Resources Conservation in Microalgae Biodiesel Production

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***Abstract*— The aim of this investigation was to reduce cost and production time of biodiesel from microalgae by conserving water, energy and chemical resources. This was done by investigating two goals. The first is conservation of fresh water by microalgae growth in treated wastewater using reduced chemical nutrients while taking advantage of the nitrates and phosphates in wastewater. The second is conservation of energy, while reducing production time and eliminating the use of hazardous hexane solvent by integrating the two steps of microalgae oil extraction and transesterification of extracted oil into a one-step in-situ process. To accomplish the first goal, the project included growth, monitoring, and harvesting Chlorella vulgaris microalgae in various reduced chemical nutrient solutions and analyzing the algae growth and the lipid production results. The added chemical nitrate and phosphate nutrients to the growth medium were reduced from the "standard" chemical recipe by a 25% step. The second goal was accomplished using a sonicator to disrupt algae cells. The in-situ process was used to obtain the biodiesel production from the dry harvested algae. The results were promising, a reduced nutrient supply, specifically the trials of 50% phosphates and 50% nitrates resulted in a higher lipid production than the "standard" full nutrients requirement.**

***Index Terms*— Biodiesel, Chlorella vulgaris, municipal wastewater, in-situ process, alternative renewable fuel, Fresh water conservation, Chemical use reduction.**

I. INTRODUCTION

A. *Microalgae Biodiesel Benefits and Challenges*

Microalgae are photosynthetic, aquatic, single-celled organisms with a high surface-to-volume body ratio. Microalgae biofuels offer many benefits to the environment over traditional fuels and biofuels from corn or soybean. They can produce more than 80 times more oil than soybean or corn per acre of land per year. Unlike corn and soybeans, algae do not require arable land hence they do not compete with food production land. In addition, microalgae are not considered a food source for human nutrition; hence, there is no food versus fuel issues when biodiesel is produced from microalgae. A comparison of petroleum diesel to biodiesel is given in Table I [1]. Key factors listed are heating values in Btu per gallon, and CO_2 emissions in pounds (lb) per gallon of the fuel.

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Table I. Heating Value and CO_2 emissions per gallon of Petroleum diesel and Biodiesel

Property	**Petroleum diesel**	**Biodiesel**
Heating value, Btu per gallon.	130,500	128,000
CO_2 emissions, lb per gallon.	26.55	5.85

Biodiesel produces virtually the same heating value as petroleum diesel. However, biodiesel produces about a fifth of the carbon emissions per gallon

Figure 1 shows the process diagram of producing biodiesel from algae. The steps include algae growth, harvesting, dewatering, oil extraction, and transesterification of algae oil to fatty acid methyl ester (FAME) or biodiesel. However, there are still technical challenges that have limited the commercial production of microalgae biodiesel. These include 1- Water Requirements, which range from 300 to 2000 gallons of water per gallon of biodiesel [1 - 10]; 2- Lipid extraction is energy intensive [11], uses hazardous hexane solvent and is the most costly step in the algae growth process; 3- The high cost of algae nutrient chemicals [12].

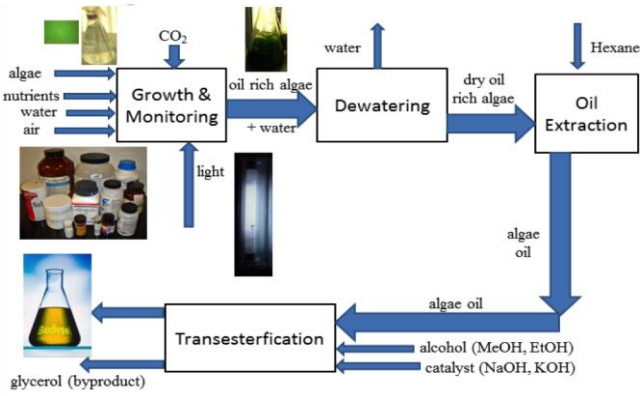


Fig. 1. Process diagram of producing biodiesel from algae.

