GCMS Analysis of an Algae's Hydrocarbon Content

INTRODUCTION

As the 22nd Century draws closer, it also brings a global energy crisis. Taking a quick glance at the breakdown of world energy usage by type, one can conclude that over 75% of global energy consumption exists as the combustion of fossil fuels. Data from the Energy Information Administration (EIA) International Energy Outlook 2010 shows that liquid fuels comprise 34.5% of the world's total energy consumption with 172.9 quadrillion BTU/year, natural gas occupies 23.5%, coal occupies 26%, nuclear occupies 5.5%, and all "renewables" occupy only 10.5% of total world energy consumption (EIA IEO2010).

This heavy dependence on petroleum, coal, and natural gas threatens the stability of all fuel markets. Due to all fossil fuels' sluggish rates of formation, industry cannot expect to burn these fuels infinitely. Coal reserves are estimated to last another 251 years; and natural gas may last 64 years, but proven oil reserves will run out in less than 41 years (Cho 2010). The EIA estimates proven oil reserves worldwide have about 1,238.892 billion barrels of petroleum left (EIA AEO2009). Although this statistic slowly rises as companies discover new oil fields, the new reserves tend to be smaller and more remote. The enormous hazards to deep sea drilling may be seen clearly in the April 20, 2010 BP oil spill.

People are conscious of problems posed by petroleum but addiction to cheap, easily accessed liquid fuel energy is still escalating out of control. As of 2009, the United States solely consumes petroleum at a rate of 18.69 million barrels per day; furthermore, the world consumes 83.62 million barrels per day. (EIA International Energy Statistics) Nearly every single modern activity revolves around the combustion of cheap petroleum - ranging from agriculture to athletics. With the growth of rapidly industrializing giants, China and India, 40 more years of oil

would be a generous estimate; furthermore, the price of these liquid fuels will become problematic in only a couple of years.

In the meantime, many alternatives just cannot compete with petroleum. U.S. barrels of crude oil remain at about $76 each, retail gasoline prices are currently at an average of $2.70/gal, and Diesel fuel is at $2.89/gal (EIA Petroleum). Research on various bio-fuels has grown exponentially in the last decade, but bio-diesel and ethanol produced from soybean, rapeseed, and other crops are grown too inefficiently. World Bio-fuel production only remains at 1.5 million barrels a day (EIA International Energy Statistics); needless to say, a superior bio-fuel crop is needed.

Comprehensive research on fuels derived from algae has been an ongoing project in the last few decades, most notably under the Aquatic Species Program (ASP). The U.S. Department of Energy focused on finding lipid concentrations in certain species of algae to create bio-diesel. Although the DOE cut funding to the program because it concluded algae could never compete with petro-diesel at 1998 prices, many economic changes have occurred since then (DOE ASP 1998). In the U.S, 1998 retail gasoline prices were about $1.03/gal and 1998 retail diesel prices were about $1.12/gal (EIA Petroleum). After adjusting for inflation through C.P.I, 1998 gasoline prices would be $1.38/gal and 1998 petro-diesel prices would be $1.50/gal in modern times. Prices have nearly doubled since the shutdown of the ASP, making algal fuel a realistic competitor to fossil fuels; furthermore, recent research has improved the growth rate and fuel content of algae.

Algae pose as a greater bio-fuel source when compared to current terrestrial crops because it has daily harvesting cycle, allowing continual output of fuel (Wagner 2007). While it is grown in an aquatic environment, algae also have ease of access to abundant water, nutrients

and CO2. More specifically, microalgae is preferable to macroalgae. Microalgae's structure is far more simplistic, allowing for more efficient solar energy conversions. Some species also have unusually fast growth rates or high oil content. Microalgae can produce more than 30x the amount of oil per unit of land compared to top terrestrial oilseed crops; in fact, some species are predicted to output more than 20,000 gallons per acre per year of fuel (Wagner 2007). Additionally, most strains are able to grow in some waste water and purify pollutant emissions from coal burning factories. A M.I.T. powerplant pumped emissions through tubes filled with algae and the resulting gas was recorded to have 40% less CO_2 and 86% less Nitrous Oxide (Clayton 2006).

Hydrocarbon producing algae strains that require minimal resources may hold the key to providing a more efficiently accessed fuel. The benefits are boundless- from the increased uptake of CO_2 to the ability to provide feedstock from the cell remains. After development, this technology could be quickly economically superior to petroleum, especially with the inevitable production decline of fossil fuels; and predicted algal output is certainly enough to fill some major global fuel demands. It would definitely be more environmentally friendly in comparison to petroleum. If this new fuel was mass marketed along with other alternatives, the imminent, future energy deficit could be avoided.

A new, unidentified species of microalgae discovered in Lake George, ND may have unique oil qualities when compared to other combustible bio-fuel crops. The purpose of my experiment was to find its relative potential in fuel production by testing the strain to discover its natural, optimum growing environment and its hydrocarbon abundance. This mystery Specie "X" may secrete a high concentration of viable hydrocarbons in at least one of the growing conditions and have the potential to provide a competitive alternative fuel to global fuel markets.

