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Producing Biodiesel from Microalgae Grown in Municipal Wastewater from Dover, NH

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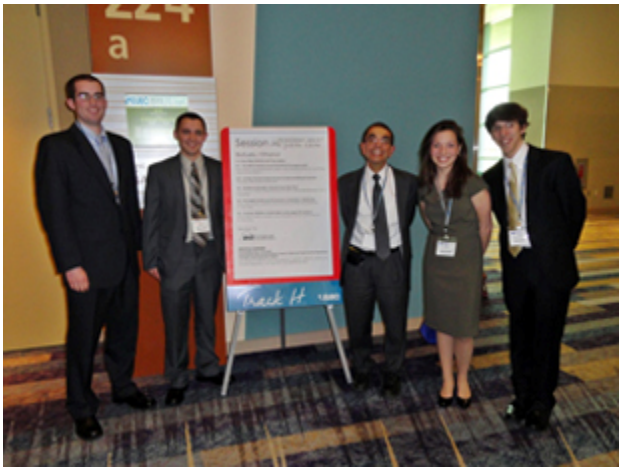
research article

Producing Biodiesel from Microalgae Grown in Municipal Wastewater from Dover, NH

—Brian McConnell (Edited by Brigid C. Casellini)

During the fall 2009 semester I took a course titled, "Energy and the Environment." I have always been interested in the ways humans affect the environment, and this course gave me the perfect opportunity to study this relationship. I soon realized that merely learning how to protect the environment is not enough; I needed to take action. Knowing that performing research on biofuels would be a productive and effective way to make a difference, I joined Dr. Ihab Farag's Biodiesel Research Group in spring 2010.

Currently in the United States and Europe, the main biodiesel feedstocks are food crops. There is a great fear that if biodiesel production from food crops is increased substantially, many people will suffer from malnutrition. Biofuels have caused food shortages and subsequent violence, such as riots, in some parts of the world. Farmers are selling their crops to the biofuel companies instead of consumers because the companies pay more. Less land is available for growing crops which puts a strain on agriculture. Using biodiesel from food crops could make the situation even worse (Brabeck-Letmathe 2008). Fortunately there are other options.



The author (far left) presenting his research at the 2011 Energy, Utility and Environment Conference (EUEC) in Phoenix, AZ, with fellow students of Dr.Ihab Farag (center).

Recent research has looked into the use of microalgae to produce oil which can then be converted to biodiesel. Microalgae are microscopic, simple, plant-like cells that use photosynthesis to turn carbon dioxide and light into energy. Dr. Farag's research focuses on growing the microalgae and extracting its oil. After working under his supervision for a semester, Dr. Farag nominated me for the Research Experience and Apprenticeship Program (REAP), funded by UNH's Hamel Center for Undergraduate Research. As a REAP fellow, I experimented with different water sources—freshwater as well as municipal wastewater from Dover, New Hampshire, each with different nutrient concentrations—to grow microalgae for biodiesel production in Dr. Farag's lab.

Microalgae as an Alternative Biodiesel Feedstock

Biodiesel is a clean-burning and environmentally friendly fuel. Because the combustion of biodiesel releases carbon dioxide into the air that was recently taken out of the air by plants, it is called carbon neutral. In fact, biodiesel releases less greenhouse gas than diesel. Biodiesel is also biodegradable, so it does not cause great harm to the environment if there is a spill (*Biodiesel Handling and Use Guide* 2008).

Another advantage of biodiesel is that it can be produced from many different sources of oil. Some of the feedstocks include grease, vegetable oil, and animal fats (*Biodiesel Handling and Use Guide* 2008). However, using oil from a food crop, like vegetable oil, has many drawbacks because crops traditionally grown for food are now being grown for fuel. This fuel- versus-food debate has caused researchers to look at alternative feedstocks like microalgae.

Algae are much more versatile than plants. Both plants and algae need water, light and carbon dioxide to grow, but plants need fertile soil while algae do not grow in the ground. “Algae can grow in salt water, freshwater or even contaminated water, at sea or in ponds, and on land not suitable for food production” (*Science Daily* 2008). Algae’s versatility makes it an attractive alternative to crops.

