Cornell Institute
for
Biology Teachers

Lab issue/rev. date: 6/26/95

Title: **Photosynthesis and Respiration in *Elodea***

Authors:

Dan Flerlage, Alternative Community School, Ithaca, New York, 14850 and Dick Ecklund, Cornell University, Ithaca, New York, 14853.

Revised by John Bartsch, Amsterdam High School, Amsterdam, NY 12010 and Glenn Simpson, Victor High School, Victor, NY, 14564.

Appropriate Level: Regents, Honors, and possibly AP-level high school courses. Also, with appropriate modifications explained in the introduction, appropriate for general level high school courses.

Abstract:

This lab involves the qualitative measurement of the changes in carbon dioxide concentration associated with both respiration and photosynthesis in the fresh water plant *Elodea*. Bromthymol blue is used as an indicator for the presence of CO_2 in solution. When CO_2 dissolves in water, carbonic acid is formed. A bromthymol blue solution, acidified to pH 6.0 by the addition of carbon dioxide produces a yellow color. The blue color is restored when the CO_2 is removed and the pH becomes higher than 7.6.

Students are responsible for the basic design of this investigation. Given a list of tasks, and the student background sheet entitled "Photosynthesis and Respiration in Elodea," they are asked to design an experiment which will allow them to demonstrate the use of CO_2 by a green plant in photosynthesis, and net production of CO_2 (by respiration) in the absence of photosynthetic activity.

Time Required: This lab requires two 45 minute periods, separated by 1 to 2 days.

Special Needs:

Living Environment: **1-Inquiry, analysis, design**: 1-Purpose of scientific inquiry: 1.1a,1.3a; 2-Research plan, hypothesis: 2.1, 2.3a-c, 2.4; 3-Analysis of results: 3.1, 3.3, 3.4all; **4-Content**: 1-Living things: 1.1a,c, 1.2a,f,g,i; 5-Dynamic Equilibrium: 5.1a,b,d-f, 5.2a, 5.3a,b; 6-Ecology: 6.1a,b

Photosynthesis and Respiration in *Elodea:*

Teacher Information

Objectives:

Through the investigation of the involvement of CO_2 in the processes of photosynthesis and respiration in an aquatic plant, this lab is designed to demonstrate the following:

1. CO_2 is consumed during photosynthesis
2. CO_2 is generated during respiration
3. Plants recycle some of their by-products
4. Indicators can be used to determine the presence or absence of specific molecules
5. Appropriate controls allow for comparison in scientific experiments

Level of Course:

This lab is appropriate for Regents high school biology classes. Honors-level and/or AP students may be challenged by this lab through the opportunity to design their own experiments. The lab can be simplified for other groups in the following ways:

1. Divide the class in half and have lab groups in each half address either "Purpose A," dealing with the use of CO_2 by *Elodea* during photosynthesis, or "Purpose B," demonstrating the production of CO_2 during plant respiration. This allows the students to focus on only one process -- either photosynthesis or respiration. A post-lab class discussion would include the sharing of information between the two groups.

2. Address "Purpose A" and "Purpose B" sequentially as two separate activities. A follow-up discussion would interrelate the two activities.

Time Requirement:

This lab requires two 45 minute periods, separated by 1 to 2 days. Do not begin it on a Friday.

Day 1: Discussion, practice use of indicator solutions, design labs and set up experiments.

Day 2: Observation, recording of results, analysis, and reports.

Student Background:

Student should be familiar with the following concepts before undertaking this lab:

1. Plants can carry on both photosynthesis and respiration.

2. Photosynthesis is dependent on the input of light energy.

3. Respiration is not dependent on light energy.

4. During photosynthesis, CO_2 is consumed by plants.

5. Respiration generates CO_2.

6. Good experimental design requires controls and experimental treatments that differ by only one variable at a time.

Advance Preparation:

1. Bromthymol Blue Stock Solution, prepared by adding 0.5 g of bromthymol blue to 500 ml of distilled H_2O. Next, add 9 drops of 1M sodium hydroxide (this will turn the solution a deep blue - you can prepare an approximately 1M NaOH solution by mixing 4g of NaOH with 100 ml of water. CAUTION: Sodium hydroxide is caustic and bromthymol blue is toxic if ingested.)

