Fueling the Debate: Exploring the Feasibility of Algae as a Biofuel in Holland, MI

Sarah Brokus, Derek Duncan, Morgan Willming
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Introduction

As concerns over energy resources and fuel prices continue to escalate around the world, investigations into alternative energy sources are an area of significant research. The current reliance on electricity generated from carbon based sources is not sustainable from an environmental or economic standpoint. New sources of energy are needed, and technology is racing to find alternatives to coal and oil. Of particular interest are biological sources of energy, such as microalgae, which can be used directly for power generation, or processed to create fuels such as biodiesels, ethanol or methanol (Becker 1994 and Illman et al. 2000). Because microalgae have greater photosynthetic rates and higher lipid content than most higher plants, they can serve as a prime source of biofuels, while remaining relatively easy to grow (Illman et al. 2000). Microalgae are also known to increase their lipid content when grown under "stressed" environmental conditions making it relatively simple to culture large quantities of algae having a high calorific, or energy, content (Hu et al. 2008). A higher energy concentration in the algae would increase the usefulness of the resulting biofuel. Since algae need a source of carbon for growth, algae bioreactors could be fueled using carbon dioxide, already generated from the air emissions of many factories or power plants and currently unused. While the potential for microalgae as a source of biofuels has been well researched, much more knowledge about the response of algae to environmental factors needs to be understood before algal bioreactors can be implemented on a large scale.

Within the Lake Macatawa watershed in Holland, MI, preliminary investigation has been done on the feasibility of using air emissions from the DeYoung coal-fired power plant as a

source of carbon dioxide to fuel an algae bioreactor. This laboratory investigation addresses the effects of two environmental factors, light intensity and the pH effects of trace air pollutants found in the power plant emissions, on the energy content of a unicellular green algal species, *Chlorella vulgaris. C. vulgaris* has been widely used as a representative algal species in biofuel research due to its potential for high lipid production (Illman et al. 2000). Higher lipid content can be induced by limiting the amount of nitrogen in the growth media, or stressing the algae, a method well-established in literature (Hu et al 2008 and Illman et al 2000).

In this study we assumed that trace emissions pollutants such as SO_x and NO_x would be emitted continuously from a power plant, and if bubbled constantly through a bioreactor would dissolve to the point of saturation lowering the pH considerably. Since most algal species prefer a near neutral pH, we investigated the effect of lowered pH on algal growth and energy content. This is also important in considering the added cost required of buffering a large amount of algae to a suitable pH as would be necessary in a large scale bioreactor. Additionally, the light intensity treatments were designed to represent seasonal variations in light intensity and how this may impact algal growth and energy content.

Methods

Experimental Design

Chlorella vulgaris (#152075) was ordered from Carolina Biological Supply along with Alga-Gro concentrated freshwater medium (#153751). Upon arrival, algae was added to a beaker of prepared Alga-Gro growth medium and placed in a growth chamber on a 16 hour light, 8 hour dark diurnal cycle at about 30º C with a light intensity of approximately 80 μmol photons/m^s/s. Carbon dioxide was delivered continuously from a gas cylinder to the algae using a gas manifold system. To create the manifold, a PVC pipe was sealed at one end and fitted with an input for an air tube at the other. Two rows of holes were drilled lengthwise on the top and into each was

glued a three way luer-lock connector. The connectors allowed some control over gas flow. Once the glue had dried, distribution tubes were locked into the luers and taped into beakers containing the algae. The manifold system was adjusted until a similar flow was observed in each beaker.

Later the algae was moved to a different growth chamber with higher light intensity (800 μmol photons/m^s/s) and set at 25° C with the same diurnal cycle. However, the initial light intensity in this incubator may have been too high since there was an observed reduction in the greenness of the algae after a few days. As a result light intensity was decreased to approximately 400 μmol photons/m^s/s and the algae recovered. Light intensities, measured as photosynthetically active radiation (PAR), were measured using an AccuPAR light ceptometer.

