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Production of methyl ester fuel from microalgae — Source link

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Production of Methyl Ester Fuel from Microalgae

by

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T-3832

A thesis submitted to the Faculty and the Board of Trustees of the Colorado School of Mines in partial fulfillment of the requirements for the degree of Master of Science, Ecological Engineering.

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Abstract

With recent concern over potential global climate changes, interest has resurfaced into renewable fuels. Efficiency in CO_2 utilization, lower pollutant emissions, national security and diversity of energy sources are just a few advantages of renewables.

Microalgae, small single cell plants, can be cultured on arid lands to produce storage lipids. These storage lipids can be chemically transformed into a liquid fuel that has many of the characteristics of a #2 diesel fuel. Microalgae from the Solar Energy Research Institute were mass cultured and harvested. Several solvents systems were evaluated for their ability to extract microalgal lipids. Several solvents were identified as being promising and these were scaled up to evaluate handling and efficiency of extraction. Lipid produced from the extraction process were transesterified into fatty acid methyl esters (FAMES). Statistical designed experiments were developed to identify and optimize the important variables in the reaction.

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Chapter 1

Introduction

Following the energy crisis of the 1970's the United States investigated a number of energy alternatives to provide renewable fuels. Energy from biomass was viewed as an attractive alternative, due to its availability and low cost.

One of the alternatives examined was production of fuel from algae. Possible fuels from algae included glycerol, ethanol, methane, pseudo vegetable oil, Mobil M-gas and fatty acid methyl esters (FAMEs). In 1985 the Department of Energy and the Solar Energy Research Institute analyzed the potential for fuel production from microalgae. The executive summary of that technology assessment indicated that microalgal-based fuels could be price competitive with conventional fuels by the year 2010.

Microalgae are small single celled plants that have a higher rate of productivity than other plants. They can double their mass three to five times per day. They are unique because they can store solar energy in the form of storage lipids. This lipid component can be converted into a premium gasoline or a synthetic diesel fuel. Under the current plan, large raceways will use saline groundwater to

grow the microalgae in the desert Southwest. Algal biomass will be harvested and converted into a liquid fuel. Theoretically, this technology could produce 100 to 200 barrels per acre per year (Neenan et al., 1985).

With concerns over the effects of increased global warming this technology has the potential to reduce carbon dioxide emissions, while providing a renewable liquid fuel. Using stack gas effluent from the power plants in Southwest microalgal farms could process 2-3% of the total United States CO_2 emissions from coal and provide 2 quads of energy per year, in the form of liquid fuel (Brown, 1989).

Significant research strides must be accomplished in order to produce a microalgal based liquid fuel, at a cost of $1.60 to $2.00 per gallon, by the year 2010 (Neenan et al., 1985). Microalgal lipids cannot be used directly in a compression injection engine. They resemble seed oils in structure and these have problems with viscosity, polymerization in the fuel tank, and high oxygen content (Kaufman et al., 1982). One of the major research goals is the extraction and conversion of the lipid component into a liquid fuel. No data exist on pilot plant studies or laboratory scale experiments for conversion of microalgae to a liquid fuel.

Research planned

While use of microalgal fuels could reduce CO_2 emissions, provide a renewable fuel and potentially reduce air pollution problems there remain several problems:

1. The lipid is contained inside the cell and must be extracted before any type of conversion takes place. The first step in this project will be to identify and test a variety of solvents that can extract the lipid fraction from the algae.
2. While a large body of literature is available on transesterification of seed oils to FAMEs, no information exist on what variables are important in the conversion of microalgal lipids to FAMEs. The next step of the project will be to identify important variables in the transesterification of microalgal lipids to FAME.
3. Research planned will be the optimization of those variables identified as significant. One of the goals of this project is to gain information to enable the Microalgae Technical Research Group, at the Solar Energy Research Institute to expand the scale of this process.

Chapter 2

Background

Production and Bioconversion of Algae

In 1953, the future was bright for mass culturing of microalgae. In a report by the Carnegie Institute of Washington, author John Burlew envisioned that microalgae would supply the world with a cheap source of single-cell protein. That future has not been realized yet.

In 1957, it was first demonstrated that microalgae could be fermented to produce methane using wastewater as a culture medium (Golueke et al., 1957). This was the first documented case of bioconversion of microalgae into methane, with reasonable efficiencies. Recycling waste water and the conversion of solar to chemical energy was an attractive concept. Problems with low growth rates, harvesting and low removal efficiencies demanded that microalgae had to be grown in intensive systems.

Macrocystis, a macroalgae, has been investigated for its potential for bioconversion to methane (Bird, 1984). This system involved two components, kelp production and anaerobic digestion of the kelp. Gellenbeck and Chapman (1984) investigated the potential of marine kelp for alginic acid production and bioconversion to methane. Problems with

harvesting, loading rates for the digester and low market value for methane prevented the project from being cost competitive.

Early experiments with the microalga Botryococcus braunii (Aaronson and Patri, 1984) revealed that the organism produced significant amounts of hydrocarbons. Over 50% of the ash free dry weight (AFDW) was lipid and 15-30 % of that portion was hydrocarbons. Glycerol production from microalgae was investigated by Nakas (1983a and 1983). Using four species of Dunaliella, yields of over 10 grams per liter of glycerol were obtained. Increasing the salinity of the growth media increased the glycerol production. Aaronson (1984) found that environmental manipulations could increase the lipid content, yet understanding what triggered increase in lipids was not known.

With the exception of the experiments done to bioconvert algae to methane, no pilot plant data exist for the conversion of microalgae into liquid fuel.

