0273-2289/90/2425—0355$02.00

# Production of Methyl Ester Fuel from Microalgae

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## ABSTRACT

Microalgae are unique photosynthetic organisms that are known to accumulate storage lipids in large quantitites and thrive in saline waters. Before these storage lipids can be used, they must be extracted from the microalgae and converted into usable fuel. Transesterification of lipids produces fatty acid methyl esters that can be used as a diesel fuel substitute.

Three solvents, 1-butanol, ethanol, and hexane/2-propanol, were evaluated for extraction efficiency of microalgal lipids. Type of catalyst, concentration of catalyst, time of reaction, temperature of reaction, and quality of lipid were examined as variables for transesterification. The most efficient solvent of the three for extraction was 1-butanol (90% efficiency), followed by hexane/2-propanol and ethanol. Optimal yield of fatty acid methyl esters was obtained using 0.6*N* hydrochloric acid in methanol for 0.1 h at 70°C.

**Index Entries:** Microalgae; methylesters; lipids; extraction; conversion.

## INTRODUCTION

World-wide energy shortages and the oil embargo of the 1970s forced many nations to evaluate energy sources and supplies. Energy from biomass is an attractive solution because it is renewable. One of the goals of the US Department of Energy and the Solar Energy Research Institute is to develop a technology base for the large-scale production of lipid-pro-

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ducing microalgae and conversion of microalgal lipids into substitutes for traditional fuels.

Neenan et al. (*1*) analyzed the production of gasoline and diesel fuel substitutes using mass culture of microalgae. Large-scale raceways of microalgae could yield 100–150 barrels of oil/acre/year. Two methods of conversion have been proposed (*1*): (1) transesterification for ester fuel production and (2) zeolite catalysis for gasoline production. Although production of methyl esters from seed crops has been studied extensively, research on methyl esters from microalgal lipids is limited. This paper will report the results of our study, which examined several solvent systems for lipid extraction from microalgae and identified the impact of major variables in transesterification of microalgal lipids.

## MATERIALS AND METHODS

### Biomass Production

Biomass was produced for lipid extraction and conversion work at two locations—the Solar Energy Research Institute Field Test Laboratory Building (FTLB), and the Solar Energy Research Institute Outdoor Test Facility (OTF) in Roswell, NM (*2*). At the FTLB, the diatom *Chaetoceros muelleri* (SERI designation CHAET9 [*3*]) was grown in five 1.4-$m^2$ ponds in a greenhouse. The ponds were filled to a depth of 20 cm with SERI Type II/10 water as a growth medium (*4*). The ponds were heated to maintain a minimum temperature of 19°C. High-pressure sodium lamps (Applied Hydroponics, San Rafael, CA) were used to maintain a minimum light intensity of 400 $\mu E \cdot m^{-2} \cdot S^{-1}$. Growth medium was pH-controlled, at an upper limit of 9.5, by carbon dioxide injection. Ambient air and water temperature and light intensity were monitored, and pH was controlled by an Apple computer using a Strawberry Tree data acquisition card (Sunnyvale, CA). Growth rates and nutrient status were monitored daily. The maximum growth rate obtained was 28 $g/m^2/d$ of ash-free dry weight.

At the OTF, the alga *Monoraphidium minutum* (SERI designation MONOR2 [*4*]), was grown in three 3.0-$m^2$ ponds outdoors. The ponds were filled to a depth of 15 cm using on-site saline ground water as a growth medium. The ponds were not heated and received only natural illumination. pH was controlled to an upper limit of 9.5.

### Harvesting

At the FTLB, biomass was harvested by pressure filtration. A filter press (Star Systems, Timmonsville, SC) with twenty plates and frames (30 cm in diameter) and 5-$\mu$m-pore-size paper filters was used to harvest the algae. Growth medium and cells were pumped through the press at a rate of 800 $L/h^1$. Filtrate (growth medium) was returned to the ponds through four cycles of growth and harvest. The biomass was collected on

the paper filters. Biomass was concentrated from an initial concentration in the ponds of 0.5% solids to a final concentration of 8.0% solids.

At the OTF, polymer flocculation (*5*) was the harvest method chosen. Biomass was concentrated from an initial density of 0.5% solids to a final density of 4.0% solids.

## Extraction

Lipid extractions were performed with a variety of solvents to determine the extraction efficiency compared with a control method. Three replicate trials of each solvent extraction were performed on *C. muelleri.* All extractions were batch runs, timed for 90 min. Temperature was varied, depending on the boiling point of the solvent. Solvent-to-biomass ratio in all trials was three:one (*see below*). All biomass percentages are expressed on a wet weight/vol basis.