After about 2 weeks of initial growth, the treatment groups were set-up according to Table 1. Three replicates were used for each treatment and each 250 ml beaker contained 15 ml of the original algal culture and 85 ml of growth media. The low nitrogen stressor medium was prepared according to Illman et al. (2000) and contained 203 mg/l $(NH_4)_2HPO_4$, 2.236 g/l KCl, 2.465 g/l $MgSO_4$, 1.361 g/l KH_2PO_4 and 10 mg/l $FeSO_4$. The pH was lowered by adding drops of 1M HNO_3 until it was at the desired acidity. Cheesecloth was placed over the beakers in the low light treatment, while all other beakers were exposed to the ambient light intensity in the growth chamber (400 μmol photons/m^s/s). The low light treatment was exposed to 200 μmol photons/m^s/s. Carbon dioxide was also bubbled continuously through the samples and media was replenished periodically due to evaporation. This experimental growth phase lasted for 3 weeks.

Table 1: Table of conditions for each treatment group.

Test	Description	Light Intensity	Nutrient Medium	pH
1	Alga-Gro (Control)	High	Alga-Gro	7
2	pH and Light Control	High	Stressor	7
3	Low Light	Low	Stressor	7
4	pH 3	High	Stressor	3
5	pH 4	High	Stressor	4
6	pH 5	High	Stressor	5

Sample Preparation and Bomb Calorimetry

After the experimental growth phase, the algae samples were mixed well in the beakers and then divided into 15 ml vials that were pre-weighed and numbered. Once divided, the vials were centrifuged until the solution was clear and contained compressed algae at the bottom. The remaining solution was then carefully pipetted off, making sure not to disturb the algae collected at the bottom. Once the vials were pipetted, the algae was covered with a single layered cheese cloth and placed upright to ensure drying by evaporation. After several days, the samples were tested for moisture content. Upon completion of drying, the algae and test tube were weighed again. Algae samples were scrapped out of the tubes into corresponding treatment weigh boats. Once every sample had been weighed a final time, each of the twelve weigh boats were dumped individually into a mortar to be ground into a fine powder. Once the contents had been completely crushed, they were brushed out into the correct weigh boat to make pellets to be used in the bomb calorimeter.

A total of twelve pellets were created using the algae powder. The process for creating

the pellet included using a mixture of roughly both 0.900 g benzoic acid and 0.100 g algal powder. A layer of approximately half the benzoic acid was placed into the bottom of the pellet press, while another quarter of the remaining acid was added to the algal powder. The powder and acid were mixed well and layered as evenly as possible into the pellet press, with the remaining benzoic acid poured on top of the algae/acid mix. The mixture was then pressed down by the stroke of the press arm with force. The pellet was then weighed. A thin wire used for firing the pellet was cut at 10 cm and weighed separately. The wire was wound around the end of a paperclip to create a small coil. This coil was then flattened and each end of the uncoiled wire was hooked to a closed circuit. Once each end had a current, the pellet was placed on a mat that centered the circuit board. The bottom of the pellet containing most of the benzoic acid was placed face up. A button opening the circuit was then held down until the coil heated and could be pressed down into the pellet. The button was released when the coil was far enough into the pellet, not to reach and burn the algae layer, but to ensure fusion within the benzoic acid. The pellet was left to cool and once again weighed. A Parr 1411 Semimicro Oxygen Bomb Calorimeter was used for the energy content analysis. In conducting the bomb calorimetry trials, the procedure was followed according to the Hope College Physical Chemistry lab manual for Fall 2008 as well as the instrument's operating manual.

Results

Throughout the growth phase there were visible differences in the algal growth in each of the treatment groups. After 1 week the pH 3 and 4 treatments were no longer green and the algae was visibly dead. There was not enough algae to continue with further analysis of mass or energy content in these samples. The pH 5 treatment had little growth and was only slightly yellow in color after 3 weeks. The Alga-Gro, stessor and low light treatments showed significant growth, with the low light samples appearing the greenest.

Dry masses of each treatment were totaled across the replicates and can be seen in Figure 1. The low light treatment had the greatest total dry mass of the treatments, while the Alga-Gro and pH 5 treatments had the least algal growth.