A review by Goldman (1979), indicated that options for fuel and single cell protein production were limited due to large amount of land and nutrients needed for growth. Seven years later, Neenan et al. (1986), indicated that microalgae could supply up to 100 to 200 barrels of oil per year per acre. The most promising fuel options were fatty acid

methyl esters (FAMEs) and gasoline produced from pyrolysis and zeolite conversion of microalgae.

Oil Extraction

Removal of oil from oil seeds was practiced by the Egyptians many centuries ago (Becker, 1978). Oil seeds are processed by crushing the seeds and filtering the oil from the seed coats. Mechanical extraction involves mechanical screws, worm drives and grinding wheels. Liquid extraction of oils from seeds has usually involves grinding the seeds with hexane in a counter current extractor. Oil is recovered by distillation and the hexane is recycled through the system. While grinding has been perfected for seed crops perfected, their use is limited for microalgae. The small size of the microalgal cell and the high concentration of water limits grinding or milling techniques.

Supercritical extraction is similar to regular solvent extraction. Instead of using an organic solvent to extract the desired material, a gas is pressurized above it's critical point and mixed with the feedstock. This can dissolve non-volatile hydrophobic materials. Separation and recovery of the material can be controlled by varying the pressure and temperature. Super critical extraction is being used on an industrial scale to decaffeinate coffee, oxidize wastes

and remove oil from the milling of iron into steel (Basta, 1984). Supercritical extraction has been used in the manufacture of vegetable oil, and this process offers better yields and lower energy costs than the traditional milling, flaking, pressing and filtering of seed oils. Supercritical extraction is capital intensive due to the special nature of the pressure reactors and piping necessary to operate at high pressures.

Microalgal lipids

Lipids are important storage and structural components of cells. Unlike proteins and carbohydrates, lipids defy classification because a they often have high degree of branching and group substitution. Soluble in organic solvents and insoluble in aqueous solutions, they are high energy compounds. Lipids generally fall into two broad classes, those which have long chain fatty acids or those derived from an isoprene unit, known as terpenoid lipids. In algae, lipids are further divided into two classes, neutral and polar lipids. Neutral lipids consist of triacylglycerols, steroids, waxes (esters of fatty acids and alcohols) and hydrocarbon. Polar lipids are usually associated with membrane and chlorophyll functions consisting of phospholipids and glycolipids (Ratledge et al., 1988).

While all algae have lipids, some are of more interest than others. Neutral lipids have higher energy content and the quantity can be increased in side the cell by manipulation of environmental conditions. Nutrient deprivation can increase quantity as well as the quality of the lipids (Roessler, 1986). Neutral lipids, consisting of free fatty acids, triacylglycerols and hydrocarbons are excellent feedstocks for transesterification. Triacylglycerols are the primary storage product in microalgae. They are very similar to the storage products found in plants. Polar lipids have a higher degree of substitution of carbohydrates for fatty acids, and these lipids could be transesterified, but the yield of methyl esters could be lower.

In exponential growth the concentration of lipid in diatoms is 18% to 25% AFDW , over 90% of the lipid fraction is polar (Nagle et al., 1986). Under silica deprivation, the concentration of lipid can increase to 40-50% of the cell's dry weight, of which over 80% of the lipid fraction is neutral. Exactly why the increase in lipid and type of lipid occurs is not known. In mass culturing of microalgae for fuel, nutrient deprivation can be used to stimulate the quantity and quality of lipid. Nutrient deprivation can slow growth rate, increase predation and lower stability of the culture. Balancing these contrary activities and main-

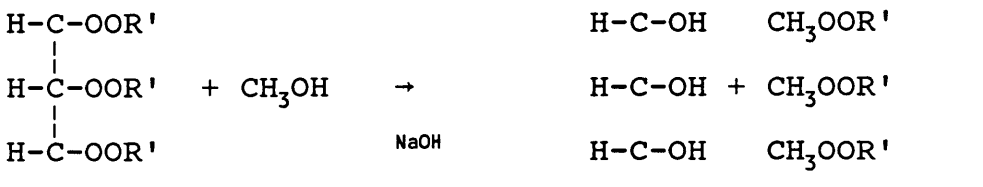
taining lipid production could be accomplished by a two stage process of maximum growth, followed by nutrient deprivation.

Transesterification

Vegetable oils will perform in compression injection engines for short periods (Harrington et al., 1984). Problems with fouling of injectors, polymerization of fuel in gas tanks and carbon deposits on cylinder heads require conversion before use. Ester fuels produced from seed oils have a long a record of successful use in compression engines. Microalgal lipids, because of the similar chemical composition could be transesterified into fatty acid methyl esters.

Transesterificaion has been studied extensively and a dozen U.S. patents have been issued to various investigators. The process involves the formation of an ester bond from the reaction of an alcohol and an acid. In this case, the alcohol is usually methanol or ethanol. The acid is supplied from a triglyceride, which is a major component of the storage lipid in microalgae.

The reaction listed below shows fatty acid methyl esters produced from methanol and a fatty acid.



*Where R'= 16 to 18 carbon chains

The stoichiometry of the reaction indicates glycerol is a byproduct and 3 moles of fatty acid methyl esters are produced for each mole of triglyceride.

