

INVESTIGATING PHOTOSYNTHESIS WITH ALGAE BEADS

Why does algae-rich water change pH in the presence of light?

Objectives

- Relate changes in water chemistry to the activity of algae.

Materials and Equipment

- Data collection system
- Wireless Spectrometer and Spectrometry software
- Cuvettes (2)
- Test tubes, 15 cm × 2 cm (2)
- Ring stand
- Test tube holder
- Beaker
- Graduated cylinder, 10-mL
- Disposable pipets (3)
- Distilled water, 8 mL
- Dilute bicarbonate indicator solution
- pH color chart for indicator
- Algae beads (20 or more, in a 15-mL culture tube)
- LED or CFL light sources (2)
- Timer
- Kimwipes®

Safety

Follow these important safety precautions in addition to your regular classroom procedures:

- Wear safety goggles at all times.

Procedure

1. Turn on the spectrometer and pair it to your device. Pairing is not necessary if your spectrometer is connected by USB.
2. Open the Spectrometry application.
3. Designate one clean pipet for each of the following solutions:

Distilled water

Spring water

Bicarbonate indicator solution

4. Use the distilled water pipet to fill one cuvette $\frac{3}{4}$ full of distilled water. This is the "blank" or calibration reference solution. Seal the cuvette.
5. Handle the sealed cuvette only by the ridged sides. Wipe the clear sides with a clean Kimwipe. Place the cuvette in the spectrometer and orient it to allow light to pass through the clear sides.
NOTE: Follow these instructions each time you insert a cuvette into the spectrometer.
6. Use the icons at the bottom-left of the screen to calibrate dark and calibrate reference.
7. Use the spring water pipet to transfer all the water from the algae bead culture tube to an empty test tube. Avoid contacting the beads with the pipet. Set the pipet and test tube in a beaker.

8. Pour the beads from the culture tube into an empty test tube. Set the test tube in the test tube holder. Attach the test tube holder to the ring stand as shown in Figure 1.
9. Measure 8 mL of bicarbonate indicator solution and pour it in the test tube with algae beads.
10. Use the bicarbonate indicator solution pipet to stir the solution, then transfer enough solution from the test tube to fill an empty cuvette $\frac{3}{4}$ full. Avoid picking up algae beads with the pipet. Cap and wipe the cuvette.
11. Place the cuvette in the spectrometer and start collecting data. When the absorbance reading stabilizes (after about 5 seconds), stop collecting data. Use the Scale to Fit button below the display to expand the curve.
12. Notice the peak yellow wavelength around 585 nm. Find the Coordinates box on the data display, which includes wavelength (λ) and absorbance (A). Select the "Enter Wavelength" area, type "585", and hit Enter. Click the blue check mark that appears next to the wavelength value.

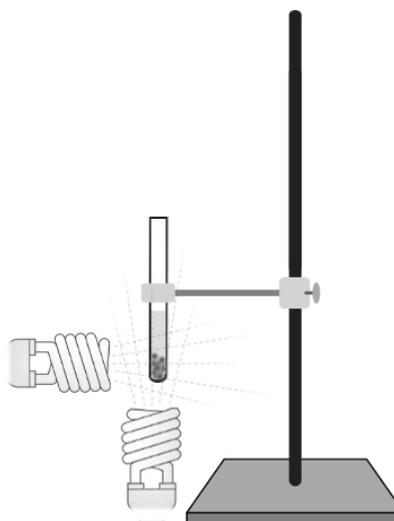


Figure 1: Light is focused on algae

Note: A vertical black line should appear on the display with the peak wavelength of 585 nm identified. Repeat steps 11-12 if the black line did not appear.