Another benefit of using algae is that microalgae “can generate 15 times more oil per acre than other plants used for biofuels” (*Science Daily* 2008). Since growing algae for biodiesel requires less land than growing crops for biodiesel, there will be plenty of land available to grow crops for food. An added benefit of algae is that after its oils have been extracted, the remaining biomass can be used as protein-rich animal feed.

Microalgae Growth in Wastewater

When Dr. Farag and I were planning this research project, we decided to focus on the water source used to grow the algae. We made this decision because the large amount of water required to grow the algae could impact production costs, the environment and society. Based on our research, it takes roughly 1000 gallons of water to produce one gallon of microalgae biodiesel. Some important issues to consider when choosing a water source is the water’s availability and nutrient content, and the side effects of taking the water from the environment.

The water’s availability is probably the most important factor to consider because water is such a precious resource. Using freshwater on a large scale would not only be expensive, it would be irresponsible. Today, about one billion people have inadequate access to clean drinking water, and if the same conditions continue, by 2025, two out of three people will struggle to find clean drinking water (World Meteorological Organization 1999). There are some parts of the world where fresh drinking water is more important than biodiesel fuel.

Wastewater, on the other hand, is readily available. In 2004, the United States alone produced approximately 35 billion gallons of wastewater per day. In 2004, the US population was approximately 295 million. Therefore, approximately 120 gallons of wastewater per person per day was produced. Using this abundant water source to grow algae would not negatively impact drinking water supplies (University of Michigan, Census Bureau; 2010).

The next factor to consider is the water source’s nutrient content. Freshwater has very few nutrients, so nutrients need to be added which adds to production costs. Conversely, wastewater already contains nutrients, so little need to be added.

Wastewater is great for growing algae because it contains large quantities of nitrates and phosphates which are two key factors in microalgae growth. Another important nutrient, carbon, is missing from the wastewater, but there is a simple solution to this problem. Carbon is added to the system by bubbling carbon dioxide through the water. By using flue gases containing carbon dioxide to grow the algae, carbon dioxide emissions will be reduced because the algae will consume the carbon dioxide and produce oxygen through photosynthesis (Greer 2009).

The environmental benefits of using wastewater are important to consider. As mentioned above, it does not impact drinking water supplies. In addition, using it to grow algae would prevent it from being released back into the environment.

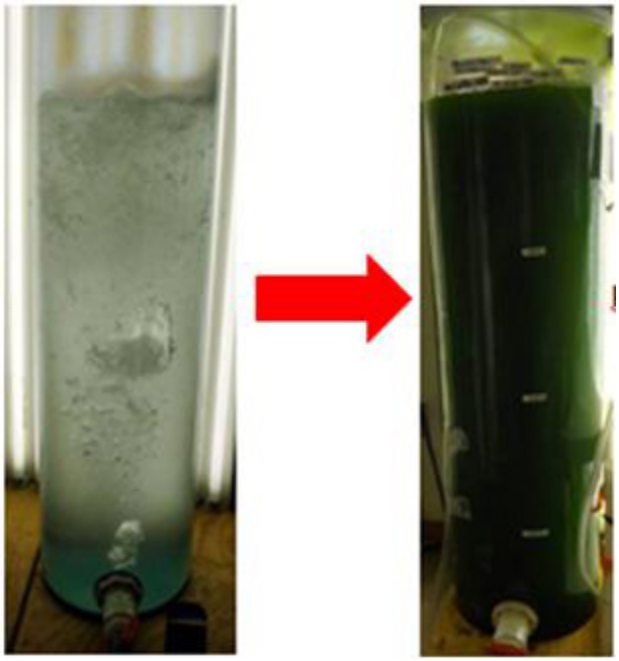
My Algae to Biodiesel Process

We knew that using wastewater had great potential, but we needed to experimentally determine whether or not it could actually be used to grow the microalgae. The algae to biodiesel process can be broken down into four main stages: growing and monitoring the algae, harvesting the algae, extracting the oil/lipids and producing biodiesel. My research explored the first three stages, with a focus on the growth of microalgae in wastewater treated with ultraviolet (UV) light.

My first objective was to determine the best nutrient medium for growing microalgae. The best nutrient medium is the one which produces the most algae in the shortest period of time. The growth is monitored by measuring the solution’s turbidity (darkness), and counting the number of cells in a known volume of sample under the microscope.

