SEAING GREEN

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1- Background

1.1 Oil Consumption in the U.S. The U.S. is one of the world's largest importers of oil. Currently the U.S. has an annual diesel demand of 65 billion gallons. The increase in oil prices provided a strong incentive to research renewable fuel sources. One research area is the area of biofuels, expected to create local jobs and offer alternatives to the U.S. reliance on petroleum based fuels.

1.2 Biofuels Challenges. Currently biofuels are being produced from corn, soy bean, canola, and sugar cane. While these fuel sources are renewable and are more environmentally friendly than petroleum fuel sources, they have their draw backs. First and foremost these crops are already being used as "food crops" for the U.S. and the rest of the world. The use of these as "energy crops" has led increase in the price of food. In addition, Plant energy crops are a dispersed source of energy requiring large land acreage to produce the required oil feedstock. For example an acre of soy beans only produces about 90 gallons of useable oil per year.

1.3 Biodiesel from Algae. One potential source of biodiesel production is to grow single celled high lipid algae. Depending on the microalgae used, the lipid content may be 20-40% (dry mass basis). The high lipid content makes these algae suitable for biodiesel production. It has been hypothesized that an acre of algae could produce anywhere from 5000-15000 gallons every year, which is much higher than traditional crops. Algae utilize photosynthesis just like plants do to store carbon and make lipids, and grow very rapidly. Algae can be grown in almost any conditions as long as they are exposed to sunlight, a nutrient solution, and air (more importantly

carbon dioxide or CO2). In addition algae do not require soil for growth and can be located on marginal land, thus avoiding the competition of land use for food crops.

1.4 Photo-bioreactors. A photo-bioreactor is a reactor in which algae is grown. It provides a light source (sunlight or man-made), nutrients, and air to help grow the organisms. When algae were first studied for use as a feed stock for biofuels, they were grown in open system (ponds) photo-bioreactors in which the algae were exposed to the environment. These open system photo-bioreactors suffered contamination. In addition, it was very hard to control many other important variables such as temperature and pH.

In recent years many researchers have been looking at enclosed photo-bioreactors in which variables like temperature, contamination, and pH can more easily be controlled, thus making it much easier to grow a strain of algae than in the open system photo-bioreactors.

1.5 Nutrients. Soluble nutrients are needed for the algae to grow. The most important nutrients are nitrates and phosphates followed by sodium chloride and silicate. A typical nutrient medium solution includes macro nutrients (sodium, phosphates, nitrates, and silicate), micronutrients (iron, sodium EDTA, Co, Zn, copper sulfate, and Mn), and vitamins (B7 and B1).

2. Goal/Objectives

The overall goal of this research project is to find a more economical technique of growing algae that not only grows the algae quickly, but also allows the algae to have high oil yields. The objective of this research project was to study the effect of salinity on the growth and oil production of two algae species X1 and X2.

3. Salinity Experiments

Saltwater was used a nutrient solution to grow algae for two reasons. First it is a very abundant resource. Growing algae in saltwater would save potable water used to make nutrient solutions.

Also the increased salt concentration would probably kill most of the contaminants, which probably are not adapted to the salinity. The testing was done in three phases.

3.1 Phase One. The two algae strains X1 and X2 were placed in a regular nutrient solution (the control), and a 0.1 M sodium chloride and nutrient solution. This was done to determine whether an increase in the amount of salt would have positive effects on the algae growth and oil content. During a two weeks growth period, the algae and lipid concentration were monitored.

3.2 Phase Two. The two algae strains were placed in pure ocean water. This was done for two reasons. First, the results from phase one indicated that the algae had grown better and produced more oil when the concentration of sodium chloride increased. Second, if the algae could in fact grow better in pure salt water than in nutrient solutions, then the process of growing the algae would be much more economical. At the end of phase two it was determined that the algae could not grow in pure salt water, because it lacked the nutrients that the algae needed to survive.

3.3 Phase Three. The algae were grown in mixture of 50% (by volume) ocean water and 50% 0.1 M sodium chloride and nutrient solution, vs. the 0.1 M sodium chloride and nutrient solution. The objective was to determine the effects a mixture of nutrient solution and pure ocean water would have on the oil content and growth of the two algae strains being investigated, X1 and X2.