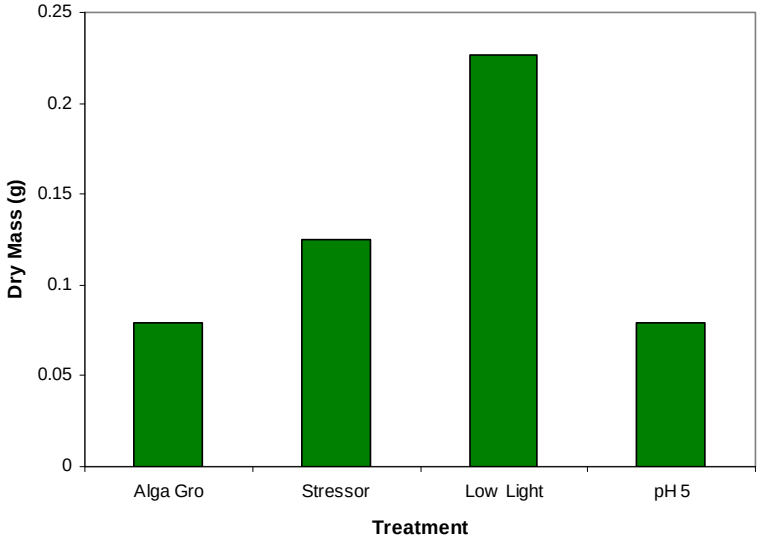

Figure 1: Total dry mass of algae in grams summed across replicates for each treatment group.

From the bomb calorimeter data, the mean energy content per dry mass the algae samples was calculated (Figure 2). The Alga-Gro treatment had the highest mean energy content at 13 kJ/g (+/-3 kJ/g) and the pH 5 treatment had the lowest at 4 kJ/g (+/-1 kJ/g). However, there was large variation in energy content across all of the replicates in each of the treatments.

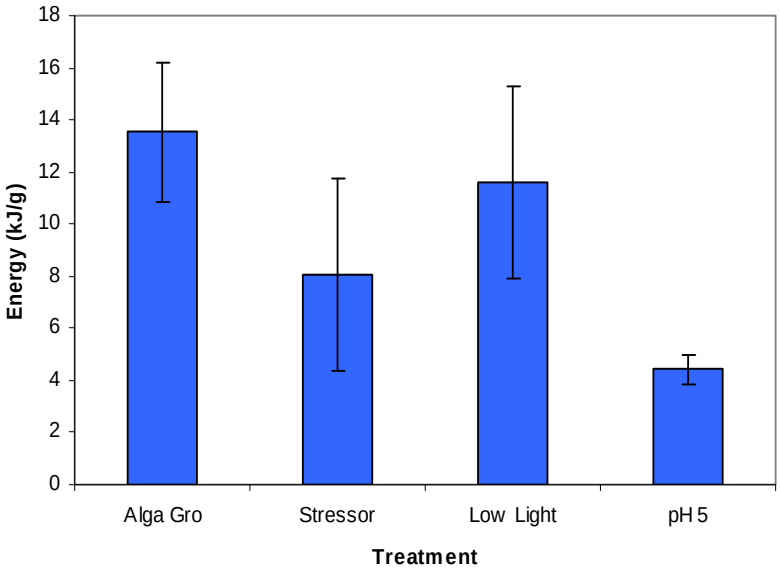

Figure 2: Mean energy per dry mass of algae in kJ/g.

To account for differences in the final dry masses across treatments, the total energy in each treatment was calculated (Figure 3). The low light treatment had the greatest total energy content with nearly 3 kJ (+/-0.8 kJ), while the pH 5 treatment had less than 0.4 kJ (+/-0.04 kJ). Even though the Alga-Gro treatment had the highest mean energy content per dry mass, since it also had the least total mass, this treatment had much lower total energy.

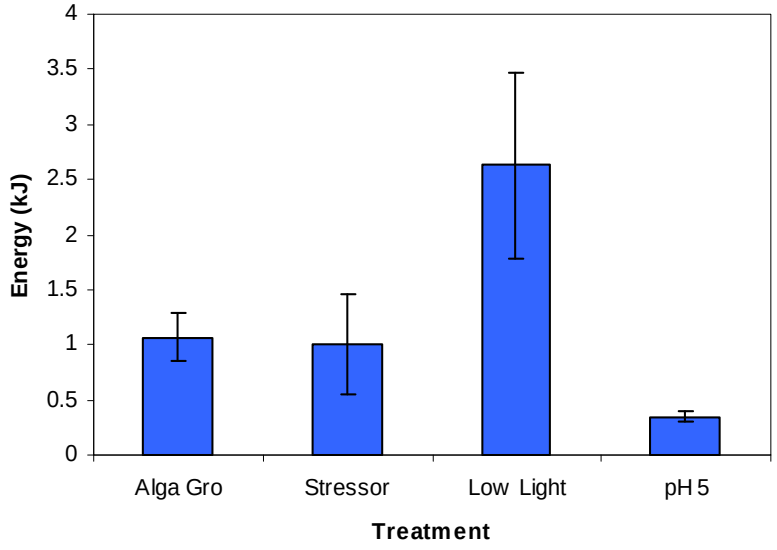

Figure 3: Total energy content of algae in kJ for each treatment group.