Optimization of the reaction has been widely investigated. Engler reported that maximum yield occurred using sunflower oil with 0.8% sodium ethoxide catalyst, with 80% excess ethanol at room temperature, for 2.5 hours (Engler et al., 1983). Freedman and Pryde (1984) studied several transesterification variables that affect yield and purity of product esters. Molar ratio of alcohol to vegetable oil, alcohol type, catalyst type (alkaline and acidic) and temperature (60C, 45C, and 32C). One of the most important variables was the ratio of alcohol to vegetable oil. A 6:1 molar ratio gave the greatest conversion to ester. Type of alcohol did not affect the overall reaction and alkaline catalysts (NaOH or Na methoxide) were more efficient at lower temperatures and required shorter reaction times than acidic catalysts. Nye and Southwell (1984) investigated

effects of important variables on transesterification of vegetable oils. Alcohol to vegetable oil ratio was very important. A 6:1 ratio gave the best conversion. Increasing the ratio did not increase ester yield. Alkaline catalysts were more effective at room temperature than acidic catalysts and the stirring rate was not significant as long as it was vigorous.

Harrington (1984) examined direct transesterification of sunflower seeds in comparison to esterification of sunflower oil. High yields were observed using the in situ method vs. the extracted oils. The esters were produced using an acid-methanol catalyst to produced the FAMES. This work was significant since it indicated that direct transesterification can be accomplished without the extraction of seed oils, a possibility perhaps for microalgae.

Large-scale processing of seed oil to methyl esters has been world wide, particular in countries with little or no petroleum reserves. Several noteworthy studies (Peterson et al., 1988) have been done to investigate the scale up of ester production from pilot to production plant. While transesterification scales well and can be used on a continuous vs. batch basis, most operations have been done on a batch basis. A new process called ATT incorporates several processes to recover free fatty acids, methyl esters, de-

gummed vegetable oil and glycerine (Stage, 1988). This process requires minimal capital investment and can allow farmers to offset poor economic conditions. This process uses a countercurrent film which pumps oil from the bottom into a reactor and methyl ester from the top. Economics indicate that diesel would have to rise to $3.18 for this process to become feasible unless emergency conditions developed. Kaufman (1984) indicated that economics of transesterification would depend significantly on by-product credits, such as glycerol.

Experimental Design

One of the approaches used in this project was to statistically design the experiments. Literature searches indicated a number of variables that could affect the yield of fatty acid methyl esters from plant lipids.

Consider the simple problem of determining what variables have the most affect on boiling an egg and how can we optimize the process. Time, temperature and perhaps the size of the egg are all important variables. You could hold temperature and egg size constant, while varying time, over several temperature ranges. You could do all possible combinations but there is a more efficient way of determining important variables. Imagine that you could frame the

solution inside of cube. This cube is constructed using the three variables as x,y and z coordinates. By having a high and low value for each variable you can construct the end points of the cube. Now rather than sample all of the possible combinations inside the cube you can sample at end points and find where the most important variables lie. This is a screening approach. The one used in this project is called the Plackett-Burman design.

To determine the effect of different agricultural practices on productivity, R.L. Plackett and J.P. Burman (1946) developed a screening protocol that would allow the main effects to be determined. These designs were developed to evaluate main effects without interactions. This approach, using two levels of variables has experimental runs in multiples of 4, ie 4, 8, 12, 16, 20....32. Each factor is scaled to -1 or +1, indicating a high or low level for each variable. The advantage of this approach is economy. Seven variables at two levels would require 2^7 or 128 runs for a full factorial design. A seven factor screening design takes only 8 runs. The main disadvantage is that second order and quadratic interactions are not observed. In choosing the variables it is important to understand that you assume the main effect lies inside your experimental region. Examination of the literature, knowledge of instru-

ment capability and past experience will help set the appropriate ranges for the independent variables.

Let's get back to our boiling eggs. We now know what the important variables are in getting a great boiled egg but how can we optimize the process and hopefully model it ? Imagine our cube. Let's change the points to include a midpoint between our high and low point along each axis. Lets also select several center points within the cube. Our cube now has mid points and center points. Experiments run at the center give us an estimate our experimental error and provide a reference point for effects that might be curvilinear. This is design is called a Box-Behnken design. The Box-Behnken design employs a subset of the points in a three-level full factorial. A three-variable, three-level experiment requires 27 runs for a full factorial experiment. A Box-Behnken uses 13 of the 27 points from the full factorial and 2 extra replicate points near the center, for a total of 15 runs. Five degrees of freedom are provided. Except for the center points, all points are at the midpoint of the edges of a cube. This design provides information about the linear, quadratic effects and secondary interactions associated with the independent variables.

CHAPTER 3

EXPERIMENTAL METHODOLOGY

Biomass Production

Biomass produced for lipid extraction and conversion was produced at two locations, the Solar Energy Research Institute Field Testing Laboratory Building (FTLB) and the Solar Energy Research Institute Outdoor Test Facility (OTF) in Roswell, New Mexico (Johnson et al., 1987). At the FTLB, a diatom Chaetocerous muelleri (SERI designation CHAET9, Johansen et al., 1987) was grown in five, 1.4 m^2 rectangular ponds (Weissman, 1986) in a greenhouse. Microalgae were circulated by paddle wheels mounted on each pond, rotating at 30 rpm. Ponds were filled with Type II/10 water (Barclay et al., 1986) and heated using 200 watt aquarium heating elements to maintain a minimum temperature of 19^0C. Type II/10 water is a synthetic water, derived from analysis of saline ground water found in New Mexico. High pressure sodium lamps (Applied Hydroponics, San Rafael, CA) were used to continuously maintain a light intensity of 400 $uEm^{-2}sec^{-1}$ at the surface of the ponds. Growth medium was controlled at an upper ph limit of 9.5, by CO_2 injection. Ambient air temperature, pond temperature, pH and light intensity were monitored by and pH controlled by an Apple computer using a

Strawberry Tree data acquisition card (Sunnyvale, CA). Growth rates and nutrient status were monitored daily.