MATERIALS and METHODS

Lake Water Collection and Algae Culture Construction

9L of Lake George water, 90cc of deep lake sediment, and 90cc of near-shore lake sediment was first collected directly from the lake and then transported to the research facility. Both the water and sediment samples were expected to have algae in them. Then all the collected water was filtered through 0.2μm paper and distributed amongst 9 uniform containment units. The filtration served to separate any larger organisms collected. 5L Erlenmeyer flasks were used as the containment units and 1-L filtered (0.2μm) Lake George water was added to each flask. The microalgae were expected to remain in the filtered water and to grow. Each 5L flask now served as an algae culture home. This experiment involved finding an ideal natural growing environment so each of the 9 flasks had different combinations of environmental conditions. 3 flasks were placed in a 4^{o}C growth chamber, 3 flasks were placed in a 10^{o}C growth chamber, and 3 flasks were placed in a 20^{o}C growth chamber. Then 3 different sediment conditions were set up for the 3 flasks in each different growth chamber. Flasks with the “Deep” condition had 30cc of deep Lake George sediment added. Flasks with the “Unfiltered” condition had 30cc of near-shore sediment added. Flasks with the “Control” condition had no sediment added. No other nutrients were added to simulate natural growth. All 9 flasks were given 12 hours of light and then 12 hours of darkness to simulate natural outdoor growth. The light was provided by a small light-bulb fixed above each flask. To account for the additional heat released from closely attached light-bulbs, 2 small fans were placed beside cultures in both the 4^{o}C and 20^{o}C growth chambers. Finally, small sheets of aluminum foil were fixed to the top of each flask to prevent contamination in the cultures. Initially all 9 environmental conditions had completely clear water

when all cultures were set up, but after they were checked upon 2 weeks later, the algae strain had changed the color of the water and could be seen (See Pictures 1-3).

Culture Sampling for GCMS Analysis

9 separately marked 20ml glass vials were then allotted to each of the algae cultures. Large 10ml pipettes were used to extract algae from each culture and placed into the appropriate corresponding vial. Whenever pipettes were used in this experiment, different ones were used for each separate sample to avoid contamination. A 5ml mixture of algae and water from the surface film of water was collected for each sample (See pictures 4-6). The outer rim of the water was the optimum area to collect algae because the strains were noticeably concentrated around the edge in the form of a thick green line. To ensure the most uniform extraction, the pipette tip was spun 360 degrees around the edge of the flask. Algae from the bottoms of the Erlenmeyer flasks were not extracted in order to avoid contamination by sediment and other organic material.

All 9 vials were then placed in a freezer overnight. This step prepared the samples to be freeze-dried; under low pressures in a vacuum, the boiling point of water decreases so all water evaporated, leaving all the dry organic material behind.

Preparing the GCMS Sample

Step 1: Sample Dissolution

After all water was removed from the 20ml vials, approximately 3.5 ml Dichloromethane (DCM), a common organic solvent, was pipetted into every 20ml vial. Because some dried vial contents clung to the sides, the pipette was also used to scrape inner vial walls to ensure that all organics dissolved. After thorough scraping and washing by DCM, the vials were sonicated for 20 minutes to better dissolve all compounds. After the sonicator finished running, the particulate matter was separated from solvent by settling at the bottom of each vial. 9 other 4ml vials were

then allotted to each corresponding 20ml vial. After the sonication was complete, pipettes were used to transfer solvent from the 20ml vials to corresponding, empty 4ml vials. Procedures starting from the beginning of *Step 1* were completed again with another 3.5ml DCM, and 3.5ml of DCM/Methanol solvent in the ratio 1:1 (Methanol is added to ensure the dissolution of all polar organics). Each time the 4ml vials received about 3.5ml of solvent, nitrogen gas was blown into them so the solvent evaporated faster; therefore, the 4ml vials could receive the 3.5ml DCM twice and the 3.5 DCM/Methanol (a total of 10.5ml solvent).

Step 2: BSTFA Derivitization and Further Dissolution

50µl Bis (Trimethylsilyl) trifluoroacetamide (BSTFA), 50µl Pyridine and 1ml Toluene was then added to every 4ml vial. The GCMS cannot handle really polar compounds so BSTFA was needed for the samples' alcohol fractions. The BSTFA displaces active hydrogens with $Si(CH_3)_3$ to ensure that all molecules could be read in the GCMS. The pyridine and toluene were added to further dissolve all organics in the samples. Once all three compounds were added, nitrogen gas was blown into every open 4ml vial for 5 seconds and tightly capped right afterwards. This step was done to ensure that no oxygen in the air entered the sealed 4ml vial (Since BSTFA oxidizes very quickly). All 4ml vials were heated at 60^{o}C for 6 hours to ensure a complete reaction. After all the vials cooled back to room temperature, nitrogen gas was blown into the vials to completely dry all vials.

