

Comparison of Freshwater and Wastewater Medium for Microalgae Growth and Oil Production

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Abstract: Biodiesel, a renewable energy source, has the potential to satisfy our energy needs. It is made from the transesterification of oils and alcohol. Oils from soybean and rapeseed food-crops are common feedstock used to produce biodiesel in the US and Europe, respectively. Microalgae oil is an alternative non-food feedstock for biodiesel. Algae can generate 15 times more oil per acre than other plants which reduces the land footprint. Algae can potentially grow in nutrient-containing wastewater effluents. This is important because of the growing worldwide scarcity of fresh water. This research aimed to evaluate the viability of algae growth in wastewater. The main objective is to compare microalgae growth and oil production in fresh water versus municipal wastewater and the use of less expensive urea to supply nitrogen nutrient instead of KNO_3 . Experiments included bench-top to pilot size photobioreactors, various water and nitrogen sources for algae growth, and various oil extraction techniques, and solvents. The results showed that urea is a cost effective source of nitrogen for algae growth and that wastewater is a viable option for growing lipid-rich microalgae with an average algae production rate in wastewater is 0.08 g/liter-day and an average lipids yield is 1.07 g /100 g of dry algae grown in wastewater.

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1. Introduction

1.1 Biodiesel Advantages and Challenges

As the world's natural energy sources become scarce, fossil fuel costs rise. Fossil fuels are a source of air pollution, water pollution, and solid waste. As a result there is a global effort to find clean and renewable liquid fuel sources.

Biodiesel is a renewable liquid fuel that can be used to replace diesel in diesel engine cars and equipment with little or no changes. Compared with petroleum diesel, biodiesel has a higher combustion efficiency, higher cetane number, lower toxicity, and higher biodegradability. Some economic advantages are domestic origin and reducing dependency on imported petroleum [1]. It is a fuel made from natural oils (edible or non-edible); commonly soybean, sunflower, and canola plants, often termed food crops [2, 3, and 4]. However, the potential biodiesel market greatly exceeds the available plant oils which places stress on those food crops. The biggest challenge for the production of biodiesel from plants is having enough feedstock to produce enough biodiesel to replace petroleum diesel [4]. Biodiesel production in the United States has traditionally relied on corn and soybeans as the primary feedstock [5, 6, 7]. With one acre of land, soybean (18% oil) can yield about 49 gallons of biodiesel/year, sunflower (44 % oil) can yield 84 gallons, and canola (43% oil) can yield 76 gallons when used as energy crops [6]. Hence, these crops are not viable options for mass biodiesel production [3].

The consumption rate of petroleum diesel in the US is roughly 40 billion gallons per year [9]. For biodiesel to replace half of the amount of diesel that the US uses, the land area required for growth of feedstock would be around 1.4 billion acres [10]. This causes deforestation, and increase in food prices. The United Nations Environmental Program (UNEP) [10] recently warned that the global rush for energy crops at the expense of food crops threatens to bring food shortages and increase poverty [11]. High-lipid microalgae loom as excellent alternate feedstock.

1.2 Microalgae

Microalgae are simple, plant-like, sunlight-driven cell factories that reproduce themselves using photosynthesis. They use sun energy and convert carbon dioxide to potential biofuels, foods, feeds and high-value bioactives [12, 13]. One acre of microalgae can produce five to fifteen thousand gallons of biodiesel and it would require only 1 to 3 million acres of land to replace half of the United States' diesel use [10]. This is non-arable land and will not affect food production.

Another issue with growing microalgae is the water requirement. Producing one gallon of biodiesel from soybean requires the use of over 15,000 gallons of water [14]. Using freshwater on a large scale to grow crops is expensive and irresponsible. Today, about one billion people have inadequate access to clean drinking water, and if the same conditions continue, by 2025, two out of three

people will struggle to find clean drinking water. There are even some parts of the world where fresh drinking water would be more important than the biodiesel fuel [15]

Microalgae growth requires about 300 to 1000 gallons water per gallon of biodiesel [14]. While this is a great improvement over soybean oil, it is not realistic to use freshwater. Therefore this study explores growing algae using ultra-violet treated municipal wastewater because of its availability and nutrient content.

