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Laboratory Experiment

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Releasing Stored Solar Energy within Pond Scum: Biodiesel from Algal Lipids

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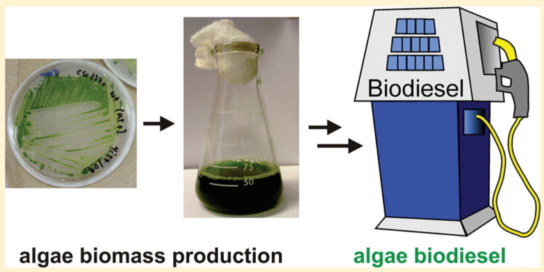
Ⓢ *Supporting Information*

ABSTRACT: Microalgae have emerged as an attractive feedstock for the mass production of renewable transportation fuels due to their fast growth rate, flexible habitat preferences, and substantial oil yields. As an educational tool, a laboratory was developed that mimics emerging algal biofuel technology, including the extraction of algal lipids and transesterification into biodiesel. First, students were introduced to algae as an energy feedstock and taught how to cultivate and harvest algal biomass. Next, students learned how to convert algal biomass to a transportation fuel using transesterification and extraction, including an experimental comparison of base- and acid-catalyzed transesterification methods. The energy content of homemade algae biodiesel was determined and compared to soybean biodiesel and petroleum diesel using a homemade calorimeter.



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Dwindling petroleum reserves and climate change caused by combustion-related CO_2 emissions illustrate the necessity for a sustainable, carbon-neutral fuel source. Nonrenewable petroleum hydrocarbons currently meet 37% of energy needs in the United States,[1] and transportation is the second largest consumer of energy in the United States, accounting for nearly 60% of our nation's petroleum use, an amount equivalent to all of the oil imported into the country.[1] Therefore, creating a renewable transportation fuel such as biodiesel has the potential to dramatically affect the future energy outlook of the United States.[2]

Biodiesel is a high-energy, lightweight fuel derived from lipids and oils through a chemical transformation called *transesterification*. In terms of energy transport and storage, biodiesel offers several advantages over competing transportation fuels such as hydrogen[3] and corn ethanol. Biodiesel has an energy density comparable to that of petroleum, while emitting less greenhouse gases, and if produced by a carbon-sequestering organism such as plants or algae, it can be rapidly produced on scale in a carbon-neutral and sustainable fashion.

Microalgae have emerged as an attractive feedstock for the mass production of renewable transportation fuels due to their fast growth rate, flexible habitat preferences, and substantial oil yields.[4] Algae efficiently store solar energy and sequester CO_2 during photosynthesis, alleviating climate change caused by the emission of greenhouse gases. Biodiesel created from microalgae does not require fertile land, the main drawback of corn-based ethanol and plant oils.[5] In fact, algae can grow in a myriad of environments, including brackish water, wastewater, near power plants, and in both extreme cold and warm environments. In addition to their fast growth rate, microalgae are capable of producing 1000−4000 gallons of oil per acre per year,[6] a much higher yield than the 48 gallons of oil per acre per year from soybeans, currently the leading biodiesel feedstock.[6] Microalgae store excess energy as fatty acid triacylglycerides (TAGs) in lipid bodies, which can be liberated and converted to their methyl ester derivatives via transesterification to synthesize biodiesel. Last discussed in this *Journal* in 1985 to demonstrate the isolation of natural products,[7] algae have recently attracted wide interest due to their potential as a biofuel feedstock. As new algal technology emerges, education will be necessary to sustain the growing industry.[8]

The production of algae biodiesel can be used to visualize chemical and biological concepts and techniques in a classroom setting. This laboratory aims to expose students to important facets of algal biofuel technology. First, students are introduced to algae as an energy feedstock and taught how to cultivate and harvest algal biomass. Next, students learn how to convert algal biomass to a transportation fuel using transesterification and extraction. Finally, the energy content of homemade algae fuel is determined using calorimetry.

■ EXPERIMENTAL OVERVIEW

This laboratory was conducted over a semester in an introductory precollege science course. The first part of the lab involved the culturing and scale-up of model algal species *Chlamydomonas reinhardtii* over a 6 week period, followed by harvesting algal biomass (Figure 1). The second part of the

