# Algal Biofuels: The Effect of Temperature on Algal Growth and Lipid Content

by

**Stephanie Marie Klenzendorf, B.A.**

**Report**

Presented to the Faculty of the Graduate School of
The University of Texas at Austin
in Partial Fulfillment
of the Requirements
for the Degree of

**Masters of Arts**

**The University of Texas at Austin**
**2009**

# Algal Biofuels: The Effect of Temperature on Algal Growth and Lipid Content

**Approved by**
**Supervising Committee:**

**Jill Marshall**

**K. Sathasivan**

**Mona Mehdy**

# Dedication

This work is dedicated to my parents Ron and Jeanne Sisco, and to my husband Brian Klenzendorf. Without their guidance, support, and love, this could never have been possible.

# Acknowledgements

I would like to acknowledge Dr. K Sathasivan, Dr. Mona Mehdy, Dr. Jerry Brand, and Dr. Martin Poenie for their willingness to provide the time and resources needed for this project. I would also like to acknowledge the other members of the above mentioned labs who were instrumental in completing this experiment including Tanya Sabharwal, Min Hui Lim, Michelle Randazzo, and Rebeccah Powell. Lastly, none of this would be possible without Mary Walker and the other UTeach professors who continue to work tirelessly to develop the very best science teacher education program in the country.

I would also like to recognize the tremendous support given by the UTeach program especially Dr. Mary Walker and Ruth Buskirk. I feel very fortunate to have had the opportunity to learn and work with these outstanding professors and the rest of my UTeach classmates. I would also like to thank my lab partner and classmate, Cesar Gutierrez, whose collaboration on the algae biofuels project added so much to this study.

August 6, 2009

# Abstract

## Algal Biofuels: The Effect of Temperature on Algal Growth and Lipid Content

Stephanie Marie Klenzendorf, M.A.
The University of Texas at Austin, 2009

Supervisor: Jill Marshall
Reader: Kanagasabapathi Sathasivan
Co-supervisor: Mona Mehdy

Replacing fossil fuels with algae, a renewable resource, is an exciting possibility. This study evaluates the algae found in South Texas brackish water ponds used for aquaculture of fish as a possible source of biofuels. Samples of algae from these ponds were cultured at varying temperatures ranging from 15.5ºC to 36.5ºC. High levels of growth were observed at 20.5ºC and the highest lipid content was measured at 23.0ºC. Temperature was also a factor in the distribution of microalgal taxa throughout the temperature gradient. This information will be added to the growing body of research investigating similar cultures of algae for future biofuel production.

# Table of Contents

# List of Tables

# List of Figures

# Chapter 1: Introduction

The need for a sustainable fuel source was recognized by scientific communities even before the onset of energy shortage concerns. Traditional fossil fuels that are used to sustain modern societies are formed only after hundreds of millions of years of exposure to pressure and heat. Recently, scientists have developed techniques that may speed this process and allow oil to be harvested from organic sources, potentially eliminating the need to drill.

While many plant sources such as corn, soybean and switch grass have been considered as possible sources for biofuel, they are met with problems such as available land use and food shortages. More recently, a new candidate has arrived that seems to present a viable solution. Microalgae are the most efficient biological producer of oil available and may soon be one of the Earth's most important renewable fuel crops (Campbell, 1997). While large scale production of algae biofuel is still many years away, this alternative could very well become common-place at some point in the near future.

The purpose of this study is to identify the optimal growing temperature of a culture of microalgae, found in one of the many brackish ponds located in South Texas. These ponds containing pre-existing algae are currently being considered as a potential location for mass biofuel production. Understand optimal growth temperature is important because valuable time and money can be lost if the algal culture is either overheated or over cooled. Before investors will be able to develop land for biodiesel production, they will need to know what algae to select and how much oil can be produced by this source as a function of daily and seasonal temperature fluctuations. This report describes relevant literature concerning this study, the design and setup of the temperature gradient experiment, and will include a thorough discussion of the data and results. The last section will present an integration of this research into the high school biology classroom.

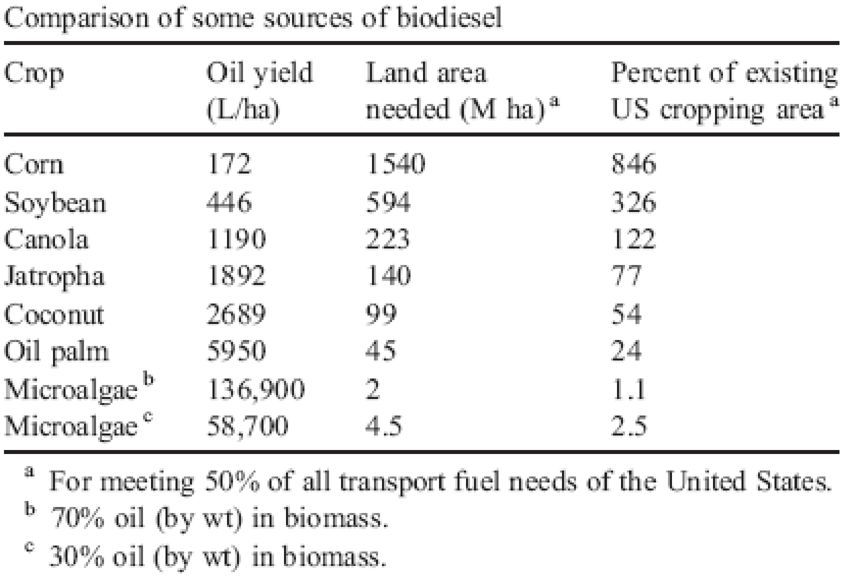
# Chapter 2: Review of the literature

## General Overview

There are many theories as to which source might be the most viable alternative for energy production, and much progress has been made in this area. Until other sources such as hydrogen, wind, and electric become more cost efficient, many scientists are focusing on renewable organic sources that can be refined into fuel for our cars. Biodiesel is recognized as being a cleaner and more renewable source of energy than traditional fossil fuels (Vasudevan, 2008). It is produced by a process of transesterification which turns extracted oils into fatty acid alkyl esters (biodiesel) in the presence of alcohol and a catalyst, with glycerol as a byproduct.

Microalgae are not the first or only organic source that has been considered for biodiesel production. Other types of oil such as waste cooking oil and animal fat are viable for energy use, but are not realistic in terms of the energy and land resources that must be used to produce them. (Chisti, 2007). Other studies also describe the feasibility of making quality biodiesel from recycled oils but identify the problems with the free fatty acids present in the raw materials. Too many fatty acids result in the production of soap in the presence of the alkali catalyst and additional steps must be taken to remove the acids before it can be used for fuel. (Vasudevan, 2008)

Other possible sources of virgin oil come from soybeans, jatropha, palm and corn. While these were initially a popular source of biodiesel fuels, oxidative instability and cold flow in northern climates limit the usefulness of a soybean oil-derived biodiesel as fuel. (Vasudevan 2008). Palm oil, one of the most productive oil crops, yields only ~5950 liters of oil per hectare (Chisti, 2007). To produce enough biodiesel to supply the U.S. with enough fuels at the current rate of consumption, oil palm would need to be grown over an area of ~111 million (M) hectares, which would equal nearly 61% of all agricultural cropping land in the united States (Chisti, 2007). This type of fuel source is not feasible as it would in turn create a shortage in food supply and is therefore not a realistic alternative. Table 1 reports some lab tested oil yield values from a few possible biodiesel sources.



