Growing Lipid-Rich Microalgae in Wastewater for Biodiesel Production

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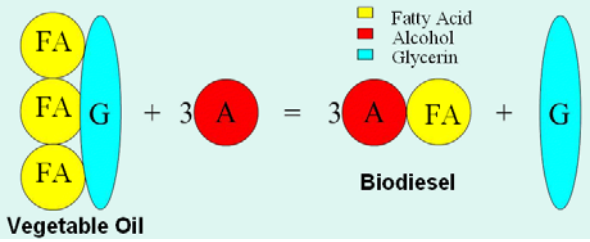
1- Introduction

1-1 Background. The public is becoming more aware of the need for alternatives to petroleum based fuels. The price of oil is increasing faster than new technologies, such as gas-electric hybrids, can compensate for. Biofuels such as biodiesel provide an environmentally safer alternative to petroleum fuels.

1-2 Biodiesel. Biodiesel is a renewable alternative to diesel fuel. It is made by the transesterification reaction of any lipid/oil with alcohol. In this process, three Fatty Acids which are bonded to a Glycerin group react with an alcohol to produce the biodiesel. The Glycerin, which is a byproduct of the reaction, could then be used in other products.



Biodiesel can be burned in existing diesel engines without any additional modifications. Also, biodiesel can be blended in any proportion with petroleum diesel. Potentially, biodiesel can be produced sustainably. It reduces the dependence on fossil fuels, produces far less carbon dioxide (CO_2) and other greenhouse gases (GHGs) than petroleum diesel, reduces other forms of air pollution such as carbon monoxide (CO) and sulfur dioxide (SO_2). Biodiesel has many advantages: It is a high-quality fuel; it can be immediately used within the existing

infrastructure, and can support local agriculture and economic development.

1-3 Food Crop Challenges. Currently, biofuels such as biodiesel are produced from food plants such as soybeans and canola. Most plants are highly inefficient in utilizing the sunlight, using only 0.5% of the incident light. Plants are not very efficient in using the available land space. For example, soybean plants produce roughly 50 gallons of biodiesel per acre per year and Canola plants produce 90 gallons of biodiesel per acre per year. Huge amounts of land would be needed to produce enough biodiesel to meet the current US demands for diesel (roughly 60 billion gallons of diesel per year). It would take over one billion acres of land used for growing soybeans to produce enough biodiesel for the United States. This strategy causes deforestation, increases food prices as corn is being used as an "energy crop" to produce oil and ethanol instead of being used as food crop, and farmers are growing more corn to supply demand for biofuels.

1-4 Microalgae as a Feedstock. The term "Algae" is used to cover a wide range of organisms. Algae may be small, single-celled organisms to multi-cellular organisms which are usually found in water bodies. They are similar to plants in that they require the same three components to grow: sunlight, water, and carbon dioxide (CO_2). Just like plants, algae undergo the biochemical process of photosynthesis in which the algae use CO_2 and sunlight to store chemical energy and release oxygen. Swamps and salt lakes are natural sources of large amounts of algae. Compared with plants, microalgae tend to have better solar efficiency utilization. This is because they can be grown in continuous culture and hence provide higher yearly productivity. It is also believed that the addition of CO_2 to microalgae is simpler than the case of plant crops. From the point of view of producing biodiesel, the most important constituents of microalgae are lipids and fatty acids. These provide the source of energy for the microalgae. Earlier research indicated that the lipids content of algae ranges from 2% to 40% based on the dry mass of algae.

Microalgae have the potential to solve many of the current problems with biofuels. They grow faster than any food crop, and can produce between 5,000 and 15,000 gallons per acre. That is about 100 times the biodiesel yield of soybeans. The left-over algae biomass (after oil extraction) can be fermented to alcohol, or can be used as a source of protein in the cattle feed. Based on an average of 10,000 gallons of biodiesel per acre, we would need 6 million acres of land containing algae to produce enough biodiesel for the United States, which would be close to the size of New Hampshire (assuming all of New Hampshire to be agricultural land). Algae also have the ability to grow in closed bioreactors, which can be situated in areas that are unfit for farming. This is attractive both on a financial standpoint and an ethical standpoint.

