

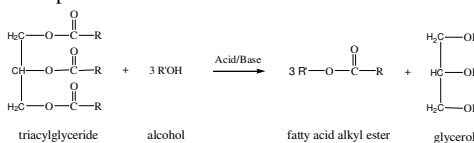
## ABSTRACT

Biodiesel (fatty acid methyl esters) derived from oleaginous microbes (microalgae, yeast, and bacteria) are being actively pursued as potential renewable substitutes for petroleum diesel. Here, we report the engine performance characteristics of biodiesel produced from a microalgae (*Chaetoceros gracillis*), a yeast (*Cryptococcus curvatus*), and a bacteria (*Rhodococcus opacus*) in a two cylinder diesel engine outfitted with an eddy current brake dynamometer, comparing the fuel performance to petroleum diesel (#2) and commercial biodiesel from soybeans. Key physical and chemical properties, including heating value, viscosity, density, and cetane index, for each of the microbial-derived biofuels were found to compare favorably to soybean biodiesel. Likewise, the horsepower, torque, and brake specific fuel consumption across a range of engine speeds also compared favorably to values determined for soybean biodiesel. Analysis of exhaust emissions (hydrocarbon, CO, CO<sub>2</sub>, O<sub>2</sub>, and NO<sub>x</sub>) revealed that all biofuels produced significantly less CO and hydrocarbon than petroleum diesel. Surprisingly, microalgae biodiesel was found to have the lowest NO<sub>x</sub> output, even lower than petroleum diesel. The results are discussed in the context of the fatty acid composition of the fuels and the technical viability of microbial biofuels as replacements for petroleum diesel.

## INTRODUCTION

Biodiesel is a renewable liquid transportation fuel that can be used to displace petroleum-derived diesel fuel without significant modifications to existing engines or fuel distribution networks.

Current biodiesel used in the United States is derived from oilseed crops, thereby competing with food products and requiring quality farmland. Traditional crops (corn or soybean) are only capable of meeting a small fraction of the US transportation fuel demand.



**Figure 1: The Transesterification Process**  
 Triacylglycerides are transesterified using an alcohol in the presence of acid or base to generate fatty acid alkyl esters and glycerol

Evaluating how biofuels from different microbes perform in engines will reveal insight on how different fatty acid profiles affect the resulting fuel and allow for selection or genetic engineering of specific microbial strains in order to produce superior fuels.

## METHODS

### Strains

Microalgae: *Chaetoceros gracilis* (UTEX# LB 2658)  
 Yeast: *Cryptococcus curvatus* (ATCC# 20509)  
 Bacteria: *Rhodococcus opacus* PD630 (DSM# 44193)

### Biodiesel production from microbial biomass

Microbial lipids are extracted and converted to biodiesel using a direct transesterification approach developed at Utah State University. Biodiesel production from yeast, bacteria, or microalgae was initiated by the addition of 0.5 to 1 kg of dried microbial biomass to methanol containing 2% (% v/v) H<sub>2</sub>SO<sub>4</sub> at a ratio of 20:1 (L:kg) in a 20 L pilot reactor. Reaction was maintained at 62°C for 6 hours with stirring at 400 rpm. Once the reaction was complete, chloroform was added. The chloroform filtrate was then mixed with the methanol and then water was added to force a phase separation. The bottom organic layer, containing the crude biodiesel was then distilled off. The FAME was recovered from the crude mixture by utilizing vacuum distillation up to a temperature of 180°C.

engine model	Kubota Z482-ES04
number of cylinders	2
engine type	Indirect injection naturally aspirated 4-stroke diesel, liquid-cooled
displacement	29.23 in <sup>3</sup>
bore	2.64 in
stroke	2.68 in
compression ratio	22.5:1
fuel injection type	Nozzle
injection pressure	1991 psi
injection timing	20° BTDC
continuous rated output	10.8 hp SAE (7.9 kW)
rated speed	3600 rpm
dynamometer type and model	eddy current absorber (#20 96V, Land & Sea, Inc.)
controller	eddy control 96-DC
control program	Dyno-max 2010 version 10.15

**Table 1: Engine Specifications**

### Engine test procedure

To begin each test, the engine was first started and idled for 30 seconds. The throttle was then increased to full (3800 rpm) and run for an additional 30 seconds. An electronically-controlled down-sweep load was then applied to the engine via the ECB to load the engine from 3800 rpm down to 2450 rpm at a rate of 150 rpm s<sup>-1</sup>. For each fuel, the test procedure was performed in triplicate. Upon completion of triplicate engine performance tests, emissions data for each fuel was collected by operating the engine without load at 3500 rpm for 60 s, using a 5-gas analyzer (CO<sub>2</sub>, CO, NO<sub>x</sub>, unburned hydrocarbon, and O<sub>2</sub>).