Comparison of some sources of biodiesel

| Crop | Oil yield (L/ha) | Land area needed (M ha)[a] | Percent of existing US cropping area[a] |
|---|---|---|---|
| Corn | 172 | 1540 | 846 |
| Soybean | 446 | 594 | 326 |
| Canola | 1190 | 223 | 122 |
| Jatropha | 1892 | 140 | 77 |
| Coconut | 2689 | 99 | 54 |
| Oil palm | 5950 | 45 | 24 |
| Microalgae[b] | 136,900 | 2 | 1.1 |
| Microalgae[c] | 58,700 | 4.5 | 2.5 |

[a] For meeting 50% of all transport fuel needs of the United States.

[b] 70% oil (by wt) in biomass.

[c] 30% oil (by wt) in biomass.

Table 1: Comparison of some sources of biodiesel. Chisti 2007

Researchers are looking for crops that can yield a high net conversion of solar energy in the form of fuel, while providing additional side benefits. The net efficiency of harnessing solar energy through photosynthesis into liquid fuels is rather low in most land crops, yet microalgae have the potential for the higher photosynthetic efficiency due to the fact that they are aquatic, and therefore have better access to water, $CO_2$ and nutrients (Vasudevan, 2008). Also, the energy inputs can be substantially minimized when growing algae compared to other sources. Microalgae contain high amounts of oil, have fast growth rates, permit the use of non-arable land and non-potable water, create far less waste and do not displace food crop cultivation (Gouveia and Oliveira, 2009). In addition their production is not seasonal and they can be harvested daily although there are wide variations in yield expected depending on season and latitutde. For these reasons, microalgae are capable of producing 30 times the amount of oil per unit area of land as terrestrial oilseed crops (Sheehan, 1998). Another added benefit is the ability of algae to utilize $CO_2$ for photosynthesis, resulting in a promising method of removing gases from power plants, thus reducing greenhouse gases (Maeda, 1995).

## Microalgal Culturing

The energy produced by microalgae can be harvested in several different ways. One such method uses methane produced by the anaerobic digestion of the algal biomass. The other method uses biodiesel derived from microalgal oil and photobiologically produced biohydrogen. This second method seems to have the most promise and seems to be the focus of most biofuels research (Chisti, 2007).

Currently, many different scientists are experimenting with ways to improve the economics of large scale production of microalgal fuels by finding the most productive species and perfecting extraction techniques in both raceway ponds and tubular photobioreactors. Raceway ponds are generally perceived to be less expensive than photobioreactors, because they cost less to build and operate, but these raceways do not produce as much energy per hectare as the more expensive photobioreactors (Chisti, 2007).

Also, open ponds and raceways present problems when growing microalgal cultures because contamination from other species must be controlled by using highly alkaline or saline selective environments (Molina, 2001). This restricts the species that can be grown with these methods. Photobioreactors that are fully closed provide opportunities for other types of algae that cannot be grown in such harsh environments yet incur other costs such as those associated with cooling, nutrients and $CO_2$.

While there are many different types of photobioreactor designs, researchers at the Department of Chemical Engineering at the University of Almeria in Spain indicate that the most promising are those with tubular solar collectors that circulate the culture by using an airlift device. This airlift device is important because it achieves circulation without moving parts and grows healthy cultures without the risk of contamination with other species (Molina, 2001). Because there are no moving parts, cell damage caused by mechanical pumping is eliminated. Finally, the air pump is able to remove the oxygen produced by photosynthesis from the system. This is essential because too much oxygen in the broth can actually halt photosynthesis.

The Aquatic Species Program has also led efforts to establish the feasibility of large-scale algae production in open ponds. The open pond in Roswell, New Mexico successfully completed a full year of operation in 1998 and although the system was able to utilize 90% of the injected $CO_2$, it has not yet been reported to achieve consistently high productivities due to the low temperature conditions at night. (Sheehan, 1998).

There are several different techniques that can be used to harvest oil produced by the algae. One such method, known as fast pyrolysis, works by exposing the substance to extremely high temperatures (400 – 600 C) for less than 2 seconds. The result is a chemical decomposition of the condensed subject that is used to extract the raw oil from the algal biomass. (Miao 2004) Fast pyrolysis is not cost efficient due to the large energy input to heat, so scientists are currently looking for lower input alternative.

## Microalgal Species

Many biofuel researchers are undertaking the lengthy process of identifying the most economical algal species to use for biodiesel. Researchers are looking for algae that not only produce large amounts of oil, but can also be grown under variable conditions such as temperature, pH, salinity, and other factors that would be difficult to control in the field. After collecting over 3,000 species of algae, researchers at the University of Hawaii were able to screen, isolate, and characterize the most useful species so that there now exists a collection of about 300 species of algae and diatoms that will hopefully be utilized for future research in this area (Sheehan, 1998).

Of the thousands of algal species, there are many that seem promising for the purposes of biofuel but are each met with challenges that must be overcome. For example *Nannochloris* and *Dunaliella* are marine microalgae, which are easy to control but are not suitable for freshwater cultivation. *P. incise* produces triglycerides that are rich in polyunsaturated fatty acids, making them less desirable for biodiesel production due to the production of soap during the refinery process (Li et al, 2008). *B. Braunni* produces lipids that are mostly large hydrocarbon molecules which are ideal for liquid biofuel production by means of liquefaction or pyrolysis but cannot be converted into biodiesel through transesterification (Li et al, 2008).

## Growth Conditions

While some research teams are working to find the most efficient species of microalgae, others are working to find the optimal growing conditions in which these species must be grown. Under some types of environmental stress, such as nutrient deficiency, some algae exhibit a response resulting in increased lipid production. (Sheehan 1998). One recent study investigated the effect of nitrogen sources and their concentrations on cell growth and lipid accumulation of *Neochloris oleoabundus* a promising freshwater microalgae species from the UTEX algae culture collection at the University of Texas in Austin (Li et al, 2008). This species of algae exhibited rather poor growth under ammonium bicarbonate conditions, while sodium nitrate was the best nitrogen source among the three tested compounds. The maximum biomass concentration obtained with sodium nitrate as the nitrogen sources was approximately 2.5 g/L. (Li et al, 2008). It was also observed that cell growth decreased when the sodium nitrate was increased further suggesting that sodium nitrate might actually be inbibitory at high concentrations (Li et al, 2008). These findings might prove to be a significant breakthrough due to the fact that sodium nitrate is less costly than other forms of nitrate. This can be an important advantage when financing industrial processing of the algae. *Neochloris oleoabundus* was also found to produce up to 56% maximum lipid content after 6 days of nitrogen depletion under nitrogen starvation without $CO_2$ supplementation, identifying this species as an adequate source of single-celled oil for use as biodiesel (Gouviea, 2009).