1-5 Photobioreactors. These are closed vessels where the microalgae can grow under optimum conditions without the concern about contamination which could consume or out-compete the selected strain of microalgae. A photobioreactor can be as simple as a lab scale conical transparent glass beaker exposed to light, to a more sophisticated industrial scale design. The challenge in photobioreactors is to be able to achieve high growth rate of the microalgae in a cost-effective manner.

1-6 Nutrients. Algae use light energy (e.g., sunlight) to obtain their inorganic compound's nutritional needs. The three key nutrients that influence the growth are nitrogen, phosphorous and carbon. The metabolism of algae is strongly affected by limitation in nutrients. It is well known that most algae stressed by nitrogen limitation would increase their lipid content. Phosphorous is an important nutrient. Phosphorous limitation would slow down the growth rate. Carbon is the only source of photosynthesis. When running algae growth experiments, it is necessary to use soluble nutrients for the algae to grow. The most important nutrients are nitrates and phosphates followed by sodium chloride and silicate. A typical nutrient medium solution

includes macro nutrients (sodium, phosphates and nitrates), micronutrients (iron, sodium, and Zn), and vitamins.

1-7 Wastewater Use Motivation. Municipal wastewater usually contains nitrogen, phosphorous and other nutrients. This may be a desirable environment for growing algae. It was hypothesized that the substances dissolved in the wastewater and effluent would serve as nutrients and support microalgae growth and lipid production. The UNH Biodiesel Group is investigating the use of municipal wastewater to grow lipid-producing algae. These algae would then be used as a feedstock for biodiesel production.

Wastewater is an attractive medium because it is inexpensive compared to chemically defined media, and is readily available wherever there is a municipal wastewater treatment facility. If wastewater proves to be an effective medium, it may be possible to build algae biodiesel production facilities that would work directly off the effluent from wastewater treatment facilities. This wastewater algae biodiesel technology will not place additional demand on freshwater supplies needed for domestic, industrial, and agricultural use.

2- Goal

The economics of producing biodiesel from algae requires the most effective utilization of the algae. The goal of this project is to improve the biodiesel process economics by using microalgae-produced lipid/oil. This is attempted by using wastewater to grow the microalgae.

3-Experimental Methods

As discussed, algae require sunlight, water, and carbon dioxide (CO_2) to grow. The light was supplied by an array of fluorescent lights. CO_2 was supplied by bubbling air into the algae solution. The water used was obtained from the Durham Wastewater Treatment Facility in Durham, New Hampshire. Water samples used in our experiments included homogeneous

untreated waste water, dechlorinated effluent (simply termed effluent) , which was a treated wastewater suitable to be released into the environment, and finally a mixture of 50% wastewater and 50% dechlorinated effluent. Our photobioreactor was a two-liter clear glass conical flask in which the algae and water solution were placed. Air was bubbled into the solution to provide a source of CO_2 and to stir the algae solution. The clear flask was placed in front of fluorescent light. All experiments were done indoors. The work proceeded in two steps.

3-1 Step 1. Wastewater Contamination. Initially, the untreated wastewater was observed for unwanted growth in the system. The solutions tested were wastewater alone (as a blank), wastewater plus algae, and finally wastewater plus algae and 0.1 Molar NaCl. It could be seen that there was contamination in the wastewater and this contamination would most likely lead to negative competition between the lipid-producing microalgae (which is highly desirable) and a contaminating algae or bacteria. Thus, the untreated wastewater would not be a desirable medium for algae growth unless the contaminating organisms could be controlled.

3-2 Step 2. Effect of Solution Media. The microalgae were grown in three solutions: a nutrient solution containing 0.1 Molar NaCl (termed Nutrient Solution in the Figures), a dechlorinated effluent solution containing 0.1 Molar NaCl (termed Effluent in the Figures), and finally the mixture of 50% effluent and 50% wastewater plus 0.1 Molar NaCl (termed Effluent/ Wastewater Blend in the Figures). The outcomes are that the nutrient solution produced the algae with the highest oil content, while the dechlorinated effluent showed a good promise for algae growth.