2. Bromthymol Blue Working Solution (to be used by students) prepared by diluting 100 ml of the concentrated stock solution with 400 ml of distilled H_20.

3. *Anacharis (Elodea)*: This fresh water plant is available year round at most tropical fish and pet stores. Obtain the *Elodea* a few days in advance of the lab and enhance growth with an artificial light source to ensure active sprigs. *Elodea* responds best when placed in non-chlorinated water. To do this let H_20 sit overnight in a container with a large surface area.

Materials:

- Clean test tubes, approx. 18 x 175 mm; about 8 per group
- Tape and marking pen or Wax Pencil
- Large Test tube rack
- Elodea plants
- Light source(s) (for illuminating selected plants, as required, during the experiment)
- Aluminum foil
- Corks (8 per group) or Parafilm (avoids contamination from reused corks)
- Clean straws
- Flasks: 250 ml (1 per group)
- 100 ml graduated cylinder
- Chemical safety goggles (for use when bubbling exhaled air into BTB solution)

Lab Format:

This lab requires two periods. The lab handout should be given to students at least a day before their first involvement. Assign them to read it in its entirety before class. Begin the first day with a discussion of the purpose of the lab. Clarify what the students are being asked to accomplish. Explain that they will make decisions regarding the design of the lab, and that this will affect the information that they will obtain. Students will become familiar with the materials for this lab while performing exercises described in "Getting Started." This preliminary familiarity with the materials will help students to design their own experiments. Be sure that the students understand how the color change associated with the bromthymol blue indicator can be useful to them. The pH range for the indicator is 7.6 (blue) to 6.0 (yellow), with green appearing around pH 7. Depending on the interests or abilities of the students, this can be dealt with on a number of levels. At its simplest, the bromthymol blue is **blue** when it is free of dissolved CO_2 gas, and is **yellow** when CO_2 is dissolved in the solution. The process actually causing the shift in color is a pH change (change in the H^+ concentration of the solution.) Carbon dioxide reacts with water in the following way:

$$CO_2 + H_2O \leftrightarrow H_2CO_3 \leftrightarrow H^+ + HCO_3^-$$

A plant in the dark respires but does not carry out all stages of photosynthesis. Because CO_2 is being generated by respiration but not consumed by photosynthesis, there is a net accumulation of CO_2. As the concentration of CO_2 increases in solution, the above reaction increases the H^+ ion concentration, thereby lowering the pH. A plant in the light can carry on all stages of photosynthesis. The CO_2 **used** in photosynthesis per unit time is much greater than the amount **generated** by respiration over the same time period (about 10-30 times greater.) Therefore in the presence of light, *Elodea* will take up CO_2, causing the concentration of CO_2 in its surroundings to drop. This causes a decrease in the H^+ ion concentration through the reversible reactions above and, therefore, the pH will increase. The following reaction takes place:

$$HCO_3^- + H^+ \rightarrow H_2CO_3 \rightarrow H_2O + CO_2$$

Two important pieces of information provided to the students under their "Getting Started" section are treated as givens and should be emphasized, since the students' design may well depend on them. The first is a "note" which informs them that when CO_2 is removed from solution, the solution turns back to its original blue color. The students are asked to prove this to themselves by blowing CO_2 into bromthymol blue in a 250 ml "test beaker" and setting it aside to be observed after a 24 hour period. The second is the assumption that bromthymol blue will not interfere with *Elodea's* normal photosynthetic and respiratory processes.

The students must have their design approved by you before proceeding to the actual set up of the lab. Allow 24 hours or longer between the first and second activities. The second day should be spent observing, recording, and analyzing results. Be sure to review the data table they are directed to prepare in the student instructions. Emphasize its use as an aid in keeping track of information.

