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Cultivating, harvesting and processing microalgae for biodiesel production

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Abstract. In recent years, sustainable aviation fuels (SAF) have been gaining popularity as an alternative to conventional jet fuel due to their smaller carbon footprint. It has been estimated that their use will result in a 65% reduction in CO_2 emissions. SAF can be produced by using waste oil, animal fat, plant-based oil or municipal waste. One promising feedstock for SAF production is alga as it grows quickly under the right conditions and may contain 50% oil. In this **student project**, algae species were reviewed, and one was chosen based on its growth speed and lipid-producing characteristics. A photobioreactor was designed and built. The algae grown were monitored and harvested. Due to the short duration of this project and difficulties in extracting the algae and a malfunctioning ultrasonic bath, the oil extracted was not sufficient for conversion into biodiesel. The heat of combustion of the produced fuel from used cooking oil was measured. The lessons learned are discussed.

Key words. Photobioreactor, Biodiesel, Microalgae

1. Introduction

The number of microalga species is estimated to be about 800,000, over 40,000 of which are currently known. They range in size from 5 to 200 μm. Among other applications, microalgae are being researched and developed for CO_2 fixation, cosmetics, pharmaceuticals, nutraceuticals, and nutrient removal from wastewater [1],[2].

Many microalga species are being studied and genetically modified for biodiesel production, primarily due to the high rate of lipid production from microalgae (third generation feedstock) compared to second- and first-generation feedstocks. Additional benefits of microalga feedstocks versus traditional feedstocks are that microalgae use less water, can be cultivated on non-arable land and in seawater, wastewater, and freshwater, do not require herbicides or pesticides, are relatively easy to biochemically modify, and can easily be utilized for CO_2 fixation, biodiesel production, and ethanol production [3],[4].

Biodiesel production is a relatively simple process. It provides sustainable fuel to be used for any application currently using traditional diesel fuels such as jet engines. Biodiesel starts off as oil collected primarily from plant matter. To produce large amounts of biodiesel, large algae bioreactors have been constructed. Depending on the species used, the yield can be 15-77% in oil from the dried algae mass. This makes alga an efficient feedstock for biodiesel production. Once the oil has been extracted, it is combined with methyl alcohol using lye as a catalyst.

This article reports the details of a student design project with the objectives of growing algae in a bioreactor, extracting the alga oil, converting the oil to biodiesel fuel and measuring its heat of combustion.

2. Microalgae Species Selection

There are many different species of alga with different attributes. For this student project, the amount of oil produced, cost, and ease of growing were of priority. Two alga species were considered: *Chlorella* and *Nannochloropsis Oculata*. *Nannochloropsis Oculata* is both a fresh water and saltwater alga. Two vials of each, *Chlorella* and *Nannochloropsis Oculata*, were ordered online.

Both species can produce the oil needed for making biodiesel. However, *Nannochloropsis Oculata* is one of the best species in terms of oil to dried weight ratio (31-68%) whereas *Chlorella* contains 28-32% of its weight as oil [5]. The reason for selecting the *Chlorella* was because it is very common and has moderate growth and oil production. The *Nannochloropsis Oculata* was selected because of its high oil to weight ratio. The *Nannochloropsis Oculata's* main drawback is that it is not solely a freshwater species and requires more of a catered environment than *Chlorella*. *Nannochloropsis Oculata* was less expensive than the *Chlorella, but* the cost savings was only USD 4; because

the cost savings were so low, cost did not play a role in the algae species selection. The combination of a higher performance alga and one that is very common allowed for the selection to happen after they were tested in the photobioreactor. The final choice, *Chlorella*, was based on its performance over *Nannochloropsis Oculata* in the trial period in which it responded much better to the added nutrients.

3. Bioreactor System Design

In large scale algae production, two major types of system design are used: closed system and open system. In an open system, an alga culture is placed in large pools of around 30-cm-deep water and the culture is left open to the atmosphere to absorb sunlight and perform photosynthesis. These pools are often gently stirred in large "racetrack" style grooves to facilitate balanced exposure to sunlight. There are also closed system designs (photobioreactors) in which algae are pumped from a central tank into transparent pipes or plates/bags where the surface area of algae exposed to sunlight is much greater and factors such as nutrients and pH levels are much easier to control.

Due to this higher level of control, a closed system was utilized for this project. The main components included a tank for adding nutrients and housing the bulk of the biomass, a solar absorption array which consisted of sixteen 3-foot-long (91-cm-long) pipes of 2-inch (5-cm) diameter to increase the surface area of algae exposed to solar rays, a water delivery and recovery system from the solar absorption array, and a frame/base to hold these components in place.