4. Measurement Methods

4.1 Measuring Turbidity/Absorbance. Two characteristics are employed when screening algae species as a viable source for biodiesel. These are the algae growth rate, and the amount of lipid produced. The algae growth rate is determined by measuring the algae concentration over time. The turbidity/absorbance of the algae solution is directly proportional to the algae concentration. By measuring the turbidity over a period of time (7 – 14 days in most experiments) it is possible to determine the relative growth rate of different algae species. The algae solutions turbidity of was measured using a Bausch & Lomb Spectronic 20 spectrophotometer at 682 nanometers.

4.2 Algae lipid content. While it is important to select fast growing algae for the production of biodiesel, it is just as important to find high lipid algae. When extracted, these lipids can then be turned into biodiesel. To measure the change in the lipid content of a given algae species over a period of time, the method of Cooksey et.al. was used in which the oil is stained with Nile Red due and the fluorescence is measured. A Varian SF-330 spectroflourometer with a Xenon Lamp Power Supply was used to measure the algae fluorescence. Algae cultures were normalized (diluted) to an equal turbidity. By measuring the peak fluorescence over a period of time it was possible to determine how the lipid content of the algae changed.

5-Results

5.1 Phase One: Fresh water vs. Salt water results. These experiments involved comparing algae grown in a regular nutrient solution (denoted by X2 nutrient in the figures) and a 0.1 M sodium chloride and nutrient solution. (denoted by X1 Sea and X2 Sea in the figures). Figure 1 shows the turbidity (which measures algae cell concentration).

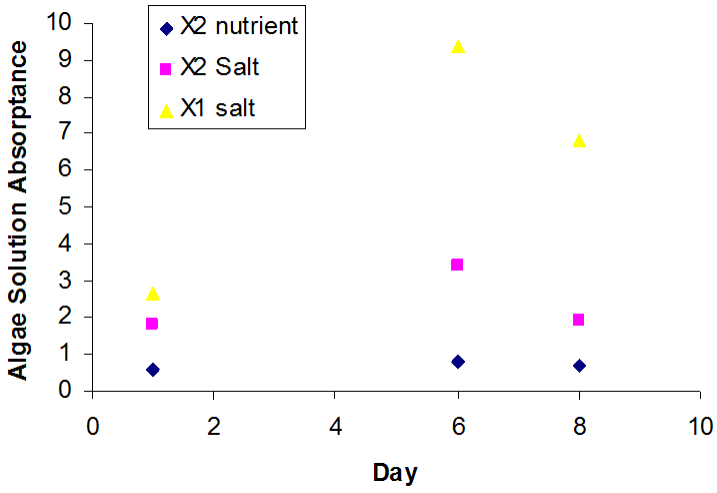

Figure 1: Effect of salt on algae growth

Figure 2 shows the peak normalized fluorescence; a measure of the lipid concentration in the algae cells.

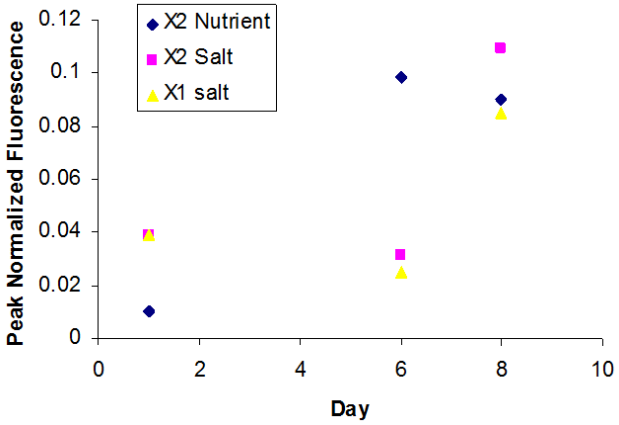

Figure 2: Effect of salt on algae lipid concentration

Figure 3 is the product of the turbidity (Figure 1) and the peak normalized fluorescence (Figure 2). It is a measure of the lipid content of the algae solution. The graphs show that algae species X1 grew faster than algae species X2 (Fig. 1), and that X1 had a higher fluorescence (Fig 2) and a higher lipid content (Fig. 3).