B. *Microalgae Growth Requirements*

There are four classes of microalgae. These are diatoms, green algae, blue-green algae and golden algae, [13]. Green algae, e.g., Chlorella vulgaris were used in the present study because they can grow in fresh water and in wastewater.

Algae growth in batch cultures experiences five different phases. These are 1- Lag: Initial period of slow growth; 2- Exponential: Rapid growth and often cell division; 3- Declining Relative Growth: Occurs when a growth requirement for cell division is limiting; 4- Stationary: Cell

division slows due to the lack of resources necessary for growth; 5- Death/ Lysis: Cells begin to die due to lack of resources. Figure 2 shows these five phases.

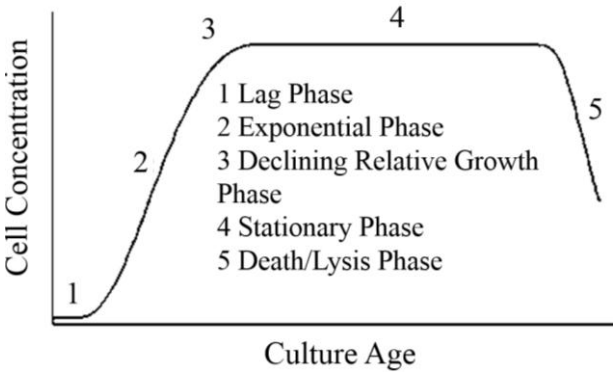


Fig. 2. Five growth phases of algae cultures [14].

In addition to the microalgae species, algae growth requires:

1- Water source: in this research two water sources have been studied; fresh (or reverse osmosis) water and municipal wastewater that has been treated with ultraviolet (UV) light to kill pathogens.

2- Light source: Algae undergoes photosynthesis. They use the light energy to convert carbon dioxide into organic compounds, e.g., lipids/oil. Lights sources could be sunlight, fluorescent lights, light emitting diodes (LEDs) and others [15, 16]. For consistency, the present research used daylight fluorescent lights in all experiments.

3- Nutrients: to make up the deficiencies in the water medium. Table II lists the "standard" nutrients used in the present work.

Table II. "Standard" Nutrients used in the Present Work

Chemical Name	**Chemical Formula**	**Initial Concen-tration**
Calcium Chloride	$CaCl_2$	0.2 mM
Boric Acid	H_3BO_3	0.13 mM
Potassium Nitrate	KNO_3	5.2 mM
Magnesium Sulfate	$MgSO_4$	5 mM
Sodium Phosphate	Na_2HPO_4	0.4 mM
Sodium Chloride	NaCl	0.1 M
EDTA	$C_{10}H_{16}N_2O_8$	26.9 mg/L
Ferrous Sulfate	$FeSO_4$-$7H_2O$	2.8 mg/L
Zinc Sulfate	$ZnSO_4$-$7H_2O$	0.288 mg/L
Molybdenum Trioxide	MoO_3	0.125 mg/L
Copper Sulfate	$CuSO_4$-$5H_2O$	0.075 mg/L
Cobalt Chloride	$CoCl_2$-$6H_2O$	0.025 mg/L
Manganese Chloride	$MnCl_2$-$4H_2O$	0.15 mg/L

Nitrates supply nitrogen to the algae proteins to function. Phosphates supply the algae phosphorous needed for algae growth, since phosphorous is an essential element in DNA.

4- Mixing and Aeration. These are very important to prevent algae sedimentation, ensure equal exposure of all algae cells to the light and nutrients and improve gas exchange between the algae medium and the air. Air is bubbled into the algae medium to provide the CO_2 needed for photosynthesis and to mix the solution.

4- pH: Most algae species grow well in a pH range between 7 and 9. The optimum pH range is usually between 8.2-8.7.