At the OTF, the alga Monoraphidium minutum (SERI designation MONOR2, Barclay et al., 1986) was grown in 3.0 m^2 ponds outdoors. Ponds were filled to a depth of 15 cm using saline groundwater as growth medium. Ponds were heated and received only natural illumination. pH was controlled to an upper limit of 9.5.

At the FTLB, biomass was harvested by pressure filtration. A filter press (Star Systems, Timmonsvile, SC) with twenty plates and frames (30 cm in diameter) with a 5 μm pore size paper was used to harvest the microalgae. Growth medium and cells were pumped through the press at 800 l h^{-1}. Filtrate (growth medium) was returned to the ponds, through four cycles of growth and harvest. Biomass was collected on filter papers from an initial concentration of 0.5% solids to a final concentration of 8.0% solids. At the OTF, polymer flocculation (Weissman, 1988) was used to harvest the ponds. Biomass as harvested at an initial density of 0.5% and concentrated to a final density of 4.0%. Biomass scraped off the paper filters was stored in plastic beakers at -10°C.

Biomass measurement

Biomass was measured by pipeting 10-50 ml of the liquid in the pond, on to a glass fiber filter, held in a filter flask. The filters were dried at 50°C, weighed, then ashed at 550°C. Ash free dry weight was calculated as mass volatilized divided by ml of liquid to arrive at mg/l ash free dry weight. The same procedure was used at Roswell, at the OTF.

Lipid Extraction

A screening program was designed to evaluate the ability of organic solvents to extract lipid, using small amounts of biomass. Ethanol, hexane, butanol, ethyl acetate, methylene chloride-methanol, hexane-isopropanol were compared to a control method. The control method was a gravimetric procedure using a five-step, water/methanol/chloroform extraction, followed by a phase separation. This method has been evaluated for its ability to extract lipids. It is considered to be a standard method for removing bulk lipids from plant cells. Sensitivity of this method lends itself well to extracting bulk lipid but may not be the best method in extracting unique or unusual lipids. It has been used extensively by members of the Microalgae Research Group (Roessler, 1986), at the Solar Energy Research Institute.

Solvents were screened by adding ten grams of harvested CHAET9 into triplicate tubes with Teflon screw caps. Thirty milliliters of each solvent was added to each tube. Samples were well mixed for one hour at 60°C in a water bath. After one hour the tubes were centrifuged and the supernatant was pipetted into a tared tube. Samples were evaporated under a nitrogen stream at 50°C until constant weight was obtained. Samples were weighed and analyzed for lipid purity using an Iatroscan.

The Iatroscan is an instrument that uses flame ionization detection with thin layer chromatography. Samples of lipids are spotted on silica rods then developed in a tank of chloroform:acetic acid (100:0.5). Rods are dried in a 100°C oven to evaporate solvent. Rods are then scanned to just above the origin in FID, for neutral lipids. Peaks are identified by comparing unknown samples against known lipids standards from SIGMA scientific company. Rods were then developed again in chloroform:methanol (50:50), then dried in the oven,and scanned under FID for polar lipids. Results from solvent extractions were compared against the control extraction to determine % of lipid extracted.

Promising solvents were scaled up to provide more information about extraction efficiency, ease of recycle and handling. Three replicate trails of each solvent were

performed using lipid from Chaetoceros muelleri (CHAET9). All extractions were batch run, timed for 90 minutes. Temperature was varied, depending on the boiling point of the solvent. Solvent to biomass ratios in all trials was 3:1. All biomass percentages are expressed on a wet weight per volume basis. Solvents used were 1-butanol, ethanol, and a mixture of hexane and 2-propanol. Hexane/isopropanol solvent system was 40% hexane and 60% 2-propanol, by volume. The control method was identical to the method used in the screening experiment.

Harvested biomass (8.0% solids) was concentrated by centrifugation to 15% solids to reduce handling difficulty and volume of solvent needed. A 400 g sample of concentrated biomass was mixed with 1200 g of solvent. The mixture was heated to near boiling and refluxed at that temperature for 90 minutes. Mixture was well agitated during the extraction, then vacuum-filtered through a glass filter to separate the solvent from the solid residue. Solvent was removed by rotary evaporation. Lipid was further purified by phase separation in chloroform/methanol/water (1:1:0.9). A second distillation was done to remove excess chloroform. The extracted lipid was weighed, then analyzed by FID thin layer chromatography to determine purity. Resulting yield was determined by multiplying the grams of lipid by the

percentage of purity of the lipid as determined by FID chromatography (Iatroscan). Efficiency of extraction was determined by first determining the amount of lipid in a 10 g sample of algal paste using the five-step chloroform/methanol/water phase separation. Once the percent lipid was determined, this number was multiplied by the weight of the algal feedstock used in the large scale extraction. This figure was considered to be 100% of the total lipid in the feedstock, which could be extracted. Dividing this number into the grams of lipid extracted for that particular solvent, determined the percent efficiency of extraction.

