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Article

Effect of Different Growth Media on Algae's Ability for Carbon Dioxide Biofixation

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SUMMARY

Carbon dioxide makes up 72% of all greenhouse gases produced, which makes it the leading source of air pollution. Certain green algal species such as *Chlorella vulgaris* fix the carbon dioxide into fatty acids present in cells in a process known as "carbon dioxide biofixation". This project tests the effect of different algal growth media on the efficiency of *Chlorella vulgaris*'s carbon dioxide biofixation. In the testing process, we added *Chlorella vulgaris* to four bottles, each containing four different substances (distilled water, Blue Green 11 medium, Bold's Basal Medium, and Guillard's f/2 medium), and cultured for eight days. Each algae and medium mixture was then divided equally into three smaller bottles and rotated for three days. To compare data, we measured the change in carbon dioxide content by subtracting the carbon dioxide content of the bottles with algae to a similar bottle without algae. The results for the average change in carbon dioxide content were 59.3 ppm for Blue Green 11 medium and algae, 50.6 ppm for Guillard's medium and algae, 22.6 ppm for Bold's Basal Medium and algae, and 10 ppm for distilled water and algae. The Blue Green 11 medium most effectively decreased carbon dioxide content of the bottles. This supported our hypothesis that algae's capacity for biofixation can be greatly enhanced through the effective use of media, a finding that has extensive real-world benefits in reducing pollution.

INTRODUCTION

Excess carbon dioxide in the atmosphere is one of the leading sources of air pollution. Carbon dioxide is a greenhouse gas, so when it is trapped in the atmosphere, the greenhouse effect causes a rise in global temperature, as the energy is not reflected back into space. There has been a 41% increase in carbon dioxide buildup in the atmosphere since 1990 due to excess air pollution. Carbon dioxide concentration has increased from 370 ppm in 1999 to just over 410 ppm in 2019, but 350 ppm is considered a safe concentration of carbon dioxide in the atmosphere (1). This excess carbon dioxide leads to ocean acidification, poor air quality, change in weather patterns, and a rise in sea levels. Air pollution is one of the world's biggest challenges to human health as well, as it kills up to nine million people annually and increases the risk of developing and aggravating lung diseases like asthma.

To avoid the devastating effects of air pollution, scientists have been researching feasible, sustainable options to reduce carbon dioxide pollution. Current research indicates that certain green algal species, such as *Chlorella vulgaris*, have the ability to sequester carbon dioxide into fatty acids present in their cells and use this carbon dioxide as a fertilizer to support more growth (2,3). Algae grow up to 50 times faster than terrestrial plants because they lack roots and shoots, which are energy sinks. Therefore, algal species have a higher photosynthetic efficiency than land plants, which have a photosynthetic efficiency of 1%. *Chlorella vulgaris* has a photosynthetic efficiency of 20% (4). Due to its high photosynthetic efficiency and growth rate, *Chlorella vulgaris* is commonly used in carbon sequestration research. Although this process of carbon dioxide biofixation is still under development, it has shown promising results of reducing air pollution up to 80%. Using algae to reduce air pollution is thus feasible and practical.

The goal of this research was to optimize the amount of carbon dioxide sequestered by the algae *Chlorella vulgaris* by altering the type of algal fertilizer or growth medium. We tested the effect of various growth media on the amount of carbon dioxide biofixation via *Chlorella vulgaris*. The growth media used in this project were Guillard's f/2 medium, BG 11 medium, and Bold's Basal Medium. All media used in this experiment are commonly used for growing freshwater algae like *Chlorella vulgaris* (5).

A higher quantity, mass, or cell density of *Chlorella vulgaris* results in higher displacement of carbon dioxide, as there would be more *Chlorella vulgaris* storing more carbon dioxide so the environmental carbon content would decrease (3). Consequentially, the medium which causes the most growth of algae would be directly responsible for the highest reduction in carbon dioxide.