6- Temperature: Most microalgae species grow in the temperature range of 16 C (61 F) and 27 C (81 F). Below 16 C (61 F), the growth will slow down growth. Temperatures higher than 35 C (95 F) may harm the algae. The experimental work in the present investigation was done at room temperature of about 21 C (70 F).

C. *Wastewater for Microalgae Growth*

Municipal wastewater contain nitrogen and phosphorus ingredients. Both of these elements are important in microalgae growth [17]. Growing microalgae in a wastewater medium would help the algae grow and at the same time remove these otherwise nitrates and phosphates harmful ingredients from the water. Wastewater is also readily available. The wastewater treatment capacity just in the US is roughly 34.4 billion gallons per day or a staggering 12.5 trillion gallons of wastewater per year [18]. The use of wastewater as the primary medium for the growth of microalgae would conserve the drinking water supply and reduce the cost of the biodiesel production.

The municipal wastewater used in the present work was obtained from the Wastewater Treatment plant, in Dover, NH [19]. It was collected after the last stage of the treatment; ultraviolet (UV) disinfection was done. This ensured that the wastewater was free of undesirable pathogens and safe for use in the lab.

II. Project Purpose and Goals

A. *Project Purpose*

Techno-economic and scale-up studies have been done for of the biodiesel production, e.g., [20]. The purpose of this research is to improve the economics of microalgae biodiesel production by 1- Conserving fresh water and chemical nutrients (nitrates and phosphates). This will be done by replacing the use of fresh water with treated municipal wastewater as the algae growth medium. Using wastewater, which contains nitrogen and phosphorous reduces the necessity for additional costly chemical nutrients, and 2- Conserving energy, reducing production time and hazardous material use by integrating the algae oil extraction and transesterification into a single step, termed in-situ process

B. *Project Goals*

1. Reduce the nitrate and phosphate chemical nutrients required to grow algae in treated wastewater, utilizing the nitrates and phosphates already present.

2. Reduce production time, energy requirements, and eliminate the use of hexane by integrating algae oil extraction and transesterification into a one-step in-situ process that produces the FAME, or biodiesel from algae.

III. METHODOLOGY

A. *Experiment Layout*

The experimental work to study the effect of reducing nitrates and phosphates chemical nutrients on algae growth and lipid production (Goal 1) was done in 4 phases. The aim of phases 1 and 2 was to establish baseline data on the performance/growth of microalgae in fresh water and in wastewater. Phases 3 and 4 were to decrease the concentrations of nitrates and phosphates in wastewater. Algae growth was done in 2L flasks supplied with air and illuminated with fluorescent lighting, as shown in Figure 3.

Figure 3. Algae growth in 2 L flasks supplied with air and illuminated with fluorescent lighting.

In-situ (Goal 2) approach combines the two-step procedure of lipid extraction from algae and transesterification of extracted algae lipid/oil to FAME into one-step. The experimental work starts by dissolving 1g of dry algae in 40 mL of KOH in methanol. This solution is then placed in a cell disrupter/ Sonicator, shown in Figure 4.

Figure 4. Model W-375 Sonicator (cell disrupter)

The sonicator runs for 10 minutes and breaks the algae cells. It was set to a power of 7-8 on the output dial and the power meter less than 75% in continuous mode.

The disrupted algae are filtered from the (methanol+ algae oil) solution using a vacuum flask. The methanol transesterifies the algae oil into FAME/biodiesel. The excess methanol is evaporated using a hot water bath. The remaining algae oil is then dissolved in chloroform to be injected into the gas chromatograph (GC) to analyze the lipid content. The GC is equipped with an integrator, which plots the data and displays different peaks. These peaks are used to identify the content and amount of the main lipids. The GC injection is done using a needle to gather 3 microliters of (lipid + chloroform) solution, this is inserted into the machine. The GC then runs for roughly 20 minutes and analyzes the solution. Because each needle's cylinder is very tiny, they can be easily clogged. When this were to happen, the following procedure was conducted; about 10 mL of solvent (methanol or chloroform) is added to 200-300 mL of fresh (RO) water in a beaker (approximate, no exact numbers). Then, the syringe is separated into parts and placed in the solution. The solution is sonicated for 10 minutes. Then all of the pieces are dried using paper towel. It was important to be careful and allow time before opening the sonicator because the chloroform odor could be strong. The GC was first tested by running pure biodiesel (B100) sample. The B100 GC results were comparable to those of [21]. Table III summarizes the methodology used in this investigation.

