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Harvesting, oil extraction, and conversion of local filamentous algae growing in wastewater into biodiesel

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Abstract
Algae are known to be a potential feedstock in the production of biodiesel fuel. Although much of the focus has been on microalgal species, macroalgae are also suitable as a source of lipids. In this study, a locally abundant (central Illinois) filamentous algae has been harvested from a water treatment plant; dried to about 10% of its initial weight; pulverized in a hammermill; and treated with methanol to extract the oil. The algae are a combination of several coexisting species including *Cladophora* sp. and *Rhizoclonium.* Oil yields ranged from 3% to 6%, by weight, of the dried mass. This oil was reacted by transesterification to yield fatty acid methyl esters (biodiesel fuel) with an overall mass conversion efficiency of 68%. A B5 blend of this algal biodiesel and petrodiesel was run in a 13.4-kW test engine. Measurements indicated similar performance compared to pure petrodiesel in terms of fuel efficiency and carbon dioxide and carbon monoxide exhaust emissions. Significantly, there was a 22% reduction in nitrogen oxides when using the B5 fuel. It has been demonstrated that filamentous macroalgae may be cultivated as biodiesel feedstock and have inherent advantages such as an ability to remove phosphorus and nitrogen compounds from wastewater, simplicity of harvesting, and natural resistance to local aquatic grazers and competing organisms.

Keywords: Biodiesel; Filamentous algae; Lipids; Macroalgae; Wastewater.

1. Introduction

Biofuels such as ethanol have long been seen as an alternative to the conventional petro-based fuels. Recently, biodiesel has become viable as a fuel for transportation applications. However, the current use in the United States of virgin soybean oil is a major drawback due to the diversion of soybeans from the food supply. As an alternative biomass source for fuel, algae are currently being extensively studied. Algae-based biofuels may be particularly suited for the development of small-scale production facilities to provide the energy needs of rural communities or populations residing in areas that are not located near large agricultural regions. More specifically, biodiesel can be produced from algae, freeing the soybean crop for the food market.

While most research is focused on microalgae, macroalgae are also an important source of feedstocks for biofuels. Freshwater filamentous algae are ubiquitous globally, and certain species grow prolifically in wastewater, which minimizes costs of providing nutrients. In addition, they have potential as a means of

treating wastewater and reducing discharge of excess phosphate and nitrate into waterways. The green filamentous algae used in this study are from the genera *Cladophora* and *Rhizoclonium* and include some epiphytic microalgae and diatoms. Such organisms have already been examined for their ability to produce biofuel.

The current study can be divided into four primary sections: (i) the collection of filamentous algae indigenous to the Rockford, Illinois, USA, area, (ii) the development of a viable lipid extraction method for obtaining oil from algal cells and analyzing its quality, (iii) increasing the quantity of algal oil to generate a reasonable amount of biodiesel fuel, and (iv) conversion of algal oil into a usable fuel.

2. Collection of filamentous oil-producing algae

The Rock River Water Reclamation District (RRWRD) in Rockford, Illinois, USA (42.225N, 89.096W) is a water treatment plant and was identified as a source for abundant filamentous algae. The algae grow submerged in multiple drainage channels (launders) leading away from effluent final settling tanks; water from the launders is directly discharged into the Rock River, or, depending on the season, chlorinated, dechlorinated, and then discharged. Figure 1 is a photograph of the algae growing in the launders. The algae are a combination of several coexisting species including *Cladophora* sp. and *Rhizoclonium*. There are also some epiphytic species of diatoms and free microalgae which are a minor part of the harvested biomass. The total algae serve a useful purpose as scavengers of particulates as well as phosphorus and nitrogen compounds, but when present in great abundance, they can slough off and elevate levels of particulates discharged in the final effluent.

