Determining The Best Lighting Wavelengths to Grow *Chlorella vulgaris* For The Use of Biodiesel

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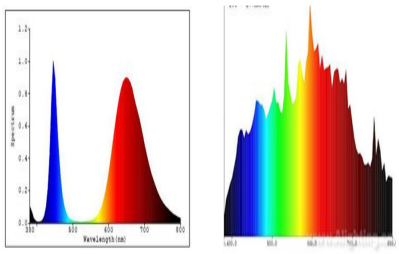
As researchers continue to look for alternative fuel sources to fossil fuels, certain strands of algae have emerged as leading candidates due to their oil content that can be chemically converted to biodiesel (*Biodiesel*). Among these algae strands, *Chlorella vulgaris,* a unicellular strand with a photosynthetic efficiency rate of 8%, is seem as a leading candidate (Chlorella). However, before *Chlorella vulgaris* can be used on a large scale basis for biodiesel production, the process of growing it and converting it to biodiesel must become more cost efficient. The goal of this research project was to determine if using a red-blue LED lighting fixture will produce significantly more algae growth and eventually biodiesel than traditional broad spectrum LED lighting fixtures will.

The reasoning behind this is that *Chlorella vulgaris* has two types of chlorophyll: chlorophyll-a and chlorophyll-b (*Biodiesel*). Chlorophyll-b primarily absorbs blue light which has wavelengths from 400 nm to 450 nm, and chlorophyll-a absorbs red light which has wavelengths from 650 nm to 700 nm (Croce). Therefore, to achieve maximize photosynthetic efficiency and algal growth blue and red light need to be provided at the greatest level of intensity. However, the broad spectrum LEDs that are often used to grow the algae in indoor facilities lack a high intensity of blue light while also emitting lots of light that cannot be absorbed by the algae. This is shown in figure one. Therefore, the hypothesis was that the blue and red LED lighting fixtures will produce a greater growth rate than the broad spectrum lighting as they will provide the light needed for photosynthesis at the greatest intensity and therefore produce more algae and eventually biodiesel than the broad spectrum lighting.

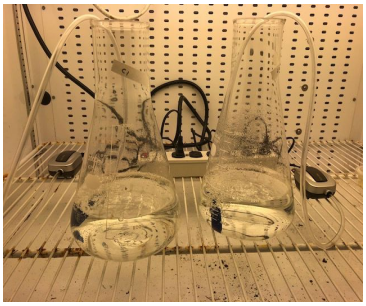
Fig. 1: Broad vs Blue Red Lighting Spectrum



The left graph displays the blue red LED lighting spectrum while the right graph displays the broad spectrum lighting display (n.d.).

The first stage of the project was to set up the growth chamber that the algae would be grown in. The Percival growth chamber was programmed to run a diurnal setting which simulated night and day temperature, humidity, and lighting changes. The lights were set to run for 12 hours, and the temperature was set to 22 degrees Celsius during the day lighting period and 20 degrees Celsius during the night period. The relative humidity was 66% during the day lighting period and 60% during the night period. Alga-Gro Concentrated Medium Tubes from Carolina were then adjusted to a pH of 7.8 and mixed with 1000 ml of spring water. The growth medium was then placed in a 2000 ml Erlenmeyer flask and autoclaved. This process was completed for two Erlenmeyer flasks. Air pumps were also placed into each flask to ensure the CO2 necessary for growth was provided. The growth chamber apparatus is shown in figure 2.

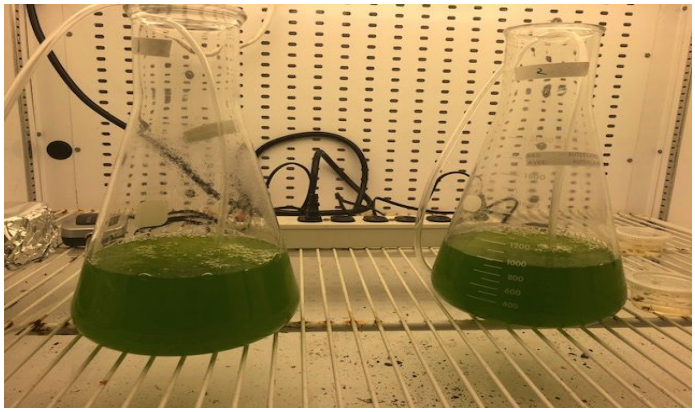
Fig. 2: Growth chamber apparatus. Control group in growth chamber



The algae growth was measured using a spectrophotometer. The Spectrophotometer used was a Spectronic™ 200 Spectrophotometer. The instrument measures growth by scanning a sample of the algae to find the light wavelength it absorbs the most. For the sample of *Chlorella vulgaris* scanned, the maximum light absorbance was at 684 nm. The spectrophotometer then emits this light wavelength into the sample of algae and measures how much of the light is absorbed. The greater the amount of light absorbed the denser the sample. That means if the daily samples of algae are increasing in the amount of light they are absorbing than more algae is present to absorb the light which indicates growth.

