Introduction

Today demand for fossil fuels is outstripping supply, while the political and environmental costs of U.S. dependence on fossil fuels are only just being realized. As a nation, we rely on inexpensive oil to keep our economy growing at the same time as we pursue costly foreign policy ventures. This reality has become clear as we continue to track the progression of climate change and other deleterious effects of our energy consumption. Increasingly, renewable liquid transportation fuels are being viewed as an important, domestically producible alternative to petroleum. At the very least, along with conservation measures, they may be viewed as bridging technology before the arrival of fuel cells, hydrogen, or other clean fuel technologies.

Biodiesel

The diesel fuel substitute biodiesel is an important renewable alternative. Made by combining any biological fat or oil with alcohol, biodiesel is clean burning, non-toxic, and carbon-neutral with respect to global warming (provided the alcohol used is not derived from fossil fuels). Biodiesel can be burned in existing diesel engines with no modifications and can be blended in any proportion with petroleum diesel. This allows for its use in the existing fuel distribution infrastructure and giving it value as a fuel extender. Despite these significant advantages, a major challenge that biodiesel faces in becoming the preferred diesel fuel is the cost and availability of the feedstock. The cost of vegetable oils most commonly used, like soybean and canola, runs greater than $2 per gallon. This, coupled with processing costs typically makes biodiesel marginally more expensive than petroleum diesel. However, cost alone is not the only obstacle; production capacity is a critical issue. The approximate yield of oil from soybeans is about 40 gallons per acre-year, or 80 from canola (Biodiesel Use in Engines 2006). Presently, U.S. demand for distillate fuel is roughly 63 billion gallons per year (U.S. Product 2006). With conventional land based crops, the U.S. can certainly not grow enough vegetable oil to meet this demand.

Microalgae as a Feedstock

Microalgae may be valuable as source of feedstock oil. In this case, the microalgae being considered are single-cell photoautotrophic organisms that have the ability to grow at very high rates with only basic nutrient requirements. Certain species of microalgae store a large portion of their energy reserves in lipids, thus making them a potential oil source. This class of algae was the focus of the National Renewable Energy Lab’s (NREL) Aquatic Species Program, with much of the work being devoted to species screening and open pond cultivation. NREL scientists concluded that there were major research challenges to overcome before algae could be grown for fuel, and that it was not at the time feasible (Sheehan et al. 1998). However, some of these challenges can be and are overcome through the use of photobioreactors.
**Photobioreactors**

Photobioreactors are closed vessels where algae can be cultured under optimum conditions without the threat of contamination from competitive species or culture collapse from predation (both significant problems in open ponds). Photobioreactors have high capital costs to build, but high rates of productivity, with the potential to yield 5,000-15,000 gallons of microalgal oil per acre per year (Brown et al. 1994). If an average yield of 10,000 gallons oil/acre/year could be achieved, the 63 billion gallon diesel demand of the US could be produced in just 6.3 million acres (for comparison purposes the land area of Texas is 167 million acres). The challenge for photobioreactors is being able to achieve those yields in sustained operations, and in a cost effective manner. In some cases, photobioreactors serve only as the first step in the algae production, where they are used to create a monoculture under near-ideal conditions, with the mature algae culture then being discharged to open ponds where a nutrient deficiency is used to increase their oil content (Huntley 2004). It is worth noting that designing photobioreactors such that they can be built on very large scales at low cost has never been a priority, which has lead to the impression that photobioreactors *have* to be extremely expensive. Lowering the capital costs of photobioreactors will surely be important to their widespread use for energy production. From a similar economic point of view, coupling algae growth with the performance of beneficial tasks, such as scrubbing CO₂ from power plant flue gases or removing nutrients from wastewater, will likely be necessary. By coupling algae production with a waste treatment or pollution control process, the economic viability of microalgal biodiesel is significantly improved. Utilizing power plant flue gases to grow algae for biofuels is already being done by GreenFuel Technologies Inc. in Cambridge Massachusetts, and by several other companies. In any such process where algae are to be produced for fuel, as shown in Figure 1, extraction of oil from the harvested microalgae is an important step to be examined.

![Figure 1: Process Diagram for Biodiesel Production from Algae](image-url)
1) Microalgae are grown in a photobioreactor, which is a closed vessel that allows light to penetrate to a growth medium consisting of water, nutrients, and algal cells.

2) Microalgae harvesting separates the oil rich algae from the water, allowing the water and some algae to recycle back to the photobioreactor.

3) Oil extraction from the harvested algae. This is most commonly done by solvent extraction, and may be preceded by mechanical cell disruption as well. The solvent is recovered and reused after this step.

4) Biodiesel production is most often done by transesterification with alcohol and a catalyst.

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**Algal Oil Extraction**

Oil extraction from biological materials is performed by chemical means, physical means, or a combination of the two. For large scale oil extraction from microalgae, the process is usually accomplished with mechanical cell disruption followed by solvent extraction. In this case, the mechanical disruption is commonly performed with either a bead mill or ultrasonication. Bead mills work by having a vertical or horizontal cylindrical chamber that houses a series of mechanically driven agitating elements. The grinding of the cells is performed by plastic or glass bead that occupy about 80% of the chamber’s volume, and this has been successfully used to disintegrate algal cells. Ultrasonication, the other main technique uses an ultrasonic probe to disrupt small volumes of cells. The probe uses a transducer to generate sound waves which in turn cause small bubbles to form, and it is the formation and cavitation of these bubbles that produces shock waves that rupture the cells. It has been found that at higher working volumes a higher acoustic power is required, which can cause larger bubble formation and decreased effectiveness. Thus, for larger scale use, specially designed disruption vessels, with a continuous flowing stream of material to be disrupted are used (Richmond, 2004, pp 232).