Figure 1. Filamentous algae growing in water treatment plant launders

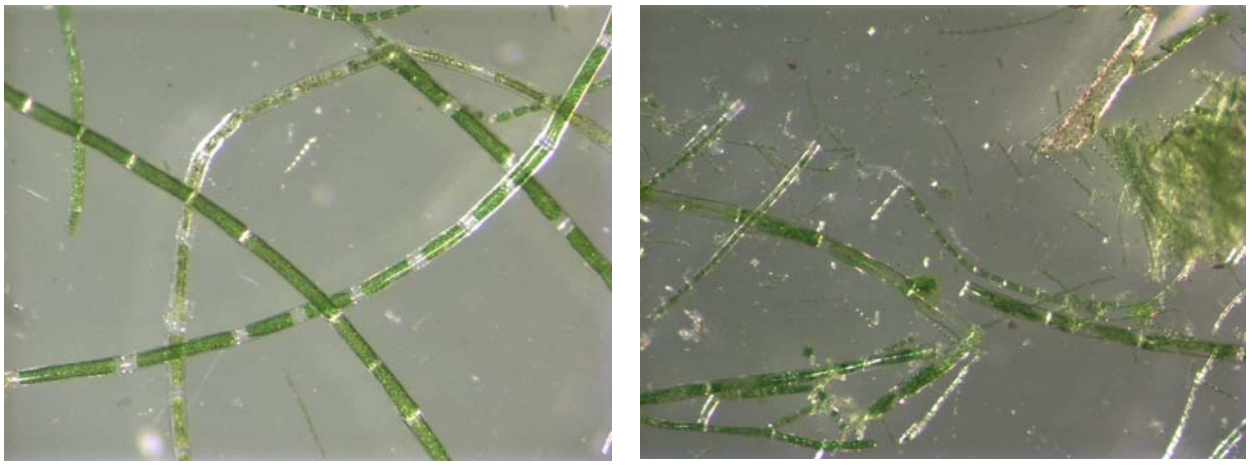
Harvesting the algae involved collection from the tertiary treatment facility using rakes. Algae growth rates vary seasonally in Illinois, with peak productivity in June, July, and August. For example, the filamentous algae yield from the surface of the RRWRD launders during the month of June (2011) showed the rate of production in wastewater to be 8.5 kg/m^2. This approximates to 0.85 kg of dry weight over that four-week period.

Mats of the algae were compressed to remove excess water, and then air-dried outdoors on racks (Figure 2) for several days until water content was less than 10% by weight. (The freshly-harvested algae are typically degraded by bacteria and fungi in less than a week if they are not dried, or stabilized by autoclaving.) The drying racks are made of wooden frames with 12-mm metal screens at the bottom. Algae were further dried to near zero moisture outdoors or in an oven at 60 ^{o}C.

Figure 2. Harvested filamentous algae spread on wooden racks for drying in the open air

3. Oil extraction from filamentous algae

Processing of filamentous algae for oil consisted of drying, powdering, solvent extraction, and solvent recovery for reuse. Since algal filaments contain many cells joined together by tough cellulose walls, the efficacy of mechanical cell disruption and breakage of the filaments was examined by different small-scale experiments: (i) freshly harvested, air-dried, and autoclaved filaments were extracted in either acetone or 1:1 (v:v) chloroform:methanol solvents for 24 hours, or (ii) were first disrupted in a Sorval blender, and then ground to a fine powder with a mortar and pestle in liquid nitrogen. Figure 3 is a micrograph of the algal cells showing the effects of pulverizing the frozen filaments.



(a) Intact filamentous algae (b) Algal filaments pulverized in liquid nitrogen

Figure 3. 20X micrographs of filamentous algae processed by grinding in liquid nitrogen

On a larger scale, dry algal filaments were hammer-milled (Model PPH130, Pellet Pros, Inc., Davenport, Iowa, USA) into a powder which consisted of some filaments up to 10 mm long, with most of the powder consisting of particles that were 1-2 mm long; the result is shown in Figure 4. The powdering step increased the surface area of algae available for oil extraction.