Step 3: Silica Gel Filtering of Samples

The sonication had separated most of the particulate matter from the solvent but still not enough for the GCMS to handle. After adding DCM to a dry 4ml vial from *Step* 2, I discovered that some mysterious powder/crystal substances would not dissolve; therefore, a filtering step was taken to remove them. I prepared 9 filters using glass pipettes, steel wool, and silica gel.

Contrary to conventional silica gel chromatography, I prepared columns serving to filter the algae samples rather than separating chemical compounds within the samples. The steel wool was first stuffed down into the thin tip of the pipette. Then a funnel was placed on top of the pipette to prevent spilling silica gel. Using a spoon, I transferred a small plug of silica gel down the funnel and onto the wool. The wool served to prevent any gel from falling out of the pipette. Samples from *Step 2* were transferred into the silica gel filter using DCM and 9 new, corresponding 4ml vials were placed beneath each respective filter. About 3 ml of ethyl acetate was used to flush the samples through the silica gel and into the new 4ml vials. All particulate matter stayed on top of the silica gel so the filtration was successful. The 4ml vials were then allowed to dry overnight.

Step 4: Preparing GCMS vials and Adding an Internal Standard

After filtering the samples, the 2ml vials used for the GCMS were prepared. Nine 2ml vials were allotted to the 4ml vials, and a 250µl insert was placed in each of the 2ml vials. The purpose of the insert was to concentrate the samples so the GCMS peaks would be more prominent. Pipettes were used to place 200µl DCM in the dried 4ml vials from *Step 3*. The pipettes were used once again to scratch the inner walls to ensure uniform dissolution of the 4ml vial contents. After the 4ml vial contents dissolved in DCM, pipettes were used to transfer all organics into corresponding 250µl inserts within the 2ml vials. Then 50µl of C_{36} Hexatriacontane in toluene solution (with a known concentration) was added in each of the 250µl inserts. The Hexatriacontane served as an internal standard for the GCMS, allowing me to quantify all chromatogram peaks. All 2ml vials with inserts were capped afterwards and placed in the GCMS. Finally, my mentor aided me in setting up the Instrument Control Parameters before I ran the GCMS.

Table 1: GCMS Machine Parameters

Inlet Parameters	Column Parameters	Injector Parameters
Mode: Splitless Initial Temp: 320ºC Pressure: 6.30 psi Purge flow: 30.0mL/min Purge time: 0.80 min Total flow: 34.1mL/min Gas type: Helium.	Capillary Column Model Number: Agilent HP5MS 28 m Max temperature: 330 ‘C Nominal Length: 28.0 m Nominal diameter: 250.00 µm Nominal film thickness: 0.25 µm Mode: Constant flow Initial flow: 1.0mL/min Nominal init pressure: 6.31 psi Average velocity: 37 cm/sec	Sample Washes: 0 Sample Pumps: 3 Injection volume: 1.00 microliters Syringe size: 10.0 microliters
Table 1: These are the GCMS parameters set up specifically for the algae samples. In other terms, this step is similar to prepping a computer program to perform a certain job.		

RESULTS

Quantitative Results and Calculations

After all 9 vials were run through the GCMS, the machine produced chromatograms for each sample. These chromatograms are shown in graphs 1-9 below. A computer program allowed me to record the retention times of all major peaks and also allowed me to integrate major peaks to find the area beneath each one. Tables 2-10 below basically show the number of prominent peaks I integrated and their area values. The listed Table retention times (R.T.) match with corresponding chromatograms beside each Table. A specific peak in the chromatogram can be spotted by matching the Table’s R.T. for a peak with the actual time values on the chromatogram’s x-axis.

Chromatograms and Peak Data:

Graph1: Vial 1 Chromatogram

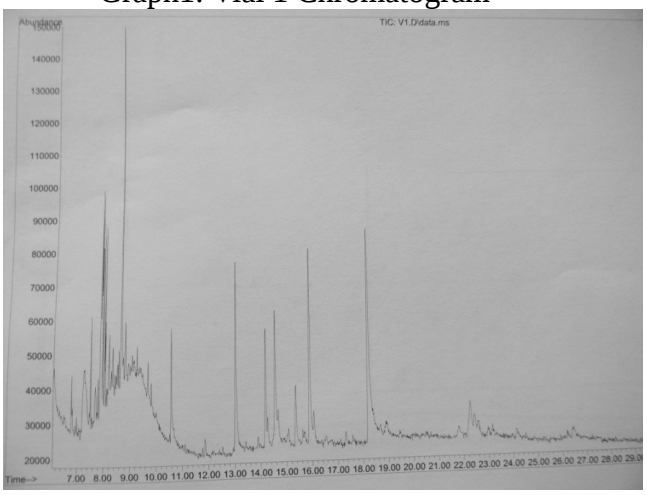


Table 2: V1 Peak Data

Peak	R.T.	Area
1	6.794	265772
2	7.276	1512020
3	7.511	283250
4	7.866	2223147
5	7.973	611145
6	8.034	404909
7	8.647	1902060
8	10.567	790858
9	13.011	1682893
10	14.171	1344192
11	14.545	1975992
12	15.352	748913
13	15.898	2620736
14	18.226	3517132
15	22.292	652752

