Lab Photosynthesis

Laboratory objectives:

- Learn about photosynthesis.
- Design an experiment to test how light affects photosynthetic rates.
- Plot and interpret the data you obtain from your experiment.

Introduction:

Photosynthesis and respiration

Photosynthesis is the process by which light energy is used to produce oxygen, glucose, and water from water and carbon dioxide. The reactions of photosynthesis can be summarized as:

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

At the same time photosynthesis is occurring, respiration is also taking place. While photosynthesis is an *energy-acquiring* process, respiration is an *energy-releasing* process.

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

Using algae to study photosynthesis

Green algae (phylum Chlorophyta) are the closest living relatives to land plants. They range from single- to multi-celled organisms, and share many cellular and physiological features with the land plants. In today's lab, we will be using the single-celled alga *Scenedsmus quadricauda* to study photosynthesis (see Figure 1). We will be immobilizing these algae into beads using calcium alginate. This method allows us to control the amount of algae in each of our experimental vials. The alginate is porous, and allows for the exchanges of gases between the algae and their surrounding environment.

Figure 1. *Scenedesmus quadricauda* forms colonies of cells (four cells are shown in this image. The terminal cells have spiny projections. Each cell is 11- 18 μm long and 3.5 -7 μm wide.

We will be placing our algal beads in vials filled with a hydrogencarbonate indicator solution. The indicator will change color in response to changes in the pH of the solution (see Figure 2). CO_2 dissolved in water forms carbonic acid, which will lower pH. As dissolved CO_2 increases and pH falls, the color of the solution changes

from red to orange to yellow. Respiration will produce CO_2, while photosynthesis will take it up. Therefore, when the rate of respiration is greater than the rate of photosynthesis, the CO_2, concentration will increase, and pH will fall. Conversely, when the rate of photosynthesis is greater than the rate of respiration, the CO_2, concentration will decrease, and pH will increase. Under these conditions, the color of the indicator will change to a deep purple.

Using this method, we can study the effect of varying light levels or wavelengths on photosynthetic rates in the algae.

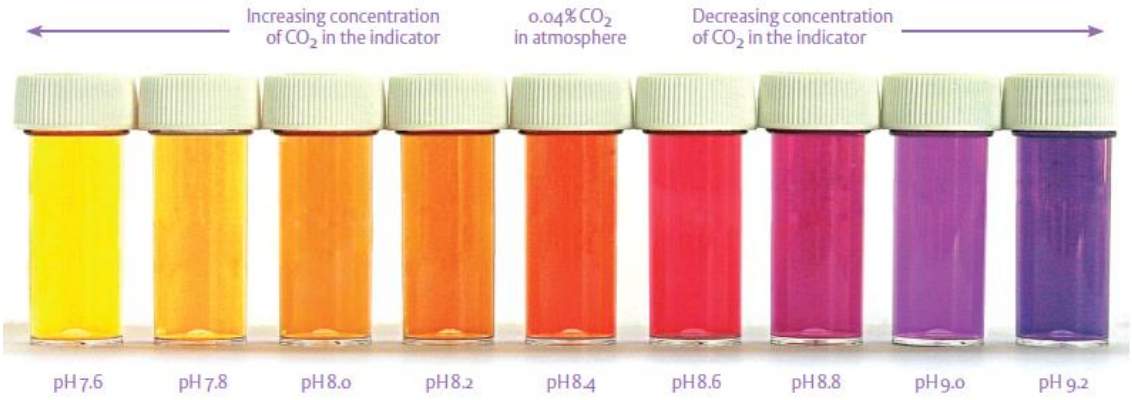
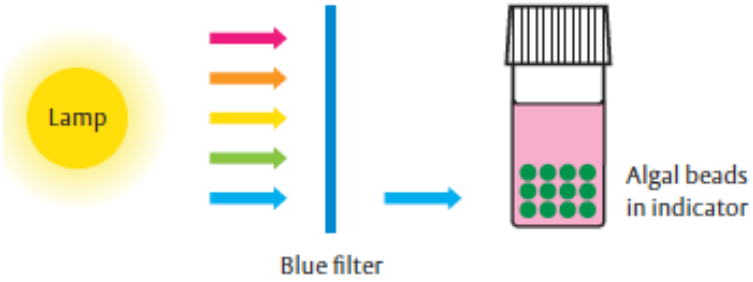


Figure 2. Color changes of hydrogencarbonate indicator solution in response to changes in CO_2 concentration. Image credit: Science and Plants for Schools, National Center for Biotechnology Education, University of Reading, UK.

Light and rates of photosynthesis

In today's lab, you will be designing an experiment to test the effects of light on photosynthesis. There are a number of possible ways these effects can be explored.

Transmittance. Colored filters can be used to control the wavelengths of light that the algae receive. In the example below, the filter is blue, permitting blue light to pass through, while other wavelengths are absorbed. Similarly, a red filter allows red light to pass.



Distance. The distance of the algae from the light source will affect the intensity of light that the algae receive. Using the inverse square law, you can calculate the relative light intensity each bottle receives based on its distance from the light source as:

$$Light\ intensity = \frac{1}{Distance^2}$$

So, for example, 2x the distance gives $\frac{1}{2^2} = \frac{1}{4}$ of the light intensity while, 3x the distance gives $\frac{1}{3^2} = \frac{1}{9}$ of the light intensity.