Discussion

Variations in light intensity played a significant role in this experiment. During the initial growth stages, the noticeable color difference between beakers in separate growth chambers led us to believe that the algae had died in the beaker exposed to the higher light intensity, while the algae in the lower light intensity growth chamber remained a vibrant green. When the beakers were left in the lower light growth chamber over night to recover, the bleached algae returned to a vibrant green color. Light intensity seemed to be the strongest of the variables in this experiment. While we expected that the algae would grow better in the higher light intensity, we discovered that the algae in fact grew better in the lower light intensity. This suggests a bell curve for the dependence of algal growth on light intensity, and we may have exceeded the optimum initially during this experiment. While this seems counter-intuitive given that natural PAR levels at this latitude can regularly exceed 2,000 μmol photons/m^s/s, a probable cause is that the white reflective walls of the incubator were amplifying the light and also delivering light from multiple directions. Comparatively in a natural environment, the only source of PAR is the sun which is more unidirectional.

When looking at pH variability between the beakers, those with pH values of less than five were clear and void of algae, while the beakers held at pH 5 had minimal amounts of algae to condense and further analyze. It appears that a solution with such a low pH will require buffering if carbon dioxide emissions containing trace pollutants are bubbled through it. Without adequate buffering, the pH may drop too low, severely limiting algal growth in such acidic conditions. This added buffering potentially factors in a large cost that would have to be included in the implementation of any large scale algal bioreactor.

From the results of our growth and energy content analysis, it appears that the low light intensity had higher total mass and energy content. However, there was large variation in energy

content across bombing runs which introduced a significant source of error into our final data analysis. Also, since the dried algae only accounted for about 10% of the total pellet mass, this further increased opportunities for errors in the analysis. Surprisingly, it appeared that the low nitrogen stressor media did not necessarily increase the energy content of the algae, but again it is necessary to note the large sources of error in our specific methodology.

The methodology described in this experiment is the result of our experiences with growing algae and there is potential for improvement to reduce the potential for error. The pellet creation, in particular, needed considerable alteration in order to work with dried algae. It could further be improved as our precision was limited by the small amount of algae we could compress into a pellet. Possible improvements are a mechanized pharmacologist pellet press which could apply enough force to cause the algae to stick together, a more accurate mass balance, or perhaps a different bombing procedure or equipment where the algae does not need to be in a pellet form but could instead be powder in a dish with the wire laying in it. Additionally, while the gas manifold worked, it could be further improved to have better control on flow rates to each tube as the current set up affords only rough control and the torque generated from turning the dial threatens to weaken the glue sealing the connection to the manifold. The algal species used in this study proved to be effective, but before implementation of large scale bioreactors, many species of algae, including specially engineered strains, need to be studied in order to achieve the best energy output and survivability.

In a future experiment, the effect of light intensity and reflection off of the walls of the growth chamber could be analyzed and modeled to hopefully find the preferred light intensity to maximize algal growth. It would also be useful to know how the algae grown under constant light intensity in the incubator compares to algae exposed to actual sunlight. Discovering temperature dependence would be another interesting set of experiments. We would like to

increase our replicates in any future work to reduce sources of error, have longer time to grow the algae, and potentially have multiple tests running simultaneously.

Algae shows promise as a source of energy, but it cannot be the sole source. In the future algae could be used to complement a broad and diverse renewable energy portfolio, but much research needs to be completed before it can be scaled up to a commercial venture. Such investigation needs to be pursued in order to maximize the amount of energy produced by a potential bioreactor, and methods of extracting the algae on a large scale need to be implemented in an efficient manner. The system also needs to be monitored, especially regarding any buffering done to maintain a desired pH, to ensure that the amount of energy and cost going into the system will not outweigh the final energy output. This experiment and others like it will continue to add momentum behind using algae as a carbon neutral source of energy.

References

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