Graph 2: Vial 2 Chromatogram

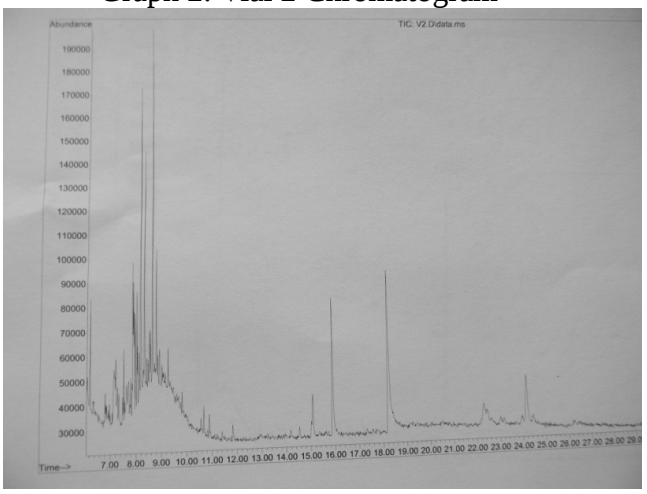


Table 3: V2 Peak Data

Peak	R.T.	Area
1	6.21	402148
2	7.865	1790840
3	7.972	468330
4	8.033	639099
5	8.167	1618243
6	8.326	1078274
7	8.643	1603057
8	8.799	652455
9	9.262	117538
10	15.077	713845
11	15.895	1917412
12	18.216	2976417
13	24.15	1179452

Graph 3: Vial 3 Chromatogram

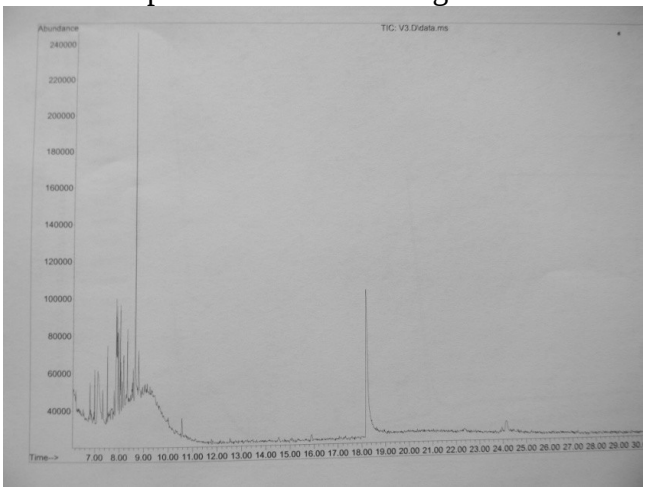


Table 4: V3 Peak Data

Peak	R.T.	Area
1	7.113	1584536
2	7.511	359146
3	7.865	1783042
4	7.973	550522
5	8.033	730932
6	8.116	442312
7	8.174	461571
8	8.326	369154
9	8.643	2280692
10	8.79	310303
11	10.567	170294
12	18.221	4132254
13	24.161	388281

Graph 4: Vial 4 Chromatogram

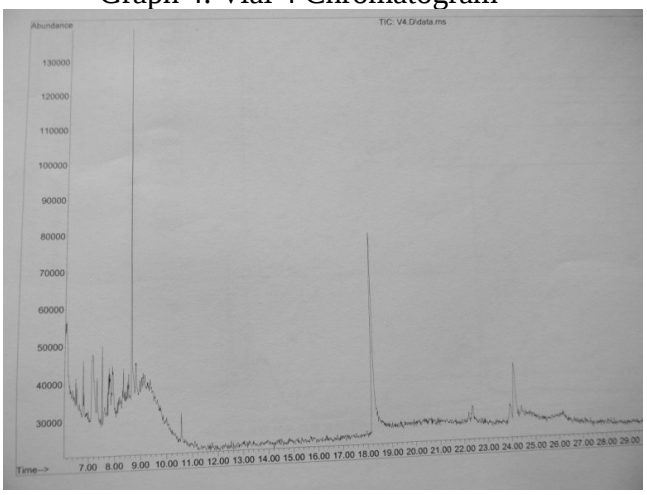


Table 5: V4 Peak Data

Peak	R.T.	Area
1	6.793	193404
2	7.149	1114573
3	7.511	180979
4	8.644	1508430
5	10.566	134290
6	18.217	2611761
7	24.149	988352

