[1]302. INVESTIGATIONS INTO PHOTOSYNTHESIS

Plants, algae and cyanobacteria carry out a form of photosynthesis that converts light energy into chemical energy (carbohydrates) and liberates gaseous oxygen.
Carbon dioxide, water, chlorophyll, and light are all needed for the photosynthetic reaction to take place (Equation 1). If any of these are absent, glucose and oxygen will not be formed. If any are scarce, the products may be formed slowly.

Equation 1.

$$6\ CO_2 + 6\ H_2O \xrightarrow[\text{chlorophyll}]{\text{light}} C_6H_{12}O_6 + 6\ O_2$$

In nature, CO_2 is present in air and dissolved in water. CO_2 and water enter the plant by diffusion. Light for the reaction is provided by the sun. Chlorophyll is synthesized within the phototroph in the presence of light.
The O_2 that is released diffuses into the atmosphere. The carbohydrate may be converted into other carbon structures to synthesize cell biomass, polymerized to form structural cellulose, polymerized and stored as starch, or oxidized through respiration resulting in the formation of ATP.
We can monitor biochemical reactions or pathways by measuring either the disappearance of substrates or the appearance of products. In photosynthesis, it is easier to detect the appearance of O_2 and carbohydrate than the disappearance of substrate. O_2 is not very soluble in water, so when photosynthesis occurs under water, bubbles of O_2 form. As one of the major end products of photosynthesis is carbohydrate stored as starch, starch accumulation also provides an indicator of photosynthetic activity. Starch is easily detected because it stains blue-black with iodine.

Text References

POH 168-179, Photophosphorylation

Study Questions

Define the terms: photosynthesis, photophosphorylation, electron transport system.
How does cyclic photophosphorylation differ from noncylic photophosphorylation? What is the role of chlorophyll a in cyclic photophosphorylation? What is the function of the electron transport system which includes Fd, pQ, Cyt and pC? Why does it require two photons of light? What happens to the electron?

Purpose

To demonstrate the dependence of photosynthesis on CO_2, light, and chlorophyll.

[1]Advance preparation required. One week before carrying out the lab, partially cover one or more leaves on a plant with foil.

The effect of CO_2 concentration on photosynthesis.

We can reduce the CO_2 concentration of water by heating it to drive off gases. The concentration of CO_2 in water can be increased by adding $NaHCO_3$, which reacts in water to form sodium hydroxide and carbon dioxide.

$$NaHCO_3 \rightleftharpoons Na^+ + OH^- + CO_2$$

Materials: 3 20-mm test tubes; 50 ml nonsterile 0.4% $NaHCO_3$ in tap water; three sprigs of *Elodea*; ring stand and test tube clamp; lamp; Bunsen burner; ice bath

Fill one tube with $NaHCO_3$ solution, the second with tap water and the third with tap water which has been boiled and cooled. *Predict on the worksheet how the rate of photosynthesis will compare in the three tubes.*

Cut three sprigs of *Elodea* 2 cm shorter than the tubes and each having a similar number of leaves. Keep the sprigs under water until you are ready to use them. Insert a sprig upside-down in one of the tubes, making sure that the ends of the sprigs are submersed. (If the sprigs will not stay submersed, try folding them into a "J".) Place the test tubes 20 cm from a lamp for 10 minutes. After this time, count the bubbles that come from the *Elodea* at 1 minute intervals for 10 minutes. (Bubble-counting may require more than one person.) *Record the results as bubbles produced per minute for each of the three plants.*

The effect of light intensity on photosynthesis.

Move the plants 40 cm from the lamp, allow them to equilibrate for 10 min, and again count the bubbles. *Record results as before.*

The dependence of photosynthesis on chlorophyll and light.

The interesting color patterns of variegated plants make them popular house and garden plants. Since areas of the leaves of variegated plants lack chlorophyll, we can use them to demonstrate the dependence of photosynthesis on chlorophyll. The dependence of photosynthesis on light can be demonstrated simply by covering part of a leaf with an opaque shield.