The algae are grown in a nutrient solution, and supplied with light and carbon dioxide. Light is provided by fluorescent lights, and carbon dioxide is supplied by bubbling air through the nutrient solution. The light and carbon dioxide are used

for photosynthesis, and the nutrients help speed up the algae growth, which is why they also are called fertilizers. I determined the best medium by analyzing water sources and nutrient concentrations; the water sources were freshwater (pure water, also called reverse osmosis [RO] water) and UV-treated municipal wastewater.



This picture shows the algae growth in the 80L photobioreactors. The trial starts off clear, and once the algae have fully grown the color is a deep green.

About a dozen chemicals are used to make the nutrient solution. Nitrogen was the nutrient of interest; therefore, I conducted experiments with two sources of nitrogen: potassium nitrate (KNO3) and urea ((NH2)2CO), and varied the concentration of nitrogen in the nutrient solution. Potassium nitrate was the standard nitrogen source, and we also tried substituting urea because of its lower cost. Potassium nitrate is about seven times more expensive than urea on an atom of nitrogen basis (one atom of nitrogen weighs 14.01g).

The first step in harvesting the algae is to separate it from water; this is done by using a centrifuge and freeze dryer. The centrifuge spins the sample at high speeds (5000rpm) causing the algae to settle at the bottom of the container. We are able to pour off roughly 95% of the water. The remaining water was removed through freeze-drying (a two-day process). Once the dry algae was obtained it could be weighed, and the algae production (grams of algae produced per liter of nutrient solution per day) could be determined based on the final algae mass, volume of nutrient medium used and days grown.

After determining the most productive nutrient medium, we scaled-up production from 2L batches to 80L batches. This helped us determine if the solution would stay homogeneous at a large scale, whether the algae would still grow and produce oil at the same rate, and if contamination was more likely. The 80L batches were grown in photobioreactors which are made of clear plastic in the shape of a circular cylinder. They are four feet tall and one foot in diameter. This method allowed us to analyze algae growth on a much larger scale. The large batch would also produce significantly more algae, allowing for further testing of lipid (oil) extractions.

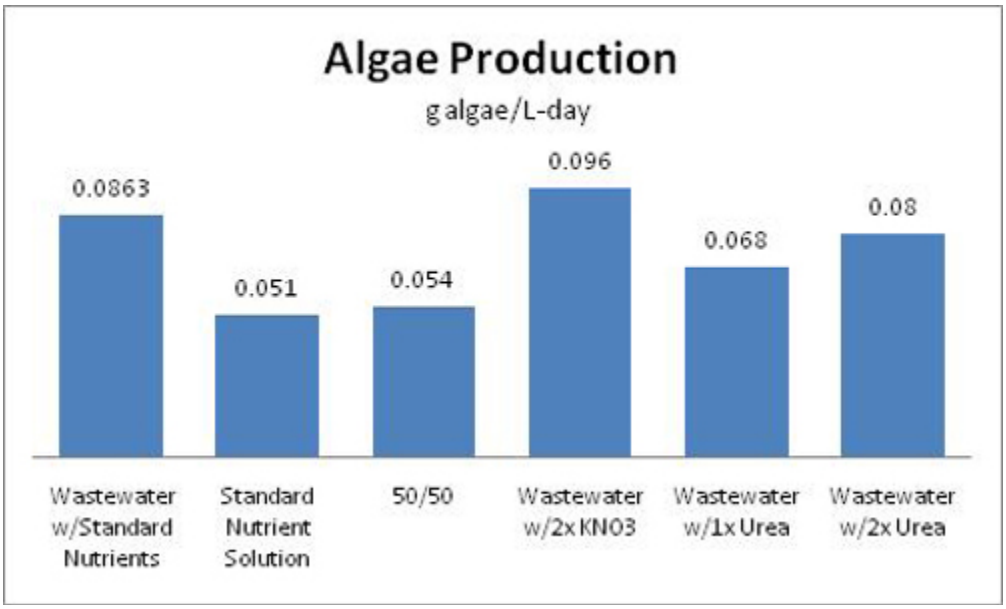
Once the dry algae were obtained, the lipids could be extracted from the cells using a solvent (hexane). I used two different extraction methods: soxhlet and flask. In both methods, the solvent is used to pull the lipids out of the algae cells. After extracting the lipids, the remaining algae, lipids and solvent had to be separated. The algae and liquid were separated by filtration. Then, the solvent was evaporated leaving the lipids (oils) behind.