Table III. Project Methodology

Phase	Description
1- Obtain baseline data	The trials of growth were done in 2L flasks supplied with air and illuminated with fluorescent lighting for consistency. There were six flasks per baseline run of varying conditions. These 6 flasks included Two Flasks with RO (fresh) water and 1- An initial nutrient and algae supply, 2- No initial nutrient or algae supply Four Flasks with wastewater and 3- an initial nutrient and algae supply 4- just an initial algae supply 5- just an initial nutrient supply 6- no initial nutrient or algae supply
2- Repeat	Repeat Phase 1 runs to ensure accuracy.
3- Reduced nutrients experiments in wastewater	Six flasks per experimental run of varying conditions. The added nitrate and phosphate levels were adjusted in increments of 25% less nutrients from the recipe of Table II, i.e., 0.4mM phosphates varying nitrates 1- 75% nitrates (3.9 mM) 2- 50% nitrates (2.6 mM), 3- 25% nitrates (1.3 mM) 5.2 nM nitrates varying phosphates 4- 75% phosphate (0.3 mM) 5- 50% phosphate (0.2 mM) 6- 25% phosphate (0.1 mM)
4- Repeat	Repeat Phase 3 runs to ensure accuracy.
5- Algae Sonication	Dissolve dry algae in a solution of KOH in methanol. Place the solution in a sonicator to disrupt the algae cells and release the oil. The methanol transesterifies the released algae oil into FAME/biodiesel. Remove algae by filtration. Evaporate methanol.
6- Gas Chromato-graph (GC) Analysis	Dissolve the FAMEs in chloroform. Inject the dissolved solution into the GC. Analyze the peaks. Use the areas under the peaks to determine the FAME production.

B. *Analytical procedures: pH and Nitrates*

It is important to measure the pH, nitrite and nitrate levels to maintain a consistent growth environment. These readings were taken every other day. The procedure was to collect 5 mL of each algae solution in a test tube then use Mardel 5 test strips.

C. *Analytical procedures: Algae Growth Monitoring*

Algae growth was monitored daily by measuring the absorptivity at 680 nm using a spectrophotometer. These readings were important in determining when the algae reached its stationary phase and needed to be harvested.

The algae growth runs varied in duration, ranging roughly from nine days to two weeks. Once the algae was harvested, they were dewatered, centrifuged, and freeze-dried. The final algae powder mass of each run was measured using a digital scale. This would allow the determination of the algae mass production per initial volume of algae solution for each run.

D. *Algae Harvesting, Dewatering and Drying*

Algae harvesting is the process of separating algae from its growth medium. The challenge is that the algae are in a dilute solution. The high water content of algae must be removed to enable harvesting. Several processes have been developed to harvest microalgae. These include using microscreens, centrifugation, flocculation, and broth filtration. High-speed centrifugation (5000 rpm for 10 minutes) was used in the present work [5], [7], [12] and [21] because it did not require the addition of flocculants, and for the ease of separating the solid from the liquid solution. Algae drying was accomplished by freeze-drying for approximately 48 hours. This produced dry algae flakes, which were crushed into powder using a mortar and pestle.