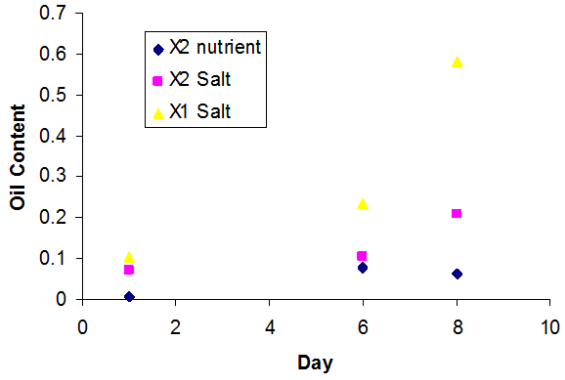

Figure 3: Effect of salt on algae lipid content

5.2 Phase Two: growing the algae in pure ocean water The second set of experiments involved growing the algae in pure ocean water. Algae species X2 was grown in a normal nutrient solution, a 50% sea water and 50% normal nutrient solution, and a pure ocean water solution. Figure 1 shows the turbidity (which measures algae

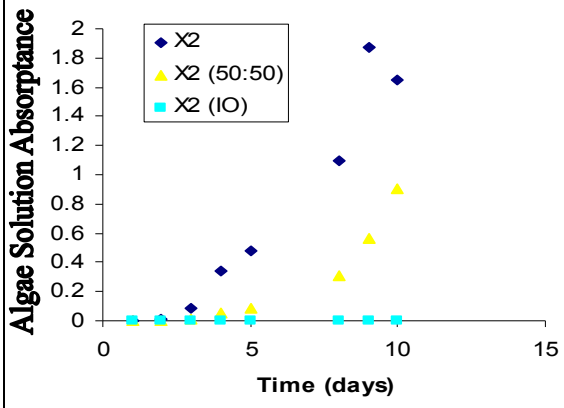

Figure 4: Effect of pure ocean water on algae growth

cell concentration).

Figure 5 shows the peak normalized fluorescence; a measure of the lipid concentration in the algae cells. Figure 6 is the product of the turbidity (Figure 1) and the peak normalized fluorescence (Figure 2). It is a measure of the lipid content of the algae solution. The graphs show that algae species. These results show that the algae do not grow well in the pure ocean water (possibly due to lack of nutrients) but they do grow well in the 50% sea water and 50% normal nutrient solution

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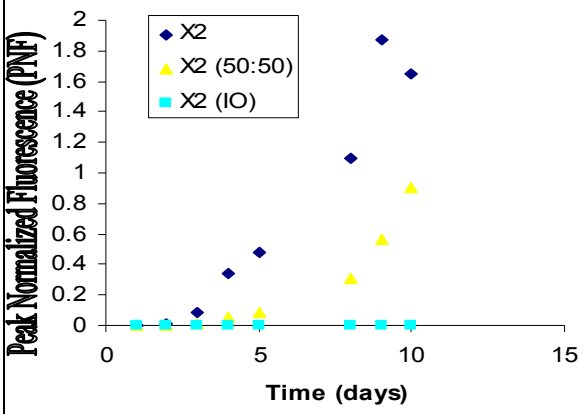

Figure 5: Effect of pure ocean water on algae lipid concentration

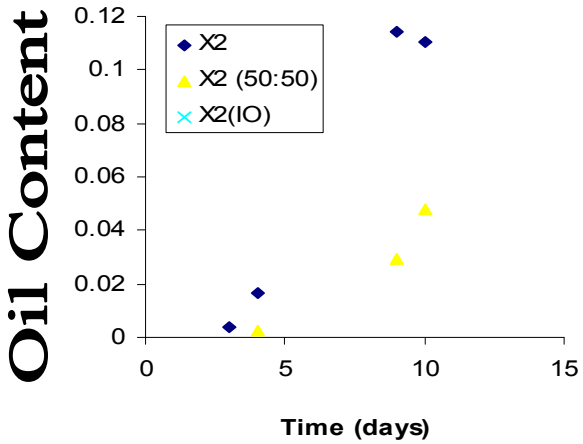

Figure 6: Effect of pure ocean water on algae lipid content

5.3 50% sea water and 50% normal nutrient solution vs. normal nutrient solution

The third set of experiments involved comparing algae grown in a normal (0.1 M NaCl) nutrient solution (denoted by X1 and X2 in the figures) versus the growing the same X1 and X2 algae in a mixture of 50% sea water and 50% normal nutrient solution. This is denoted X1 Sea and X2 Sea in the figures. Figure 7 shows the turbidity (which measures algae cell concentration). Figure 8 shows the peak normalized fluorescence; a measure of the lipid concentration in the algae cells. Figure 9 is the product of the turbidity and the peak normalized fluorescence. It is a measure of the oil content of the algae solution. From the above graphs it was again observed that algae species X2 grew faster than algae species X1 (figure four), but from figures five and six it was clear that algae species X1 had a higher fluorescence and a higher oil content in both the 50:50 mixture of ocean water and 0.1 M sodium chloride nutrient solution and the 0.1 M sodium chloride nutrient solution than algae