3-3 Monitoring Algae Growth Rate. In order to reach the above outcomes, the microalgae strain was tested at room temperature for two parameters over a time period of about 7-14 days per trial. The first parameter was growth rate, which was determined by measuring the solution absorbance using a Bausch & Lomb Spectronic 20 spectrophotometer. Absorbance is used to

measure turbidity, and higher absorbance reading indicated higher algae concentration.

3-4 Monitoring Algae Lipid Concentration. The second parameter is the lipid/oil concentration in the microalgae. This was found using Cooksey's method. All solutions were first normalized to an equal turbidity then Nile Red dye was added to stain the lipids in the algae. The solution was then placed in aVarian SF-330 spectrofluorometer to measure the fluorescence of the dyed lipids. When recording the data from the spectrofluorometer, it was observed that the fluorescence (of the normalized sample) gradually increased to a maximum value then decreased. The data was recorded in 5 minute increments. When the data was analyzed, the highest and lowest fluorescence values were subtracted from each other to find the peak normalized fluorescence of the sample. It is assumed that the peak normalized fluorescence is proportional to the algae lipid concentration.

4- Results

Figure 4.1 displays the algae cell concentration (as measured by the absorbance/turbidity of the solution) vs time for the three media. Clearly, the best algae growth takes place in the nutrient solution. Some growth took place in the effluent/ wastewater blend. The growth of algae in the effluent is encouraging.

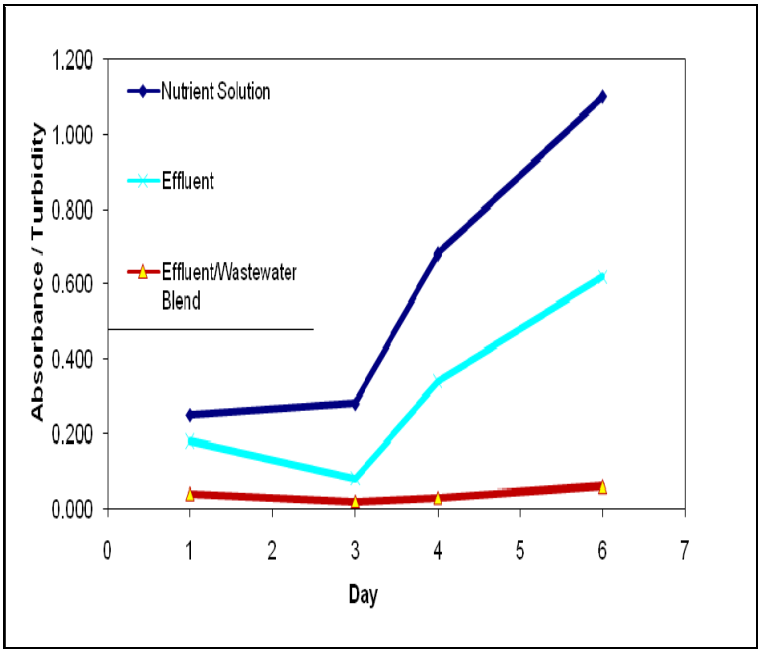


Figure 4.1 Effect of solution media on algae concentration.

Figure 4.2 displays the algae lipid concentration (as measured by the peak normalized fluorescence) vs time. The algae oil concentration in the effluent media and the effluent/ wastewater blend had about 20% of the nutrient solution.