Graph 5: Vial 5 Chromatogram

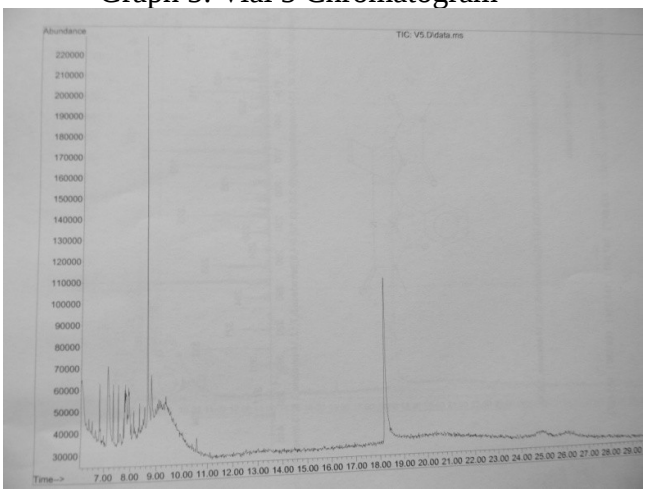


Table 6: V5 Peak Data

Peak	R.T.	Area
1	6.793	328566
2	7.109	1855782
3	7.318	316956
4	7.511	226511
5	8.644	2611318
6	8.791	444682
7	10.568	109861
8	18.223	3861816

Graph 6: Vial 6 Chromatogram

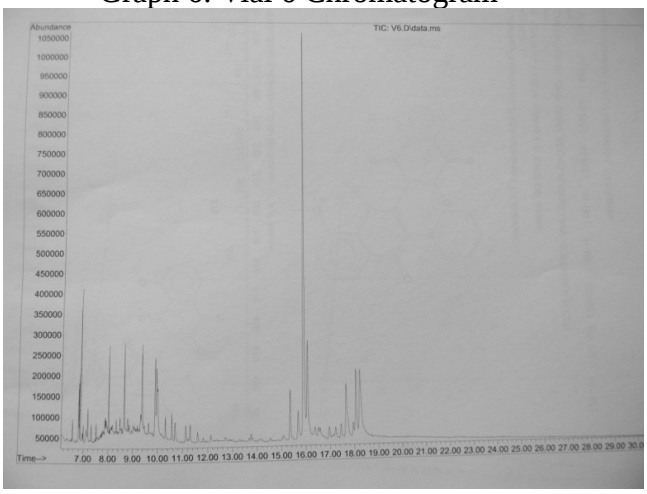
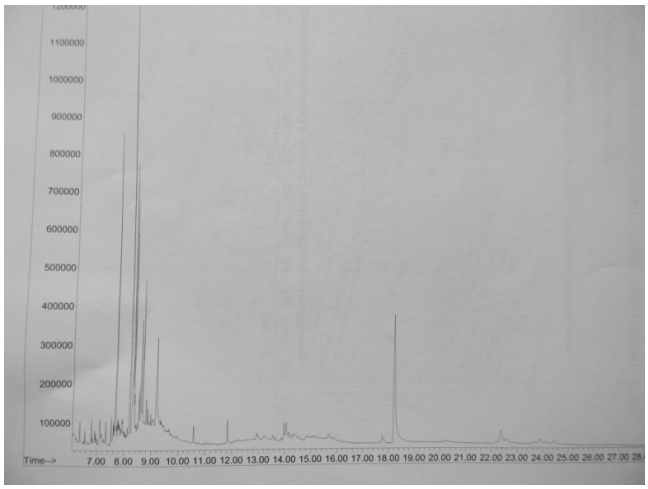


Table 7: V6 Peak Data

Peak	R.T.	Area
1	6.818	2863356
2	6.864	3886482
3	8.01	2044237
4	8.643	2407012
5	9.359	4622632
6	9.891	9953573
7	15.345	3466044
8	15.859	3849047
9	17.656	6249747
10	18.061	5285769
11	18.227	7407121

Graph 7: Vial 7 Chromatogram

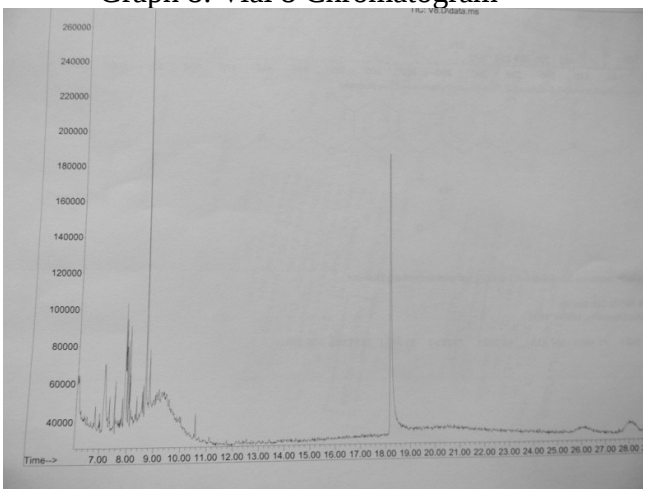
Table 8: V7 Peak Data



Graph 8: Vial 8 Chromatogram

Peak	R.T.	Area
1	7.657	7573816
2	8.169	13981183
3	8.278	16144662
4	8.572	3493186
5	8.642	3100694
6	9.155	4173292
7	10.564	563838
8	11.825	1028308
9	14.054	2323253
10	18.224	13864414