E. *Calculation of Oil yield from GC Measurements*

The GC Integrator generated graphs (chromatograms) of each mixture injected into it. The graphs contained peaks that corresponded to each algae biodiesel fatty acid in the injected mixture. A series of standard FAME cocktails were prepared, injected into the GC and the results were analyzed [21]. The GC calculations of the in-situ biodiesel determined the ratios (area of produced peaks to specified standard) [21]. This was done after matching the in-situ biodiesel peaks to the appropriate FAME standard. The results the composition, concentration, and mg of FAME per gram of algae.

F. *Measurements and Metrics*

The measured variables and purpose/metrics calculated are given in Table IV.

Table IV. Measured Variables and Metrics Calculated.

Measurement	Purpose/ metrics
Daily Algae Solution Absorbance (using Spectrophotometer)	Algae growth, from absorbance/turbidity.
pH, nitrite and nitrate levels with a Mardel 5 in 1 test strip.	Nutrient concentration and if there is nutrient depletion or starvation
Algae mass after harvesting and drying (using a balance)	Algae production (g dry algae/L)
Mass of algae Oil after extraction (using a gas chromatographer)	Algae oil yield (g oil/100 g dry algae)
Algae biodiesel production from the one-step in-situ process	Algae FAME/biodiesel production (g FAME/L)

IV. Results/Discussion/Accomplishments

A. *Algae Growth and Monitoring Results*

Figure 5 shows the absorbance/turbidity of the algae growth solution measured over the growth period. The data covered several "growth solution" cases to establish the confidence in the data. These cases include fresh (RO) water, RO water with algae and nutrients, wastewater with algae, wastewater with nutrient, and wastewater with algae and nutrients. For fresh (RO) water with no nutrient and no algae, the absorbance remains at zero, as expected.

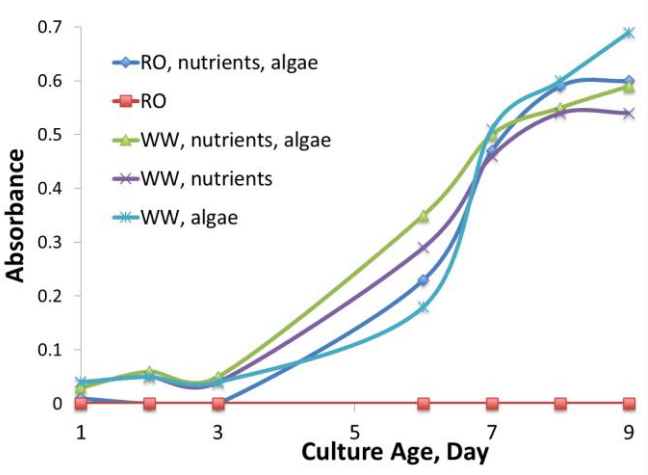


Fig. 5. Algae Monitoring; Absorptivity versus age of culture in days for different growth media.

B. *Nitrate, Nitrite and pH variation during Algae Growth.*

Figure 6 shows the nitrate measured over the growth period. It indicates that the trials without an added nutrient solution lack nitrates over time. The solutions with an added nutrient solution have the nitrates remain rather consistent around the maximum amount the test strips read and that infers the nutrient is likely present in excess.

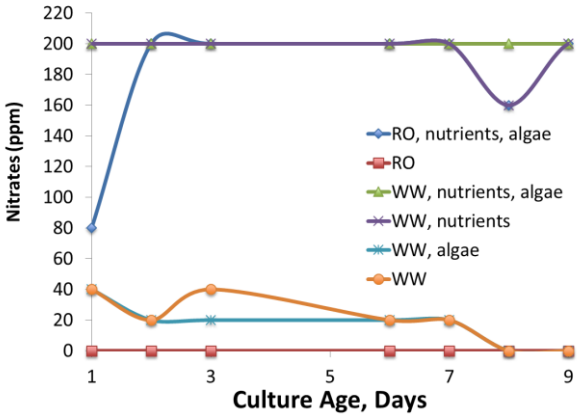


Fig. 6. Nitrates concentration (ppm) variation with culture age (days) in different growth media.