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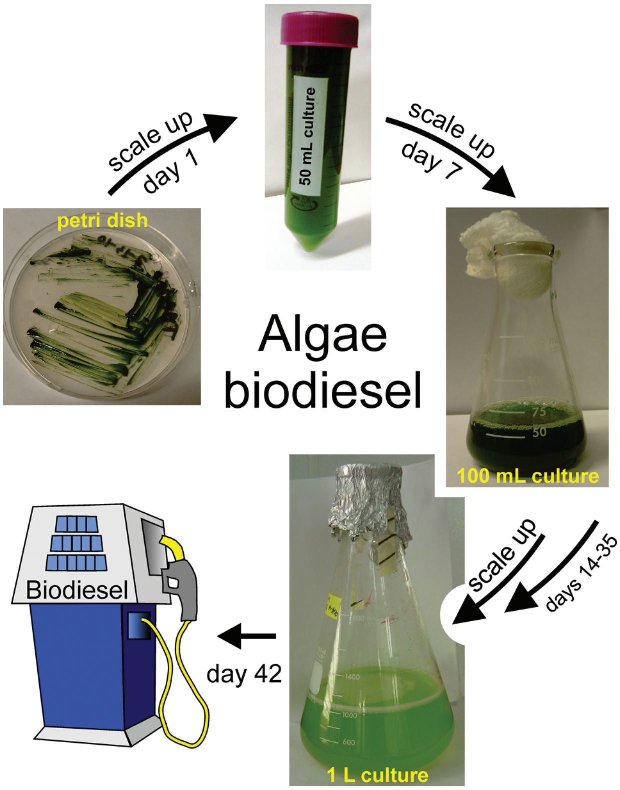

Figure 1. Timeline of algal growth. Algal biomass was cultivated over a 6 week period, beginning from a single algal colony on a TAP-agar plate (day 1). Students monitored the algal culture daily, and when cells were grown to high density, they were used to inoculate a larger volume of TAP media. The process continued until students had obtained a culture volume of 1 L. Centrifugation yielded a concentrated algal biomass pellet. Acid-catalyzed esterification converted algal lipids to biodiesel.

laboratory involved the synthesis, characterization, and utilization of algae biodiesel. Because industrial-scale biodiesel production utilizes base catalysis due to rapid reaction rates and high yields, base-catalyzed transesterification of soybean oil was carried out and compared to a recently developed acid-catalyzed method that converts lipids within algal biomass to biodiesel.[9] Students quantified the amount of biodiesel obtained from algae and compared it to that prepared from soybean oil, a plant-based feedstock. Energy content of each fuel was measured using calorimetry and compared to petroleum diesel. Finally, students were able to observe the efficacy of their homemade algae biodiesel, combusting it to power a diesel engine.

■ EXPERIMENTAL DETAILS

Algal Culturing, Timeline, and Harvesting of Biomass

C. reinhardtii strain csc137c was grown initially on an agar plate enriched with tris−acetate−phosphate media (TAP, see the Supporting Information).[10] A sterile 50 mL liquid TAP starter culture was inoculated from a single, robust algal colony. After 7 days of agitation under constant white light illumination, the algal culture was used to inoculate a flask containing a larger volume of media. Cells were inoculated every 7 days or when the culture became a dark green color. This process was repeated until a 1 L culture was obtained (Figure 1). When 1 L of a dense, dark green *C. reinhardtii* culture was observed, algal biomass was harvested via centrifugation. The algal paste was transferred to a 50 mL conical tube and stored at −20 °C.

Acid-Catalyzed Transesterification of Algal Biomass

To convert algal lipids to their methyl ester derivatives,[9] 10 mL of a 1 M methanolic HCl solution was added to the dry algal biomass. The algae cell pellet was resuspended to homogeneity in the methanolic acid solution and the mixture incubated at 65 °C for 30 min (Figure 2).