Expected Results:

The teacher should not spell out a specific experimental design or set-up. Students should be encouraged to create their own protocol. Teachers might even allow students to conduct experiments which lack controls, but the problems resulting from this design error should certainly be part of the post-lab discussion.

A well designed investigation might utilize 8 test tubes, and be set up as depicted in the following chart. (Expected results are also shown.) Do not provide this chart to the students.

***Elodea* Experiment - Data Table**

Test tube	**A**	**B**	**C**	**D**	**E**	**F**	**G**	**H**
Light treatment	light	dark	light	dark	light	dark	light	dark
Test tube treatment	no plant	no plant	no plant, CO_2 added	no plant, CO_2 added	*Elodea*	*Elodea*	*Elodea* CO_2 added	*Elodea* CO_2 added
Color of bromthymol at start	blue	blue	yellow	yellow	blue	blue	yellow	yellow
Color of bromthymol after 24 hours	green to blue	green to blue	yellow	yellow	dark blue	green	green to dark blue	darker yellow

By comparing tubes "A" and "B," it can be seen that the presence or absence of light does not affect the bromthymol blue, although the air trapped inside the test tubes may cause a small shift toward a lower pH due to a small degree of acidification.

By comparing tubes "C" and "D," it can be seen that the presence or absence of light does not affect the bromthymol blue after it has been charged with CO_2.

By comparing tubes "E" and "F," it can be seen that the CO_2 concentration increases somewhat in the "dark" tube, while it decreases in the "light" tube. This indicates that the *Elodea* is behaving differently in the two tubes. In “E,” both photosynthesis and respiration are taking place, but the rate of photosynthesis is greater than that of respiration. In “F,” the light dependent reactions of photosynthesis cannot take place due to the lack of light. Therefore, the shift is due to the plant's respiratory processes only.

By comparing tubes “B” and “F,” it can be seen that while the bromthymol blue itself may have tended to acidify slightly, the tube with the *Elodea* (going through the respiratory process) showed a greater shift from blue to yellow. This indicates that the color changes observed in tube “F” is not due to a characteristic of bromthymol blue itself.

By comparing tubes “G” and “H,” it can be seen that the color change from yellow (with CO_2) to blue (no CO_2) is not only dependent on the presence of *Elodea*, but specifically on the *Elodea* being supplied with light.

By comparing tubes “G” and “C” it can be determine that the color shift is indeed due to some process going on within the *Elodea*, and is not a characteristic of the chemistry of the bromthymol blue itself.

Photosynthesis and Respiration in *Elodea*

CIBT
Student Laboratory Exercise

Background Concepts:

Plants can carry out both photosynthesis and respiration simultaneously. During photosynthesis, plants are using the energy of the sun to build molecules which effectively store this energy (glucose). Chemically, the photosynthetic reaction looks like this:

$$6CO_2 + 6H_2O \xrightarrow{light} C_6H_{12}O_6 + 6O_2$$

During respiration, plants are using this stored energy (glucose), to fuel their metabolic processes. Chemically, the respiratory process looks like this:

$$C_6H_{12}O_6 + 6O_2 \longrightarrow 6CO_2 + 6H_20 + \text{energy}$$

Remember that plants respire all the time.

Among other things, the converted energy from respiration is used to synthesize molecules, move materials around within the organism, grow (create new cells) and reproduce. Notice that in **photosynthesis**, CO_2 (carbon dioxide) is being used up as it is "fixed" into glucose molecules. During **respiration** the opposite is true. As the plant releases the energy stored in glucose by breaking it down, CO_2 is being given off into the surrounding water or atmosphere. The relationship between these two processes is special in that it allows plants to recycle some of their by-products. (While CO_2 is being given off during respiration, it can be re-utilized during photosynthesis.)

In this lab, you will try to demonstrate the net change in carbon dioxide when the common fresh water plant *Elodea* is placed under different conditions. You will be using a **chemical indicator**, **bromthymol blue**, as a means of determining the presence or absence of CO_2. This solution changes color when CO_2 is introduced. Bromthymol blue changes color due to a change in pH. When CO_2 is dissolved in water, it forms carbonic acid. This lowers the pH of the solution and causes the bromthymol blue to change its appearance.