Conversion of Microalgal Lipid

Initially, the focus of the experiment was to produce fatty acid methyl esters from microalgal lipids, for the Department of Energy. Documented methods were adapted for converting lipid to oil. Using a protocol for converting of rapeseed oil to methyl esters (Melville et al., 1983), 4 g of algal lipids were mixed with a hydroxide catalyst, consisting of 1.0 g methanol and 40 mg potassium hydroxide. The mixture was heated to 60°C for one hour and mixed well by vortexing. After 48 hours no phase separation occurred as was described in the protocol by Melville. Lipids were then

extracted by addition of hexane-water in a 3:1 ratio. No lipid was extracted and the experiment was repeated increasing the catalyst from 1.0 ml to 2.0 and 3.0 ml of potassium hydroxide/methanol. The resulting mixture was an oily soap, which resisted phase separation with hexane/water.

At this point it was obvious that algal lipids were not amendable to transesterification by conventional methods and the important variables in this reaction would not be identified by this protocol. A statistical approach was designed to answer the following questions:

1. What are the most important variables in the conversion of algal lipids to methyl esters?,
2. Are there important interactions between variables?,
3. Does the species of microalgae affect the conversion of lipid to ester?, and
4. What is the maximum yield of FAMES that can be achieved?

A 12-factor Plackett-Burman screening design (Box et al., 1978; Murray 1984) was used to screen major variables that affect transesterification. Variables examined were type of catalyst (hydrochloric acid-methanol vs sodium hydroxide-methanol), concentration of catalyst (0.12 N vs 0.6 N), time of reaction (0.1 and 3.0 hours), temperature of

reaction (20° and 70°C) and type of feedstock used (lipid extracted from C. muelleri vs M. minutum). A 250 mg sample of the designated lipid was weighed into a Teflon screw cap test tube, 5 ml of the catalyst-methanol solution was added and incubated at the appropriate temperature and time as prescribed by the screening design. Samples were agitated every 10 minutes. Fatty acid methyl esters were extracted by addition of 5 ml of petroleum ether followed by vortexing and centrifugation. The top layer was drawn off and evaporated under nitrogen at 50°C and weighed.

Each variable was scaled to "+" or "-", representing a the high or low value for each variable. The dependent variable in the regression model was called YIELD, which was the amount of FAME produced by the individual treatment. FAME identification was done using the Iatroscan using a chloroform/toluene/methanol (45:45:12) development by (Ranny, 1984). FAME identification was done by matching the retention time from the unknown peak against FAME standards from Sigma Chemical.

A Box-Behnken response surface design was developed based on the information gained in the screening experiments to determine secondary effects between major variables and to optimize yield. Three levels for each variable were chosen, centered with respect to one another. Temperature

of reaction (20°,45°,70°C), time of reaction (0.1,1.6,3.1 hours) catalyst concentration(.12N,.32N,.6N) were chosen as variables, entered into a statistical software package and a worksheet was generated for the different combinations of time, temperature and concentration of catalyst. A 250-mg sample of lipid was weighed into a test tube with a teflon screw cap. Five mls of the hydrochloric acid-methanol catalyst was added to the tube at the appropriate concentration and the tube was incubated for the length of time and temperature prescribed by the experimental design. FAME extraction and analysis was done identically as before. The dependant variable in the regression equation was again YIELD, the amount of fatty acids methyl esters (FAMEs) produced by the treatment.

Methylation of Algal Lipids

While the yield of FAMEs was examined by comparing the yield from different strains of microalgae it was recognized that yield could depend on the type of lipid as well as the amount present in the cell. To compare the yield of FAMEs from lipids found in the microalgae, lipids standards were obtained from SIGMA chemical company representing those lipids found in plants. Palmitic acid, triolein, phosphatidyl choline, phosphatidyl glycerol, monogalactosyl

diglyceride and lipid from C. muelleri were refluxed with 5 ml of hydrochloric acid-methanol catalyst (0.6 N) for 1.5 hours at 70°C. FAME yield was determined in the same manner as before.

Comparison of in situ transesterification

To compare the yield of FAMEs from transesterification of extracted lipid versus transesterifcation of the whole microalgae, an experiment was done using freeze dried cells and lipid extracted from C. muelleri. 500 mg of extracted lipid was transesterified at 1.5 hours at 70°C, using the hydrochloric acid-methanol catalyst at 0.6N concentration. FAMEs were recovered and analyzed in the same manner as before.

In situ transesterification was accomplished by adding 10 ml of methanol:benzene (3:2) to freeze dried cells, followed by mixing and addition of 10 ml of methanol:acetyl chloride (100:5). Mixture was refluxed at 100°C for one hour. Mixture was cooled, then 10 ml of water was added, followed by 10 ml of hexane. Mixture was vortexed and centrifuged, top layer was removed and evaporated to dryness under nitrogen. Sample was analyzed using the Iatroscan. Percent efficiency was determined by dividing the amount of

FAME weighed by the amount of lipid present in the original sample.

Feedstock Analysis

Fatty acids from C. muelleri were analyzed by gas liquid chromatography. Extracted lipid was methylated by refluxing 100 mg of lipid with 4 ml of 5% hydrochloric acid/methanol for 1 hour at 70°C. Methyl esters were extracted by addition of 5 ml of petroleum ether followed by centrifugation and the top layer was removed and used for injection into the gas liquid chromatograph. A Shimuzdo GLC was set to analyze samples with oven conditions at 180°C and detector set at 200°C. Data was analyzed by an Shimuzdo integrator. Methyl ester standards were obtained by Sigma Scientific Co. to compare fatty acid length against the lipid extracted from C. muelleri. Lipid groups were identified using the Iatroscan in conjunction with Sigma lipid standards. Lipid from CHAET9 was developed once in a chloroform:Acetic acid solution (100:0.5), analyzed on the Iatroscan for neutral lipids then developed again in a Chloroform:methanol solution (50:50) for polar lipids.