1.3 Wastewater Availability, Pros and Cons

Global annual water use for domestic purposes during 1987-2003 was estimated at 325 billion m³ (roughly 86 trillion gallons) annually. Water usage for industrial purposes was estimated to be 665 billion m³ (176 trillion gallons) [15]. Most of this water turns into wastewater which pollutes the environment and creates health hazards. But if only 50% of the wastewater used for industrial purposes (88 trillion gallons) could be used for algae production it would generate approximately 247 million tons of algal biomass (roughly 2.8 kg algae per 1000 gallons or 0.7 g per liter of wastewater) and 37 million tons of algae oil (roughly 0.42 kg or 0.13 gallons of algae oil per 1000 gallons of wastewater, with yield of 15 g algae oil per 100 g dry algae) [16]. Treated wastewater can be obtained from a number of industrial facilities or municipal wastewater treatment plants. This medium can then be used to grow the microalgae instead of using freshwater. It has been claimed that most microalgae flourish and multiply exponentially in wastewater [7]. This is due to the leftover nutrients in the wastewater which allow the algae to absorb more nutrients.

There are many pros to using wastewater as the primary medium for microalgae growth, the main ones being: reduction in wastewater affecting the environment, reducing the demand on freshwater supplies, low costs, and more nutrients for the algae at no added cost [15, 17]. Some cons of using wastewater is making sure that it is sanitary enough for humans to handle and the toxicity is low enough for the algae to survive. For this reason this study used ultra violet (UV) treated municipal wastewater from Dover, New Hampshire.

1.4 Aim and Objectives

The aim of this research is to evaluate the viability of algae growth in wastewater. The main objectives are to; 1- assess replacing KNO₃ with urea as a nutrient nitrogen source; 2- compare microalgae growth and oil yield in fresh water versus municipal wastewater,; and 3- evaluate techniques and solvents

for the safe and cost-effective extraction of oil from algae.

2. Material and Methods

2.1 Experimental Design:

Briefly, the algae to oil process involves the following steps; 1- algae growth in water in the presence of nutrients, air and energy for photosynthesis; 2- algae harvesting; 3- algae dewatering and drying; and 4- solvent extraction of oil from algae. This process takes about 3 weeks. The objectives of this research were accomplished by growing algae in a wastewater medium, a freshwater (distilled water) medium, and a 50/50 mixture of the two. The growth of each individual run was done in a 2L photobioreactor flask. Algae production and oil yield studies were also done in a pilot size 80 liters photobioreactor. Algae growth was monitored using cell counts and turbidity readings.

2.2 Wastewater and Lighting:

The samples of UV treated wastewater used were collected from the Wastewater Treatment facility in Dover, New Hampshire. The experiments were done at room temperature range around 20 C. Lighting was provided for 24 hours per day, 7 days/week with 18W fluorescent lamps. The measured light intensity was 14,000 lux (lumen/m²) or 20.5 W/m² (1 W/m² = 683 lux).

2.3 Nitrogen Sources

Nitrogen (N) is an important nutrient for algae. Two possible sources of N chemicals were compared. These are potassium nitrate and urea. Urea has the advantage of lower cost per mole of N added.

2.4 Data Analysis

Algae were harvested when they reached the stationary growth phase, usually after 12-14 days of growth. They were dewatered using a centrifuge then dried to powder using a freeze dryer. The measured dry biomass yield was reported as g dry algae/L-day. The lipids were then solvent extracted from the dry algae by either Soxhlet or boiling method. The mass of extracted lipids was measured and reported as g lipids/100 g dry algae. Two different methods were used to extract the lipids; Soxhlet and flask boiling. Two solvents were used for the extraction; hexane and ethanol. The dry algae were characterized by measuring the heat of combustion before and after lipid extraction. A Parr bomb calorimeter was used.