Factors that affect the growth of *Chlorella vulgaris* include light, temperature, and pH levels. Light and temperature are kept at a constant, but different media have different pH levels. *Chlorella vulgaris* grows the best under media with higher pH levels, with the optimum pH for growth around 8.0 (5). Nutrients cause the differences in pH. Guillard's f/2 medium has a pH of approxiamately 7.5 to 7.9. BG 11 medium has a pH of 8.0. Bold's Basal Medium has a pH of 6.6. Distilled water (dH_2O) has a pH of 6.8, as after absorbing carbon dioxide from the air it becomes slightly acidic. Distilled water served

pH of media			
Medium	**Initial pH**	**pH after 7 days**	**pH after turning on the rotator**
BBM	6.65	7.28	7.36
BG 11	8.00	8.10	10.92
f/2	7.90	7.88	10.70
Distilled Water	6.90	7.50	6.53

Table 1: This table shows the change in pH levels over the entire experiment. As shown, the average pH of all of the mediums except the control distilled water is much lower initially than after turning on the rotator. The pH of the BG-11 medium in particular rises considerably post-rotation. It changes from a 8.0 to a 10.9. On the other hand, the control group's pH went down from 6.90 to 6.53.

as our control because it has no other minerals or nutrients. Therefore, I hypothesized that the medium with the highest pH will cause the highest growth in the algae and therefore, the highest reduction in carbon dioxide.

RESULTS

To test the hypothesis, I designed a procedure where I grew the algae in its appropriate medium under optimal circumstances, then transferred it to smaller, sealed bottles, and finally rotated it in these smaller bottles on a bottle rotator.

There were four stages to the experimental design. The first stage was building the culturing mechanism. The culturing mechanism consisted of four soda bottles with holes in the caps and tubing that connected the bottles to an air filter and air pump. The second step was making the growth media. I mixed in the appropriate amount of media with distilled water inside of each soda bottle and added *Chlorella vulgaris* for each media. For the control trial, I mixed the distilled water with the same amount of *Chlorella vulgaris*. The third stage was growing the algae in the culturing mechanism for eight days under optimum conditions. The final stage was to transfer the algae of each media into smaller sealed bottles and to rotate

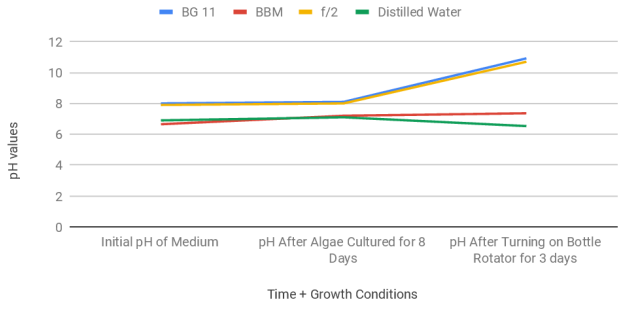

Figure 1: Effect of Time and Growth Conditions on pH Value of All Algae + Medium Mixture. This figure displays the correlation between the amount of time passing and the growth medium type on the pH value of the media and algae mixture. As seen in the graph, the pH increases for all of the trials except through the course of the experiment. The BG-11 medium that has the ratio that follows the Redfield Ratio the closest has the highest pH, while control groups' pH decreases during the experiment.

Average Cell Count of Each Medium + Chlorella vulgaris mixture	
Medium Name	**Average Approximate Total Cell Count (cells)**
BG 11	9.90×10^5
BBM	3.35×10^5
Guillard's f/2	8.25×10^5
Distilled Water	2.25×10^5

Table 2. This table shows the average cell count of each medium and *Chlorella vulgaris* mixture. The BG-11 medium had the highest cell count, and it was especially dramatic in comparison to the distilled water trial. The BBM's cell count was more similar to the distilled water trial, while the Guillard's f/2 medium's cell count was similar to the BG 11 medium's cell count. This pattern repeats with the other tables.

these bottles under the same conditions as before. The pH was measured by a pH meter and the carbon dioxide content was measured by a CO_2 gas sensor.