The tank used held about 10 gallons (38 liters) of water/algae solution, while the array of transparent pipes held about 8 gallons (30 liters), The water recovery system from the pipe array was custom designed to also include a gravity aeration fitting which introduced oxygen into the central tank as the water fell back into it from the solar absorption array, via the Venturi effect. This component also served to improve the ease of filling the system with water, for air bubble elimination within the tubes, and the potential for air filtration with a half 2-liter bottle attached to it as seen in the upper-left-hand corner of Fig. 1. In addition, a flow-limiting ball valve was added to the exit of the solar array to be able to prevent the water solution from draining too quickly in the event of too little flow from the pump.

This design allowed for easy addition of nutrients either into the 2-liter bottle funnel or directly into the lower tank. This tank had a clear acrylic sheet cover to better control what would enter the system and to reduce water losses due to evaporation. The pump was able to run 24 hours a day for four weeks during the experiment without any problems, and a waterproof covering was provided for its plug end. This plug ran off extension cord power from a nearby building. However, it is entirely possible to run this pump off solar power provided by a photovoltaic (PV) panel and energy storage system such as a battery. The extension cord was used as a simple and effective means to begin the experiment and to confirm its effectiveness at growing algae.

Fig. 1. Photobioreactor with algae

The frame was made of treated lumber and scrap wood for strength and simplicity. The fittings which connect the tubes of the array together, and to adapt the clear pipes to the smaller tubing for the pump, were 3D-printed in PETG. These fittings were 3D-printed due to the lack of compatible standard fittings with a 2-inch (5-cm) inner diameter required to fit onto the transparent pipes of the upper array. The material PETG was selected due to its toughness and strength in addition to its UV resistance compared to PLA plastic. This complicated and extended the assembly process as the parts took time to print. Extra time was required for post-processing and sealing efforts to provide a connection that would not leak. Silicone sealant and Flex-Seal branded rubber spray were used to completely seal the printed parts. This allowed for the final assembly to have no leaks.

4. Cultivating Microalgae

Within days of receiving the algae, they were placed in open systems; the *Chlorella* was placed in a 10-gallon (38-liter) fish tank with a small aerator and the *Nannochloropsis Oculata* was placed in a 5-gallon (19-liter) bucket with an underwater pump for agitation. These were temporary systems to begin algae growth while the closed system photobioreactor was being constructed.

There was a quarter of the algae left in each of their respective vials which were kept indoors as backup cultures. Since two vials of each species were ordered, there was a side experiment ran regarding nutrients. One vial of *Chlorella* received some liquid aquarium nutrients (an amount in line with the nutrient instructions) while the other vial did not receive any nutrients. Both vials were placed indoors by a window and periodically shaken for agitation. This was repeated with the *Nannochloropsis*

Oculata. The *Chlorella* seemed to enjoy the nutrients, slightly increasing in density compared to the vial without nutrients. However, it did not increase as much as originally hypothesized. The *Nannochloropsis Oculata* did not do well with the nutrients; it died after about a week. The vial without nutrients slightly decreased in density over this same period. This could be related to the nutrients being formulated for freshwater species while the *Nannochloropsis Oculata* is a freshwater/saltwater species.

While in the temporary open systems, the algae were closely monitored for growth (and degrowth). Nutrients were also added on an irregular basis; 0.5 mL was added every few days for a total of about 5 mL of nutrients for each of the systems for 2.5 weeks. After about 2.5 weeks, the *Chlorella* had visually increased in density while the *Nannochloropsis Oculata* had dramatically decreased in density; only a few small clumps remained. This decline is attributed to either too much nutrients, too much agitation, and/or too little sunlight (only 4-6 inches of water was in the opaque bucket).

After 2.5 weeks, the *Chlorella* was chosen to be placed into the completed closed system (photobioreactor) and the *Nannochloropsis Oculata* was disposed of. Once up and running, the system was largely left alone. 1-2 mL of nutrients were added irregularly, deionized water was added as needed (quite frequently until a sheet of clear plastic material was added to the top of the tank to reduce evaporation), and the whole system was aggressively shaken periodically to provide more agitation and loosen algae which had become stuck on the inside of the transparent tubes. About one gallon of pond algae was added one week before the algae were harvested. This addition was intended to stimulate more growth and ensure that there were sufficient algae to harvest for oil collection.