3. Results and Discussion

3.1 Algae Growth and Harvesting

The growth of the algae was measured quantitatively by two methods, cell counts and

absorbance readings. Based on these readings, the algae growth curve is formed, as shown in Fig. 1. Algae growth is characterized by four main stages: lag phase, exponential growth, stationary phase, and lysis. The stationary phase is when the algae are harvested. If the algae are not harvested they go into the lysis phase, which is where they start dying due to a lack of nutrients. This stage should be avoided. Figure 1 shows the first three stages.

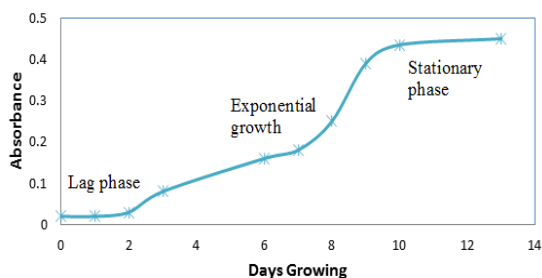


Figure 1: Growth curve of a turbidity graph for freshwater growth. Once the stationary phase is reached then it is time to harvest.

Once the stationary phase is reached the algae were harvested using a Damon/IEC B-20A centrifuge. The algae samples were spun in the centrifuge at 5000 rpm for at least 15 minutes. After centrifugation, the samples were freeze dried at -80°C under vacuum for 48 hours using a Labconco Freeze Dryer 5. To shorten the harvesting period the algae were flocculated using aluminum sulfate. About 0.2 g/L of aluminum sulfate [17] were added to the solution to flocculate the algae. This reduced centrifugation time from 36 hours to about 6 hours.

3.2 Water Sources

The specific medium factors that were compared were, Freshwater (RO) vs. wastewater, wastewater vs. 50/50 mixture of freshwater and wastewater. Figure 2 shows a comparison of different runs grown in a wastewater medium and freshwater medium. The production rate for the freshwater runs was about 0.051 g algae/L-day. For the wastewater, the average production rate was 0.08 g dry algae/ L-day. If algae are grown for 14 days the production will be 1.12 g/L. This compares well to the 0.7 g per liter of wastewater reported by Chinnasamy [7]. This lead to the conclusion that wastewater is a viable option to grow algae in.

Based on the results in Figs. 2 and 3, it was decided that wastewater would be used for testing the effect of nutrient nitrogen sources in the medium.

3.3 Nitrogen Sources

Three sets of experiments were done to study the effect on algae production.; 1- doubling the

KNO_3 concentration from the normal (1X) to twice as much (2X) with results shown in Fig. 4, 2- replacing KNO_3 with Urea (with the same nitrogen concentration in the medium), results shown in Fig. 5, and 3- doubling the urea concentration from 1X to 2X, results shown in Fig 5.

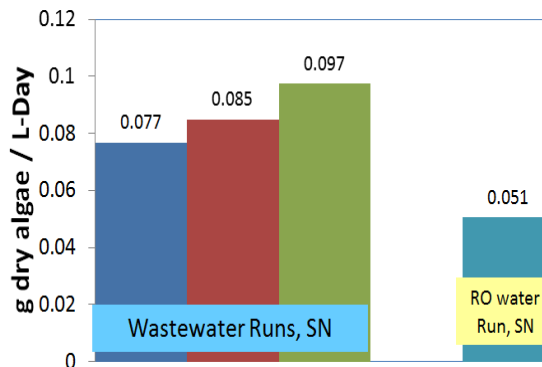


Figure 2: Comparison of different runs grown in wastewater and freshwater. The left side has wastewater runs and the right has freshwater runs.

Figure 3 compares the average algae production rate in wastewater and a 50/50 mixture of wastewater and fresh water. It shows that the average production rate of the wastewater is about 0.08 g algae/L-day. The production rate of the 50/50 mixture is 0.054 g algae/L-day, significantly less than the wastewater. It can be concluded wastewater is more effective than the 50/50 mixture.