As indicated earlier, open raceway ponds are susceptible to foreign contamination and one way to control this is to farm species that are able to grow in waters with more extreme conditions. *Nannochloropsis sp.* is a marine microalgae that can tolerate waters with higher salinity than potential contaminators. These algae produce up to 28.7% oil content in natural conditions and ~50% oil content when grown under a nitrogen limited condition (Gouveia, 2009).

Nitrogen-limited growth can increase oil productivity by stimulating the cells to produce more lipids per cell since protein biosynthesis is limited (Suen, 1987). This discovery can play an important role in maximum oil production. However, while nitrogen deficiency can promote higher oil content, nutrient restrictions unfortunately limit growth, which in turn limits photosynthetic efficiency. (Vasudevan, 2008). Researchers are currently looking for ways to balance the desire for high growth and high oil production to maximize the total amount of oil produced.

Other conditions that can influence the growth of microalgae include temperature, salinity, and pH. Each of the thousands of different species of algae has a different set of optimal conditions that must be met for maximal oil production. This information can be quite valuable to potential biofuel production companies that plan to use tubular photobioreactors. In this type of arrangement, algal broth from the column is continuously pumped through a solar array, where sunlight is absorbed. Cooling water must be pumped through a heat exchanger coil in the degassing column to keep the temperature from becoming too high during the day and too cold at night (Chisti, 2007). Also, during the night, as much as 25% of the biomass produced during daylight might be consumed depending on the light level under which the biomass was grown and the growth temperature both during the day and at night. (Sanchez 2000). The advantage of these tubular photobioreactors is that, although they are costly, temperature conditions can be controlled whereas it is nearly impossible to do so in an outside raceway pond.

Other researchers are looking into the possibility of obtaining biofuel from heterotrophic species of algae such as *Chorella protothecoides.* These algae can be grown under culture conditions with an addition of glucose as a carbon source and a decrease of inorganic nitrogen in the medium (Miao, 2004). This method of controlling the metabolic pathway of *C. protothecoides* though heterotrophic growth and fast pyrolysis results in a high yield of bio-oil compared to other sources of biomass. To be specific, their yield was 3.4 times higher from the traditional autotrophic algal sources (Miao, 2004). In addition, the fast pyrolisis of heterotrophic algae resulted in a bio-oil that was more comparable to fossil oil and therefore more easily transformed into actual biofuel.

## The Effects of Temperature

While there is limited research detailing the effects of temperature on the lipid content of algal production, several studies have focused on algal growth. Heat and cold can harm/slow the growth of algae, yet some algae are able to undergo physiological changes that allow adaptation to more extreme temperatures. One such experiment reported changes in species diversity of diatoms when exposed to differing salinities and pH. Specifically, the spatial distribution of diatom taxa was clearly linked with salinity and the temporal distribution is mainly determined by the temperature (Resende, 2005). They observed that certain species of diatoms such as *Chaetoceros eibenii, C. subsecundus, Nitzschia palea and Stauroneis smithii* showed a preference towards higher temperatures while others such as *Nitzschia acicularis, Nitzschia insignis, Skeletonema costatum and Surirella robusta* showed more growth at lower temperatures (Resende, 2005). Each of these species contains different adaptations that allow their cellular processes to operate on a faster scale at the optimal temperature. Other investigations have sought to determine whether temperature is a controlling factor in the occurrence of blue-green algae in Lake Mendota near Madison, Wisconsin. The results of the experiments with natural samples indicate that temperatures below 15ºC do not limit blue-green algal growth and at 40ºC, the amount of photosynthesis was usually 50% of that at the optimal temperature (Konopkat 1978). Researchers were then able to conclude that the springtime lake temperature per se does not explain the absence of blue-green algae in this period and hypothesized that other factors such as nutrients and species competition played a larger factor.

**Microalgae Manipulation**

Another group of researchers from facilities across Europe have examined the existence of a sophisticated stress surveillance system in diatoms and perhaps other phytoplankton such as algae. This mechanism is thought to trigger the production of sub lethal quantities of poisonous chemicals when cells are in trouble. The types of stresses that could initiate an algal response could include light intensity, nutrient availability and even other phytoplankton competitors or predators. These chemicals then act as a warning to other algal by triggering the production of hormones that result in resistance to the abiotic or biotic stress in the community and compromises the hatching success of the grazers (Vardi, 2006). This reaction could be artificially induced by biofuel producers by releasing these same stress hormones into the system without fear of losing valuable algae to any added heterotrophs. The researchers propose that this production and sensitivity to lethal aldehydes by diatoms may determine the fitness and succession of phytoplankton communities in the marine environment through mechanisms regulated by intracellular calcium and Nitric Oxide signals (Vardi, 2006).

Researchers have also been looking for breakthroughs in molecular biology and genetic engineering. The ASP (Aquatic Species Program) was the first program to isolate the enzyme Acetyl CoA Carboxylase (ACCase) from a diatom (Sheehan, 1998). This enzyme was found to catalyze a key metabolic step in the synthesis of oils in algae. The gene that codes for the production of ACCase was in turn isolated and cloned. Scientists have been working to utilize the transformation of algae a way to increase oil production by causing the algae to over-express the ACCase gene. This finding has been a major milestone but has not yet led to an actual increase of oil production in algal cells (Sheehan, 1998). Other researchers are looking into the possibility of manipulating autotrophic species of algae to grow heterotrophically and be able to thrive on glucose in the absence of light through the introduction of a gene encoded with a glucose transporter (Miao, 2004). This could potentially decrease the economic costs of microalgae biofuel production making it a viable alternative to fossil fuel.

# Chapter 3: Methods

## Experimental Growth Conditions

The microalgae used in this study originated from pond #53 in the South Texas ponds near the Laguna Madre bay area. These ponds are already being used the production of tilapia food fish but also have a high microalgal content. These microalgae were cultured in open ponds in the greenhouse at the University of Texas at Austin with needed fertilizers and natural light conditions. The samples collected from this pond include several species but are predominately composed of a crescent-shaped algae identified as *Ankistrodesmus sp.*, a small spherical algae tentatively identified as *Nannochloropsis sp.*, and an oval centric diatom that has not yet been identified. See Figure 1. The culture, which was being grown in a large tub in a greenhouse, was not sterile and was most likely contaminated with multiple species of bacteria.