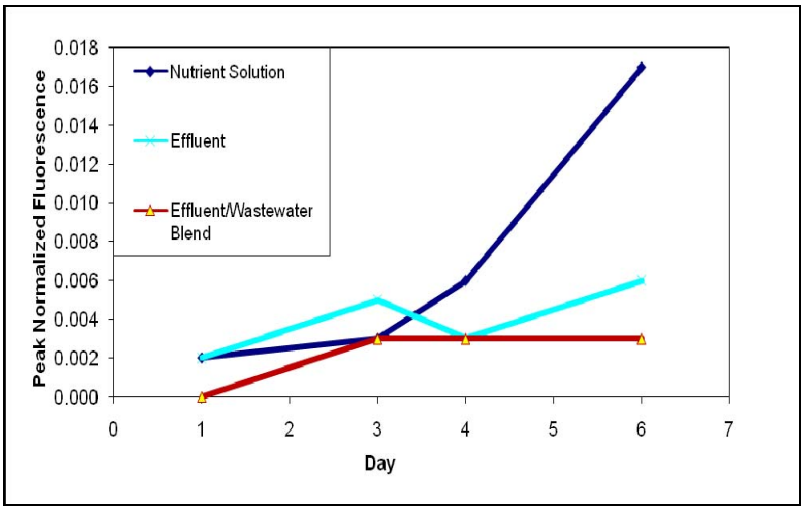


Figure 4.2 Effect of solution media on oil concentration in algae.

The oil content of the algae solution was obtained by multiplying the absorbance/turbidity (algae concentration in the solution) times the peak normalized fluorescence (lipid concentration in algae). The oil content is plotted vs time in Figure 4.3. The results show that the mixture of 50% effluent and 50% wastewater had an oil content of about 5% that of the nutrient solution, while the dechlorinated effluent was about 19.9% that of the nutrient solution.

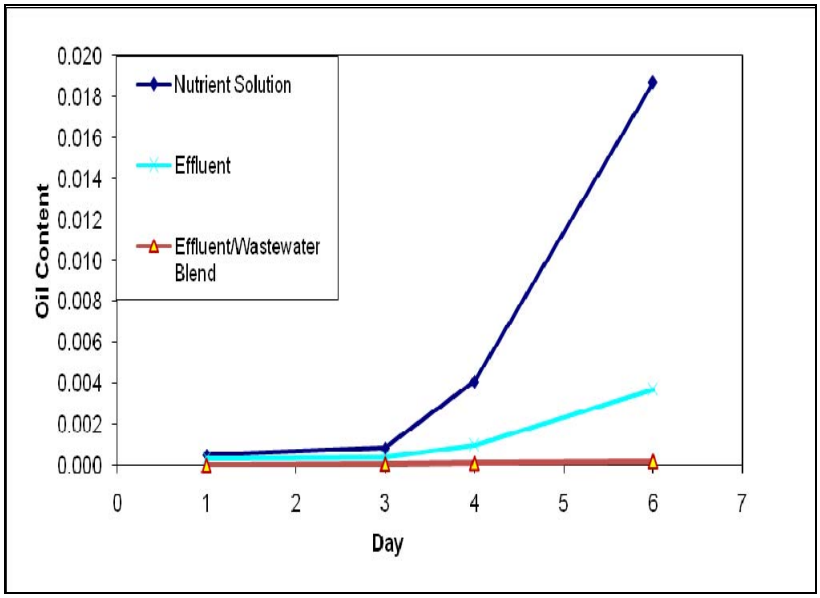


Figure 4.3 Effect of solution media on oil content of algae solution.

After testing the algae for oil content, the operation of the photobioreactor had to be adjusted. After 3-4 days of algae growth, approximately half of the solution had evaporated. To help in decreasing this evaporation, which was exacerbated by the air being bubbled in to mix the algae,

the air flow rate was lowered. Also, various reactor designs were produced by other researchers in the UNH Biodiesel Group. Although these were successful in producing algae, contamination was still a major problem. New photobioreactors are still being developed.

5- Conclusions

Microalgae grown in nutrient and salt solution produced the greatest oil yield while the microalgae grown in effluent showed good promise. More tests will be taken with the various wastewaters to determine the best method of growing algae for the conversion to biodiesel. These results will be used in future planning for microalgal biodiesel research.

6- Future Plans

New photobioreactor designs will be tested in order to find a better system. During the experiment, it was noted that the wastewater had contaminants which may affect algae growth. To prevent biological contamination, ultraviolet irradiation of the wastewater will be studied..

Acknowledgment:

The authors would like to acknowledge the support of the UNH Hamel Center for Undergraduate Research and the UNH Chemical Engineering Department.

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