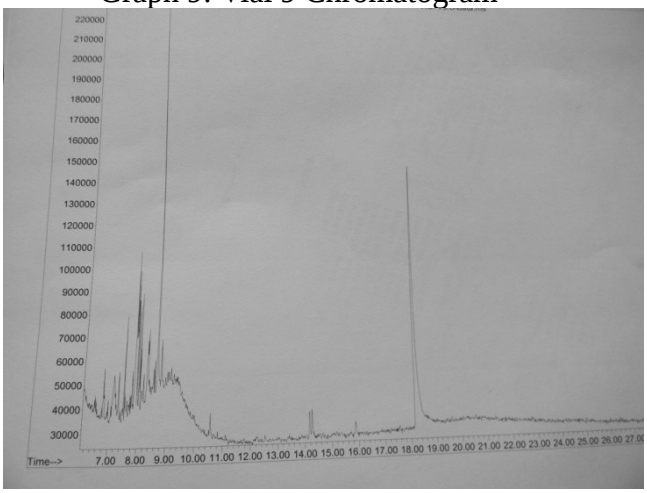
Table 9: V8 Peak Data



Graph 9: Vial 9 Chromatogram

Peak	R.T.	Area
1	7.104	1845721
2	7.509	300059
3	7.865	1994627
4	7.972	520384
5	8.033	728592
6	8.643	2697040
7	10.565	143449
8	18.222	7597752

Table 10: V9 Peak Data



Peak	R.T.	Area
1	7.318	165131
2	7.51	404893
3	7.865	2100195
4	7.972	513941
5	8.028	220060
6	8.324	473788
7	8.643	2256777
8	8.791	299183
9	10.564	154021
10	14.172	271778
11	14.263	235009
12	18.221	6000847

The addition of the internal standard (C_{36} Hexatriacontane) is seen clearly on all chromatograms (Graphs 1-9) at a retention time of about 18.2 minutes. The standard peaks are the relatively large peaks isolated towards the right side of the graph. The integration to find peak areas is important because the mass of a substance in the 250µl insert is proportional to the area below the peak. Since I know both the concentration of standard added and the area of the standard peak, the abundance of all other hydrocarbons can be quantified. The initial concentration of the standard was 2.802µg C_{36}/ 40ml toluene. Since I added 50µl of the standard solution to each 250µl insert, I can use a ratio to find the mass of C_{36} in the insert. (2.802µg C_{36}) / (40ml) = (X mass C_{36}) / (50µl). After solving for X, I now know that the total mass of standard in each vial is 3.5025µg; this mass is also directly proportional to the standard peak's area in each chromatogram. Now I can use the ratio:

$$\frac{\text{3.5025µg } C_{36} \text{ Standard}}{\text{Area of Standard Peak}} = \frac{\text{X Mass of Algae's Hydrocarbons}}{\text{(Area of All Peaks) – (Area of Standard Peak)}}$$

Solving for X will show the mass of all hydrocarbons in the algae. Then to determine the concentration of hydrocarbons in relationship to the volume of sample collected, the mass is divided by 5ml (Since 5ml samples were taken from each culture). This allowed me to calculate the concentration of hydrocarbons in every 1L algae culture. The data and results of all calculations are shown in Table 11 below.

Table 11: Hydrocarbon Concentration Calculated from Chromatogram Peaks

Vial	Standard Peak Area	Total Peak Area	Hydrocarbon Concentration in µg per L
1	3517132	20535771	3389.567585
2	2976417	15157110	2866.727158
3	4132254	13563039	1598.707362
4	2611761	6731789	1105.032051
5	3861816	9755492	1069.061819
6	7407121	86592149	7488.619683
7	13864414	66246646	2646.614095
8	7597752	15827624	758.7804045
9	6000847	13095623	828.198184

Based on experimental results, all of the hydrocarbon concentration values are very low; Table 11 shows that the only culture with over 7mg of hydrocarbons is Culture #6. In fact, Culture #6 has more than double the concentration of any other Culture. Other cultures such as Culture #8 may have hydrocarbon masses below even 1mg so there was definitely great deviation between cultures.

To find the optimum growth conditions, I arranged values from Table 11 in Chart 1 below. It shows hydrocarbon concentrations of samples in relationship with varying temperatures and sediment conditions.