The solvents used were 1-butanol, ethanol, and a mixture of hexane and 2-propanol. The hexane/2-propanol solvent system was 40% hexane and 60% 2-propanol, by volume. The control method was run in triplicate. This was a five-step water/methanol/chloroform extraction (*6*).

Harvested biomass (8.0% solids) was concentrated by centrifugation to 15.0% solids in order 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 maintained at that temperature for 90 min. The mixture was well-agitated during the extraction, then vacuum-filtered through a glass fiber filter to separate the lipid and solvent from the solid residue. Next, the solvent was removed by distillation. The lipid was further purified by a chloroform/methanol/water phase separation. A second distillation was run to remove the solvent from the purified lipid. The extracted lipid was then weighed. Purity was quantitatively determined (*7*) using a thin-layer chromatography scanner with flame ionization detector (Iatron Laboratories, Inc., Tokyo). The resulting yield of the purified lipid was compared with the control yield to arrive at the relative efficiency for each extraction.

## Conversion of Microalgal Lipid

A Plackett-Burman screening design (*8,9*) was used to screen major variables that affect transesterification. Variables examined were type of catalyst (hydrochloric acid–methanol and sodium hydroxide–methanol), concentration of catalyst (0.12 and 0.6*N*), time of reaction (0.1 and 3.0 h), temperature of reaction (20 and 70°C), and type of feedstock used, (lipid extracted from *M. minutum* and *C. muelleri*). A sample of 250 mg of the designated lipid was weighed into a Teflon™ screw-cap tube, 5 mL of the catalyst–methanol solution was added, and the tube incubated at the appropriate time and temperature. The samples were agitated every 10 min. Fatty acid methyl esters (FAME) were extracted by adding 5 mL of petroleum ether, followed by vortexing and centrifugation. The top layer was

drawn off, evaporated at 50°C, and weighed. The dependent variable in the regression model was called YIELD, which was the amount of FAME produced by the individual treatment. FAME identification was performed using the Iatroscan. The efficiency of methylation of known algal lipids was determined using individual lipid standards at conditions chosen using the Plackett-Burman screening design.

## RESULTS

### Extraction

For *C. muelleri,* the highest and most consistent exraction efficiency and lipid purity was obtained by using 1-butanol. Average extraction efficiency for the butanol trials was 90% (w/w), with a relative standard deviation (RSD) of 3%. The fraction of pure lipid in total extractables was 0.94.

Ethanol (95% ethanol, 5% propanol, v/v) gave an average lipid extraction efficiency of 73% (RSD of 13%), and a pure lipid fraction of 0.90. The hexane/2-propanol solvent system gave an extraction efficiency of 78% (RSD of 26%), and a pure lipid fraction of 0.90 (*see* Table 1). All efficiency and purity results are averages of three "large-scale" (150–400 g of biomass feedstock) extraction trials. Each large-scale trial was run with three small-scale (1.0–1.5 g of biomass) controls to determine theoretical 100% yields.

One large-scale extraction was run on the alga *M. minutum. M. minutum* was grown outdoors at the Roswell NM OTF. The solvent used for this extraction was 1-butanol. Extraction efficiency was 81%, with a pure lipid fraction of 0.96.

### Conversion

Variables examined in the Plackett-Burman screening design were time, temperature, type of catalyst, concentration of catalyst, and type of lipid used. Results indicate (Table 2) that type of catalyst was the most important variable. Time, concentration of catalyst, lipid type, and temperature of reaction were not as important. The explained coefficient of determination ($R^2$) was 74%. Type of catalyst alone could explain over 55% of the variation in the model. The maximum yield of 170.3 mg of FAME from 250 mg of lipid was achieved at the following conditions: 0.6*N* hydrochloric acid–methanol catalyst for 0.1 h at 70°C, using the extracted lipid from *C. muelleri.*

In comparison, only 3.3 mg of FAME were produced when sodium hydroxide was used as the catalyst at the same conditions that gave maximum FAME yield. The maximum yield using sodium hydroxide as a catalyst was 80.4 mg of FAME from 250 mg lipid. Conversion yields of known algal lipids (Table 3) indicate that fatty acids had the highest yield (93%), whereas monogalactosyl diglyceride had the lowest yield, 46%.