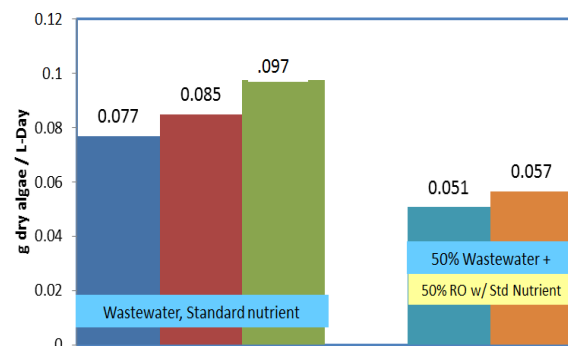


Figure 3: Comparison of wastewater medium vs. 50/50 mixture of wastewater and freshwater as a medium.

Figure 4 shows a slight increase in algae production due to doubling the KNO_3 concentration. However, it does not appear to be a cost-effective. Figure 5 compares 1x KNO_3 and 1x urea. Urea costs about \$27/kg which equals about \$0.82/ mol N, while KNO_3 is about \$58/kg which equals about \$5.90/ mol N. The 1x KNO_3 runs produced an average of about 0.085 g algae/ L-Day, while the 1x urea produced about 0.06 g algae/ L-day. When 2x urea is used, the

algae production is about 0.085 g algae/ L-Day Thus; urea could be used as a possible nitrogen source.

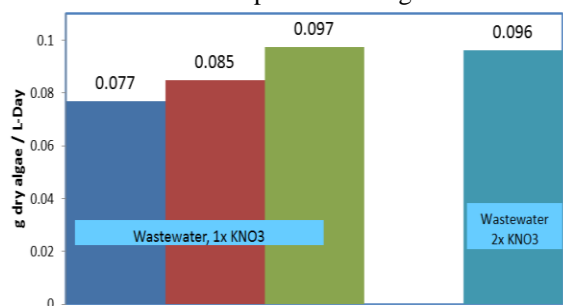


Figure 4: Comparison of one times concentration of KNO_3 vs. two times concentration of KNO_3 using wastewater as a medium in 2L photobioreactor.

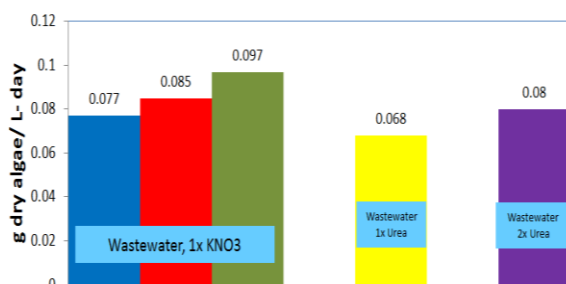


Figure 5: Comparison of one times concentration of KNO_3 , one times concentration of Urea, and two times concentration of Urea using wastewater as a medium in 2L photobioreactor.

3.4 Lipid Extraction and Water Source

Figure 6 shows the effect of the water source used to grow algae on the lipid yield. Algae grown in RO water produced about 1.8 g lipids/ 100 g algae while algae grown in the wastewater medium produced about 1.07 g lipids/100 g algae. Clearly, algae grown in wastewater have potential to produce lipids.

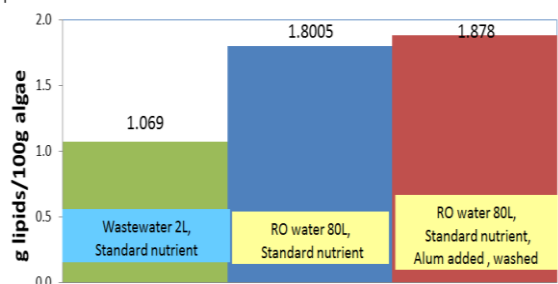


Figure 6: Effect of the water source used as a medium on lipid extractions when done in a Soxhlet and using hexane as the solvent. Each of the runs here contained the same nutrients and algae but a different medium.

3.5 Lipid Extraction Solvent

Two extraction solvents were used; hexane and ethanol. Hexane is the traditional solvent, but it is a hazardous chemical. Ethanol is a green solvent meaning it is safer for humans to handle, nontoxic, and better for the environment. Figure 7 shows the effect of the solvent and the water source on lipid extractions. It is a plot of the measured extracted algae oil yield results for the base case, ethanol extraction of RO water grown algae and ethanol extraction of wastewater grown algae. The run with the RO water medium and a hexane solvent is the base run with a yield of 4.6 g lipids/ 100 g algae. For ethanol the yield was about 10.2 g lipids/100 g algae. This illustrates that ethanol extracts more material from the algae than hexane. Using ethanol to extract lipids from algae grown in a wastewater medium resulted in a yield of about 17 g lipids/100 g algae. The wastewater grown algae produce more lipids than those grown in RO water which confirms the viability of using wastewater to grow algae for biodiesel production.