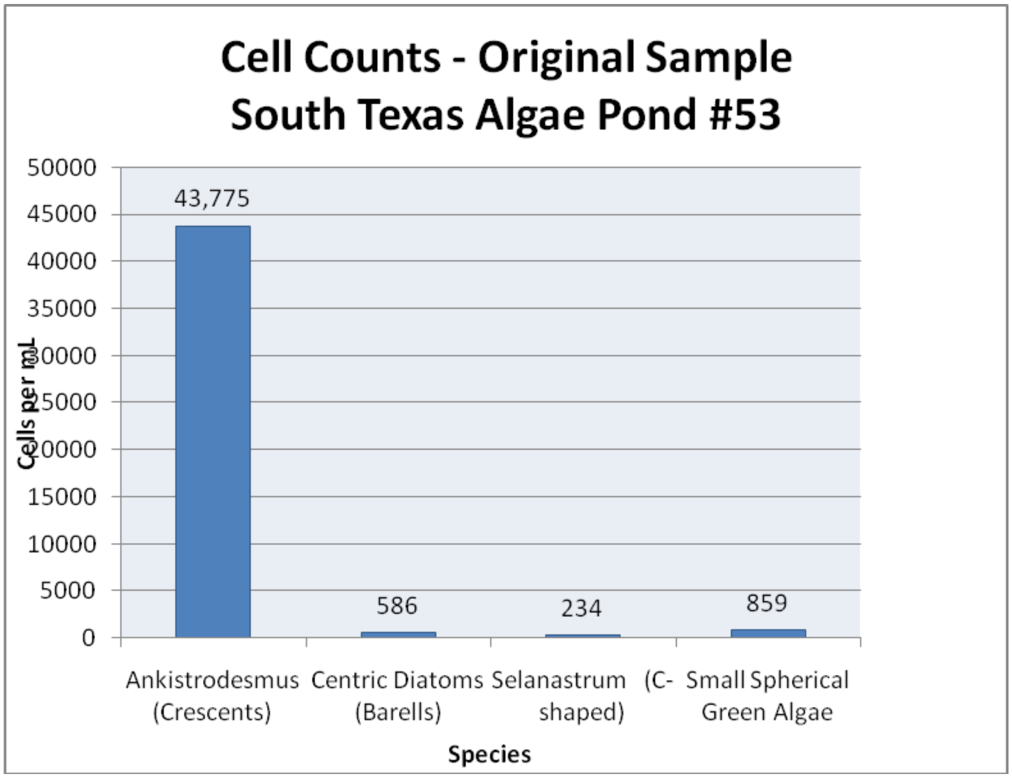


Figure 1: Original Cell Counts

Experimental samples were inoculated on June 12, 2009 and July 3, 2009 and were harvested on the 10th day thereafter under laboratory conditions. The inoculation was performed under sterile conditions with one mL of algae added to 40 mL of media. The media used was a mixture of 1 part F/2 to 2 parts Allens with a resulting salinity of ~10 ppt. The samples were then placed in a controlled temperature gradient with 9 temperatures ranging from 15.5ºC – 36.5ºC. Each trial contained two samples at each temperature for a total of 4 data sets which were used to calculate mean and standard error. All tubes were connected to a constant $CO_2$ bubbler set at approximately 1-3% $CO_2$ and the light was set at a 12 hour on, 12 hour off cycle. Illustration 1 shows the basic set up for the temperature gradient.

Figure 2: Temperature Gradient Setup

## Growth Evaluation

Growth parameters were measured using optical density (OD), and dry weight. In addition, cell counts and species identification were measured throughout the 10 day growth cycle. Light absorbance was measured at a fixed wavelength of 678 nm and was taken daily at approximately 9:00 A.M. from Day 3 after inoculation through Day 11 (except for Day 8). Dry weight was measured on day 13 of growth, and was calculated by centrifuging a 1 mL sample of each tube and evaporating in a vacuum centrifuge for approximately 1 ½ hours. An analytical balance was used to measure the weight of the tube before and after the sample was added and evaporated.

Cell counts were taken for each experimental sample using an Olympus BX60 at 400x magnification and included data on each type of cell observed in each condition. These cell counts were taken on Day 2 and Day 9 of the experiments and cell identifications were based on preliminary identifications by Dr. Jerry Brand, Michelle Randazzo, and Rebekah Powell at the UTEX Algae Culture Collection.

## Oil Extraction

Lipid extraction from the algae was based on a modified version of the Bligh-Dyer method which is used to extract nonpolar lipids such as Triacylglycerol (TAG). (Bligh and Dyer, 1959). One mL samples of algae were centrifuged at 10,000 rpm for 3 min. The supernatant was removed and the algal pellet was resuspended in 1 mL of distilled water. Chloroform (1 mL) and methanol (2 mL) were added, mixed and left undisturbed for 10 minutes. One more mL of chloroform was added, mixed and left undisturbed for 10 minutes. One mL of distilled water was added and mixed. The mixture was heated gently until there was a clear separation of the lipid layer (about 15 minutes). The lipid layer was extracted with a micropipette and dried in a vacuum centrifuge. The dried lipid was resuspended in 200 mL of a 6:1 chloroform to methanol mixture and stored in the freezer.

## Lipid Content

To determine the lipid content of each raw material, the oil samples were analyzed using high-performance liquid chromatography (HPLC) at the Dr. Martin Poenie lab at the University of Texas at Austin. HPLC utilizes a column that holds chromatographic packing material (stationary phase), a pump that moves the mobile phases through the column, and a detector that shows the retention times of the neutral lipid molecules. The graphs provided by the HPLC show peaks for each type of lipid present in the sample including TAG (Triacylglycerol). TAG is the type of lipid that contains the highest amount of stored energy due to the three long fatty acids with a glycerol backbone. TAGs are easily converted into biodiesel fuel (alkyl ester) through a reaction with simple alcohols, a process known as transesterification (Sheehan, *et al*., 1998). The other reported hydrocarbons, which are not yet identified, were added together to determine percentage of total neutral lipids in each extraction. Polar lipids were not included in these measurements. All reported measurements were converted to mg/mL of extracted oil using standard procedures.

# Chapter 4: Results and Analysis

## Algal Growth

Microalgal biomass growth data of the tested culture under the nine experimental conditions are depicted in Figure 1. Culture plots show the most growth for the algae grown at 20.5 °C with a maximum dry weight at 3.33 µg/mL (SE 0.51). Comparing this culture with the same grown at 28.8 °C, it can be observed that the former attained a 2 fold increase in biomass production after 9 days. (Fig. 1)