Similarly, Figures 7 and 8 show the variation in nitrites concentration (ppm) and pH of the nutrient medium in different growth medium.

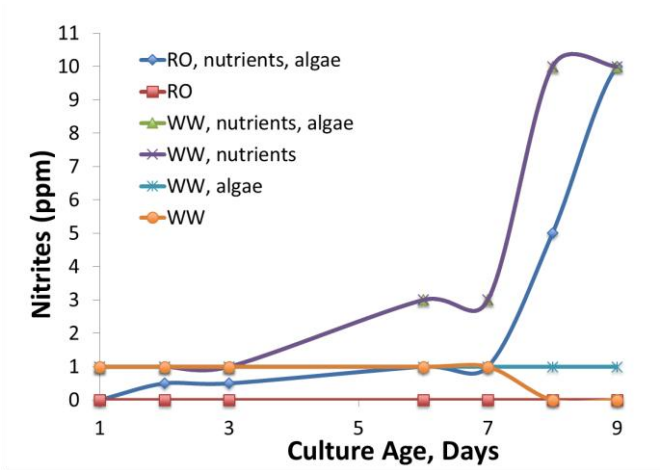


Fig. 7. Nitrites concentration (ppm) variation with culture age (days) in different growth media.

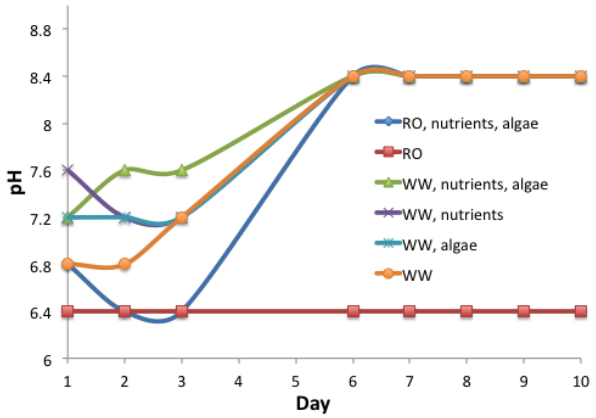
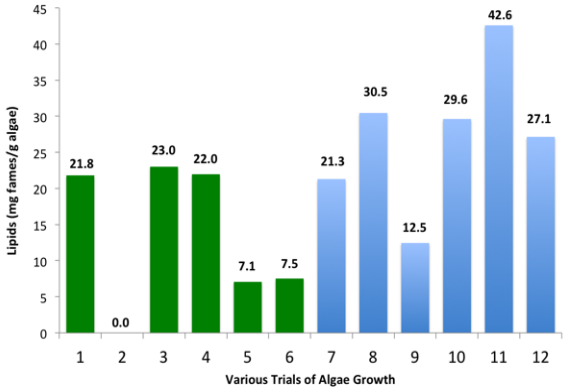


Fig. 8. pH variation with culture age (days) in different algae growth media.

Figure 8 indicates that when the algae grows the algae growth media reaches pH of 8.4. This is within the optimum algae growth pH range of 8.2-8.7.

C. *Algae Mass Production*

Figure 9 displays the algae mass production in grams per liter of growth medium under different conditions. A key is provided below the figure to correspond the number labels of the x-axis to its growth conditions (WW abbreviating wastewater and RO abbreviating reverse osmosis fresh water). The green bars are for growth solutions with 100% of chemical nutrients nitrates and phosphates. The blue bars are for growth solutions with reduced chemical nutrients (25%, 50% and 75%) nitrates and phosphates. The average algae production of the six blue lines is 0.751 g/L. The algae production with reduced nutrients are within 25% of the average algae production rate of all runs with reduced chemical nutrients.



1- RO, nutrients, algae, 2- RO, 3- WW, nutrients, algae, 4- WW, nutrients, 5-WW, algae, 6- WW, 7- 25% nitrates, 8- 50% nitrates, 9- 75% nitrates, 10- 25% phosphates, 11- 50% phosphates, 12- 75% phosphates

Fig. 9. Algae mass production (g of dry algae per liter of growth medium) of the base run trials (green bars) compared to the experimental trials (blue bars).