The cell count was measured with a hemocytometer after the bottles' rotation on the rotator. As portrayed in **Tables 1-4**, the BG 11 medium condition had the highest average cell density of 1320 cells per mL, the highest average cell count of 990,000 cells, the highest average change in carbon dioxide of 59.33 ppm, and the highest resulting pH level of 10.92. The BBM condition had an average cell density of 446 cells per mL, a cell count of 335,000 cells, an average change in carbon dioxide of 22.67 ppm, and a resulting pH level of 7.36. The Guillard's f/2 medium condition had an average cell density of 1100 cells per mL, a cell count of 825,000 cells, an average change in carbon dioxide of 50.67 ppm, and a resulting pH level of 10.7. The distilled water control condition had an average cell density of 200 cells/mL, a cell count of 225,000 cells, an average change in carbon dioxide of 10 ppm, and a resulting pH level of 6.53. Enough trials were not conducted for statistical analysis because lab was closed due to ongoing pandemic.

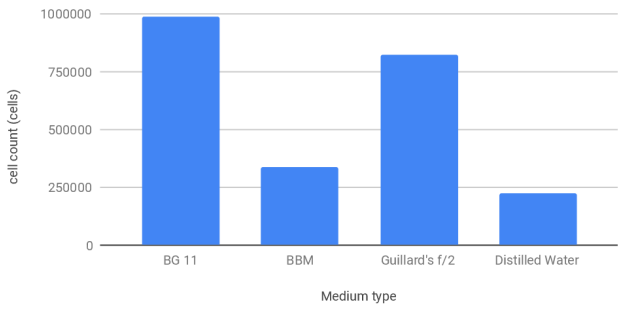

Figure 2: Average Cell Count of All Algae + Medium Mixtures (avg. of all trials) This figure displays the average cell count produced by each of the media and algae mixtures. The cell count is the highest for the BG-11 medium, which fosters the largest growth due to its nutrient levels. The control group has the lowest cell count due to the absence of nutrients. This pattern repeats for the **Figures 3 & 4**.

Average Cell Density of Each Medium	
Medium Name	**Average Cell Density (number of cells/mL of media)**
BG 11	1320
BBM	446
Guillard's f/2	1100
Distilled water	300

Table 3. This table depicts the average cell density of each medium and *Chlorella vulgaris* mixture. Again, the BG-11 medium has the highest average cell density, while the control had the lowest cell density. The BBM trials followed close to the control's cell density, and the Guillard's f/2 was similar to the BG-11's cell density.

Average Change in CO_2	
Medium Type	**Change in CO_2 (ppm)**
BG-11	59.33
BBM	22.67
Distilled Water	10.00
f/2	50.67

Table 4: This table portrays the average change in CO_2 content in the water bottles influenced by the medium and Chlorella vulgaris mixture. The BG-11 medium has the greatest change in CO_2, while the f/2 is similar but does not produce as great of a difference. The distilled water has the lowest change in CO_2, and the BBM follows close to this change.

DISCUSSION

The presence of carbon dioxide lowers the pH of media, as interaction between carbon dioxide and water creates carbonic acid. If the algae stores more carbon dioxide, then the carbon dioxide content of the water will decrease, so the pH of the water will increase. We used this as an indicator to see how effective each medium was at decreasing carbon dioxide content. If the pH was high after rotating on the bottle rotator, then the algae likely decreased the carbon dioxide content. We observed this result, particularly with the BG 11 medium, which had a pH of 10.97 in pH units, while the distilled water had a pH of 6.53 in pH units (**Figure 1**).

BG-11 media is more alkaline. An optimum pH for most algae is around 8.2-8.7 (5). The starting pH of the BG-11 culture medium was 8.00 in pH units; of all of the media, this pH was the closest to the optimum pH, which might have influenced more algal growth (6).

Both N (nitrogen) and P (phosphorus) boost the growth of *Chlorella vulgaris*. N and P can raise the pH of the solution. They are present in water in the form of ammonia, nitrates, and phosphates, usually sourced from fertilizer and pesticide runoff into rivers or other water bodies. It is important to note that cell viability was not tested; this is a limitation to this research that should be considered in this discussion. It would be interesting to consider and test this out in future tests. The results from this research as seen in **Figures 2 - 4** are suggestive but ultimately inconclusive because statistical analysis was unable to be conducted (due to too few trials and lab closure from ongoing pandemic), so a further study must be conducted to confirm the results.

