The Influence of Carbon Dioxide on Algae Growth

The first objective of this experiment is to show that increased atmospheric concentrations of carbon dioxide, CO_2, can stimulate algae growth. The second objective of this experiment is to demonstrate to middle school students that dissolved CO_2 can move from one liquid reservoir through the air to another liquid reservoir.

This experiment uses a liquid reservoir in a closed system that can be manipulated to change the amount of CO_2 in the air. The closed system incorporates a beaker of fresh water algae, *Closterium*, in growth media sitting inside a 1/2 gal container partially filled with a water reservoir (Figure 1A). Once the system is set up, *Closterium* algae is grown in the presence of light from a fluorescent light bulb on 12 hours light-dark cycle. Additionally, the system can be set up to collect oxygen gas released from the algae by adding a glass funnel and an inverted test tube filled with growth media (Figure 1B).

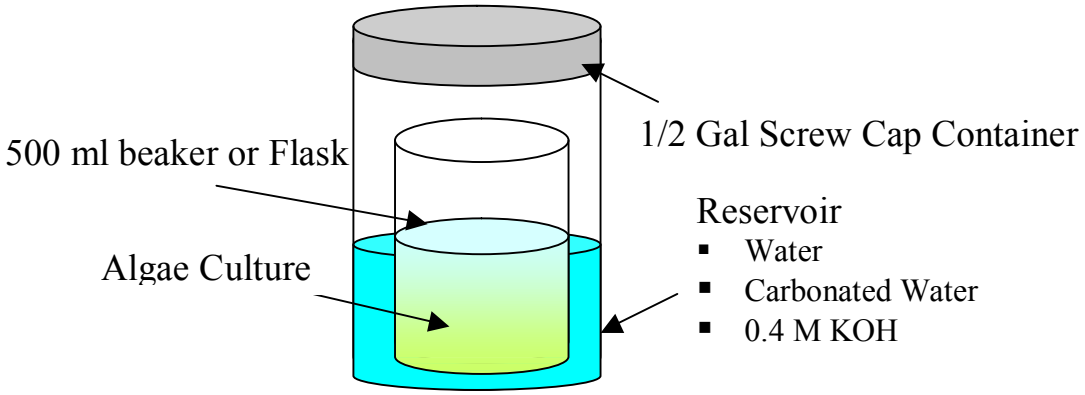

Figure 1A: Closed system for showing the affect of carbon dioxide on algae growth.

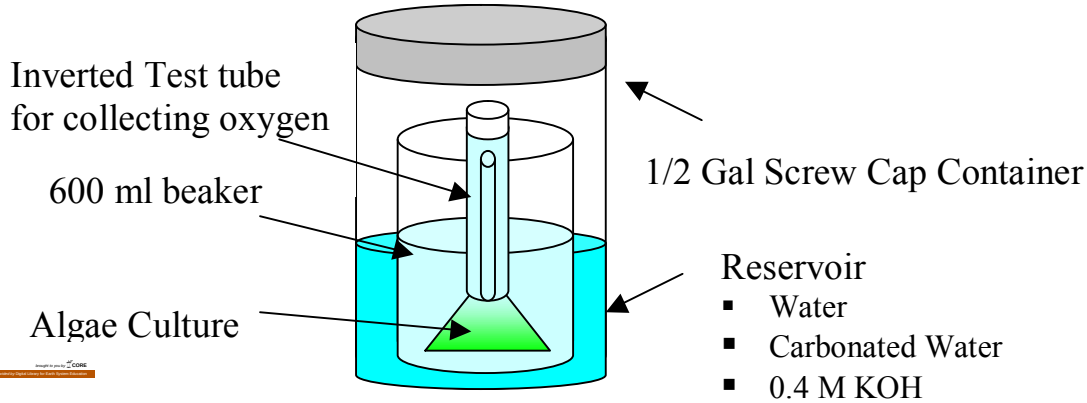

Figure 1B: Closed system for collecting release of oxygen gas by algae.