Algae Growth and Oil Production Results

During the research project six types of nutrient mediums were tested: freshwater (or RO water) with standard nutrient solution (SNS); wastewater with standard nutrients (WWSN); a mixture of 50% wastewater and 50% freshwater with standard nutrients (50/50); wastewater with twice the concentration of nitrogen from potassium nitrate (WW2xKNO3); wastewater with urea substituted for potassium nitrate (WW1xUrea); and wastewater with twice the concentration of nitrogen from urea (WW2xUrea). In addition to these trials, each time wastewater was collected, a sample of wastewater alone was supplied with light and air to test if any organisms grew in the wastewater.

The only difference between SNS and WWSN is the water source; SNS used distilled water whereas WWSN used wastewater. The 50/50 mixture had the same nutrient composition as SNS and WWSN, but the 50/50 was half wastewater and half distilled water. When urea was substituted for potassium nitrate, the nitrogen concentration was the same.

After conducting several trials with each type of nutrient medium, we determined the best medium was wastewater with twice the standard concentration of potassium nitrate (WW2xKNO3), because it produced 0.096g algae per liter per day. The medium which produced the least amount of algae was the freshwater standard nutrient solution (SNS), which produced 0.051 g algae per liter per day—close to half as much as the best wastewater trials. Stating it differently, WW2xKNO3 produced 88% more algae than freshwater SNS.



This graph shows the daily algae production for each type of nutrient medium used during this project. Wastewater results in high algae productivity, with the highest daily production obtained using wastewater with twice the standard potassium nitrate concentration. Freshwater with standard nutrients results in the lowest algae productivity.

These results are promising because they show that wastewater is an effective water source for growing algae. However, there was one problem with using wastewater; it provided very inconsistent results. Several times throughout the summer a wastewater batch would be growing well, and the next day it would collapse. It was very hard to predict when the batch was going to collapse because some batches collapsed after a couple days and some lasted over a week. One time, four batches used wastewater which was collected at the same time, and two of the runs collapsed after a few days while the other two produced a good amount of algae.

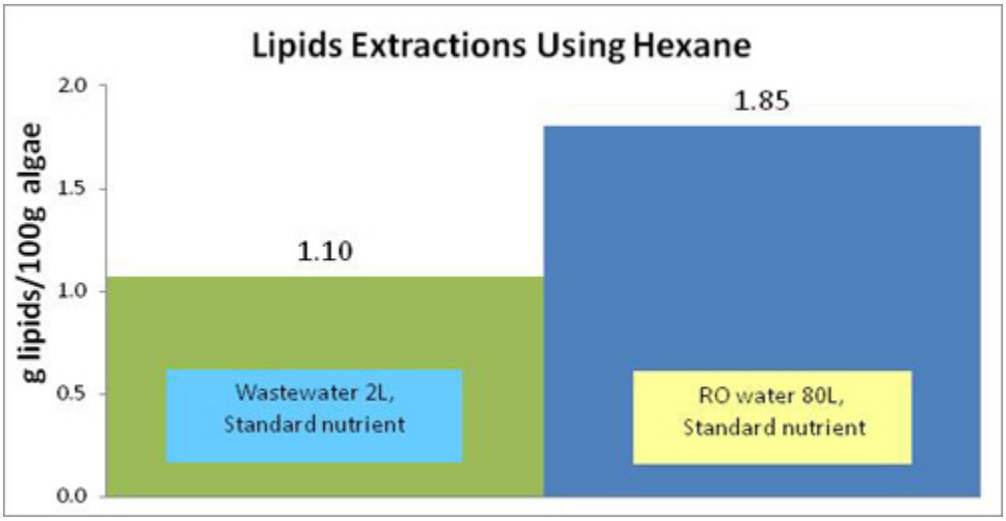
The source of the inconsistency is that some microorganisms were introduced into the system by the wastewater. While performing cell counts, I could see foreign species swimming around and eating the desired (good) algae. Usually the runs that collapsed quickly had more foreign species, so we concluded that the foreign species were to blame.