Chart 1: Hydrocarbon Concentrations in Relationship to Environmental Conditions

	Deep	Unfiltered	Control	Average
4°C Chamber	V1: 3390µg/L	V2: 2867µg/L	V3: 1599µg/L	2618µg/L
10°C Chamber	V4: 1105µg/L	V5: 1069µg/L	V6: 7489µg/L	3221µg/L
20°C Chamber	V7: 2647µg/L	V8: 759µg/L	V9: 828µg/L	1411µg/L
Average	2381µg/L	1565µg/L	3305µg/L	

A quick glance at the averages in Chart 1 show that the temperature promoting the highest hydrocarbon production is 10°C (average of 3221µg/L), and the optimum sediment condition is “Control” (average of 3305µg/L). These values seem to be rather inconsistent with individual vial data though. Vial 6’s relatively huge concentration of hydrocarbons boosts the averages of both the “Control” condition and the 10°C condition; however, the 4°C chamber seems to have a higher consistent hydrocarbon concentration with all 3 cultures above 1500µg/L. The “Deep” sediment condition also seems to have a higher consistent hydrocarbon concentration when looking at all 3 “Deep” cultures in Chart 1.

DISCUSSION

Optimum Growing Conditions

To reinforce my proposal that the optimum growth conditions occur in “Deep” sediment conditions and at 4ºC, I compared concentrations to the relative colorations of the algae cultures. These algae colorations can be view in Pictures 1-3. I noticed that both sediment conditions and the temperature conditions had a predominant effect on the algae’s hues. Cultures with deep sediment tended to have a much darker hue of green and a thicker concentration of algae in the water; furthermore, Chart 1 shows that “Deep” cultures have consistently higher hydrocarbon concentrations than the other sediment conditions. This reasoning makes sense because algae grown beside lake sediment would have more nutrients to feed on, while the “Control” cultures are only grown on sunlight and plain lake water. The effect of temperature on algae coloration is seen clearest in the comparisons between algae grown in 4ºC and 20ºC chambers. Picture 3 shows that algae grown at 20ºC have an orange hue while picture 1 shows that algae grown at 4ºC have a drastically healthier, green hue. Samples grown at 10ºC also have a green hue and this is reflected in hydrocarbon concentration values in Chart 1. The average concentration of the 4ºC samples is 2618µg/L, the average of the 10ºC samples is 3221µg/L, but the average of the 20ºC samples is only 1411µg/L. This data reinforces my deduction that the orange hue of 20ºC samples reflects plant death and limited hydrocarbon production. Thus with the exception of Culture #6, this algae strain’s optimum, natural growth environment is around deep sediment and at a temperature of 4ºC.

Commercial Use of this Algae Strain

Even at optimum, natural growing conditions, this algae strain cannot be grown for commercial oil production. None of my algae cultures had hydrocarbon concentration values to

serve any industrial fuel production purposes. A concentration of 7mg hydrocarbons per liter is still way too low when compared to that of industrial, algae growth projects. The Tubular PhotoBioReactor (TPBR), an algae growth mechanism, can easily produce “1g of dry algal biomass per liter of medium within 12 days, with a lipid content of 12% approximately” (Mulumba 2010). A leading company Solazyme commercially grows algae within steel vessels and has reached productivity levels of “several hundred grams per liter of dry cell weight and 75% oil within that dry weight” (Schwartz 2010). Although my statistics are very small in comparison to these companies’ oil concentrations, my experiment uniquely emphasized natural algae growth rather than pumping the strains full of abundant nutrients. From my hydrocarbon concentration statistics themselves, I can safely conclude that my strain of algae cannot meet fuel demands if it is grown without additional nutrients. Regardless of the resources saved by growing this strain in the wild, it would never even comprise 1% of the current fuel market because its hydrocarbon concentrations would be far too low.

CONCLUSION

There may have been errors in my quantitative experimentation that invalidate the exact nature of my results. Integration was quite difficult because the quality of the chromatograms were very poor. In Graphs 1-9, all the chromatogram baselines rise up significantly towards the beginning like a hill instead of remaining flat. Thus, the lines I used for integration were slanted and may not have given accurate areas. Some peaks were also split as well so I had to integrate a peak that may have been 3 separate ones. I also skipped over some smaller peaks which may significantly affect the total hydrocarbon concentrations in every sample.

Algae sample collection would also contribute to the inconsistency of my results. The concentrations of hydrocarbons in each 1L culture was derived solely from the data of mere 5ml

samples. There was no way to uniformly collect 5ml samples from each culture to ensure consistent results. Some areas in the cultures themselves may have higher or lower concentrations of hydrocarbons. This would have most likely contributed to the relatively huge concentration found in sample #6.

My results were also mostly based off of how many hydrocarbons the algae released into the water rather than how much was in the actual cells. In some industrially grown algae, the cell walls are broken by a press to release the oil. Then researchers can find the percentage of hydrocarbons by dry mass of algae collected. This certainly could be further research for the strain I tested.