Table 1
Efficiency of Lipid Extraction from *C. muelleri* for Several Different Solvents[a,b]

| Solvent | Gross efficiency | Purity | Net efficiency |
|---|---|---|---|
| 1-butanol | 94% (3) | 0.94 (3) | 90% (3) |
| ethanol | 82% (12) | 0.90 (3) | 73% (13) |
| hexane/2-propanol | 87% (25) | 0.90 (6) | 78% (26) |

[a]All extractions were heated to near-boiling and run batchwise for 90 min. All results below are the averages of three trials, with coefficients of variation in parentheses. Gross efficiency is actual lipid yield divided by theoretical yield from control extractions. Net efficiency accounts for the purity of extracted lipid. Purity is determined by thin-layer chromatography (IATROSCAN).
[b]One extraction trial was run with *M. minutum* feedstock and 1-butanol solvent. The results were gross efficiency of 84%, purity fraction of 0.96, and net efficiency of 81%.

Table 2
Regression Coefficients for Yield of Fatty Acid Methyl Esters
Using a Plackett-Burman Screening Design[a]

| Coefficient | Catalyst | Std. Error | Confidence Coef. |
|---|---|---|---|
| 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% |

[a]Lipids from *C. muelleri* and *M. minutum* were transesterified at different combinations of time, temperature, catalyst, and catalyst concentration. Type of catalyst was identified as the most important, having the largest coefficient of determination.

Table 3
Yield of FAME from Standards Representing
Lipids Found in Algae[a]

| Lipid | Yield |
|---|---|
| Palmitic acid | 93.4% |
| Triolein | 87.6% |
| Phosphatidyl glycerol | 54.4% |
| Phosphatidyl choline | 65.1% |
| Monogalactosyl diglyceride | 46.6% |
| Digalactosyl diglyceride | 55.8% |
| Chaetocerous muelleri lipid | 68.8% |

[a]All yields are the mean of two samples of lipid standards. Reactions took place using 5 mL of 0.6*N* hydrochloric acid in methanol at 70°C for 1.5 h. Yield is based on mass of FAME produced divided by mass of starting material.

## DISCUSSION

### Extraction

Solvents were selected for analysis based on several criteria. These were low carcinogenicity, ease and safety of handling, volatility, availability and cost, and previous research. Ethanol, 1-butanol, and hexane/2-propanol appeared to be the most promising solvents.

All solvents were fairly effective at extracting a pure lipid product at reasonably high efficiencies, with 1-butanol obviously being the best of the three. Butanol extractions were also the most consistent from trial to trial, in efficiency and purity. Although all sovents were relatively easy to handle, the consistency of the butanol results indicates a lower sensitivity to changes (or errors) in extraction procedure. This may be a considerable benefit as the process is scaled up.

The source of biomass (*C. muelleri* vs *M. minutum*) does not appear to significantly affect extraction efficiency. Although only one extraction trial with *M. minutum* was run, its efficiency was not significantly different from the *C. muelleri* extractions.

All conversion studies were run with lipid extracted with 1-butanol. All three solvents provided comparably pure lipid, but downstream effects (conversion efficiency) were not investigated as a function of extraction method. Future work may include studies of the downstream effects of extraction solvent choice.

### Conversion

Transesterification of seed oils has been well documented (*10*). Freedman et al. (*11*) optimized the variables for ester formation from triacylglycerols. The feedstock should have less than 0.5% free fatty acids, 1% sodium hydroxide–methanol should be used in a 6:1 molar ratio of alcohol to oil, and the mixture should be refluxed for 1 h at 60°C. Freedman found that yields from seed oils using acid catalyst were lower, the reaction took longer, and higher temperatures were required than when hydroxide catalyst was used. Freedman (*11*) and Nye (*12*) recommended acid catalyst when starting materials are low grade or have a high concentration of free fatty acids; the fatty acids would deactivate an alkaline catalyst. The concentration of free fatty acids in the lipid from *C. muelleri* and *M. minutum* was 24.6%; thus an acid catalyst would be more effective than a base catalyst. The high confidence coefficient for the hydrochloric acid–methanol catalyst over the sodium hydroxide catalyst confirms this. The negative coefficient for time suggests that longer incubation times are deleterious to FAME yields. Temperature was not significant. An explanation might be related to the high concentration of free fatty acids in the algal lipid. The lower conversion yields from the phosphatidyl choline, phosphatidyl glycerol, and mono- and digalactosyl diglyceride esters can

be explained by the substitution of fatty acid groups by carbohydrate and phosphate groups.

## ACKNOWLEDGMENTS

This research was supported through the Aquatic Species Program of the DOE Biofuels and Municipal Waste Technology Division under BF71 (656-87). We would like to thank Joseph Weissman of Microbial Products, Inc., for cultivating and harvesting of *M. minutum* for this project.

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