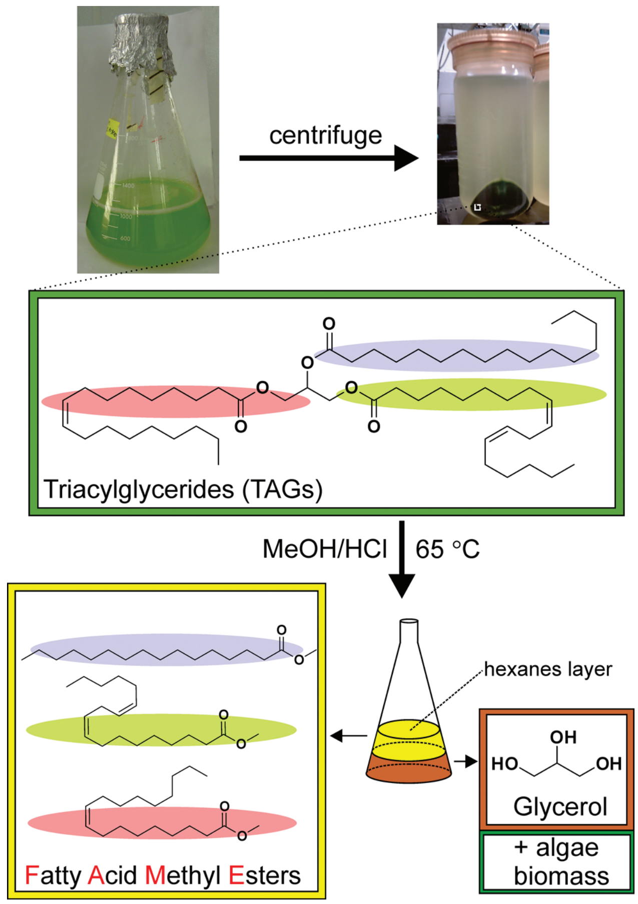

Figure 2. Biodiesel from algal lipids. After harvesting algal biomass, acid-catalyzed transesterification was performed directly on concentrated algal biomass to convert triacylglycerides to their methyl ester derivatives (biodiesel). Fatty acid methyl esters were extracted from the mixture with hexanes to afford biodiesel.

Liquid−Liquid Extraction

Fatty acid methyl esters were extracted from the mixture using hexanes. Hexanes, 2 mL, were added directly to the reaction vessel immediately upon cooling and the mixture was vortexed or shaken vigorously for 1 min. To break emulsions, a 1 min centrifugation step was carried out. The top organic layer was removed and transferred to a preweighed vial. The hexane extraction was repeated 6 times, each with 2 mL of hexanes. The hexane extract was analyzed for fatty acid methyl ester content and composition by GCMS (see the Supporting Information, Figure SI-1).

Base-Catalyzed Transesterification of Soybean Oil and Soybean Biomass

To compare methods and feedstocks, base-catalyzed transesterification of soybean oil was performed.[11−16] The temperature of the stir−hotplate was raised to 65 °C and 5 mL of soybean oil was added to a flask containing 5 mL of a sodium methoxide solution. The reaction proceeded for 30 min, stirring

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magnetically at 65 °C. The biodiesel was washed with distilled water to remove all basic residue.

In parallel, base-catalyzed transesterification was carried out directly on soybean biomass as a more equivalent comparison to algae biomass. Soybean biomass, 2 g, was added to a 50 mL conical tube and resuspended in 5 mL sodium methoxide. The reaction was placed in a heat bath set to 65 °C for 30 min. Biodiesel was extracted from the mixture using 12 mL of hexanes (6 × 2 mL extractions).

Calorimetry

A calorimeter was built from simple materials[17] to measure the energy density of three fuels: algal biodiesel, plant biodiesel, and petroleum diesel. The temperature change of 100 mL of water upon heating by burning 50 drops of diesel on a cotton ball is used as an indication of the energy density (details are available in the Supporting Information). In addition, the length of time that each fuel burned in an ethanol lamp was recorded (Table 1).

Table 1. Energy Density Comparison of Algal Biodiesel, Plant Biodiesel, and Diesel[a]

Biodiesel Source	Biodiesel Yield from 2 g Biomass (%)	Time Fuel Burned in Lamp (min)	Temp Change/ °C[b]	Heat Released kJ[c]
Algae	5	19	12 ± 3	5.0
Soybean (oil)	12	22	13 ± 3	5.4
Soybean (biomass)	5	20	13 ± 3	4.6
Diesel	NA	21	16 ± 3	6.7

[a]Data from 50 students. [b]Final water temperature − initial water temperature in the calorimeter. [c]$q_v = C \times dT \times m$, where q_v is the heat flow, or change in internal energy (J); C is the heat capacity of water, 4.18 J/(g °C); dT is the temperature change, $T_f - T_i$ (°C); m is the mass of water (g).

Viscosity

The viscosity of each biodiesel fuel was measured and compared to the oil from which it was synthesized using a Pasteur pipet and bulb as a makeshift viscometer (Table 2). The time each fuel drained from the pipet was measured and recorded.

Table 2. Viscosity Measurements of Synthesized Biodiesel Fuels and Standards

Fuel or Oil	Viscosity/(min:s)[a,b]
Motor oil	6:21
Soybean oil	3:21
Soybean biodiesel (oil)	0:26
Soybean biodiesel (biomass)	0:03
Algae biodiesel	0:03
Standard biodiesel	0:13
Diesel	0:11

[a]Data from 50 students. [b]Time it took for fuel to drain from pipet.

Gas Chromatography−Mass Spectrometry (GCMS)

The composition of each fuel was determined using GCMS. Soybean and algae biodiesel were compared to commercially available B-20 biodiesel.

Smell Test

A smell test was conducted on algae biodiesel, soybean biodiesel, and diesel. The preferred smell was reported by each student (see the Supporting Information, Table SI-2).

HAZARDS

Safety goggles, gloves, and laboratory coats should be worn at all times. Methanolic acid and base solutions are corrosive and contact with skin should be avoided. Hexanes, methanol, and ethanol are extremely flammable. Do not swallow or inhale any of the chemicals. Sodium hydroxide is caustic. Handle all substances with care.