Neutral density. Neutral density filters reduce the amount of light passing through them across the entire visible spectrum. They can be used to reduce the amount of light that is transmitted into the algal vials. Experiments using neutral density filters and distance from the light source can be used to determine the light compensation point, which is the light level at which photosynthesis and respiration are in balance. Below this point, light levels are low, and the rate of respiration exceeds that of photosynthesis. Above this point, photosynthesis exceeds respiration, and the algae are able to accumulate sugars via photosynthesis.

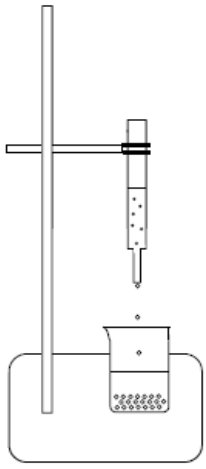
Protocol

1. Observe the algae

- Look at the algae under the microscope.
- Observe its structure, using Figure 1 for reference.

2. Immobilize the algae

- The first step is to create your algal beads.
- Pipette ~3 mL of the algal suspension into a small beaker. Make sure that you get only the most concentrated algae from the bottom of the beaker.
- Add 6 mL of 3% (*w/v*) sodium alginate solution. Screw the top on the vial and shake gently until the algae are evenly distributed.
- Fix a 10 mL syringe to a ring stand.
- Place a beaker containing 100 mL of 1.5% (*w/v*) calcium chloride solution under the syringe.
- Pour the algae/alginate solution into the syringe. Beads of algae should slowly drop into the calcium chloride.
- Leave the beads sit in the calcium chloride for 5-10 minutes so that they harden.
- Pour the beads into a tea strainer (it is OK to dump the calcium chloride solution in the sink). Rinse the beads with distilled water.



3. Design your experiment

- As a group, decide on your experimental setup to test the effects of light on the rate of photosynthesis of the algae. You need to list the following aspects of your experiment:
  - Design an experiment that tests the effects of light on rates of photosynthesis
  - Predict the outcomes
  - Identify the variables that will be manipulated

        - Identify the variables that will be kept constant
        - Identify the data that will be collected (this will be the pH that will be measured)
        - Identify how the data will be presented graphically
    - Ask your TA for help, and obtain approval before starting your experiment.

4. Prepare the vials

- Take the small vials that you will need for your experiment
- Rinse each vial with a small amount of hydrogencarbonate indicator.
- If your experiment involves using filters, cover the vials with the appropriate filters.
- Count 30 algal beads into each of the vials.
- Fill each vial ~2/3 full with hydrogencarbonate indicator. Replace the lids.

5. Photosynthesis

- Lay each vial down in front of the light source.
- If your experiment involves using different distances from the light, measure the distances. Make sure that none of your vials is shading any of the others.
- Wait 60 minutes for photosynthesis to take place.

6. Make measurements

- First, observe the color changes in the vials. As described earlier, the pH of the solutions will be low at low rates of photosynthesis (more CO_2 in the solution, so more carbonic acid forms), while pH values will be high when there are high rates of photosynthesis.
- Using the pH probes, measure the pH of each of your vials. Gently remove the probe from the protective covering. Insert it into the vial, and wait for the value to stabilize. Rinse with DI water before measuring the next vial, and before returning to the protective covering.

7. Plot and summarize your results

- Plot a graph showing the relationship between pH and your experimental variable.
- Summarize the results of your experiment.

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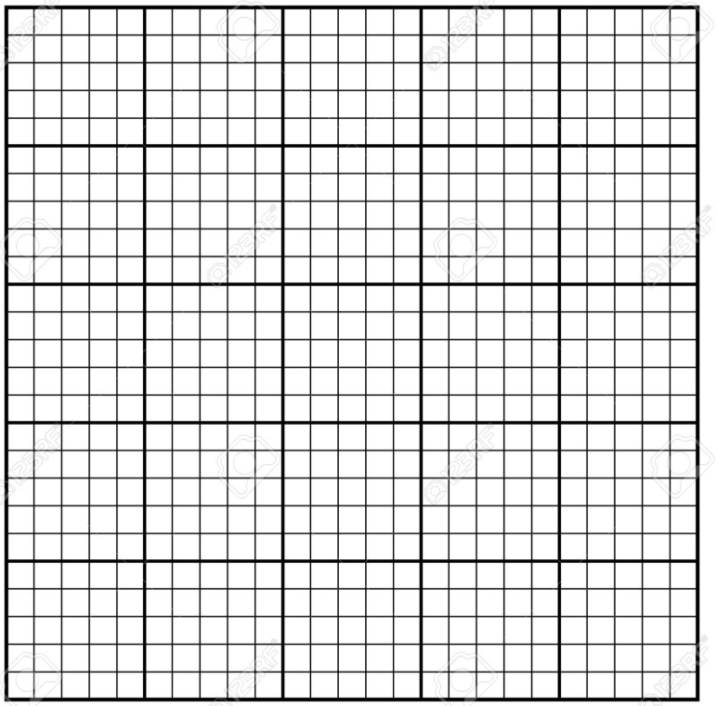
Adapted by Dr. Susan Lambrecht and Lars Rosengreen.

Name:

Questions

1. Plot your results either in the space below or in Excel. Be sure to label your axes.



2. Summarize your results. What did you observe? Were the results expected?