The solvent extraction of oil which typically follows mechanical disruption of algae cells can be performed with a two solvent system. At the lab scale, where often no mechanical disruption precedes, this can be done by the method of Bligh and Dyer (1959). This process uses a polar and a non-polar solvent to extract the corresponding lipid fractions from the cells. The drawbacks to using solvent extraction on a commercial scale are that it requires extra energy input (to distill the solvents) and it has the potential to contaminate the algae solids, thereby restricting options for their end use.

**Goal**

The goal of this research project is to improve the process economics of producing biodiesel from microalgal oil. This will be attempted by eliminating the extraction step altogether with an in-situ biodiesel production method.

**Experimental Design**

Six strains of high-oil algae from the SERI-NREL microalgae culture collection at the University of Hawaii were acquired to start the project. These included two *Nannochloropsis* sp. strains, *Dunaliella tertiolecta*, *Dunaliella salina*, *Chlorella salina*, and *Chlorella sp*. These six strains were grown under various conditions, including nutrient sufficiency/deficiency, hypo/hyper osmotic stress, and high light intensity. Samples were then screened to determine the highest oil producing combination. Special attention was paid to the nitrogen sufficient and
nitrogen limiting conditions since it is known that some species rapidly increase oil production while suffering from nitrogen deficiency (Suen et al. 1987). It is important to note that the specific growth conditions and optimal oil content were not the focus of this project; the goal was only that a reasonable amount of oil be available for extraction (10-50% of the algae dry mass). Screening for the relative oil contents was done with a Varian SF330 Spectrofluorometer by the method of Cooksey et al. (1987) on cell cultures that were diluted to equal concentrations, as checked with a Bausch & Lomb Spectronic 20 Spectrophotometer. Based on relative fluorescence readings and growth rates, the decision to grow larger amounts of Chlorella sp. was made. Although not the highest oil producer Chlorella sp., or CHLOR1 by the SERI-NREL designation, produced a reasonable amount of oil according to the screening and also grew at a very fast rate.

The experimental procedure, consisting of nine steps, is shown in Figure 2. 1- Batch Algae Production: Two photobioreactors were built to produce larger volumes of Chlorella sp. 2- Algae Settling: Cultivated algae samples were separated from solution by settling in a large glass vessel. The settling was facilitated by the addition of the surfactant polyoxyethylene sorbitan monooleate (Tween 80). 3- Algae Centrifugation: The settled algae were centrifuged with a Damon IEC B-20A centrifuge. The fluorometric screening technique was used to verify that oil content of the separated algae did not change significantly during the settling/centrifugation period. 4- Freeze Drying: Samples of the centrifuged algae were then freeze-dried to preserve them. 5- Samples Pooling: Cells from each weekly harvest were pooled together into 30 gram batches. These batches were divided into six samples of five grams of dry algae. 6- Solvent Extraction of Algae Oil: Three of the six samples were treated using a chloroform/methanol two-solvent oil extraction technique, similar to the method of Bligh and Dyer. The lipid fractions yielded by this method were considered to be the baseline oil content of the cells. 7- Transesterification: The extracted oil was transesterified using methanol and KOH to produce biodiesel. 8- In-Situ Processing: The other three replicates from the 30 gram batch were used to examine the effectiveness of an in-situ transesterification process. They were mixed with methanol and potassium hydroxide and homogenized with a 375 Watt Heat Systems Ultrasonics W-375 Sonicator. The samples were heated at 100°C for one hour then allowed to sit at room temperature for 24 hours before being centrifuged to separate them into cell debris, excess methanol, byproduct glycerin, and biodiesel. 9- Glycerin Analysis: The biodiesel from both extraction methods were analyzed in a Hewlett Packard HP-5890 Series II gas chromatograph equipped with a Flame Ionization Detector for free and total glycerin.
Figure 2: Experiment Flow Diagram

1) Batch algae production in 4 ft x 12 in and 5ft x 18 in diameter Kalwall tanks
2) Settling in glass vessel with the aid of Tween 80, a surfactant
3) Centrifugation to concentrate cells into a pellet
4) Freeze drying
5) Samples pooled into 30g batches then split into six 5g replicates
6) Three replicates from each batch treated with chloroform/methanol solvent extraction
7) Extracted Algal Oil is transesterified to produce Biodiesel and glycerin (need to add a box for this)
8) Three replicates from each batch mixed with methanol and potassium hydroxide and sonicated
9) Biodiesel yield is determined on a gravimetric basis and the biodiesel is analyzed for free and total glycerin

Results

In Figure 3, the highest performing three of the six species that were screened are shown with their average peak fluorometric readings under the normal or control growth conditions. A higher reading indicates higher lipid content. Although *Chlorella sp.* gave the lowest relative amount of oil, its growth rate was far higher than both *Nannochloropsis sp. I* and *Nannochloropsis sp. II*, the other top oil producers.
In Figure 4, the fluorometric readings for *Chlorella sp.* are shown changing over time. The designations C1-1, C1-2, and C1-3 refer to samples that were control, samples treated with 24 hours of hypo-osmotic conditions, and samples subjected to nitrogen deficient conditions. The fluorometric readings generally increased for a brief period, reached maximum value, then decayed with time. It can be seen that six of the seven samples reached their peak reading within four minutes while one required six minutes to peak. For the *Chlorella sp.* strain the control or normal growth conditions seemed based on the conditions of the first round of screenings to produce the most oil.
The results from the solvent extraction and the in-situ transesterification process will be available in November 2007.

References