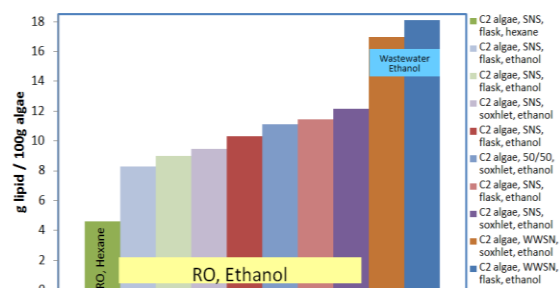


Figure 7: Effect of solvent on lipid extractions in different medium. The legend on the left describes the medium, extraction method and solvent. SNS means standard nutrient solution (freshwater, standard nutrients). 50/50 means half freshwater, half wastewater with standard nutrients. WWSN means wastewater with standard nutrients.

3.6 Lipid Extraction Method

Two methods were used to extract lipids from the dry biomass; Soxhlet and flask boiling. The boiling method has the advantage of shorter extraction time (1.5 hours) whereas for the Soxhlet takes about 5-6 hours. Flask boiling is followed by filtration. Figure 8 compares the lipids yield of the two methods. It shows that the extraction method does not have a strong effect on the lipid yield. Flask boiling (B) and Soxhlet(S) methods were virtually the same. Taking the times into account (1.5 h for boiling, 5-6 h for Soxhlet) it is concluded that the boiling method should be used as the primary method for extractions due to its advantages.

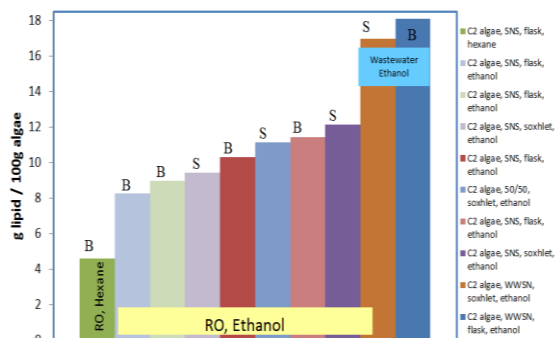


Figure 8: Effect of extraction method. The bars with a B over them were done using the boiling method while the S is the Soxhlet method.

3.7 Heat of Combustion

The heat of combustion of dry algae was measured before and after lipids extraction. Figure 9 shows the results and the comparison to literature value of diesel and B100 biodiesel.

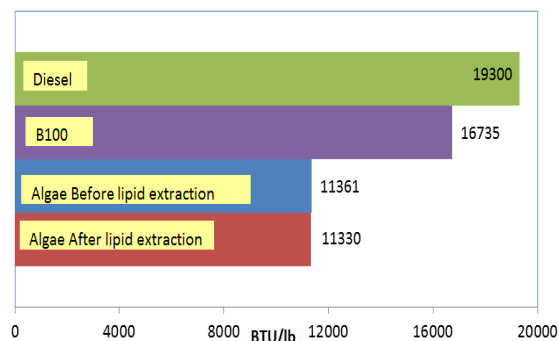


Figure 9: the heat of combustion of algae with and without lipids compared to literature values of diesel and B100.

The heat of combustion for algae drops slightly after the lipids are extracted. This is expected since lipids have a higher heat of combustion. Assuming that the lipids heat of combustion is around 15,000 BTU/lb (slightly less than B100), the difference in the algae heat of combustion before and after lipid extraction would indicate that roughly 2g lipids are extracted per 100 g dry algae. This is close to the base case of hexane extraction of algae grown in RO water in which the lipids were 4 g/100 g dry algae.