RESULTS

During the first part of this laboratory, students grew and cultivated algae biomass over a 6 week period beginning from a single colony of model algae *C. reinhardtii*. This required daily monitoring in which students took detailed notes and observations about the color of the algae, the temperature, and the light source. Students made sure the cells were agitated, taking turns to shake the flasks each day. At the end of 6 weeks, students had acquired a substantial amount of algal biomass, approximately 2 g/L of culture. Using a homemade centrifuge from a salad spinner (see the Supporting Information), students harvested 1 L of algae biomass via centrifugation and prepared it for a one-step conversion to biodiesel.

The second part of this laboratory focused on the synthesis and characterization of biodiesel from algal biomass. Direct acid-catalyzed transesterification of 2 g of algae biomass yielded 100 mg of biodiesel. Base-catalyzed transesterification of soybean oil and soybean biomass afforded 12% and 6% soybean biodiesel, respectively. Students built a calorimeter, burned each fuel, and measured heat released (Table 1). Both biodiesel from algae and soybean biomass gave comparable energy changes to that of conventional diesel. Compared to petroleum diesel, biodiesel burned cleaner (i.e., less thick black smoke was produced), longer, and yielded more heat energy. In the qualitative energy density test, each fuel was burned in an ethanol lamp and the length of time the wick stayed lit was measured (Table 1).

Viscosity measurements and GCMS analysis confirmed that biodiesel was synthesized from soybean oil, soybean biomass, and algae biomass (Table 2 and Table SI-1 in the Supporting Information).

The response from students during the smell test was that the algae fuel smelled like plants and chemicals, soybean biodiesel smelled like French fries, and diesel smelled like chemicals (Table SI-2 in the Supporting Information). Algae biodiesel was the preferred smell of all fuels.

DISCUSSION

This laboratory introduces students to the current research of algal biofuel technology and teaches basic chemical and biological techniques involved.[18] This series of experiments is unique and powerful in that students are able to observe and play an active role in the entire flow of energy, emphasizing the law of conservation of energy. Students are trained in the art of culturing algae, as solar energy is harvested by algae via photosynthesis. Students learn the chemical process by which triacylglycerides are converted into biodiesel as they extract the chemical energy stored within the algal biomass through acid-catalyzed transesterification. At the end of the project, students can use the algal fuel to power a small propeller equipped with a diesel engine, demonstrating the conversion of chemical energy to mechanical energy.

Biodiesel from soybean is commercially produced using catalytic base because it is faster than the acid-catalyzed pathway, requires lower temperatures and pressures, and is the most economical process to treat vegetable oils.[19] However, base-catalysis produces soap as a side reaction, reducing the overall yield of biodiesel and complicating the subsequent purification process.[20] Soap formation can be avoided using strong acid catalysts to convert free fatty acids into their corresponding methyl esters through acid-mediated esterification. It has recently been shown that fatty acid methyl esters can be recovered in high yields through direct acid-catalyzed methanolysis of dried microbial biomass.[9] Therefore, traditional base-catalyzed methodology was used to synthesize biodiesel from soybean oil and the more recent direct methanolysis procedure was conducted on algae biomass. By demonstrating both acid- and base-catalyzed transesterification, students realize that the same biodiesel product can be obtained through two different chemical pathways (Schemes 1 and 2 in the Supporting Information).

Commercial production of algal biodiesel is in its infancy. Following hexane extraction and evaporation, algal biodiesel can be mixed with petroleum diesel, such as in current B-20 biodiesel blends, or it can be deoxygenated to produce conventional diesel in a process called hydrotreatment. Algal biodiesel can also be used as a feedstock for traditional petroleum refineries, allowing it to be easily incorporated into the existing infrastructure.[21]

■ CONCLUSION

Exposing the next generation of scientists and citizens to renewable energy is critical, especially when they are developing basic inquiry and observation skills. Following completion of this laboratory, students acquire an appreciation of algae biodiesel, develop awareness about current renewable energy issues, and are exposed to future energy prospects. Throughout this exercise, students are provided with many opportunities to formulate new ideas about the concept of energy. These experiments evoke scientific inquiry, foster the development of laboratory skills, and elicit a novel perspective about the energy challenges we face in the future.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

A student handout; an instructor protocol and notes; an instructional Powerpoint presentation (in PDF format); full list of materials; protocol for base-catalyzed preparation of biodiesel; protocol for direct acid-catalyzed transesterification of biodiesel from algal biomass; calorimetry design and protocol; salad spinner centrifuge design; TAP media recipe and algae culture conditions. This material is available via the Internet at http://pubs.acs.org.

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