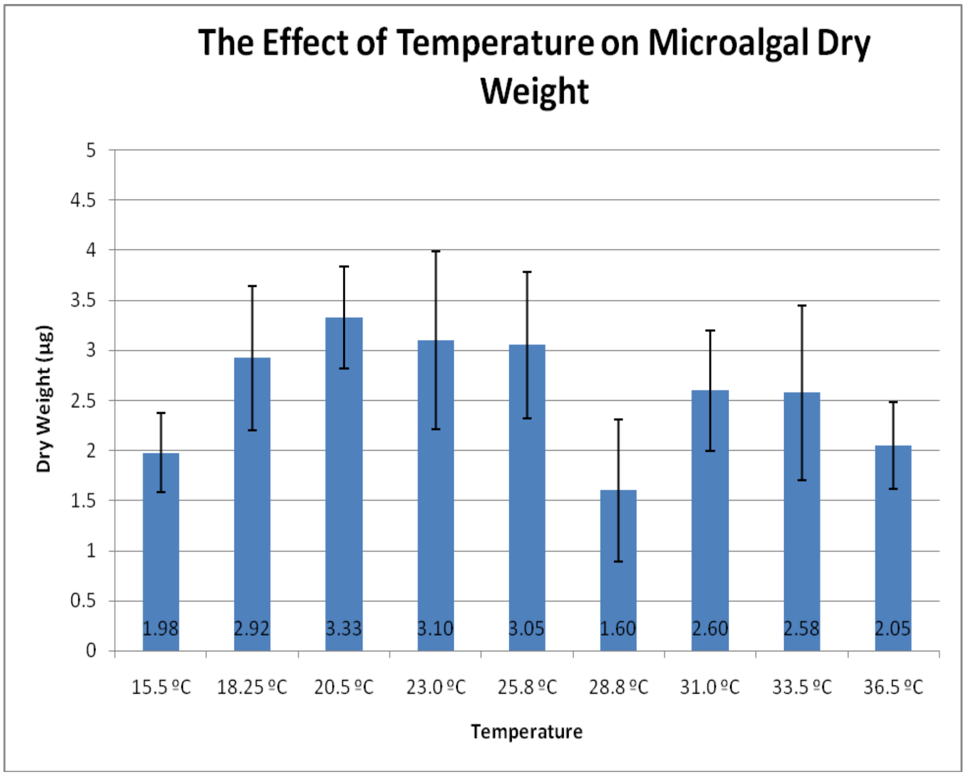


Figure 3: The Effect of Temperature on Microalgal Dry Weight µg/mL

Specific growth rates are represented by the absorbencies shown in Fig. 2. Higher (steeper) slopes mean high specific growth rates, which were observed in the cultures grown at 20.5 °C with a maximum absorbency of 0.947 at 678 nm. Comparing this culture with the same grown at 33.5 C, which had a maximum absorbency of 0.459, it can be observed that the former also attained a 2 fold higher growth rate that the latter. (Fig. 2) There does not seem to be a direct correlation between temperature and growth rate though there is a trend towards higher growth rates in cooler temperatures. Standard error is shown for the highest and lowest growth rate to show that there is a statistical difference between the two. However, standard error is larger than desired due to the fact that the second trial did not grow as well as the first, skewing the averages to a lower than expected outcome. All conditions were kept the same, but for an unknown reason, several of the cultures in Trial 2 started declining after day 2 of the experiment. One possible reason for this decline could have been the fact that the cultured used in this study was not sterile and therefore each tube may not have been inoculated with the same amounts of each species.

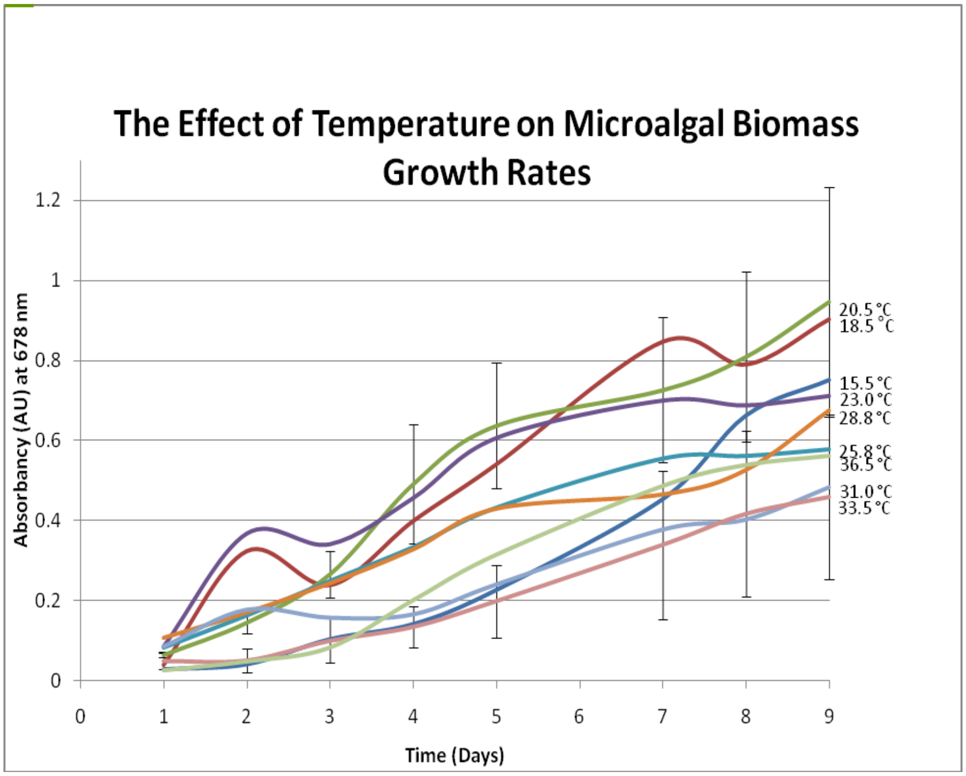


Figure 4: The Effect of Temperature on Microalgal Growth

**Species Identification**

One variable that could not be controlled for in this experiment was the fact that it was a mixed species sample. However, it was a good system to study how such mixed population responds to temperature variations. On initial observations, the sample was mostly composed of an unidentified species of *Ankistrodesmus,* a crescent shaped alga. Other species of algae that were observed in the original culture were small spherical algae tentative identified as *Nannochloropsis sp*., a boat shaped diatom belonging to the genus *Nitschia*, a C-shaped algae known as Selenestrum sp. and an unidentified species of oval shaped centric diatoms. Numerous other species were observed but were not seen in large enough numbers to report. See Illustration 2 and 3 below.