## RESULTS

### Fuel Quality

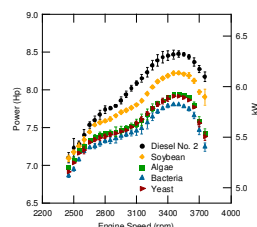
Biodiesel Fuel	Fatty Acid Chain Length (% of Total Fatty Acids)						Degree of Unsaturation (% of total fatty acids)		
	C14	C15	C16	C17	C18	C20	Mono	Poly	+
							Poly		
Soybean <i>G. max</i>	0	0	11	0	88	<1	24	61	85
Yeast <i>C. curvatus</i>	0	0	16	0	83	<1	60	6	66
Bacteria <i>R. opacus</i>	2	5	43	22	27	0	51	0	51
Microalgae <i>C. gracilis</i>	10	<1	72	0	11	6	34	28	62

**Table 2: Fatty acid composition of biodiesel fuels**

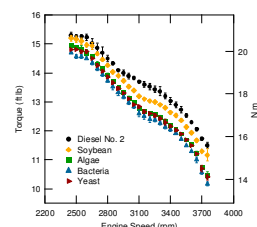
Fuel	Fuel Properties					
	Density at (15°C) (g cm <sup>-3</sup> )	Kinematic viscosity at 40°C (mm <sup>2</sup> s <sup>-1</sup> )	Heating value (kJ g <sup>-1</sup> )	Volometric energy density (kJ cm <sup>-3</sup> )	Cetane Number (minimum value)	Biodiesel Cetane Index
Petroleum diesel ASTM standard (D975)		1.9 – 4.1			40	
No. 2 (This study)	0.818	2.1 (±0.06)	46.10 (±0.036)	37.7		
Biodiesel ASTM standard (D6751)	0.86 – 0.90	1.9 – 6.0	NA		47	
Soybean <i>Glycine max</i>	0.884	3.9 (±0.1)	39.97 (±0.095)	35.3		54
Microalgae <i>C. gracilis</i>	0.885	3.4 (±0.06)	39.51 (±0.006)	35.0		51
Yeast <i>C. curvatus</i>	0.876	4.5 (±0.1)	39.33 (±0.289)	34.5		67
Bacteria <i>R. opacus</i>	0.895	4.1 (±0.05)	37.31 (±0.252)	33.4		41

**Table 3: Properties of fuels**

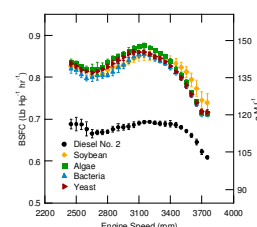
### Fuel Performance



**Figure 1: Diesel engine power output**



**Figure 2: Diesel engine torque output**



**Figure 3: Diesel engine BSFC**

### Emissions

Fuel	Emission Parameters				
	CO <sub>2</sub> (%)	CO (%)	HC (ppm)	NO <sub>x</sub> (ppm)	O <sub>2</sub> (%)
Petroleum Diesel #2	3.700 (±0.000)	0.109 (±0.006)	28.96 (±1.08)	25.71 (±1.302)	15.3 (±0.000)
Biodiesel					
Soybean <i>G. max</i>	3.881 (±0.000)	0.077 (±0.005)	17.38 (±0.59)	31.67 (±1.433)	15.4 (±0.000)
Yeast <i>C. curvatus</i>	3.800 (±0.000)	0.048 (±0.004)	11.86 (±0.48)	39.67 (±1.394)	15.6 (±0.040)
Bacteria <i>R. opacus</i>	3.891 (±0.032)	0.050 (±0.000)	9.87 (±0.49)	46.76 (±1.283)	15.5 (±0.000)
Microalgae <i>C. gracilis</i>	3.797 (±0.016)	0.090 (±0.006)	19.75 (±0.94)	21.87 (±1.817)	15.6 (±0.000)

**Table 4: Emissions for biodiesel fuels and petroleum diesel**

## CONCLUSIONS

- All microbial biodiesel fuels were found to generate similar power and torque outputs compared to soybean
- Microbial biodiesels do not show an increase in BSFC relative to soybean
- Hydrocarbon and CO emissions are reduced compared to diesel #2 levels for microbial and soybean biodiesel
- CO<sub>2</sub> levels are increased for the biofuels indicating improved combustion
- NO<sub>x</sub> emissions for soybean, yeast, and bacterial biodiesel were higher than the measured levels for diesel #2
- Microalgal biodiesel produced the lowest NO<sub>x</sub> emissions of any fuel tested

## ACKNOWLEDGMENTS

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