I was also limited to simply quantifying the amount of hydrocarbons rather than qualitatively determining the individual compounds. Though I had a computer program which suggested chemical compounds for each peak by comparing the mass spectrums, I easily discovered that many of the compounds the machine suggested were wrong because the suggested compounds usually had a mass much lower than the one given by the chromatogram. Ultimately, most of the compounds in the program's chemical library are compounds found in the pharmaceutical industry as they try to discover or create new drugs; therefore, many unidentified fuel compounds would have to be manually identified through literature research. The amount of specific compounds a strain produces could certainly effect its usefulness in the biofuel industry. For example, if my strain produced alkanes, then the collected hydrocarbons would not have to go through trans-esterfication, an extensive process used to produce bio-diesel (Aquatic Species Program 1998). Some strain's hydrocarbons could be harvested and directly combusted in an automobile engine. Finding specific compounds within each species would definitely constitute important future research.

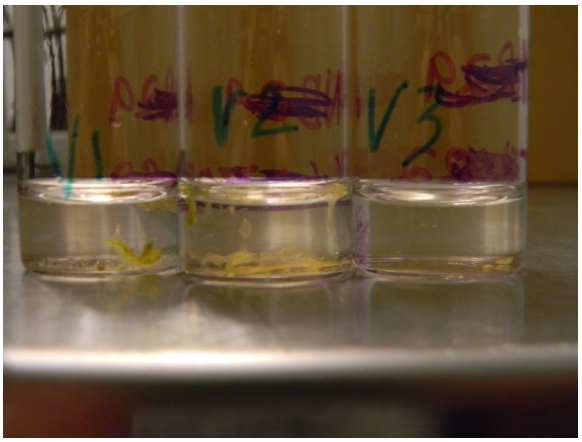
To better compare my strain of algae with others used industrially, I could continue my experiment by pumping all cultures with vast amounts of CO_2 and nutrients. Then I would be able to know for certain whether this strain is superior in any manner of oil production.

Though some people may have hopefully theorized that harnessing the fuel of wildly grown algae can solve our energy crisis, my results reveal the folly of such thinking. Like any other crop, algae require abundant nutrients in order to ensure maximized production. Even if the algae were given surplus nutrients, it would be far more efficient than crops such as corn. Water is a huge concern in the bio-fuel industry, and water conservation for mass production is optimized if the amount of oil per liter of water used is maximized. This is one area in which my algae does excel though. My cultures produced significant amounts of hydrocarbons in salt water so the need for freshwater is eliminated during fuel production. With further testing, this oil secreting strain could certainly prove to become important to the fuel industry, especially if its genome for oil secretion was isolated. Genetic engineering could produce a strain of algae that secretes a large amount of viable oil and grows very fast. Global markets certainly need a dominant renewable energy source to replace the fast depleting petroleum reserves, and algae bio-fuels pose as a promising solution. After all, the vast reserves of petroleum were formed from algae thriving millions of years ago (Oilgae).

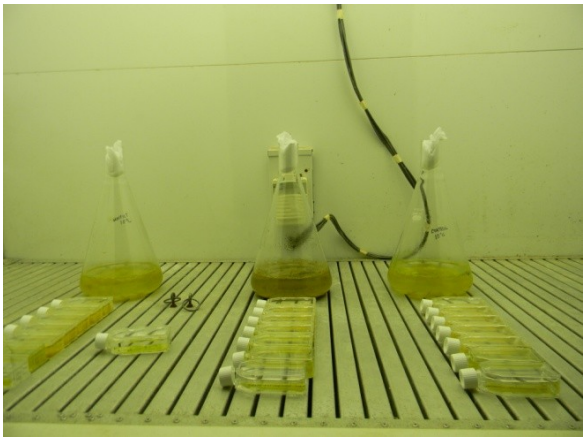
ILLUSTRATIONS

*Note: "(in the order)" is from left to right in the picture

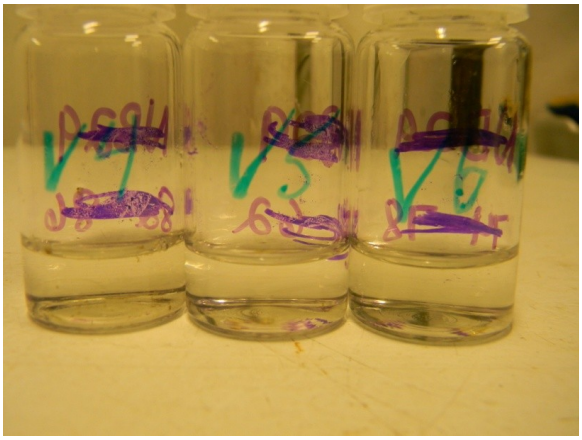
Picture 1: 4°C Growth Chamber
(In the order: Control, Deep, Unfiltered)



Picture 4: 4°C 5ml Samples
(In the order: Deep, Unfiltered, Control)



Picture 2: 10°C Growth Chamber
(In the Order: Unfiltered, Deep, Control)



Picture 5: 10°C 5ml Samples
(In the order: Deep, Unfiltered, Control)

Picture 3: 20°C Growth Chamber
(In the Order: Deep, Control, Unfiltered)

Picture 6: 20°C 5ml Samples
(In the order: Deep, Unfiltered, Control)

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