The experiment consists minimally of three groups; 1) the normal control with a reservoir of distilled water, 2) the experimental group with a reservoir of carbonated water, and 3) the negative control with a reservoir of 0.4 M potassium hydroxide. The normal control of distilled water represents the natural condition of dissolved gases in

water. The experimental group is a 1:2 mixture of bottled carbonated water to distilled water, which is enriched with CO_2 gas. The negative control with 0.4 M potassium hydroxide depletes the air of any CO_2 and shows that some CO_2 is required for algae growth. Carbon dioxide reacts with water to form carbonic acid.

$$CO_2\,(g) + H_2O\,(l) \rightarrow H_2CO_3\,(aq)$$

Potassium hydroxide reacts with carbon dioxide, CO_2, to form K_2CO_3 (s).

$$2\ KOH\,(aq) + CO_2\,(g) \rightarrow K_2CO_3\,(aq) + H_2O\,(l)$$

The presence of KOH effectively removes CO_2 from the atmosphere of the closed system. This activity requires an adult to set up the experiment, because of the glassware manipulations and the basic potassium hydroxide. In our classes, we had the students make hypotheses, observe the algae cultures, conduct parallel simulations, collect and analyze data.

Materials

- (6) ½ gallon Rubbermaid® plastic containers with lids
- (3) 600 ml glass beakers
- (3) 500 ml glass flasks
- (3) glass funnels
- (3) 12 milliliter glass test tubes
- (36) 15 milliliter glass test tubes
- (3) test tube racks
- 2.0 liter distilled water
- 0.5 liter of carbonated water
- 1.2 liter 0.4 Molar potassium hydroxide solution
- 1 tube 50X Alga-Gro® : (Carolina Biological Supply #HT-15-3751)
- 10-15-10 Plant Food
- Closterium Algae: (Carolina Biological Supply #HT-15-2115)
- Parafilm
- Plastic wrap
- Glass marking pen
- Fluorescent light
- Electric timer

Experimental Setup

A week prior to setting up the experiment, establish under artificial or natural light an actively growing *Closterium* algae culture in 100 ml of 1X Alga-Grow medium. The day before the experiment, obtain carbonated water, make 0.4 M potassium hydroxide (26.9 g of KOH in 1.2 L of distilled water), and clean glass beakers. Beakers for culturing the algae were washed with dish soap and then rinsed three times with distilled water to insure that contaminates would not inhibit algae growth.

Algae Growth System

The day of the experimental setup for algae growth, label each of three large plastic containers: normal control, experimental group, and negative control. Into the normal control container, transfer 600 ml of distilled water. Into the experimental group

container, transfer 200 ml of fresh carbonated water and 400 ml of distilled water. Into the negative control container, transfer 600 ml of O.4 M KOH solution. Set up the algae cultures by labeling each of three glass 600 ml beakers normal control, experimental group, and negative control. Fill each beaker with 400 ml of Algal-Gro medium. Add 1 drop of 10-15-10 plant fertilizer. Before transferring the algae, stir vigorously to suspend the algae and then transfer 30 ml of actively growing algae to each beaker. Carefully place the beaker inside the large plastic container, close the lid, and seal tightly with parafilm. Place the cultures next to a fluorescent light on a 12 hour on/off electric timer. Let the cultures grow for 7 to 10 days.

Oxygen Collection System

To collect oxygen gas, the set up is basically the same except after transferring the algae to the beaker wait 10 minutes for the algae to settle to the bottom. Slowly submerge an inverted glass funnel into each algae culture until the funnel rest on the bottom of the beaker. Make sure the glass stem is partially submerged beneath the growth medium. Next, fill a test tube completely to the top with growth medium, stretch the parafilm over the opening, and tightly wrap the parafilm around the side of the test tube. Invert the tube and there should be no air bubbles inside the tube, or leakage. Place the inverted tube with parafilm over the glass stem, quickly and carefully puncture the parafilm, and place the opening of the test tube beneath the surface of the medium. There should be a column of growth medium with a minimum amount of air. Mark the amount of air in the tube with a waterproof pen. Close the container and wrap with parafilm. Repeat for the other two groups and place in front of a light source. Also, let the cultures grow for 7 to 10 days.