Model Validation

To verify the model for methyl ester production, 250 mg of CHAET9 lipid were held at the optimal reaction time

and temperature using the appropriate catalyst and catalyst concentration. FAMEs were recovered and analyzed in the same manner as before. The amount of methyl esters produced was than compared to the amount estimated by the model. This experiment had seven replicates to check the variability of the experimental conditions.

Chapter 4

Results

Biomass Production

Chaetoceros muelleri grew consistently and under illumination of 300 $uEm^{-2}sec^{-1}$, at a growth rate of 27 $gm^{-2}day^{-1}$. Roughly 15% of the microalgal paste, scraped off the paper filters, was lost due to impregnation on the paper filters. The growth medium was recycled through the filter press for four cycles. No adverse affects were noted from the recycling and the medium was reused for six to ten cycles. Initially, four 210-liters pond were harvested three times per week or upon reaching a density of 500 mg l^{-1}. Nutrient deprivation, which worked in small lab-scale experiments weakened the culture and allowed protozoans and wild algae to invade into the ponds. Ponds were increased from 15 to 20 cm depth, an increase of 40 liters per pond. Another pond was set up to provide an inoculum for the four production ponds. This allowed us to completely harvest each pond and avoid restarting the ponds with weakened algae. Lipid content of C. muelleri in laboratory experiments (Nagle, 1986) was determined to be 23 to 25 % during exponential growth, 32% under silica deprivation and 30% under nitrogen deprivation. Analysis of the lipid content after two days

of silica depletion was 25%. Growth was severely limited by the cold winter temperatures, that dropped below 0°C at night, 200-watt heaters in each pond maintained the temperature range from 19° to 22°C. Light had to be augmented by artificial means because the greenhouse was covered with snow on several occasions.

Lipid Extraction

Several promising solvents were selected for scale up after the results from the screening program (Table 1). The control method using the methanol/chloroform/water phase separation, was assumed to extract 100% of the lipid from 10 g of algal paste. This was considered to be maximum amount of lipid available from the algae. All other solvents were compared with the control method. Butanol was the easiest solvent to handle and gave a clear, well-formed phase separation in a 3:1 solvent to feedstock ratio and recovered 90% of the total lipid available. Methylene chloride-methanol extracted 89% of the lipid available, but it was difficult to phase separate and recover the top portion. Hexane alone did not mix with the aqueous phase of the feedstock and extracted little lipid. Hexane/isopropanol extracted 83% of the lipid and phase separated well. Butanol and hexane/isopropanol were selected for scale-up.

TABLE 1

Results from Solvent Screening Experiment
Amount of Lipid Recovered

Solvent	mg of Lipid	% Lipid Extracted
Hexane	23	14
Butanol	210	91
Hexane/Isopropanol	200	87
Control Method	232	100

While ethanol was not screened, it was included in the scale up process based on Paul Roessler's recommendation. Solvents used in the large scale extraction of algal lipid were all very successful in removing significant amount of lipids. For C. muelleri, the highest and most consistent extraction efficiency and highest purity was obtained using 1-butanol. Average extraction efficiency for the butanol trials was 90% (w/w), with a relative standard deviation (RSD) of 3%. The extracted material was 94% lipid 9 (Table 2).

Ethanol (95% ethanol, 5% propanol, v/v) gave an average extraction efficiency of 73% (RSD of 13%), and was 90% lipid. The hexane/2-isopropanol solvent system gave an extraction efficiency of 78% (RSD of 26%), and was 90%

TABLE 2

Efficiency of Lipid Extraction from *C. muelleri* for Several Solvents

Solvent	Gross Efficiency	Purity	Net Efficiency
1-butanol	94%(3)	0.93(3)	90%(3)
Ethanol	82%(12)	0.90(3)	73%(13)
Hexane/ 2-propanol	87%(25)	0.90(6)	78%(26)

Note: Results are the average of three trials, with coefficient of variation in parentheses. Gross efficiency is actual lipid yield divided by the theoretical yield from control extraction. Net efficiency = Gross efficiency * sample purity.

lipid. All efficiency and purity results are the average of three "large-scale" (150 to 400 g of biomass feedstocks) extraction trials. Each large-scale trial was run with three small-scale (1.0 to 1.5 g of biomass) controls to determine theoretical 100% yields.

One large-scale extraction was run on the algae M. minutum. M. minutum was grown outdoors at the Roswell, New Mexico Test Facility. Solvent used for this extraction was 1-butanol. Extraction efficiency was 81%, with a pure lipid fraction of 0.96.

Lipid extraction of the remaining residue indicated that very little lipid remained in the sample.

Conversion of Microalgal Lipids

Variables examined in the Plackett-Burman screening design were time, temperature, type of catalyst, concentrati on of catalyst, and type of lipid used. Results indicated (Table 3) that greatest amount of methyl esters produced, 147 mg were achieved using CHAET9 lipid with a 5% acid catalyst, at 20°C for 3 hours. The most important variable (Table 4) was the type of catalyst. Hydrochloric acid/ methanol was superior to sodium hydroxide/methanol. Time, temperature, concentration of catalyst and type of lipid were not as important. The explained coefficient of

TABLE 3

Yields of fatty acid methyl esters (mg FAMEs) from the various variable combinations prescribed by the Plackett-Burman screening design.

