III) with 20.87% and the second was Osmotic Pressure (Method IV) with 13.40%.

**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 year’s 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 processes (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.** The 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 organism’s 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 coaxes from water?
- **The speed, amount 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.”**

**Statistical Analysis**

**Figure 2:** Percent Total Lipid Mass Lipid Yield

<table>
<thead>
<tr>
<th>Method of Extraction</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
<th>11</th>
<th>12</th>
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<tbody>
<tr>
<td>Baseline Method</td>
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<tr>
<td>Fungi Electroporation</td>
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</tr>
</tbody>
</table>

**Figure 3:** Lipid Yield Improvements from Baseline Method

**Figure 4:** Lipid Yield per Billion g of Algae

**Discussion**

- **Next, the percent lipid yields were compared to the baseline method the Bligh and Dyer Chloform/Methanol (Method IV) in Figure 3 as lipid yield improvement.** The Fungi Electroporation Method had the highest percent yield improvement at 120%.

**Applications and Conclusions**

- **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 yield.**
- **The results showed that the fungus electroporation method (critical point #11) had the greatest time (11,530 seconds).** This is due to the two-hour incubation time needed to allow the fungus to grow.
- **When amplifying the cost of each method to include the Growth, Harvesting, and Extraction steps combined, the cost of algae biomass is significantly reduced.** This is due to the Fungi 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 Fungi 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 proportion of costs is still comparable due to the ANOVA test results. However, they may not represent the equivalent costs of the project if it were done on a large scale.
- **One limitation of the data is the type of lipids was not analyzed, therefore further research into the Fungi-Assisted Method should be done to measure its potential for success on industrial scale.”**

**References**