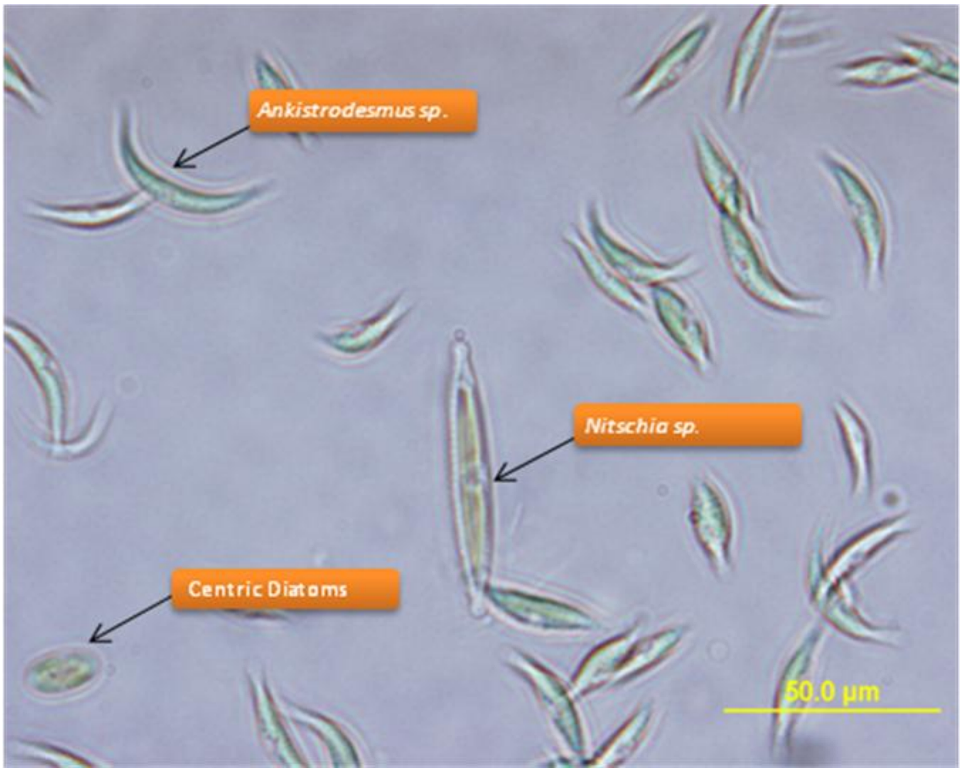


Figure 5: Microalgal Species Identification. Original Sample. 400X

Figure 6: Microalgal Species Identification. Original Sample. 400X

## The Effect of Temperature on Microalgal Species and Growth

Temperature played a large role in the species growth throughout the different temperature conditions. There was a strong relationship between the decline of *Ankistrodesmus sp*. and an the increase in temperature. The opposite was observed with the small spherical green algae as their numbers grew higher as temperature increased. (Fig. 3). It can also be observed that at cooler temperatures there was a higher diversity of algal species. At 15.5°C there were at least 4 species present while at 36.5 °C there were only the small spherical green algae. The small spherical green algae seemed to be the only species that could grow in the more extreme temperatures.

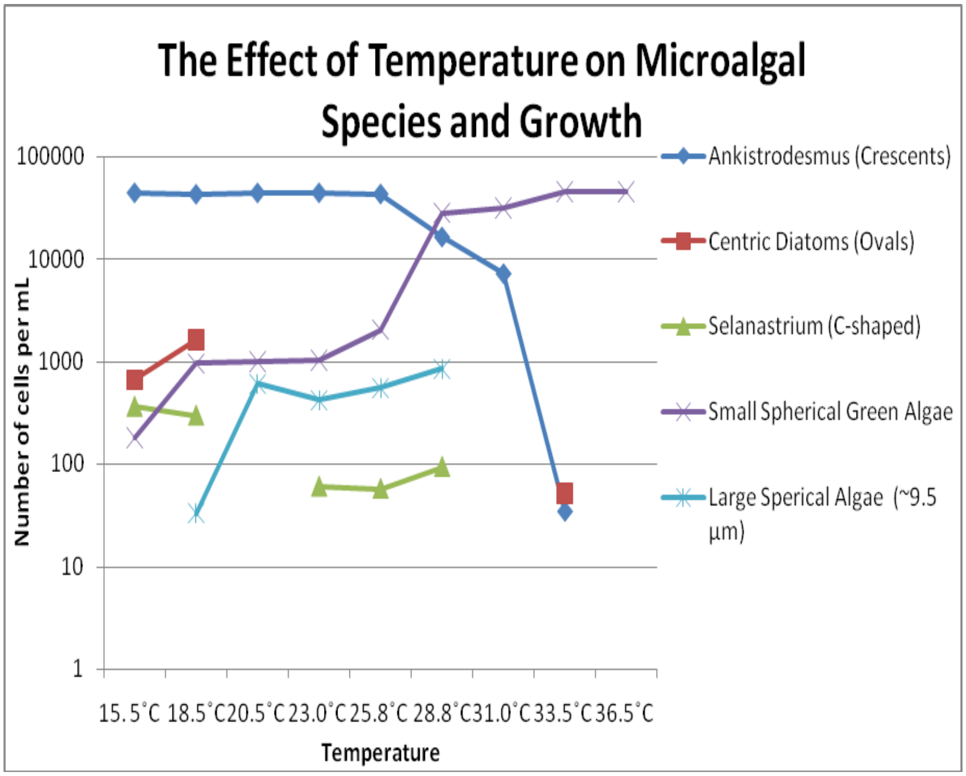


Figure 7: The Effect of Temperature on Microalgal Species and Growth

**Lipid Content**

In order to determine the lipid composition in biomass, samples (~200 µg) were evaluated through High Performance Liquid Chromatography. Samplings and lipid evaluation were performed in duplicate. Figure 4 shows both the percentage of triacylglycerol (TAG) present in each sample as well as the percentage of lipids. Standard error bars are shown and again they are larger than desired due to the low vitality seen in the second trial of this experiment. The highest TAG content of 1.53% was observed in the coolest temperature condition of 15.5°C while the lowest of 0.44% was seen at 33.5°C.

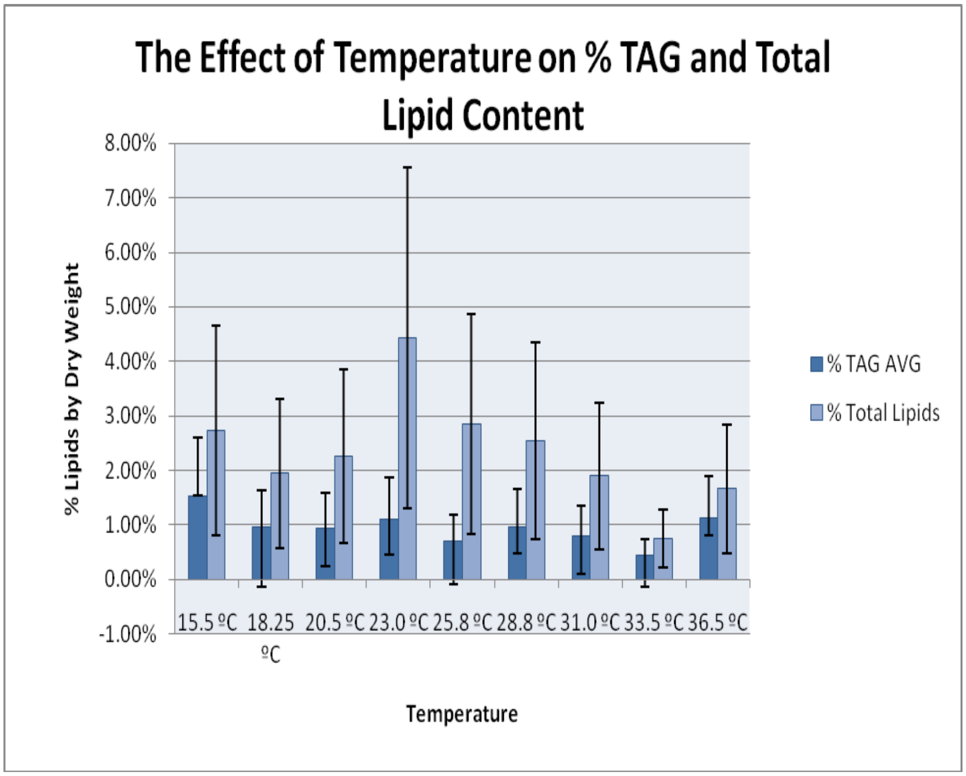


Figure 8: The Effect of Temperature on % TAG and Total Lipid Content

## Chapter 5: Conclusions

### Preliminary Conclusions

Microalgal biofuels research is an exciting frontier for those wishing to alleviate worldwide energy concerns, yet much work still needs to be done before it can become a viable alternative. The findings from this study are still in their preliminary stages and it will be necessary to conduct more trials before more reliable conclusions can be made. However, by interpreting the data collected through this study, several observations can be made about the culture of microalgae found in the brackish South Texas ponds.