	Controlling Factors					
Run#	Lipid	Temp	Time	Cata-lyst	Conc	Yield
1	1.000	20.0	3.0	1.00	0.10	47.0
2	-1.000	70.0	3.0	1.00	0.10	42.0
3	-1.000	70.0	0.10	1.00	5.00	152.0
4	-1.000	70.0	3.0	-1.00	5.00	4.0
5	-1.000	20.0	0.10	1.00	0.10	84.0
6	-1.000	20.0	3.00	-1.00	5.00	3.0
7	1.000	20.0	3.00	1.00	5.00	147.0
8	1.000	70.0	0.10	1.00	5.00	145.0
9	1.000	70.0	3.00	-1.00	0.10	41.0
10	1.000	20.0	0.10	-1.00	0.10	4.0
11	1.000	70.0	0.10	-1.00	0.10	43.0
12	-1.000	20.0	0.10	-1.00	0.10	43.0

TABLE 4

Regression coefficients for yield of fatty acid methyl esters using a Plackett-Burman screening design

Coefficient	Term	Std Error	Confidence Coefficient
8.2	Lipid	11.1	49.4%
0.3	Temp	0.4	49.9%
-10.7	Time	7.7	77.9%
39.9	Catalyst	11.1	98.5%
5.2	Conc	4.5	69.5%

determination (R^2) was 74%. Type of catalyst alone could explain over 55% of the variation in the model. The low R^2 was a result of choosing a linear fit for the model. Using only two levels for each variable prevented use of the quadratic fit. The confidence coefficient is (1-p) the percent chance that equation is nonzero.

In comparison, only 3.3 mg of FAMEs were produced using sodium hydroxide/methanol catalyst at the same conditions that gave maximum FAME yield, using an acid catalyst. Maximum yield of FAMEs using sodium hydroxide as a catalyst was 80.4 mg of FAMEs from 250 mg lipid.

Results from the Box-Behnken Response surface design indicated that the 128 mg of FAME could be produced using

(Table 5) CHAET9 lipid, 5.0% acid catalyst, at 45°C for 0.1 hours. Concentration of catalyst is the most important variable in the reaction (Table 6). The $conc^2$ term, indicating a curvilinear effect, was almost as significant as the concentration alone. It should be noted that the $Conc^2$ term is not a synthetic variable, it represents the amount of curvature in the equation. Time, with a coefficient of -6.40 at a confidence level of 94.% was the next significant variable. The negative term indicates that increasing time decreases yield. Temperature was not significant nor were any combinations of factors significant. The model used was the quadratic fit and the R^2 was .98. Once the model was revamped to include Conc, $Conc^2$, Time, and temperature the R^2 dropped from .98 to .89. The model was set to optimize the conditions for FAME yield within the conditions given, gave the following results:

Maximum Yield

Time	10 minutes
Temperature	46°C
Concentration	2.9% catalyst

(Catalyst used was hydrochloric acid/methanol.)

A yield of 132 mg of FAMEs from 250 mg of lipid was predicted.

TABLE 5

Worksheet for the Box-Behnkin Surface Design with Results

Run	Time (hr)	Temp (°C)	Conc (%)	Yield (mg)
1	1.55	20	0.10	27
2	0.10	20	2.55	108
3	3.00	20	2.55	96
4	1.55	45	2.55	106
5	1.55	70	5.00	101
6	3.00	45	0.10	23
7	0.10	45	0.10	28
8	0.10	45	5.00	128
9	3.00	70	2.55	107
10	1.55	20	5.00	111
11	3.00	45	5.00	107
12	0.10	70	2.55	120
13	1.55	45	2.55	112
14	1.55	70	0.10	30
15	1.55	45	2.55	114

TABLE 6

Regression Coefficients from the Box-Behnken Experiment

Coefficient	Term	Confidence Coef. (%)
-6.40	Time	94.6
2.17	Temp	56.4
42.63	Conc	99.8
-.25	Time*Temp	18.3
-3.90	Time*Conc	66.7
3.15	Temp*Conc	57.2
0.72	$Time^2$	23.3
-3.66	$Temp^2$	62.1
-39.77	$Conc^2$	98.7

Model Validation

A yield of 171.4 mg of FAMEs from 250 mg of lipid was achieved using the conditions dictated by the model for maximum yield. Results from the replicate samples were very close. For seven samples the mean was 171.4 mg, with a standard deviation of 5.4 mg, less than 4% experimental error.

Feedstock Analysis

Results from the Iatroscan indicated that the lipid from CHAET9 is composed of 50.2% neutral lipids and 49.8% polar lipids. Of the 50.2% neutral lipids over 36.4% are in the form of free fatty acids and 13.7% are triglycerides. Analysis by gas chromatography identified the fatty acid composition as 14.7% 14:0, 9.3% 16:0, 35% 16;1, 7.5% 16:2, 17.0% 16:3 and 26.4% 20:5.

Methylation of algal lipids

Transesterification of known algal lipids indicated that palmitic acid, a neutral fatty acid had the highest yield of FAMEs produced from the reaction (Table 7). Mono-galactosyl diglyceride, a polar lipid had the lowest yield

of FAMEs produced. Yield of FAMEs from CHAET9 lipid was 68.8%.

TABLE 7

Yield of FAMEs from Known Algal Lipids

Lipid	Yield (%)
Palmitic acid	93.4
Triolein	87.6
Phosphatidyl glycerol	54.4
Phosphatidyl choline	65.1
Monogalactosyl choline	46.6
Digalactosyl diglyceride	55.8
Chaetocoeros muelleri lipid	68.8

Chapter 5

Discussion

Biomass Production

Production of biomass was very successful. Considering the constraints the cold weather had put upon our project high productivity rates were achieved, a monoculture was maintained containing a lipid fraction of 25 to 30%. CHAET10 and MONOR2 were selected for this project because results from our screening program indicated that they were fast growers with decent lipid production and they were easy to handle. During the course of the project it was noticed that our efficiency of harvesting was decreasing. Microscopic examination of the microalgae revealed that the average size of the microalgae was decreasing. Selective pressure from the filter press was discriminating for smaller microalgae. We solved this problem by filtering each pond to completion and maintaining a separate pond of microalgae to reinoculate the harvested ponds. The main effect of augmenting natural daylight with the sodium lamps was that it increased the productivity of the ponds. No effect was observed of the artificial light on the quantity or quality of lipid produced.