Another problem we encountered was that the batches of only wastewater (no algae or nutrients) would produce undesirable algae which formed a thin film around the flask. These undesirable algae were different from the good algae being grown and must have been introduced to the system by the wastewater. Sometimes this film would also form on the wastewater batches in which good algae were being grown. This was a problem because it would limit the amount of light that reached the good algae in solution, thereby limiting photosynthesis. Also, the foreign algae consumed some of the nutrients, leaving less for the good algae.

After obtaining the dry algae, I performed lipid extractions to determine the algae's oil content. Based on the extractions, the average lipids (oil) content for algae grown in wastewater was about 1.10g lipids/100g algae, and for freshwater the lipids content was 1.85g lipids/100g algae. This means that the lipids productivity for wastewater algae was 1.06mg lipids per L of water per day, and for freshwater the productivity was 0.94mg lipids per L of water per day. The wastewater will produce about 11% more oil than the freshwater.

The Soxhlet and flask methods produced the same results, but the flask method only takes one to two hours whereas the Soxhlet method takes five to six hours. The flask method will be used in the future because of its time savings ability.

Because the wastewater runs were inconsistent and the oil/lipid productivity was not much higher, we did not choose wastewater to scale up in the 80L photobioreactors. We decided that the freshwater, standard nutrient



This graph shows samples of the algae's lipids content. The lipids content of wastewater grown algae was 41% less than the lipids content of freshwater grown algae; however when factoring in that wastewater produced 88% more algae than freshwater, the lipids productivity of wastewater grown algae is 11% higher than that of freshwater grown algae.

solution was the best option because it was reliable. Even though it produced fewer algae, the algae growth was predictable. Production of algae in the big reactor decreased slightly from the 2L reactor, but that was to be expected because the much larger quantity of algae in solution decreased light penetration and thus photosynthesis.

Personal Gains

The goal of REAP is to allow first-year, UNH Honors students to explore topics of interest, develop a foundation of knowledge in their field of study and learn more about the approaches, theories, methodologies and techniques employed by faculty in their own work. As a 2010 REAP fellow, I was able to achieve all these goals.

By spending twelve weeks working in the laboratory, I learned that a research project is a *process*. You need to conduct literature research, conduct preliminary experiments, learn from the experiments, brainstorm, conduct more experiments and finally try to form conclusions or figure out if further work must be done. In addition, I was able to present this research at the 2011 Energy, Utility and Environment Conference (EUEC) in Phoenix, Arizona. This was an excellent learning experience because I was able to present at a professional conference in front of a large group, address their comments and questions, and see other areas of research.

Most importantly, my REAP experience has prepared me for further research in the area of biofuels, given me the ability to conduct experiments in future courses and enhanced and enriched my learning abilities. My goal is to continue this line of research in summer 2011, studying the growth of microalgae in saltwater.

I would like to thank Dr. Ihab Farag for mentoring and supporting me throughout this research project, The Hamel Center for Undergraduate Research for funding this project, and Nancy Whitehouse, John Newell, Dr. Lee Jahnke and Arnold Powers for their help at various stages of this project.

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Author Bio

Brian McConnell *is a sophomore chemical engineering major and University Honors Program student from Peabody, Massachusetts. After taking a course called "Energy and the Environment," Brian became interested in alternative energy and joined the team in Dr. Ihab Farag's biodiesel research lab. Shortly thereafter, he was one of eight freshman honors students selected as a 2010 Research Experience and Apprenticeship Program (REAP) fellow. "This project has helped me prepare for future advanced-level research," says Brian, who hopes to attend graduate school to continue this line of research.*

Mentor Bio

Brian's mentor, ***Dr. Ihab H. Farag****, has been a professor in the UNH Chemical Engineering Department for 35 years. He conducts extensive research into the areas of microalgae biodiesel, bio-oil quality improvement, energy efficiency, and pollution prevention. Professor Farag is no stranger to mentoring undergraduate students, but working with Brian McConnell was his first time nominating and mentoring a REAP student. "Having a well-motivated student, like Brian, I used a mix of the different mentoring approaches," says Farag. "This helped me to provide Brian with a strong learning and research experience... supervising Brian's research also helped me sharpen my mentoring and teaching skills."*