13. Record the absorbance (A) value shown in the Coordinates box for time = 0 in Table 1.
14. Remove the cuvette from the spectrometer and record your observations of solution color and pH in Table 1. Pour the indicator solution from the cuvette back into the test tube with algae beads.
15. Place both light sources as close as possible to the test tube to maximize the amount of light reaching the beads as shown in Figure 1. Turn both light sources on and start the timer. The lights must remain on and the timer must run continuously for the remaining steps.
16. After 5 minutes have passed, transfer enough solution to fill the cuvette $\frac{3}{4}$ full. Place the cuvette in the spectrometer and start collecting data. When the absorbance reading stabilizes, stop collecting data. Record absorbance, color, and pH in Table 1, then pour the solution back into the test tube with algae beads.
17. Toggle Comparison Mode to display all runs at once, then use the Scale to Fit button below the display to expand the curve.
18. Repeat steps 16-17 every 5 minutes for a total of 30 minutes.
19. After 30 minutes, turn off the light sources. Pipet the indicator solution into the graduated cylinder. Return the algae beads and spring water to the culture tube and seal it.

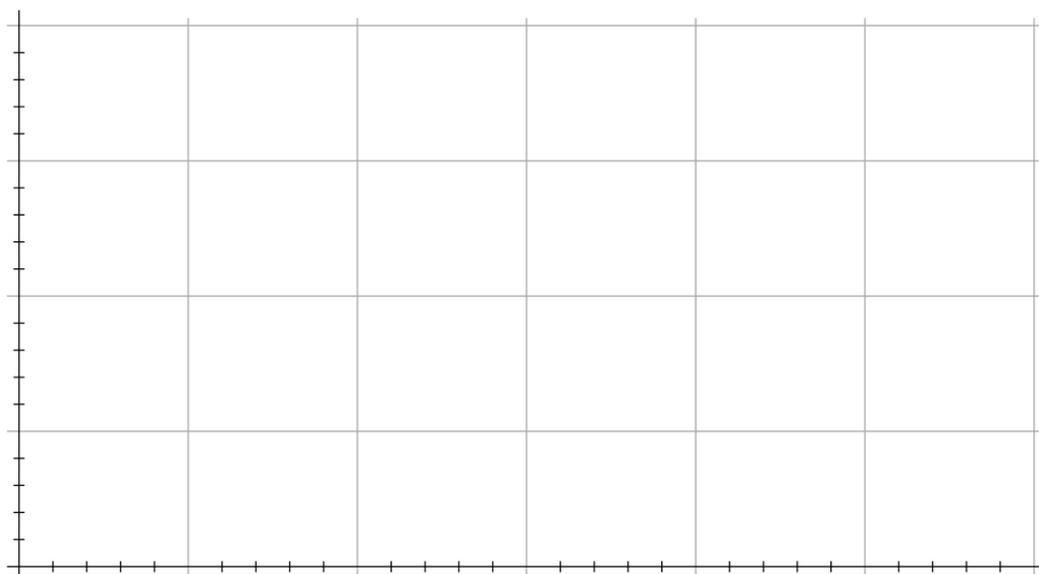
Data Collection

Table 1: Observations and absorbance at a wavelength of 585 nm over time

Time (min)	Absorbance	Solution Color	Approximate pH
0			
5			
10			
15			
20			
25			
30			

Plot a graph of *Absorbance* versus *Time* in Graph 1. Label both axes, include units, and use the correct number scale.

Graph 1: Change in indicator solution absorbance at 585 nm over time



Questions and Analysis

1. What happened to absorbance at 585 nm over time? What happened to the color of the indicator solution as the absorbance changed?

2. What happened to solution pH over time? Does this mean the solution became more acidic, or more basic?
3. The bicarbonate indicator changes color as pH changes. The pH change is due to changes in carbon dioxide concentration. Why was the carbon dioxide concentration changing in the solution; where was the carbon dioxide going?
4. Do algae change the pH of the water they live in? Support your answer with data from this investigation.
5. Explain how you would expect the absorbance, solution color, and solution pH results to change if you placed the test tube in complete darkness, taking absorbance measurements every 5 minutes over 30 minutes. Assume the absorbance will be measured at 585 nm and the solution color and pH will start with the same values you observed at time = 0 minutes for this investigation.
6. When the test tube is placed in the dark, what will happen to the carbon dioxide concentration? What causes the change in carbon dioxide concentration, and will this make the solution more acidic, or more basic?