The first five days of growth were spent running a control trial meaning no algae culture was introduced into the flask. This was done to ensure that no additional organisms would grow in the flask and impact the data. As expected, there were no measured changes or any sort of observed growth in the control flasks. Following this, the broad spectrum lighting growth period was completed. Two Erlenmeyer flasks with algal growth medium in them had a 50 ml living culture of *Chlorella vulgaris* introduced into. The flasks were then placed in the growth chamber and the algae was grown for ten days under the broad spectrum lighting. This is shown in figure 3. Daily measurements of growth were made using the spectrophotometer

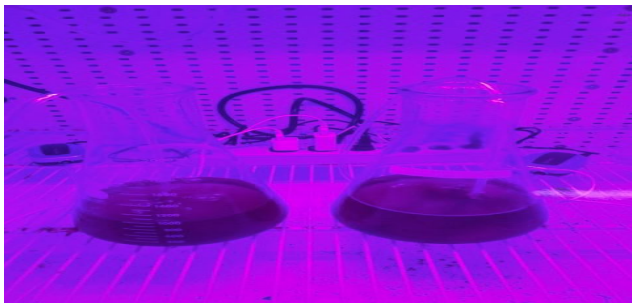
Fig. 3: Algae growing under broad spectrum lighting



Chlorella vulgaris being grown in the growth chamber under broad spectrum lighting. This growth period took ten days.

After the broad spectrum growth period, the blue and red LED lighting growth period was completed. Two 14 watt LED light bars, each containing 16 red bulbs and 9 blue bulbs, were mounted onto the top of the growth chambers. The light bars had to be manually turned on and off daily, but the same diurnal program was ran but without the broad spectrum LEDs that were used in the first growth period. 50 ml cultures of *Chlorella vulgaris* were introduced into the two growth medium containing 2000 ml flasks and the growth period lasted for ten days. This can be seen in figure 4. Daily measurements were taken using the spectrophotometer.

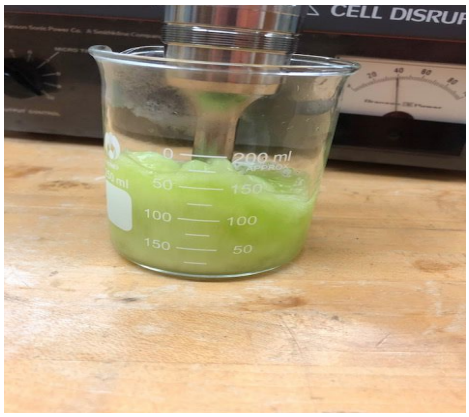
Fig. 4: Algae growing under blue and red LED lighting



Chlorella vulgaris is being grown under blue and red LED lighting. This growth period lasted for ten days.

The next stage of the project was to convert the algae grown during each growth period into biodiesel. The first step of this process was to extract the lipid content, or oil, from the rest of the algae. This was done by using a Branson Sonifier Cell Disruptor 185. The sonifier was set to a max output of 10, and the algae from each growth period was sonified 50 ml at a time for five minutes. This is shown in figure 5.

Fig. 5: Algae being sonified



The sonifier horn pictured here emits ultrasound waves causing thousands of small bubbles to form in the solution. The force of the bubbles breaking is enough to break open the algae cells and separate the oil form the rest of the algae biomass

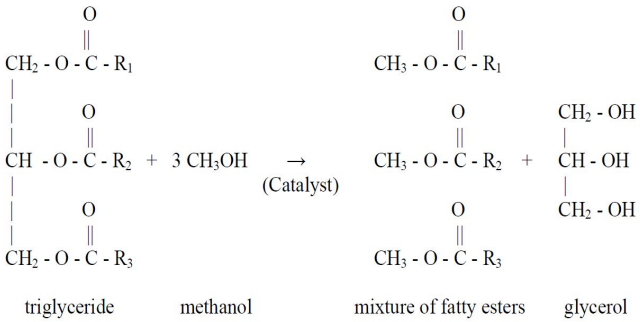
After the algae was sonified, the algae from each growth period was poured into a separatory funnel and allowed to distill for 24 hours. This allowed for the denser water and remaining algae biomass to go to the bottom of the flask and the less dense oil go to the top. The water and algae biomass were then drained out while the oil was poured into a beaker. The algae from the broad spectrum growth period produced 47 ml of oil, and the algae from the blue and red growth period produced 71 ml of oil. The separation funnel can be seen in figure six.

Fig. 6: Separation Funnel

The lipids, water, and remaining algae biomass are all separating by density so they can be separated.