In previous literature, the amount of carbon dioxide biofixation by *Chlorella vulgaris* in 72 hours was an average of 38 ppm (7). In this study, the *Chlorella vulgaris* grown in BG-11 had an average carbon dioxide biofixation amount of 59.33 ppm, which is about 20 ppm higher than the amount present in the literature search. Therefore, this research did provide information on how to increase carbon sequestration to reduce more carbon dioxide pollution.

All three medias used sodium nitrate as the source of nitrogen. The Guillard's f/2 medium contained 1 gram of sodium nitrate. BG 11 medium contained 1.5 grams of sodium nitrate. Bold's Basal Medium contained 0.25 grams of sodium nitrate. This concentration of sodium nitrate corresponded with both the algal growth and the change in carbon dioxide. The relatively high nitrogen content of the BG 11 medium aided photosynthesis and reproduction of *Chlorella vulgaris*, so the *Chlorella vulgaris* grew faster. BG 11 medium had the highest average cell count of 9.90×10^{5} cells and cell density of 1320 cells per mL and therefore the highest change in carbon dioxide.

A source of error in this project could be the variability of each cell of algae used in the project. Each *Chlorella vulgaris*

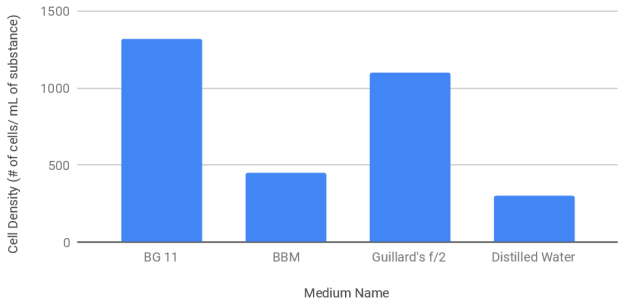

Figure 3: Average Cell Density of All Algae + Medium Mixture (avg. of all trials) This figure displays the average cell density of each of the media and algae mixtures. The cell density is highest for the BG-11 medium and lowest for the distilled water trials. The different is slightly less dramatic in comparison to the **Figure 2** but BG-11 still has the highest level of cell density and the control the lowest.

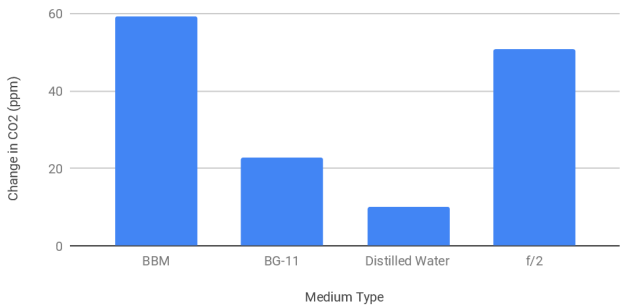

FIgure 4: Average Change in CO_2 Concentration of All Medium + Algae Mixture. This figure displays the average change in carbon dioxide by each of the different algae and media mixtures. This graph is what demonstrates the main objective of this study. The BG-11 medium produced the greatest change in CO_2 content, as it had the greatest cell count and densities, while the control led to the least change, having the lowest cell count and density.

Medium Name	Redfield Ratio (N:P)
BG 11	37.5:1
BBM	1:1
Guillard's f/2	1:1
Distilled water	N/A (no known nutrients added)

Table 5: This table shows the Redfield ratios of the different mediums. As seen, only the BG-11 medium is remotely close to the 16:1 ratio. The control group does not have any added nutrients, while the Guillard's f/2 and BBM have the same ratios.

cell is different so it could lead to differences in growth and therefore a lower or higher carbon dioxide biofixation. Additionally, sometimes the optimal conditions could change because it was difficult to make sure that the conditions were consistently the same; this could affect algal growth as well as the carbon dioxide biofixation. To maintain the conditions as close to optimal throughout the project, the light intensity and temperature were constantly checked.