After this, bulk extractions with methanol were employed using a 1:3 (w:v) ratio; typically 2 kg of the powdered algae was extracted in six liters of methanol. After two days at room temperature the extract was first filtered through a Buchner funnel covered with nylon screen (approximately 1 mm mesh size). The pulp and nylon screen were then placed on three layers of metal screens (5-mm mesh) mounted near the center of a steel cylinder. A metal disc was screwed down to compress the pulp, and additional methanol extract was collected beneath the screens. After the extract was filtered, oil was concentrated with a rotary evaporator (IKA Model RV10, Wilmington, South Carolina, USA). The recovered methanol was used for additional extractions.

Figure 4. Powdered filamentous algae after hammermill processing

As a consequence of membrane breakdown, chlorophyll pigment was released from the algal cells, and was used as an indication of the extent of solvent-associated cell breakage [1]. An absorption spectrum for chlorophyll was established by reading algal chlorophyll samples on a spectrophotometer (Ultrospec III, GE Healthcare, Bucks, England, UK) between the wavelengths of 400 and 700 nm in increments of 10 nm. The peak absorption of light in the red portion of the spectrum was between 650 and 680 nm, and light at 660 nm was selected as the wavelength to monitor chlorophyll pigment extraction (Figure 5).

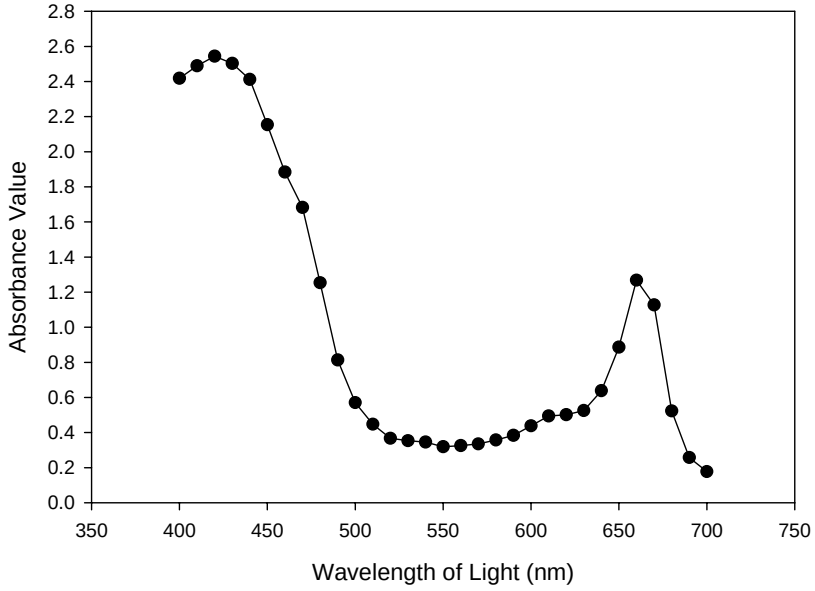


Figure 5. Chlorophyll absorption spectrum for methanol extracts of filamentous algae

Subsequent analysis of the extract was performed by thin layer chromatography (TLC) on 20 cm x 20 cm silica gel TLC plates developed in a 9:1 (v:v) hexane:acetone solvent. The separated lipids including triglycerides were visualized by exposing the plates for 15 minutes in a sealed chamber containing vapor from 8 grams of solid iodine [2].

The time required to solubilize maximal levels of lipids from filamentous algae during extraction by methanol was examined by monitoring the yield of chlorophyll after four successive solvent incubations over 172 hours at room temperature. Much of the methanol extract was retained in the algal pulp and needed pressing and filtration for recovery, as already described. The extraction required over 100 hours to release most of the chlorophyll. Subsequent successive extractions with fresh methanol solvent increased the chlorophyll levels, and oil retrieved from the algal filaments represented between 3% and 6% of the initial dried weight. Analysis of methanol extracts by TLC showed varying proportions of free fatty acids and triglycerides present in the extracts. The triglyceride fraction is the most important for subsequent processing to biodiesel.