3.8 Agreement with published data

After collecting the experimental data, the next step was to compare with published data. The first set of data was the carbon dioxide, air, water, and nitrogen requirements to produce one kilogram of dry algae. Because this work did not provide the algae with pure carbon dioxide, the carbon dioxide number is low. This work used air as the source of

carbon dioxide and for mixing, so the air requirement is greater than the PBR Facility and Raceway Pond. This work's water requirement is closer to the Raceway Pond than the PBR Facility, which means the algae productivity is close to the Raceway Pond. Also, this work's nitrogen requirement is close to the PBR Facility. Table I shows these comparisons.

Table I: Comparison of this work's results with other works' results. The data with an asterisk is from Chisti [11] and the double asterisk data is from the Bioteking Facility in the Netherlands.

Component required per kg dry algae	This work	PBR Facility*	Raceway pond*
CO ₂ m ³	0.037 (from air)	1.86	2.86
Air m ³	96	4.7	0
Water L	1600	255	1330
Nitrogen, g	83.7	81**	

The next set of data needed to be compared was the biomass results. This work's final biomass concentration was 0.67 g/L. This was greater than the 0.5g/L produce by the Raceway Pond, but less than the 4.0g/L produced in the PBR Facility. This work's biomass volumetric production (kg/m³-day) and area productivity (kg/m²-day) were less than both the PBR facility and the Raceway Pond. Table II shows these comparisons.

Table II: Comparison of this work's results with Chisti [11] study.

Variable	This Work	PBR Facility*	Raceway Ponds*
Biomass final concentration kg/m ³ = g/L	0.67	4.0	0.5
Biomass Volumetric production, kg/m ³ -day	0.06	1.5	0.1
Area productivity kg/m ² -day	0.007	0.048	0.025

Third, the overall efficiency of light transmission of the PBR and conversion to algae biomass for this work was compared with the work of Zemke, et al [18]. The overall efficiency of light transmission of the PBR and conversion to algae biomass is the percent of light that travels through the PBR wall and is then converted into biomass. This work had a 1.24% conversion which was lower than

Zemke's Pond Bioreactor and Concentrator Bioreactor.

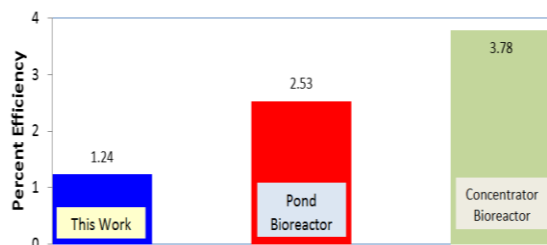


Figure 10: Comparison of the Overall Efficiency of Light Transmission of the PBR and Conversion to Algae Biomass.

The final comparison is the specific growth rate of our algae. The specific growth rate (μ_{max}) shows the increase in cell mass per unit time. As is shown in Table III our work is comparable to the values provided by Carpenter and Goldman [19] for the two algae types used.

Table III: A comparison of specific growth rates for the Chlorella and Dunaliella algae strains.

Algae Type	This Work	Literature value
Chlorella	1.62 day ⁻¹	1.88 day ⁻¹
Dunaliella	.75 day ⁻¹	.80 day ⁻¹

4. Conclusions

Lipid-rich microalgae were cultivated at room temperature in fresh reverse osmosis (RO) water and in UV treated municipal wastewater. Energy for photosynthesis was provided by fluorescent lights and the growth medium was provided with chemical nutrients. Experiments were to run to study the replacement of KNO₃ and the nitrogen nutrient source with urea, compare the algae growth and lipid yield in wastewater and fresh water. Experiments were done to test two different lipid extraction methods, Soxhlet and flask boiling, using two different solvents, hexane and ethanol. The results show that (1) there is a great potential using municipal wastewater to cultivate high-lipid microalgae, in terms of the algae production rate and the lipid yield; (2) less expensive urea could replace KNO₃ as a nitrogen source in the nutrient medium, (3) flask boiling extraction of lipids from algae is very effective and energy efficient compared to Soxhlet extraction; (4) algae heat of combustion measurement confirm the solvent extraction of lipids.

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