D. *One-stage in-situ Biodiesel/FAMES Production*

The conditions of each run and the resulting algae production (g dry algae per L of initial medium solution), algae oil concentration (mg oil per g dry algae) and algae oil production (mg FAMEs per L of initial medium solution) are summarized in Table W.

The algae oil production (mg FAMEs per L of initial medium solution) is obtained by multiplying the algae production (g dry algae per L of initial medium solution) and the algae oil concentration (mg oil per g dry algae), i.e., 1.595 * 21.816 = 34.8 (mg FAMEs per L of initial medium solution).

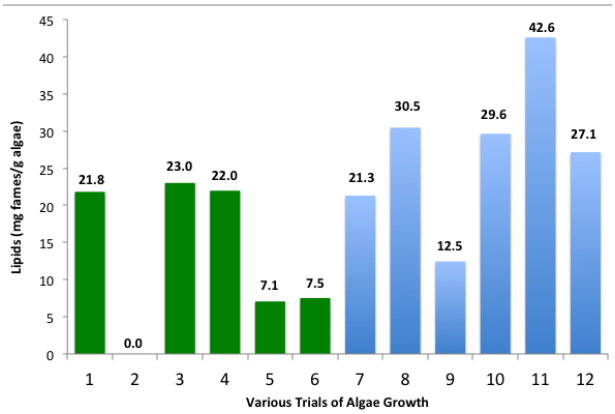
Assume the algae oil to have a density of 0.86 g/ml = 860 mg/ml = 0.86 mg/microL. This permits calculating the algae oil production in mircoL per liter of initial medium solution. Consider trial 1, with algae FAME production of 34.8 mg/L)/ (0.86 mg/microL = 40.46 microL/L). This value is entered in column I of Table II.

Table V. Oil production for Chlorella Vulgaris cultures under different algae growth conditions. (RO = Reverse Osmosis Fresh Water, WW= Wastewater)

A	B	C	D	E	F	G	H	I
1	RO	Yes	100	100	1.59	21.82	34.8	40.5
2	RO	No	No	No	0	0	0	0
3	WW	Yes	100	100	0.76	23.0	17.5	20.3
4	WW	No	100	100	1.35	21.97	29.6	34.4
5	WW	Yes	No	No	0.21	7.065	1.48	1.72
6	WW	No	No	No	0.19	7.528	1.45	1.68
7	WW	Yes	25	100	0.79	21.31	16.9	19.6
8	WW	Yes	50	100	0.58	30.46	17.6	20.5
9	WW	Yes	75	100	0.77	12.46	9.53	11.1
10	WW	Yes	100	25	0.75	29.63	22.2	25.8
11	WW	Yes	100	50	0.89	42.59	37.9	44.0
12	WW	Yes	100	75	0.73	27.15	19.9	23.2

Column Headings:
A Trial Number
B Water Source (RO or WW)
C Algae present (Yes or No)
D Nitrates, % of "standard" nitrates feed
E Phosphates, % of "standard" phosphates feed
F Algae Production (g dry algae/L of initial solution)
G Algae Oil concentration (mg FAMEs/g algae)
H Algae Oil Production (mg FAMEs per L of initial medium solution)
I Algae Oil Production (microL FAMEs per L of initial medium solution), or L FAME/million liters on initial solution.