The species found in this sample show a preference towards different temperature conditions. The crescent shaped *Ankistrodesmus sp.* grew well in temperatures ranging from 15.5°C – 25.8°C with a sharp decline in numbers as temperature increased further. The small spherical green algae tentatively identified as *Nannochloropisis sp*. exhibited their highest growth rate at 36.5°C with a sharp decline when temperatures went below 28.8°C. This information may be instrumental in determining oil production in these ponds knowing that the dominant species will most likely change with the seasons.

The samples grown at 20.5°C were determined to have the fastest growth rate and largest dry weight of the group. Algae grown at 33.5°C were observed to have the slowest growth rate and smallest dry weight. It can be said that based on these results, the crescent shaped *Ankistrodesmus* produced a higher lipid content than the unidentified spherical green algae.

### Sources of Error

Lipid content did not correspond directly with growth rate and dry weights. The largest percentage of TAG was found in the sample grown at the coolest temperature of 15.5°C while the highest total lipid percentage was found at 23.0°C. It is difficult to determine the reasons for these results as there are many variables in a heterogeneous condition that cannot be controlled for, such as variation in initial species inoculation. Also, an uneven distribution of heterotrophs, which were sporadic in their appearance across the temperature gradient, could have played a major role in these results as well.

## Future Research

To increase the validity of these experiments, more repetitions are needed and other variables should be tested such as pH, salinity, light and a gradual change in temperature. In addition, the individual species found in this pond should be isolated and tested separately in order to get a better picture of which one is producing the greatest oil content. After such work is done, it would be advisable to expand the research to include field studies at the location where these algae originate. It would be interesting to know how the oil content from algae grown in the natural setting compares to algae grown in the same conditions in the lab.

As stated before, algae are believed by much of the scientific community to be the only renewable source of bio diesel that can realistically displace fossil fuels. However, the economics of producing microalgae biodiesel need to improve substantially before it will be competitive with petro diesel. Many small studies such as those described here are together helping to contribute to the vast amounts of knowledge that must be collected in order for this to one day become a possibility.

# Chapter 6: Applications to Practice

## General Overview

With gasoline prices at times reaching more than $4 a gallon during 2008, the race is now on for a new form of fuel. Replacing fossil fuels with algae, a renewable resource, to make biodiesel is an exciting possibility. I plan to use my experiences in the lab this summer as a base for students to work from throughout the year. My plan is to help students set up and monitor an algae biofuel production station in the classroom using two native strains of algae. Students will culture and analyze the algae, assess the thermodynamic properties of traditional hydrocarbons, design small-scale bioreactors, produce biodiesel from algae-oils, test their bio-diesel in an automobile, identify the most cost efficient algae used, and engage in a critical discussion of carbon neutrality and ecological equity. The costs associated with this program will ideally be covered by the Toyota Tapestry Grant, a $10,000 classroom education program, in which I am currently in the process of applying.

While large scale production of algae biofuels is still many years away, it is extremely important for today's youth to be aware of alternative fuels that very well could become common place at some point in their lives. Linking such a relevant issue as energy to the school environment will both educate students on this very important issue and give them ownership over what they are learning in the classroom.

In today’s ever changing world, it is sometimes difficult to motivate students to think about science beyond what can be found in a textbook. Science is so much more than formulas and vocabulary, and we must continually find ways to hook students’ interests by showing them how science affects their everyday lives. The growing need to find alternative energy sources is one that most every student has heard about yet few are given the opportunity to do anything about. With this project, students will begin to see that real science can be done in their own classroom.

This project will begin with a visit to one of the leading research labs in biofuels at the University of Texas at Austin. Here they will hear from a researcher who specializes in this topic about the many advances in biofuels and the specific projects that are currently in progress. They will also tour the algae cultures room to see the many arrays of species that are being considered as possibilities. This tour will conclude with a live demonstration on how algae can be extracted using current methods to force the cells to break open and release the raw oil.

Back in the classroom, students will culture and extract their own biofuel using a photobioreactor that they make themselves. The raw oil will be processed with the help of the university and the resulting biofuel will be used to power a vehicle from our high school campus to the University of Texas and back, showing that high school students can in fact change the world in which they live.

**Description**

Through this project, students will specifically investigate and identify cellular processes including homeostasis, permeability, energy production, transportation of molecules, disposal of wastes, function of cellular parts, and the synthesis of new molecules. Students will be involved in all aspects of this project including set up, cultivation of the algae, collection, pressing, extraction, lipid identification and conversion. Students will read an article from the National Geographic Magazine entitled "Green Dreams" *by* J.K. Bourne JR, and R. Clark, published October 2007. Students will also read more technical scientific articles from the most recent publications such as "Biodiesel from microalgae" by Yusuf Chisti, published in Biotechnology Advances.

This project will investigate the utility of a photobioreactor with oil-producing algae as a means of reducing carbon emissions generated from burning fossil fuels. The bags will be stacked on top of one another and connected by a series of tubes that pump the green organic slime around a stack of fluorescent lamps to encourage photosynthesis. Teams of biology students will work in class and occasionally afterschool to collect and cultivate the algae in the classroom and refine it down to fuel. Almost all the work will be done on-site, though at one point the solution will be taken to the University of Texas for final separation, an event which will allow students a chance to see a fully operational lab. After spending a large portion of their year working through the process, students should able to create enough fuel to power a vehicle the full distance to the university.

The strains of algae that will be used for this experiment are not yet tested for biofuel performance and the data gained through this study will be communicated to the local university. The algae used in this study will be native to South TX which has a mild climate and a good potential for algal growth both independently or with the tilapia ponds that are already there. This relationship will lend to a major discussion on ecological concepts such as food chains and webs, nutrient cycles, and the idea that exotic algae are not approved for outdoor growth because their effect on the local ecology cannot fully be known.

**Rationale**

One of the biggest issues facing this generation of students is the impact, both environmental and political, of our dependence on foreign sources of fuel. According to the UN Human Development Reports, the US emits 21% of the world's carbon dioxide despite having only 4.6% of the world's population. Using these issues as a starting point, this project will address the problems while at the same time providing an educational and compelling experience for the students.

Biodiesel created from microalgae seems to be the only renewable biofuel that has the potential to completely displace petroleum-derived transport fuels without adversely affecting supply of food and other crop products. This issue is increasingly becoming more and more relevant as our dependence on expensive foreign oil increases.

One major aspect of this program is the potential to create a school environment that fosters a love for learning and research. I hope to not only get my students excited about this experiment, but the whole school and community as well. Also, because many of the materials purchased through this program are non-consumables, I hope to extend this experiment with many generations of students to come.

Naturally, the goals for this project are embedded in the TEKS that are required for all biology teachers to cover throughout the year. By developing their own biofuel in the classroom, students can deepen their understanding of the subject far more than they do by studying for multiple-choice exams. In addition, this project will give them ownership over their studies in biology and real world buy in with the potential of biofuels.