5. Dewatering and Harvesting Algae Oil

The process for harvesting algae involved running the algae through the system and using a filter bag for collection. This process extracted all the large alga colonies and some of the smaller ones. While using the filter bag, algae were scraped from the sides of the tank to be collected. The process of harvesting the algae took place after one month of growth in the closed system and two weeks of growth in an open system. The algae were restricting the flow of the pump because of buildup, hence a reasonable time to harvest.

This harvesting process was chosen as it would allow for extraction of larger colonies without removing all of the algae from the system. Removing all of the algae would mean future algae growth would need to start from another sample being added. Also, the filter bag extraction method was chosen as the bag could be put over the exit hose and then taken out and emptied while the system was running. The system also does not need to be drained to harvest in this fashion which is beneficial for the continued production of algae. Because the algae extraction method does not interrupt the growth of algae that is not harvested, the harvest can be performed at any time.

The extraction of oil from the harvested algae was done through two methods: Blending and Grinding. Both methods then relied upon the oil settling to the top of the algae sample diluted with deionized water. The blending process was done by placing algae in a kitchen blender for several minutes until the algae appeared to be of fine consistency. Grinding the algae was done by using a mortar and pestle with a damp algae sample. Neither of these methods worked well to break the algae's cells. After letting the sample settle for over 24 hours, there was too little oil separated out to continue the biodiesel production process.

These methods were used because the original extraction method, an ultrasonic bath [6], was not available due to malfunctioning lab equipment. The ultrasonic bath was initially chosen because its frequency would cause cavitation bubbles to be produced in the water/algae solution. Algae would be broken evenly and would not require drying beforehand. The ultrasonic bath would also have allowed the oil to separate from the water, reducing settling time. Breaking the cells through blending and grinding posed as the next-best methods to continue processing the alga samples, particularly since the biomass was not dried.

6. Biodiesel Production

The biodiesel was produced in a lab environment, taking appropriate precautions to ensure safety around the chemicals and equipment [7]. The production process was done under a fume hood to ensure any dangerous fumes were extracted from the work area (Fig. 2). As an alternative, and due to the absence of extracted algae oil, 200 mL of used cooking oil was substituted. 200 mL was used based upon the best estimate of oil that would have been extracted with an ultrasonic bath from the algae harvested. The process of converting biological oils to a viable fuel source is known as transesterification; the following ingredients were required to perform this chemical reaction, in addition to the 200 mL of oil.

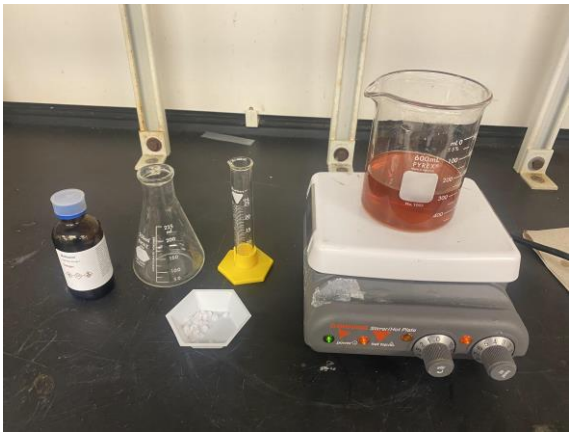

Fig. 2. Biodiesel production components

4.024 g of sodium hydroxide (lye) was dissolved into 50 mL of methanol using a magnetic stirrer for approximately 15 minutes. While waiting for the sodium hydroxide to dissolve, the used cooking oil was brought up to a temperature not exceeding 64.7 °C (148.5 °F) on a hot plate. This is important because if the methanol and sodium hydroxide solution is mixed into the used cooking oil at or above this temperature, it will become unstable as this temperature exceeds the boiling point of methanol. This poses an explosion/boiling hazard.

Once the sodium hydroxide was fully dissolved in the methanol and the used cooking oil was brought up to a temperature not exceeding 64.7 °C, the sodium hydroxide and methanol solution was mixed into the heated oil. The oil was then kept at temperature and mixed with a magnetic stirrer for approximately 10 minutes. The solution became much darker as the bonds were broken. After 10 minutes, the magnetic stirrer was turned off and the solution was poured into a separating funnel and allowed to sit overnight to separate into the biodiesel and byproduct. The next morning results are shown in Fig. 3.