Lipid Extraction

We accomplished our goal of extracting the lipid portion from microalgae. All solvents worked well in extracting lipid but butanol was the most efficient solvent. While this process works well for batches under 50 liters, the economics may not be profitable for large-scale operation. In a report to Carbonic International, (Nagle and Brown, 1989) the amount of butanol required to extract the lipid from microalgae for a 10 acre facility was calculated at 11,103 lbs/hr. Large expenditures of energy are required to recover the butanol. Even at a 95% recovery of butanol, significant amounts of fresh solvent would be required for continued operation. Additional research needs to be conducted on different types of solvents as well as physical treatments that could extract lipids from microalgae. One possible alternative could be supercritical extraction. The economics of the process might be promising enough to justify the capital cost associated with supercritical extraction.

One of the problems that needs to be addressed is the large volume of water that is carried along in the processing of the microalgae. During harvesting the percent of

solids starts out as 0.5% and increases to 10-11%. Centrifugation increases this to 18-20%, yet 80% of the feedstock stream is water. This adds additional cost to the extraction process. One possibility is to dry the microalgae before lipid extraction. This raises questions of economics, handling and how this would affect the conversion process downstream.

Conversion of microalgal lipid

One of the surprising results from this project was the successful conversion of microalgal lipids into methyl esters, using an acid catalyst with a short reaction time at low reaction temperature. Most of the work done on producing methyl ester based fuels used sodium hydroxide as the catalyst and seed oils as a feedstock (Freedman and Pryde, 1984). Acid catalysts required longer reaction time and higher reaction temperatures to achieve reasonable yields with seed oils. Nye (1985) achieved high conversion efficiencies using an acid catalysts and spent frying oil. Acid catalysts work better with heterogenous than homogenous substrates and microalgal lipids are very heterogenous.

This process should scale up well. Reaction time is short, reaction temperature is moderate and the catalyst is

a commodity chemical. Two problems that need to be addressed are:

1. Algal lipids are very viscous, causing mixing problems between the lipid and catalyst. This problem was solved at bench-scale by having an excess of methanol in the reaction. On large-scale operation another solution would be required.
2. Algal lipids have a high concentration of chlorophyll. Chlorophyll is carried over in the separation of the methyl ester from the residue. Chlorophyll is an octane enhancer and would need to be removed before use in a diesel engine.

Methylation of algal lipids

Results indicated that neutral lipids (palmitic acid and triolein) have higher yield of FAMEs than polar lipids. As phosphate groups were substituted for fatty acids, like in phosphatidyl choline, the yield of FAMEs decreased. This result was parallel with carbohydrate substitution with mon and digalactosyl diglyceride. This would be a strong case for stimulating neutral lipid production using nutrient deprivation during mass culture, because neutral lipids make better FAME feedstock.

Error Analysis

From an experimental standpoint the major sources of error were due to the Iatroscan and the viscosity of the algal lipid. The Iatroscan identifies methyl esters based on the retention time of a known methyl ester standard. The strength of the signal is dependent upon a number of variables, development time, the individual rod, type of solvent used and integrator response. Most of the error associated with the rods are controlled by using fresh or regenerated rods and by keeping them together in a set. The developing time was consistent as well as the solvent system used. Yet variation between replicate could be as high as 10%. This was controlled by doing at least four replicates for each sample. The viscous algal lipids created a mixing problem. They needed to be stirred constantly to assure good contact between the catalyst and the lipid. The magnitude of the error could not be calculated except to say that inadequate mixing gave low yields, thus variation in mixing would cause variation in yield. The model calculated a yield of 132 mg of FAME from 250 mg of algal lipid. When running the reaction at the optimal conditions we were able to achieve 171.4 mg (normalized to 250 mg sample) from a 250 mg sample.

While the amount predicted was lower than what was measured by the model, additional manipulations could improve it's accuracy. The experimental error was less than 5% and the reaction was very reproducible from sample to sample.

Chapter 6

Conclusion

The purpose of the Aquatic Species Program at the Solar Energy Research Institute has been to develop the technology for microalgal based fuels. During its seven year history the actual product existed only on paper. Now, fuel from microalgae has been produced. Research in the program should be focused on how to manipulate the microalgae to increase production of lipids for FAME production. This work is also significant in the way it has changed the assumptions of how lipid would be extracted from microalgae and how that lipid would be converted into a fuel. No doubt that this work will continue to evolve, it now provides a link between the other parts of the program in that it can be seen how each segment in the program affects the final product.

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Appendix A

Yield of Fatty Acid Methyl Esters Obtained from Model Validation

Tube 1	154 mg
Tube 2	159 mg
Tube 3	150 mg
Tube 4	159 mg
Tube 5	155 mg
Tube 6	152 mg
Tube 7	166 mg

Mean = 156.4 mg FAMEs

Std. deviation = 4.9

The samples were held at 45°C, for 10 minutes in 2.9% acid-methanol catalyst. FAME measurement and identification was identical to earlier work.