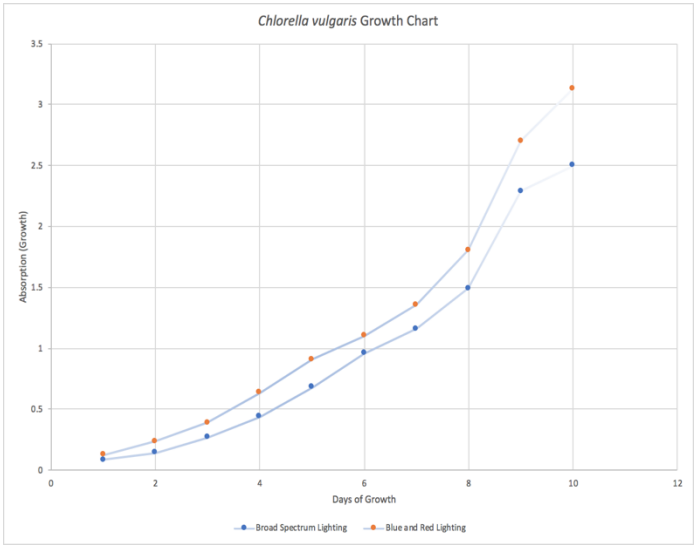
The last stage of the project was to convert the extracted oil into biodiesel. This is a chemical process known as transesterification. In this process, the triglycerides, or algae oil, are reacted with an alcohol and a catalyst to form glycerol and fatty acid methyl esters, which is the biodiesel (Biodiesel from Triglycerides). The alcohol used in the reaction was methanol and the catalyst was NaOH. Ten ml of methanol and .5 g of NaOH were used for every 50 ml of oil. The reaction is shown in figure 7.

Fig. 7: Transesterification reaction (Biodiesel from Triglycerides)



This is the reaction that was used to convert the algae oil, or triglycerides, into fatty esters, or biodiesel.

Fig. 8 : Scatter plot of growth periods



The scatter plot displays the mean light absorption of the two flasks from each growth period.

Overall, the algae grown under the blue and red LED lights outgrew the algae grown under broad spectrum lighting. The final absorption reading from the blue and red lighting was 3.13 while it was only 2.50 for the broad spectrum lighting. This difference indicates a significant difference in overall growth between the two lighting fixtures. However, it is also important to note that during certain periods, such as between days 5 and 6, the slope of the broad spectrum lighting is greater than the slope of the blue-red lighting. This indicates that either the growth rates vary from day to day, or there was an error made in recording the data on that day. More trials will be needed in the future to determine this.

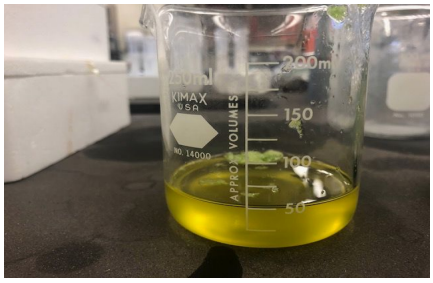
As expected, both curves display what looks like exponential growth with the biggest jump being between days eight and nine. This was mostly likely the algae bloom, and the growth rates would be expected to level out in the following days. However, as each growth period was only ran for ten days, there is no evidence in the data to support this assumption. Therefore, the growth periods will have to be extended in the future.

Fig. 9: Oil and Biodiesel Yields

Lighting	Oil Extracted (ml)	Biodiesel Synthesis (ml)
Blue and Red LED	71	83
Broad Spectrum	47	52

As seen in figure 9, 52 ml of biodiesel was synthesized from the 47 ml of oil extracted from the algae grown under broad spectrum lighting, and 83 ml of biodiesel was synthesized from the 71 ml of oil extracted from the algae grown under blue and red lighting. The biodiesel synthesized from the broad spectrum grown algae is seen in figure 10.

Fig. 10: Biodiesel synthesized from the broad spectrum grown algae



This image displays some of the biodiesel synthesized. The chunks in the biodiesel are the glycerol byproduct of the transesterification reaction and need to be filtered out before this biodiesel can be used in any sort of diesel engine.

Clearly, significantly more biodiesel was produced from the algae grown under blue and red lighting compared to the broad spectrum lighting. This was expected as the data from the growth periods suggested there were greater amounts of algae grown during the blue and red lighting growth period. Larger amounts of algae grown translated into more oil available for extraction and therefore more biodiesel produced from that oil. However, it is important to note that there was some sort of error made when synthesizing the biodiesel from the broad spectrum algae oil. The reason for this is the significantly lower percent yield observed when comparing the amount biodiesel made from the broad spectrum lighting algae to the blue and red lighting algae. There was most likely a loss of biodiesel when separating the glycerol out from the fatty acid methyl esters, or biodiesel, made from the broad spectrum algae. Although this is not too significant, it is important to note.

Overall, the original hypothesis that the blue and red LED lighting fixtures will produce a greater growth rate than the broad spectrum lighting as they will provide the light needed for photosynthesis at the greatest intensity and therefore produce more algae and eventually biodiesel than the broad spectrum lighting could not be rejected. The supporting evidence for this conclusion was the significantly higher growth rate of the algae grown under blue and red lighting than broad spectrum lighting. This growth rate evidence was later supported by the significantly larger amount of oil extracted from the algae grown under blue and red lighting compared to the broad spectrum lighting.

Moving forward, this research can be implicated into any sort of indoor growth facility that relies on artificial lighting. In particular it has great potential to boost the viability of biodiesels made from algae as a replacement to fossil fuels. If greater amounts of algae can be grown at faster rates by using blue and red lighting rather than broad spectrum lighting, then significant breakthroughs in increasing the affordability of biodiesel production can be made.

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