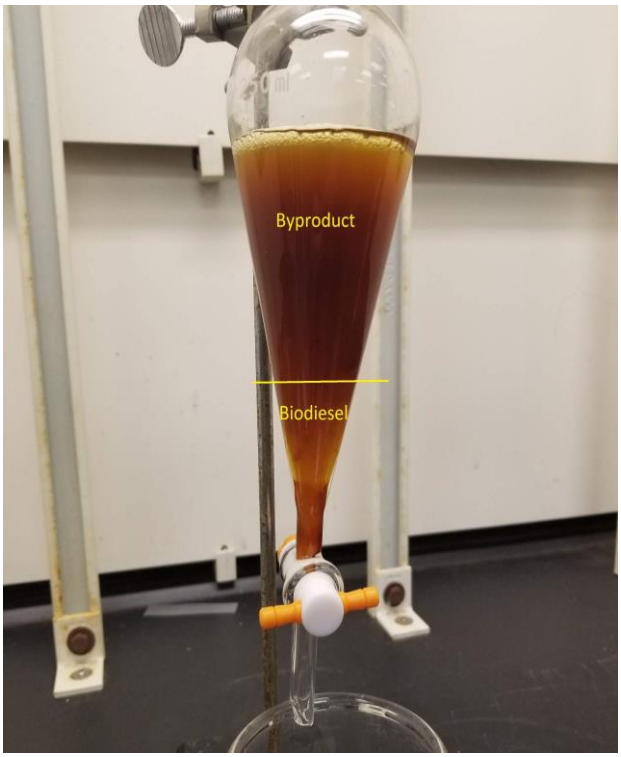

Fig. 3. Separating funnel with biodiesel

To measure the energy content of the biodiesel produced compared to standard biodiesel fuel, a Parr bomb calorimeter [8],[9] was used. Using this calorimeter, 0.87 g of fuel was combusted. The water surrounding the bomb calorimeter achieved a maximum temperature of 27.6°C. With the temperature data, the net corrected temperature rise was calculated according to the bomb calorimeter equation supplied. The fuel provided only about 76% of what was anticipated. A suspected reason for the 24% loss is that the biodiesel still contained some glycerol, thereby decreasing its heat of combustion.

7. Conclusions and Lessons Learned

The photobioreactor constructed for this project allowed for a culture of *Chlorella* algae to grow efficiently and provided a suitable environment for it plus a supplemental amount of pond algae to thrive. Over the course of one month of operation, the apparatus produced a significant volume of algae. The gravity aeration system was able to achieve acceptable levels of oxygenation which allowed for these species of algae to grow. It also decreased the electrical power input compared to running an additional aeration “bubbler” stone in the tank. The frame and tubes withstood temperature fluctuations and heavy rainfall during this experiment and performed as well at the collection stage as they did at the beginning of the project.

The speed at which the pump was able to push water through the system was very low, and even a slight increase in flow rate resulted in minor leaks from the 3D-printed fittings. These fittings represent a major weakness to the design as 3D-printing technology is not available everywhere in the world and was not able to provide a total seal without additional materials. Additionally, the photobioreactor was relatively small. An increase in the number of pipes in the solar collection array would vastly improve the efficiency of the system by increasing the surface area which encounters direct sunlight.

The largest limitation for this system was the lack of ability to collect all or most of the algae from the tank/pipe array. Large colonies of the microalgae could be collected via the use of filters and careful water drainage, but the majority of the algae remained suspended in the excess water left over after harvesting. A finer mesh screen may have improved these results slightly, but the best potential method for algae extraction would likely be to evaporate the water away from the tank in order to collect the biomass. In addition, a significant volume of algae was left within the pipes of the solar collection array after draining. This volume was not possible to fully remove using the system as designed.

Regardless of the lack of collection capabilities, the final yield was still a significant volume of microalga colonies suspended in a viscous slurry. The most difficult aspect of converting this slurry to biodiesel was the extraction of oil from the algae itself. Methods of crushing, blending, and settling were used, but none provided a significant amount of oil which could be used in the transesterification process. The method described by Kumar [6] of using ultrasound from a sonic transducer was considered. However, the resources were not available at the time of extraction. This method may have proven more effective at extracting the oil from the algal biomass. As a substitute, used cooking oil was used to produce a biodiesel product which approached heating values of commercial grade biodiesel.

Finally, this technology can be used in a variety of applications, from cleansing of wastewater to producing

biodiesel fuels to power the world. Closed system photobioreactors, such as the one built and tested in this project, may provide a carbon neutral alternative to fossil fuels.

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