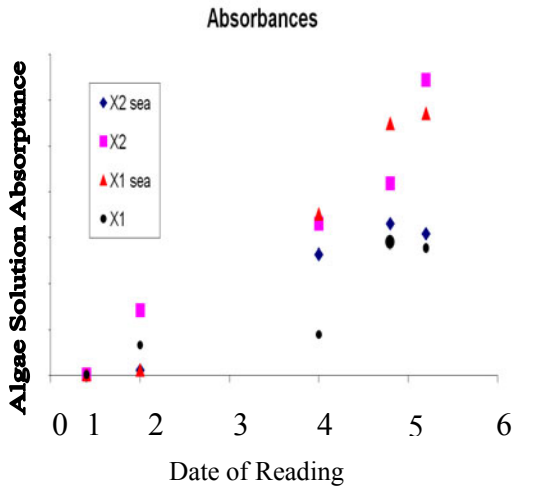

Figure 7: Effect of 50% sea water and 50% normal nutrient solution on algae growth

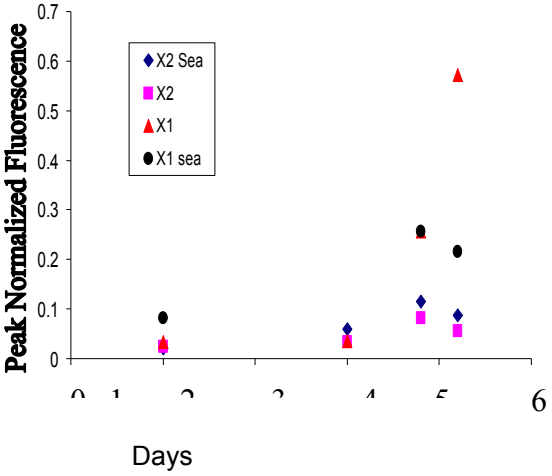

Figure 8: Effect of 50% sea water and 50% normal nutrient solution on algae lipid concentration

species X2

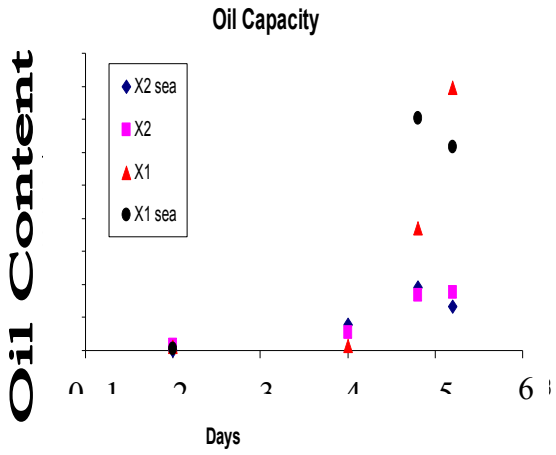

Figure 9: Effect of 50% sea water and 50% normal nutrient solution on algae lipid content

5-Conclusions:

The purpose of this research project was to determine the viability of using ocean water as a possible nutrient source to grow the two freshwater algae species that were studied. While the results of the screening process for experiment show that algae species X1 performed better than algae species X2, these results are qualitative, and more experiments must be done to determine whether the trends that were observed in these experiments can be replicated. The results of experiment number two helped us to realize that pure ocean water can not be used as a sole nutrient source, because it lacks a lot of crucial nutrients that algae, both freshwater and saltwater, need in order to grow and flourish. Even more promising were the results seen in

experiment three in which algae species X1 was able to grow rather well in the 50% sea water and 50% normal nutrient solution thus making the use of ocean water as a nutrient source a possibility.

Also based on the above results the UNH Biodiesel program plans to begin performing research with salt water algae in an attempt to find a species that can either grow well in pure ocean water, or in a nutrient solution in which ocean water is the stock source, instead of using potable water. We are very excited to continue researching growing algae in the hopes that it can be used as an alternate feedstock to produce biodiesel.

Acknowledgment:

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