Materials: nonvariegated (green) and variegated plants; foil; paper clips; microwave oven; 1 50-ml beaker; 30 ml 95% ethanol; variegated leaves; 2 Petri dishes of D'Antoni's iodine; forceps (student-supplied)

A week in advance of this experiment, partially cover a leaf with foil by folding the foil over a leaf and fastening it with a paper clip.

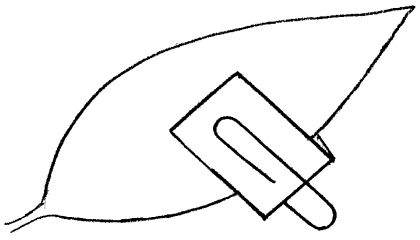

Figure 1. Foil-covered leaf.

Carefully sketch the leaf and the position of the foil. Also sketch the variegated leaf, recording the positions of the chlorophyll-containing areas.
Remove the foil from the covered leaf and place both leaves into a 50 ml beaker containing 95% ethanol. Microwave the leaves at high power for 30 seconds or until the ethanol turns green. Using forceps, carefully remove the leaves from the ethanol and place them in Petri dishes of iodine. Rinse the iodine from the leaves under slowly running water. Examine the leaves. If starch is present, the iodine will produce a blue-black color. *Sketch the leaves again, indicating the areas which test negative for starch. Compare these sketches with your previous ones.*

Cleanup

Save the Petri dishes of iodine for use by other groups. Discard the plant material. Wash the test tubes and beakers. Put away the ring stand and Bunsen burner.

Additional Experiments

Determine the optimum light intensity for growing *Elodea*. Determine the effects of other environmental parameters such as light wavelength on photosynthesis. Compare the apparatus for measuring oxygen production by *Elodea* shown in Figure 2 with the procedure you used.

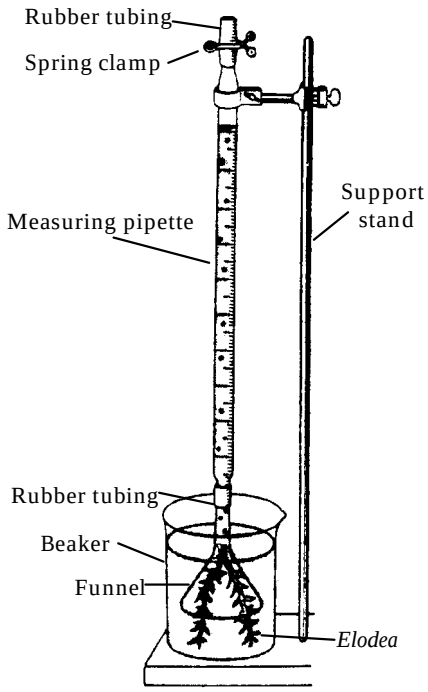

Figure 2. Apparatus for measuring photosynthetic rate of *Elodea.*

Recipes

D'Antoni's Iodine

Dissolve 1 g KI in 100 ml H_2O. Add 1.5 g iodine crystals and stir. Dilute to 1 liter with distilled H_2O.

302. INVESTIGATIONS INTO PHOTOSYNTHESIS (15 PTS)

Name . Lab day and time _____

PRELAB PREPARATION:

1. Procedural outline:

2. Predict how the rate of photosynthesis will differ in tubes containing boiled water, tap water and $NaHCO_3$ solution.

3. Predict the effect of light intensity on photosynthesis.

4. Set up tables in which to record your data.

RESULTS:

5. Data table for the effect of CO_2 concentration on photosynthesis:

6. Data table for the effect of light intensity on photosynthesis:

7. Carefully sketch the leaf and the position of the foil. Also sketch the variegated leaf, recording the positions of the chlorophyll-containing areas.

8. Sketch the leaves again indicating areas that tested positive and negative for starch.

9. How did the varying the CO_2 concentration affect the rate of O_2 production?

10. How did the varying the light intensity affect the rate of O_2 production?

11. Did the effects of CO_2 concentration and light on O_2 production match your predictions? Explain any discrepancies.

12. In which leaf areas was starch absent? Account for the lack of starch production in these areas.

13. Suggest possible sources of error in this experiment.