Purpose:

Your lab group is asked to design, execute, and analyze an experiment that tries to accomplish the following two tasks:

A. Demonstrate that environmental CO_2 is used during photosynthesis in *Elodea*.

B. Demonstrate that there is a net production of CO_2 when *Elodea* respires in the absence of photosynthesis.

Supplies and Equipment Available:

- *Elodea* plants (vigorous stems, each with an end bud)
- Large clean test tubes
- Tape and marking pen or wax pencil
- Large Test tube racks
- Corks or Parafilm for sealing test tubes
- Safety goggles
- Aluminum foil
- Bromthymol blue working solution
- Straws
- Flasks: 250 ml (1/group)
- 100 ml graduated cylinder
- light source

Getting Started:

1. Your group should obtain a solution of bromthymol blue. This solution changes color when CO_2 is introduced. Bromthymol blue changes color due to a change in pH. When CO_2 is dissolved in water, it forms carbonic acid. This lowers the pH of the solution and causes the bromthymol blue to change its appearance.

   Remember that you are a living generator of CO_2. Put on your **safety goggles** for this part. Use a straw to gently exhale into a 250 ml flask containing 20 ml of bromthymol blue. Continue exhaling into bromthymol blue solution for about one minute.

   CAUTION: Be careful not to swallow any bromthymol or splash it in your face. It is toxic if swallowed.

Record your observations on your Lab Report Sheet. Do not discard this solution. You will need it in Step 3.

What happened when you exhaled into the bromthymol solution? Why?

2. When CO_2 is removed from bromthymol blue solution, the solution turns back to its original color (blue). To confirm this, label the 250 ml flask into which you have just exhaled CO_2. Set it aside for 24 hours with the top uncovered. The CO_2 in solution will eventually achieve equilibrium with atmospheric CO_2. The air around us contains relatively little CO_2. Therefore most of the CO_2 molecules bubbled into the solution should leave.

   Your group will use sprigs (pieces) of *Elodea*, a water plant. The bromthymol blue will not interfere with respiration or photosynthesis in *Elodea*. You have test tubes and corks at your disposal, as well as other materials, including aluminum foil (which is an excellent way to provide a plant with a totally dark environment). You may use as many test tubes as are needed for you to design a controlled experiment.

Procedure:

Day 1: Now that you are familiar with the behavior of bromthymol in relation to dissolved CO_2, you are ready to use it in designing and executing your lab. Go back and read the original "Purpose." The *Elodea* sprigs can be cut to fit into the test tube provided. This allows you to give the *Elodea* a variety of controlled conditions. The test tubes may be filled with solutions containing abundant CO_2 or very little. (Where can you get some CO_2 gas?) You may choose to include *Elodea* in some but not all test tubes. The aluminum foil can be used to control light. This allows you to set up different test tubes for comparison. **Remember to include Controls. You may need more than one test tube for your controls.** You may use corks or Parafilm to seal off each of your tubes. Discuss within your group what design might best accomplish the tasks described under "Purpose." Once your group settles on a design, fill out the Experimental Design sheet and **have your teacher approve it.** After your design is approved by your teacher, use the supplies available to set up your experiment.

NOTE:

To determine the amount of bromthymol solution should be used in each of your test tubes pour a filled test tube into the graduate cylinder. Fill a 500 ml flask with all of the Bromthymol Blue that you will need to turn Yellow. Then carefully blow CO_2 into the flask to make your yellow solution. This will ensure that an equal amount of CO_2 is in each test tube. As you set up your experiment, label the test tubes. On your Data Table Design Sheet design a data table to record the contents and appearance of each tube. Remember to include space to record any changes you may observe on Day 2. Your teacher must approve your Data Table Design. Start by recording the original appearance of the tubes. Use a label or tag to identify your test tube rack. Your test tube rack needs to be exposed to a light source wherever your teacher indicates.