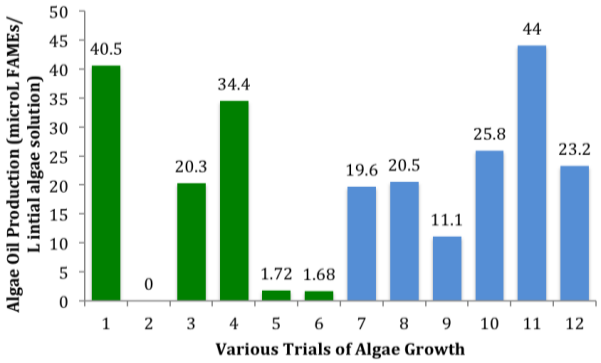
Figure 10 compares the oil/lipid production (mg of FAME/g of algae) for the different growth conditions. It shows how the different conditions affect lipid/oil production



1- RO, nutrients, algae, 2- RO, 3- WW, nutrients, algae, 4- WW, nutrients, 5-WW, algae, 6- WW, 7- 25% nitrates, 8- 50% nitrates, 9- 75% nitrates, 10- 25% phosphates, 11- 50% phosphates, 12- 75% phosphates.

Fig. 10. Algae Oil production (mg of FAME/g of algae) of the base run trials (green bars) compared to the experimental trials (blue bars).

Figure 11 compares the oil/lipid production (microL FAMEs/L of initial growth solutions) for the different growth conditions. It shows how the different conditions affect lipid/oil production



1- RO, nutrients, algae, 2- RO, 3- WW, nutrients, algae, 4- WW, nutrients, 5-WW, algae, 6- WW, 7- 25% nitrates, 8- 50% nitrates, 9- 75% nitrates, 10- 25% phosphates, 11- 50% phosphates, 12- 75% phosphates.

Fig. 11. Algae Oil production (microL of FAME/L of growth medium) of the base run trials (green bars) compared to the experimental trials (blue bars).

Figures 10 and 11 indicate that almost all of the experimental trials in wastewater with reduced nitrates or phosphates chemical nutrients (Runs 7 – 12 with blue bars) had higher lipid production per g of algae and per L of initial growth solution than the base trials of both RO water and wastewater with 100% of the "standard" nitrates and phosphates chemical nutrients (the green bars in Figure 10 and 11). In both cases, for the phosphates and nitrates, the trials with 50% of the original chemical nutrient had the higher FAME production. The highest FAMES production was obtained with 50% phosphates, Trial 11. This trial show lipid production of 42.59(mg FAMEs/g algae), 37.9 (mg FAMEs per L of initial medium solution), 44 microL FAMEs per L of initial medium solution). Nonetheless, all of the cases of reduced nutrient solutions look promising.

E. Production time and Energy use in two-stage vs. one-stage in-situ Biodiesel/FAMES Production

One of the main advantages of the in-situ process is the reduction time in biodiesel production. In the two-step method, the microalgae oil extraction would take 2 hours alone, plus evaporation and oven requirement time, roughly another 40 minutes. The extracted oil still needs to be traansestrified to biodiesel. Each in-situ would only take 10 minutes plus roughly 20 minutes to run through the gas chromatograph. In addition, the in-situ process avoids the use of a large amount of water, which is required by the flask condenser during lipid extractions. In addition, the in-situ process does not require the use of a hot water bath and oven, which are both energy intensive. Finally, in-situ avoids the use of a hazardous solvent, hexane, which is required for lipid extractions from the dry harvested algae.

V. Conclusion

Successful fresh (RO) water conservation was achieved by growing Chlorella vulgaris in municipal wastewater as the

growth medium. The base data were obtained for both growth medium with standard chemical nutrients of Table II.

This investigation indicates that 50% of the nitrate and phosphate chemical nutrients in the growth medium are conserved and the biodiesel production is increased when microalgae are grown in wastewater.

The in-situ process was also successful in reducing biodiesel production time, conserving energy and water. It also eliminated the use of hexane, a hazardous solvent used in the extraction of oil from dry microalgae.

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Dr. Farag received a number of prestigious awards, e.g., the US EPA Environmental Merit award, the Coast Guard Meritorious Commendation, the US Most Valuable Pollution Prevention (MVP2) Program award, and the UNH award for Excellence in International Engagement. In addition, he received several Outstanding Teaching awards at MIT and UNH.