Recent research indicates that nitrogen is a more effective algal fertilizer than phosphorus because nitrogen influences more reproduction (8,11). The Redfield Ratio, a ratio for nutrients present in various algae and in ocean chemistry, indicates a mass ratio of 16:1 for nitrogen:phosphorus for optimal growth, so nitrogen must be available in higher quantities compared to phosphorus (11). As shown in **Table 5**, BG 11 medium contained 1.5 g of sodium nitrate and 0.04 g of potassium phosphate, a ratio of 37.5:1. BBM contained 0.25 g of sodium nitrate and 0.25 g of potassium phosphate, a ratio of 1:1. The Guillard's f/2 medium contained 1.0 g of sodium nitrate and 0.74-1 g of monosodium phosphate, with a ratio of around 1:1. So, the BG 11 medium had a much higher ratio of nitrogen to phosphorus, and grew the fastest and reduced the most carbon dioxide content. The ratio was more than double of the Redfield ratio, but this was most likely due to the BG 11 being created for small aquariums or for a few batches of algae, not for the ocean (10).

My findings indicated that the BG 11 medium was the most effective at reducing carbon dioxide through *Chlorella vulgaris*. In three days, *Chlorella vulgaris* grown in BG 11 medium in a 250 cm3 volume was able to remove 59.3 ppm from the air (**Figure 4**). In one year, *Chlorella vulgaris* grown in BG 11 medium in a 250 cm3 volume may be able to remove almost 7200 ppm from the air if kept in ideal conditions. Extrapolating that finding, an algal farm with *Chlorella vulgaris* grown in BG 11 medium of the size of a football field with area 10800 meters at a depth of 3 meters can remove almost 2 million ppm from the air in one year. This is based purely on calculations, but it is an area to potentially explore.

Chlorella vulgaris grows better when supplied with more nitrogen and phosphorus, both of which are found in waste or sewage material (9). So, if algal farms are supplied waste material, the algae will serve a dual purpose of reducing air pollution as well as water pollution. Keeping the algae at optimum conditions will certainly lead to high maintenance costs, but it would be important to examine whether there is a trade-off between the maintenance costs and the reduction of air pollution to see whether this is a viable and practical solution to this ever-growing issue. Another area to explore in the future would be to find the optimum ratio of nitrogen: phosphorus that would be beneficial for reducing air pollution through carbon sequestration by algae.

METHODS

To make the culturing mechanism, I connected the following items in order: an air pump, 10 cm of 0.64-cm inside diameter (ID) piping, 25.4 cm of the 0.64 cm outside diameter (OD) piping, an inline air filter, 25.4 cm of the OD piping, an elbow hose fittings (screwing the other end of the barbed adaptor to the back of the 4 port manifold). I screwed four elbow hose fittings to the front of the four-port manifold and attached a 50.8 cm of the OD piping to each of the four barbed adaptors. I drilled 0.64 cm holes to the caps of 4 soda bottles, and pushed the opposite end of the 50.8 cm of the OD piping into the hole for each of the pipings attached to the 4 barbed adaptors on the front of the four-port manifold. (**Figure 5**).

Making the different growth media

To make the growth media, I added 0.529 g of the Bold Basal Powder mixture to 750 mL of distilled water. I added 1.0 mL of 0.1 M sulfuric acid solution, and then adjusted the pH to 6.6 with potassium hydroxide. Finally, I added 37.5 mL of *Chlorella vulgaris* algae solution to the media, closed the cap of the bottle, and pushed the 50.8 cm of 0.64 cm outside