### Methods

The set up will include at least 10 giant bags with 5 gallon capacity filled with one of the many cultures from the South Texas ponds. For this project, it will be critical to have a large supply of cheap nutrients using some commercial fertilizers supplemented with additional nutrients. We will provide the algae with a constant supply of carbon dioxide to facilitate photosynthesis with an attached air pump. Optimal growth conditions should allow the algae to grow to saturation level within two weeks. The starter culture should be relatively sterile so bacterial growth is not a foreseen problem; however, we will have some antibiotics on hand that can kill bacteria without negatively affecting the algae. Other protists could potentially be a problem but can be prevented by keeping the algae growing fast enough to out compete protists or keep it in a closed and monitored system.

This project will include numerous cycles of growth and extraction. The extraction will be done by students as we go with multiple lots of growth so that they can change conditions and see effects on oil yield. Calculations will be made by students based on lipid content and growth to determine the desired quantity of biodiesel. Currently, the algae dry matter yield is approximately 2 g per liter of culture in two weeks. This can be increased under more optimal conditions and with better strains. The oil yield from this is 0.5 to 1 g per liter in two weeks (assuming 25 - 50 % oil content). Students will use these numbers to calculate how much volume is needed for harvesting the algae every two weeks for the two semesters culminating in enough volume to drive a car from Stony Point High School to the University of Texas at Austin and back.

## Potential Impact

This project supports the TEKS (Texas Essential Knowledge and Skills) that are required in Texas public schools in many ways. The ability to conduct field and laboratory investigations using safe, environmentally appropriate, and ethical practices is a huge component that is often overlooked through traditional teaching. This project provides an opportunity for students to do real science that is separate from traditional cookbook labs. By learning about biofuels from algae, students will also have an opportunity to plan and implement investigative procedures by asking questions, formulate testable hypotheses and select equipment and technology. They will be required to collect data and make measurements with precision as well as organize, analyze and make inferences from their data. In addition, students will need to communicate valid conclusions to the mentoring university.

This project will also give students a reason to learn much of the factual biological information necessary to pass our state exam. Through this experiment, students will identify the parts of prokaryotic and eukaryotic cells while separating the oil from the cellular mass, as well as investigate and identify cellular processes including homeostasis, permeability, energy production, transportation of molecules, disposal of wastes and synthesis of new molecules. Students will compare the structures and functions of different types of biomolecules such as carbohydrates, lipids, proteins and nucleic acids and compare the energy flow in photosynthesis to the energy flow in cellular respiration. Due to the need to provide our algae with nutrients and carbon dioxide, students will analyze the flow of matter and energy through different trophic levels and between organisms and the environment. In addition, students will analyze the flow of energy through various cycles including the carbon, oxygen, nitrogen and water cycles and identify and illustrate that long-term survival of species is dependent on a resources that may be limited.

To further maximize the effectiveness of this program I will be reaching out to other departments at my school to plan ways in which this subject can become cross-curricular. It is my belief that social studies teachers and math teachers can incorporate the study of biofuels and energy policies in their classes as well.

The most important aspect of this project is its applicability to the real world. Students will become very familiar with the current situation concerning energy resources and should be able to see the applications of our classroom experiment. By comparing the lipid content of two different strains of algae, students in my classroom will be contributing to the growing knowledge of data on algae biofuels.

# Bibliography

Bligh EG, Dyer W.J. 1959. A rapid method of lipid extraction and purification. Can. J. Biochem. Physiol 37:911-917

Campbell, C.J. 1997. The coming oil crisis. Multi-science Publishing Company and petroconsultants S.A, Essex, England

Canale, R.P., and A.H. Vogel. 1974. Effects of temperature on phytoplankton growth. J. Environ. Eng. Div. Am. Soc. Civ. Eng. 100:229-241

Chisti, Yusuf. 2007. Biodiesel from microalgae. Biotechnology Advances 25: 94-306

Gouveia L, Marques A, Lopes da Silva T, Reis A. 2009. Neochloris oleabundans UTEX #1185: a suitable renewable lipid source for biofuels production. Journal of Ind Microbiol Biotechnology 36:821-826

Guoveia L, and Oliveria, A. 2009. Microalgae as a raw material for biofuels production. Journal Of Microbiology Biotechnology. 36: 269-274

Konopkat A., Brock T. 1978. Effect of Temperature on Blue-Green Algae (Cyanobacteria) in Lake Mendota. Applied and Environmental Microbiology. Oct. 1978, Vol. 36, No. 4p. 572-576

Li, Yanqun, Horsman, Mark, Wang, Bei, Wu, Nan. 2008. Effects of nitrogen sources on cell growth and lipid accumulation of green alga Neochloris oleoabundans. Applied Microbiology Biotechnology 81: 629-636

Maeda K, Owada M, Kiura N, Omata K, Karube J. 1995. CO2 fixation from the flue gas on coal—red thermal power plant by microalgae. Energy Convers Manage 36: 717-720.

Miao, Xiaoling, Wu, Qingyu. 2004. High yield bio-oil production from fast pyrolysis by metabolic controlling of Chlorella protothecoides. Journal of Biotechnology 110: 85-93

Molina, E., Fernandez, J. 2001. Tubular photobioreactor design for algal cultures. Journal of Biotechnology 92: 113-131

Resende, P., Azeiteiro,U., Jorge, M. 2005. Diatom ecological preferences in a shallow temperate estuary (Ria de Aveiro, Western Portugal) Hydrobiologia (2005) 544:77–88

Sanchez Miron, A. et al. 2002. Growth and biochemical characterization of microalgal biomass produced in bubble column and airlift photobioreactors: Studeis in fed-batch culture. Enzyme Microb. Technol. 31, 1015-1023

Sheehan, John, Dunahay, Terri, Benemann, John, Roessler, Paul. July 1998. A Look Back at the U.S. Department of Energy's Aquatic Species Program: Biodiesel from Algae. National Renewable Energy Laboratory.

Suen H, Subbard JS, Holzer G, Tornabene T.G.1987. Total lipid production of the green alga Nannochloropsis sp QII under different nitrogen regimes. J Phycol 23: 289-296.

Vardi, Assaf , Formiggini, Fabio, Casotti, Raffaella, De Martino,Alessandra, Ribalet, Francios, Miralto, Antonio, Bowler, Chris. Mar 2006. A Stress Surveillance System Based on Calcium and Nitric Oxide in Marine Diatoms. PLOS Biology. Volume 4. Issue 3

Vasudevan, Palligarnai , Briggs, Michael. 2008. Biodiesel production – current state of the art and challenges. Journal of Industrial and Microbiology Biotechnology 35:421-430

# Vita

Stephanie Klenzendorf was born in Dallas, TX to the parents of Ron and Jeanne Sisco. She currently lives in Pflugerville, TX with her husband Brian and has been a high school biology teacher at Stony Point High School in Round Rock, TX for the past 5 years. She was awarded a bachelors of arts in biology from The University of Texas in 2004 and is currently completing the last semester of the UTeach Master of Science Education program.

Permanent address: 915 Kirtomy Loop, Pflugerville, TX 78660