Day 2: The test tubes of your experiment have now had approximately 24 hours to carry on respiration and perhaps photosynthesis. The original CO_2 concentration of the test tubes has been altered by these two processes. Today you will make observations of your test tubes, record changes, and interpret the data you have collected. Fill in the Data Table with your 24 hour observations and your explanations for the color changes.

Putting it all together:

(The questions below should be answered in complete sentences on the Lab Report Sheet or in a more formal Lab Report.) After completion of the lab, as

Your Lab Report must include the two tasks you were trying to accomplish.

1. To provide evidence for Tasks A and/or B you must compare 2 test tubes. To do this pick two test tubes and indicate how they help to provide evidence to support Task A. Are there other tubes that when compare to each other provide evidence to support Task A. Pick two test tubes and indicate how they help to provide evidence to support Task B. Are there other tubes that when compare to each other provide evidence to support Task B.

2. If your lab results and analysis did not allow you to prove both Task A and Task B, then you need to redesign a follow-up experiment which would provide the missing data. Explain and illustrate this follow-up experiment in your report, and explain how it could provide additional information.

Related Material/Extending the Concepts

(The questions below should be answered in complete sentences on the Lab Report Sheet or in a more formal Lab Report.) After completion of the lab, as part of your laboratory report, please answer the following questions:

1. Plants in the presence of light carry on both photosynthesis and respiration. Write out the equations for Photosynthesis and Respiration (given on the first page of this handout). From the results of your investigation, which process is occurring more in a plant that is being supplied with sunlight? What evidence have you used to come to this conclusion?

2. One of the implications of this investigation is that plants can recycle some of their "waste" products. CO_2 is clearly an example of a material that can be recycled by plants. What other gas might plants generate as a "waste" through one metabolic process, but re-use in a subsequent process?

3. Describe what was in each of your control test tubes?

4. Choose one control test tube and one experimental test tube. Use these two test tubes to discuss the role of a “control” in your scientific investigation.

Photosynthesis and Respiration in Elodea

Experimental Design Sheet

Restate the two tasks of your experiment.

Task A -

Task B -

Draw the design of your experiment. Include "Controls" in your design. Also include:

1. the test tube
2. whether there is a plant in the test tube or not
3. the starting color of the bromthymol blue solution
4. whether the plants will be exposed to light or not

Team Members:

____

____

____

____

After you have filled out your **Experimental Design Sheet,** hand it to your teacher for approval.

____

Approval Signature of Teacher

Photosynthesis and Respiration in Elodea

Data Table Design

Your Data Table must include space for each test tube showing:

- the presence or absence of light
- the presence or absence of *Elodea*
- the original color of the bromthymol blue solution
- a space for the color of the bromthymol blue solution after 24 hours
- an explanation of why the test tube changed color

After you have designed your Data Table, have your Teacher approve your design.

____

Approval Signature of Teacher

Photosynthesis and Respiration in Elodea

Lab Report Sheet

Getting Started Questions:

Restate the two tasks of your experiment.

Task A -

Task B -

1. What happened when you exhaled into the bromthymol blue solution? Why?

Putting it all Together Questions

1. Explain which tubes provide evidence for Task A?

Explain which tubes provide evidence for Task B?

2. If your lab results and analysis did not allow you to prove both Task A and Task B, then you need to redesign a follow-up experiment that will provide the missing data. Explain and illustrate this follow-up experiment in your report, and explain how it will provide additional information.

Related Material/Extending the Concepts

1. Plants in the presence of light carry on both photosynthesis and respiration. Write out the equations for Photosynthesis and Respiration (given on the first page of this handout). From the results of your investigation, which process is occurring more in a plant that is being supplied with sunlight? What evidence have you used to come to this conclusion?

2. One of the implications of this investigation is that plants can recycle some of their "waste" products. CO_2 is clearly an example of a material that can be recycled by plants. What other gas might plants generate as a "waste" through one metabolic process, but re-use in a subsequent process?

3. Describe what was in each of your control test tubes?

4. Choose one control test tube and one experiment test tube. Use these two test tubes to discuss the role of a "control" in scientific investigations.