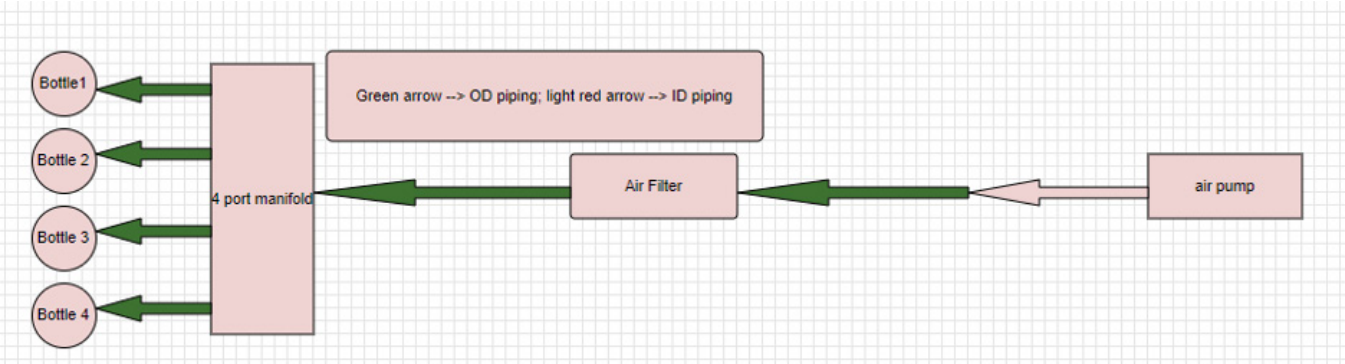

Figure 5: Schematic for assembling the culturing mechanism.

diameter tubing so that it was halfway in the middle of the media and algae mixture. Then, I added 1.26 g of the Blue Green 11 Powder mixture to 750 mL of distilled water. Next, I adjusted the pH to 8.0 with potassium hydroxide. I added 37.5 mL of *Chlorella vulgaris* algae solution to the media. I closed the cap of the bottle and pushed the 50.8 cm of the 0.64 cm outside diameter tubing so that it is halfway in the middle of the media and algae mixture.

I added 0.375 mL of the Guillard's f/2 solution to 750 mL of distilled water. I also added 37.5 mL of *Chlorella vulgaris* algae solution to the media, closed the cap of the bottle, and pushed the 50.8 cm of the 0.64 cm outside diameter tubing so that it is halfway in the middle of the media and algae mixture. Lastly, I added 750 mL of distilled water to the last soda bottle.

I also added 37.5 mL of *Chlorella vulgaris* algae solution to the media. I closed the cap of the bottle and pushed the 50.8 cm of the 0.64 cm outside diameter tubing so that it is halfway in the middle of the media and algae mixture.

Growing the algae

Then, I began the process of growing *Chlorella vulgaris*. I made sure that the temperature inside of the bottle was around 22˚C with a thermometer and that the light amount was approximately between 3000 to 3900 lux with a light meter. This is the optimum growth conditions for *Chlorella vulgaris* (4). Then, I grew the algae on the 16-hour light and 8-hour dark cycle and used the culturing mechanism system to aerate the algae for 8 days. While it grew, I checked every 5-6 hours to make sure that the light, temperature, and aeration is optimal at all times.

Rotating the algae

After the algae grew for 8 days, I measured the pH of each soda bottle with a pH meter. For each type of medium, I labelled three 500 mL water bottles with the trial number and medium name for a total of 12 bottles. Also, I made three more 500 mL bottles filled with 250 mL of distilled water but with no algae. Next, I mixed the algae in the soda bottles to evenly distribute the algae and added 250 mL of each medium including the control group into each of the three 500 mL bottles for the medium. For the control group for the water bottles, I added 250 mL of distilled water without any algae to the 500 mL water bottles. I put cling wrap around the opening of the bottles to avoid air release and close the lid and repeated this process for each of the media. I then fit the water bottles into the holes of the bottle rotator. I also made sure the environment has about 22˚C temperature and 3900 lux of light intensity and that the light dark cycle is 16-hour light and 8-hour dark. I grew the algae for three days on the bottle rotator.

After growing the algae for 3 days on the bottle rotator, I removed the water bottles and measured each of the water bottles' pH with a pH meter. Then, I calibrated the carbon dioxide gas sensor, set it at 0-100,000 ppm, inserted the sensor through the cling wrap of each bottle, and measured the carbon dioxide levels in ppm. I repeated this process with each water bottle.

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