4. Conversion of algal oils to biodiesel fuel

For the synthesis of biodiesel from algae oil, transesterification is the chemical process converting fatty acids to fatty acid methyl esters (FAMEs). This reaction targets triacylglycerols; specifically the three fatty acid chains attached to a glycerol backbone. Upon introduction of the base catalyst, fatty acids are removed, producing FAMEs. Figure 6 summarizes the chemical reaction utilizing sodium methoxide to react with the fatty acids at 60 ^{o}C.

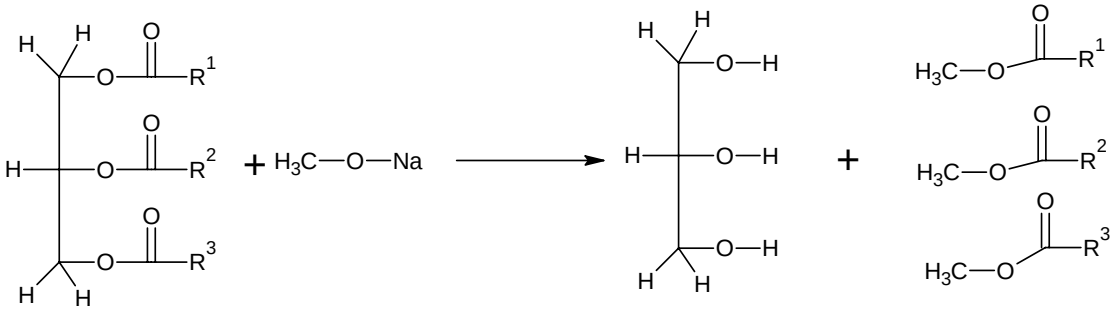


Figure 6. Fatty acids converted to fatty acid methyl esters via a transesterification reaction

The sodium methoxide dissociates into alkoxide and sodium ions. The latter have a role in the reaction as a protonated catalyst and the alkoxide performs a nucleophilic attack on the carbonyl group of the triglyceride making an alkyl ester bond and an anion of diglyceride. The diglyceride anion deprotonates the Na^+ ion and further reactions continue until all three fatty acid chains of the triglyceride have been converted to FAMEs. The glycerol backbone remains. Depending on the number of hydrocarbon tails attached to the glycerol group, between one and three FAMEs can be obtained. These FAMEs may have hydrocarbon chains of the same length, and typically have either saturated C-C bonds, or one to three unsaturated C=C bonds. The by-product glycerol is still suspended in the mixture and centrifugation is used to collect it. The supernatant is now considered biodiesel and can be used as a source of fuel.

The crude algal oil was combined with a mixture of methanol and NaOH (concentration of 1.0 M) at a ratio of 10:56 (w:w), placed in 125-mL flasks, which were heated in a shaking water bath (Model 2876, Thermo Fisher Scientific Inc., Waltham, Massachusetts, USA) at 60 ^{o}C for 90 min to conduct the transesterification reaction. Following the reaction, samples were centrifuged (Model CL2, Thermo Fisher Scientific Inc., Waltham, Massachusetts, USA) for 10 min at 4000 rpm, and the supernatant was run through filter paper (Grade 1, 11 μm, Whatman, GE Healthcare, Piscataway, New Jersey, USA) under vacuum. Liquid samples were then pooled into separatory funnels, small quantities of water were added, and then glycerol and unreacted components were removed by draining via density separation.

The resulting biodiesel had a density of 873.8 g/L, which was slightly lower than the typical biodiesel density of 880 g/L. To investigate why this may have occurred, gas chromatography was used to quantify the fatty acid composition of the biodiesel. Less than 1% of the finished product was composed of free fatty acids. Thus, it appears that the resulting density may have been due to unreacted methanol and NaOH which had not been completely removed during the separation process. In terms of overall efficiency, considering both the algal oil as well as the chemical reactants used, approximately 68% of the input algae oil was converted into finished biodiesel.

