2.2) DOSE-RESPONSE TO CARBON DIOXIDE

Objective: In this laboratory exercise you measure the effect of dissolved carbon dioxide on algae growth.
Video instructions: https://vimeo.com/70628944

Introduction: The role of carbon dioxide or human activity in climate change is hotly debated, but almost everyone agrees that drastic reductions in fossil fuels use can be very disruptive to the economy. Consequently, some scientists concerned about carbon emissions are looking to photosynthesis as a means for reducing carbon in the atmosphere.

One proposed strategy of carbon capture consists of rerouting the fumes from power plants into vats containing concentrated cultures of microalgae. Thanks to an oil content that far exceeds that of plants, algae raised under these conditions can produce a form of biodiesel that does not burden agricultural lands.

A private company called "Green Fuel" recently set up a pilot project at the Redhawk power plant near Phoenix, Arizona (*1*), but for this to become a cost-effective biofuel, breakthroughs for reducing the energy costs of harvesting these microorganisms from the water are needed.

Literature Cited:

1. McKibben, B. 2007. *Carbon's New Math*. National Geographic. October 2007, 33-59; Retrieved on June 28, 2014 from http://ngm.nationalgeographic.com/2007/10/carbon-crisis/carbon-crisis-text

Procedure:

1) Prepare two large containers with 2-4 liters of nutrient-rich water. Set one of your containers aside. This is your control medium.
2) Fill to the brim a 250-mL flask with the water from the other container and invert the flask into one of the containers of nutrient-rich water so that the water does not pour out of the flask. Insert a hose into the inverted flask for carbon dioxide collection (Fig. 1). Place a rubber stopper in the water for the purpose of sealing the flask in the next step (Fig. 1).
3) Fill the inverted flask about a third of the way with carbon dioxide produced by an effervescent tablet dropped into the test tube. While the flask is underwater, remove the hose and insert the stopper to contain both the gas and the water (Fig. 2).
4) Shake the flask vigorously for 20 seconds to maximize CO_2 absorption (Fig. 3). This is your 100% CO_2-saturated media. Prepare another flask in the same way to make sure you have enough CO_2 saturated media.

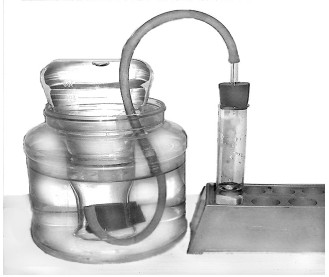

Fig. 1

Fig. 2

Fig. 3

5) Clean and prepare the four plastic bottles (remove all labels that block light). Fill two of the bottles halfway with 100% CO_2-saturated media (Fig. 4).

6) Fill <u>one</u> of the two bottles near the top with control media (from the beaker that was set aside in step 1) so that this bottle ends up with equal amounts of 100% CO_2-saturated media and control media. This results in 50% CO_2 saturation (Fig. 5).
7) Shake the bottle 5 seconds to obtain a uniform mixture (Fig. 6)

Fig. 4

Fig. 5

Fig. 6

8) Pour half of the contents of this bottle into <u>one</u> of the two empty plastic bottles as indicated (Fig. 7).
9) Fill this third bottle near the top with the control media (Fig. 5) so that this bottle ends up with equal amounts of 50% CO_2-saturated media and control media. This results in 25% CO_2 saturation.
10) Shake the bottle 5 seconds to obtain a uniform mixture (Fig. 6). Discard half of the contents of this bottle.
11) You should now have three bottles that are half-filled with the following levels of CO_2 saturation: 100%, 50%, and 25%. Fill the <u>last</u> empty bottle half-way with your control media.
12) Fill each bottle to about 3-5 cm from the top with algae culture or hypereutrophic pond water. *If your algae culture is very dense, use much less*. Label bottles and place them by a window (Fig. 8).
13) Shake each bottle twice daily to discourage settling of the algae (Fig. 6). Note the color every 2-3 days.
14) After 3 to 4 weeks, note the color and measure the turbidity using the kit supplied by the instructor and record the values in terms of JTU (Jackson turbidity units). Organize your data into a table (Table A) and graph it.

Fig. 7

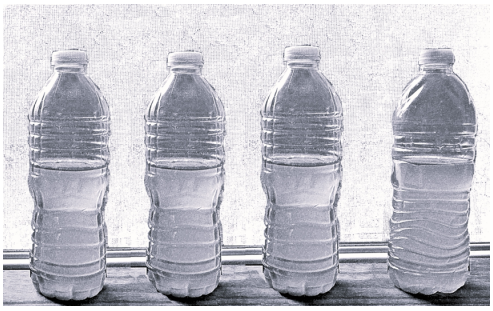

Fig. 8

Chlorophyll Extraction (optional): Your instructor may want you to measure relative chlorophyll content because it is much more accurate than measuring turbidity.

1) Shake each bottle and collect a sample from each bottle in a test tube designed to fit into a centrifuge. Make sure that all your samples have the same volume (at least 10 mL). Centrifuge these samples for at least 1 minute. After this, each test tube should have a green pellet on the bottom.

2) Using a pipette, carefully remove most of the water above each pellet, taking great care not to disturb the pellet. This water can be discarded.

3) In order to extract the chlorophyll from the pellet, add about 3 mL ethanol to each pellet and place each test tube in a beaker with scalding water for at least 5 minutes. Caution: Do not place the samples in boiling water because the ethanol may boil away!

4) Centrifuge each test tube again for at least one minute and remove a sample of the supernatant (alcohol-based chlorophyll extract) from each test tube, taking care not to disturb the pellet.

5) Measure the light absorbance of each sample extract with the spectrophotometer or colorimeter set to a wavelength of 430 nm. Organize your data into a table (Table B) and graph it.

Table A: Measuring Algae Density

CO_2 saturation	Turbidity
Atmospheric (control)	
25% CO_2 saturated	
50% CO_2 saturated	
100% CO_2 saturated	

Table B: Measuring Chlorophyll

CO_2 saturation	Absorbance
Atmospheric (control)	
25% CO_2 saturated	
50% CO_2 saturated	
100% CO_2 saturated	

Questions:

1. How many weeks did it take until you noted a color difference between the bottles?
2. Why did you shake the bottles after they were sealed and placed by the window?
3. Why did you add "plant food" to the water? What is "plant food."
4. Based on your data, what was your limiting nutrient, the plant food or the CO_2? How do you know?
5. Which bottle developed the highest algae concentration? Why did it happen this way?
6. Do you think that higher concentrations of carbon dioxide will always result in thicker algae blooms? Why or why not?
7. Some species of phytoplankton have an oil content that far exceeds that of plants. Why is this oil not being harvested on a commercial basis? *Hint: Re-read the introduction.*
8. "Green Fuel" is using carbon emissions from a power plant to grow algae. Under what circumstances is it impractical to capture carbon dioxide at its source?
9. Some climate scientists are concerned about excess CO_2 emissions. Is there such a thing as CO_2 "deficiency"? What would happen if CO_2 is completely eliminated from the atmosphere?

Assignment Checklist:

1. Did you answer all the questions?
2. Did you graph the data the Table(s) with CO_2 dose on the x-axis?