Data Collection

Students observed the color intensity of the growing cultures and recorded their observations on data sheets. After about a week the students conducted two simulations of serial dilutions of food color and filtration. First, to prepare the students for limiting serial dilutions, students serially diluted out green food color by powers of 10. The students were given four 15 milliliter test tubes and instructed to transfer 9 milliliters of water into each test tube. The students were then given a test tube with 10 drops of green food color diluted in 10 milliliters of water. The students were instructed to remove 1 milliliter from the concentrated food color, transfer to the first of the four test tubes, and mix thoroughly. The students repeated this process by removing 1 milliliter from the test tube they just prepared and transferring it to the next test tube. This process was continued until the food color disappeared. The students were able to see with each 1:9 dilution, the color intensity of the dye decreased until it disappeared.

Limiting serial dilutions were done to determine the number of algae growing in each container. Limiting serial dilutions employs power of ten dilutions to determine the number of algae per milliliter in the culture. At about the same time the students are doing their serial dilutions, 12 test tubes are labeled and filled with 9 milliliters of Alga-Gro medium for each group. Remove one milliliter with a bulb pipette from the algae culture, dilute in the first test tube, and mix. Next, remove one milliliter from the test tube containing the algae that was just diluted and transfer to the second test tube. Repeat this process through the next 12 test tubes. When finished, lightly wrap the top of the test

tubes with plastic wrap to prevent dust and spores from entering the culture. Place the cultures in front of the light source for 7-10 days. The cultures can be observed every other day to see if the algae are growing in the cultures.

Next, the students filtered prepared cultures of algae to see how different amounts of growing algae will produce different color intensities on filter paper. Students were given circular filter paper and shown how to fold it into a cone. Groups were given 10 milliliters of undiluted algae culture, 10 milliliters 1:3 diluted algae culture, 10 milliliters 1:10 diluted algae culture, and 10 milliliters 1:100 diluted algae culture. The students then label their filter paper and filter the algae through their filter paper cones.

The amount of oxygen gas released by each culture was determined by removing the lid from the container. Before removing the inverted test tube, a marker for writing on glass is used to mark the change in gas volume on the test tube. The test tube is then removed from the inverted funnel, allowing any remaining growth medium to be poured out. The volume of the gas is represented by finding the volume of water that is added back into the test tube at the pre-marked lines.

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Results

The students observed that the experimental group with carbonated water grew better as the cultures were greener and produced more oxygen. The negative control of 0.4 M KOH had little detectable algae growth or oxygen gas production. The serial diluted cultures indicated that the experimental group had $1x10^9$ algae per milliliter of growth medium, compare to $1x10^7$ algae per milliliter for the normal control and $1x10^5$ algae per milliliter for the negative control. The amount of oxygen gas collected also correlated with algae growth with 1.6 milliliter for the experimental group, compared to 0.6 milliliter for the normal control and no oxygen for the negative control. Each group was filtered and the qualitatively supported the above findings.

Table 1: Summary of Experimental Results

	Normal	*Experimental*	*Negative*
Growing Algae Color Intensity	*Green*	*Dark Green*	*Light Green*
Algae Concentration (Cells/ml)	*1×10^7*	*1×10^9*	*1×10^5*
Oxygen Gas (ml)	*0.6*	*1.6*	*0*
Filter Algae Color Intensity	+++	+++++	+

Conclusion

This experimental system shows the positive influence carbon dioxide has on algae growth. It shows that carbon dioxide dissolved in water can diffuse out of water into the atmosphere of the closed system and into the growth medium of the algae culture. The increased amount of carbon dioxide had a positive growth affect on the closterium algae has evidenced by Table 1. It produced 100-times more algae per milliliter than did the normal control. In addition, the system shows the essential requirement for carbon dioxide for algae growth. The potassium hydroxide solution removes carbon dioxide from the air by forming $K_2CO_3(s)$, which inhibits algae photosynthesis. This system

allows students to see that CO_2 is essential for photosynthesis and has positive influence on algae growth.

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