5. Biodiesel engine testing

Tests were conducted using a blend of standard #2 petrodiesel and the algal biodiesel. A separate series of tests using pure petrodiesel was run to establish baselines. The petrodiesel was obtained from a fuel distributor (SPEX CertiPrep, Inc., Metuchen, New Jersey, USA) and certified to be 100% free of any biobased content. A B5 algal fuel was prepared by splash blending. All runs were conducted at full loading and 2500 rpm with a 13.4-kW, inline, three-cylinder, direct-injection diesel engine. To load the engine, a dynamometer is coupled to the rotating shaft of the flywheel, and water flows through to act as the engine load.

The engine is instrumented for performance and exhaust emissions measurement: CO_2, O_2, CO, unburned hydrocarbons (HC), and NO_x. The system consists of a probe that is inserted into the exhaust pipe and connected to a gas analyzer. For biodiesel fuels, the focus is on NO_x, the combination of nitric oxide (NO) and nitrogen dioxide (NO_2), which often is greater. Additionally, CO and hydrocarbons

present in the exhaust represent incomplete combustion and/or excess fuel in the cylinders. To run a test, the engine was started, run at idle under full load for approximately 10 minutes, and shut off to weigh the fuel tank. The tank was quickly reinstalled, the engine was restarted, set to 2500 rpm, and three sets of data were taken, each at a 10-minute interval. The data were averaged over the three sets. Fuel consumption was measured by weighing the fuel tank after the 30-minute period.
In terms of fuel consumption, the B5 blend was slightly less efficient, consuming 7% more mass of fuel. The CO_2 and O_2 emissions showed no variation, within experimental uncertainty, while CO was negligible for both fuels. Elevated unburned hydrocarbons indicate inefficiencies in the combustion process. The B5 yielded significantly less hydrocarbons (7.0 ppm vs. 9.6 ppm) compared to the petrodiesel standard. Additionally there was a reduction in exhaust emissions of NO_x from 209 ppm to 162 ppm for the petrodiesel and algal B5, respectively. Significant decreases in NO_x are findings consistent with other algae-fuel testing [3].

6. Concluding remarks

The potential production of filamentous algae has been reported to be very high, with net specific growth rates of 0.6 per day, meaning a 60% increase in dry biomass per day [4]. As algal species utilized for oil extraction, filamentous algae typically contain lower proportions of neutral lipids than microalgae. For example *Rhizoclonium* sp. were found to contain 11.5% total lipids of their dry weight, of which neutral lipids represented 58% of the total; neutral lipid proportions (used for biodiesel conversion) comprised monoacylglycerol 22%; diacylglycerol 25%; triacylglyccerol 7% [5]. For *Cladophora fracta*, Demirbas [6] has reported lipid composition of up to 14% of dry weight extracted using hexane solvent; the lipids were converted to biodiesel by transesterification using a supercritical methanol process. Another approach utilized pyrolysis to release oils from *Cladophora fracta* with the yield increasing at higher temperatures [7].
Aside from their occurrence in wastewater, filamentous algae (*Cladophora* species) grow aggressively in freshwater lakes and rivers to the point of being "nuisance species" [8]. Harvesting of these algae from shoreline areas of lakes (e.g., Lake Michigan, USA) could be an option for additional sources of biofuel feedstocks. The benefits of using filamentous macroalgae as feedstocks include: (i) simplicity of harvesting by scraping or screen filtration; (ii) their potential to grow in wastewater with the added benefit of water treatment; (iii) they are a naturally occurring species community, not out-competed by invasive species, as can be a problem with microalgal growth operations; and (iv) once oil has been extracted, the filamentous algae can serve as a source for cellulosic ethanol production.

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