

# LAB: Photosynthesis in Leaf Disks

## HONORS BIOLOGY: UNIT 3

**Introduction:** Photosynthesis is a process in which plants convert light energy (sunlight) into usable chemical energy (carbohydrates). Photosynthesis involves two simultaneous processes: the **light dependent reactions** and the **light independent reactions** (Calvin Cycle). In the light dependent reactions, light energy is captured and converted to high energy ATP and NADPH molecules. In the light independent reactions these high-energy molecules are used to reduce CO<sub>2</sub> and eventually form carbohydrates such as glucose.



Overall reaction:  $6\text{CO}_2 + 6\text{H}_2\text{O} + \text{light energy} \rightarrow \text{C}_6\text{H}_{12}\text{O}_6 + 6\text{O}_2$

In this experiment, the spaces in the spongy mesophyll of leaf disks are filled with a sodium bicarbonate solution, which causes them to sink in the solution. The leaf disks are then exposed to light and observations are made as the cells undergo photosynthesis.

### Objectives:

By the end of this activity you should be able to:

- Describe the reactants and products of photosynthesis and the source of reactants from the environment.
- Explain the relationship of photosynthesis to the observations made during the experiment.
- Identify another variable that might affect photosynthesis and design an experiment that uses leaf disks to test your ideas.
- Create hypotheses about the effects of environmental variables on the rate of photosynthesis.

### Materials:

- 1.5 g sodium bicarbonate (baking soda)
- Liquid dish soap
- Eyedropper
- Plastic syringe (20-65 mL)—no needle!
- Plastic spoon or straw (for stirring)
- Leaf material
- Hole punch
- 1 large beaker or plastic cup
- 2 small beakers or plastic cups
- Timer or clock with second hand
- Light source
- Paper towels



**Procedure:** Solutions are safe to handle without gloves. Wear Goggles!

1. Using a one-hole punch, cut 20 leaf disks from young actively growing leaves.

2. Prepare a 0.2% solution of sodium bicarbonate ( $\text{NaHCO}_3$ ) and water in the large beaker or clear plastic cup, by adding approximately 1.5 g sodium bicarbonate to 300 mL of water. Stir until dissolved. Use an eyedropper to add about 2 drops of dish detergent to the solution and stir gently. There should be no bubbles afterward.

3. Remove the plunger from a large clean syringe (no needle). Place 20 leaf disks into the body of the syringe. Be sure the leaf disks are near the tip of the syringe as you re-insert the plunger so as not to damage the disks. **(A)**



4. Insert the tip of the syringe into a beaker of 0.2% sodium bicarbonate solution and draw 15-20 mL into the syringe. The leaf disks should be floating at this time. If your syringe is smaller than 60ml fill it about one third full.

5. Hold the syringe tip upward and expel the air by depressing the plunger carefully. Stop before solution comes out the tip.

6. Seal the tip of the syringe using the index finger of your left hand and hold tightly. Pull back on the plunger creating a partial vacuum within the syringe. If you have a good seal it should be hard to pull on the plunger and you should see bubbles coming from the edge of the leaf disks. Hold for a count of ten. **(B)**



7. Simultaneously, release your index finger and the plunger. Some of the leaf disks should start to sink. Tap the side of the tube or shake gently to break any bubbles on the edges of the disks.

8. Repeat steps 6 and 7 until all the disks sink. Do not overdo these steps!! You have been successful if the disks sink to the bottom. Don't repeat "just to be sure" as it is possible to damage the cells of the leaves.



9. Remove the plunger from the syringe and pour the solution containing the disks into 2 plastic cups or beakers and add the remainder of your solution equally to both beakers. There should be 10 disks per cup. Make sure they sink to the bottom.

10. Cover ONE of the beakers to block light from the leaf disks. Place the second beaker under a light source, approximately 6-8 inches below the light. The lights are best held in a clamp on a ring stand. Begin timing the experiment as soon as the light is turned on. Record your observations on page 2.

11. Notice what is happening to the leaf disks as photosynthesis proceeds. Continue to record your observations in the chart on page 2. After each time check, tap the side of the beaker to make sure disks are not "sticking" to the container walls. *Check the covered beaker quickly to avoid light exposure. When instructed, clean the lab equipment and dispose of solutions in the sink drains.*

**Data Table: Number of Leaf Disks Floating**

| <b>Time (Minutes)</b> | <b>Number of Disks Floating (Light)</b> | <b>Number of Disks Floating (Dark)</b> |
|-----------------------|---|--|
| <b>0</b>              |   |  |
| <b>1</b>              |   |  |
| <b>2</b>              |   |  |
| <b>3</b>              |   |  |
| <b>4</b>              |   |  |
| <b>5</b>              |   |  |
| <b>6</b>              |   |  |
| <b>7</b>              |   |  |
| <b>8</b>              |   |  |
| <b>9</b>              |   |  |
| <b>10</b>             |   |  |
| <b>11</b>             |   |  |
| <b>12</b>             |   |  |
| <b>13</b>             |   |  |
| <b>14</b>             |   |  |
| <b>15</b>             |   |  |

(After 15 minutes consider the experiment over and that no more disks will rise.)

**Lab Analysis and Questions:**

1. Use the graphing grid on page 5 and graph the results from the **light** and **dark** treatments.
2. What are the **dependent** and **independent variables** in the experiment?
  
3. What problem/question did you answer in this experiment?

4. Why was **detergent** added to the solution?

5. Why was **sodium bicarbonate** ( $\text{NaHCO}_3$ ) added to the solution?

6. Explain why it was important to keep one beaker covered during the experiment.

7. Describe and explain the relationship between the number of disks floating and time, as shown on the graph.

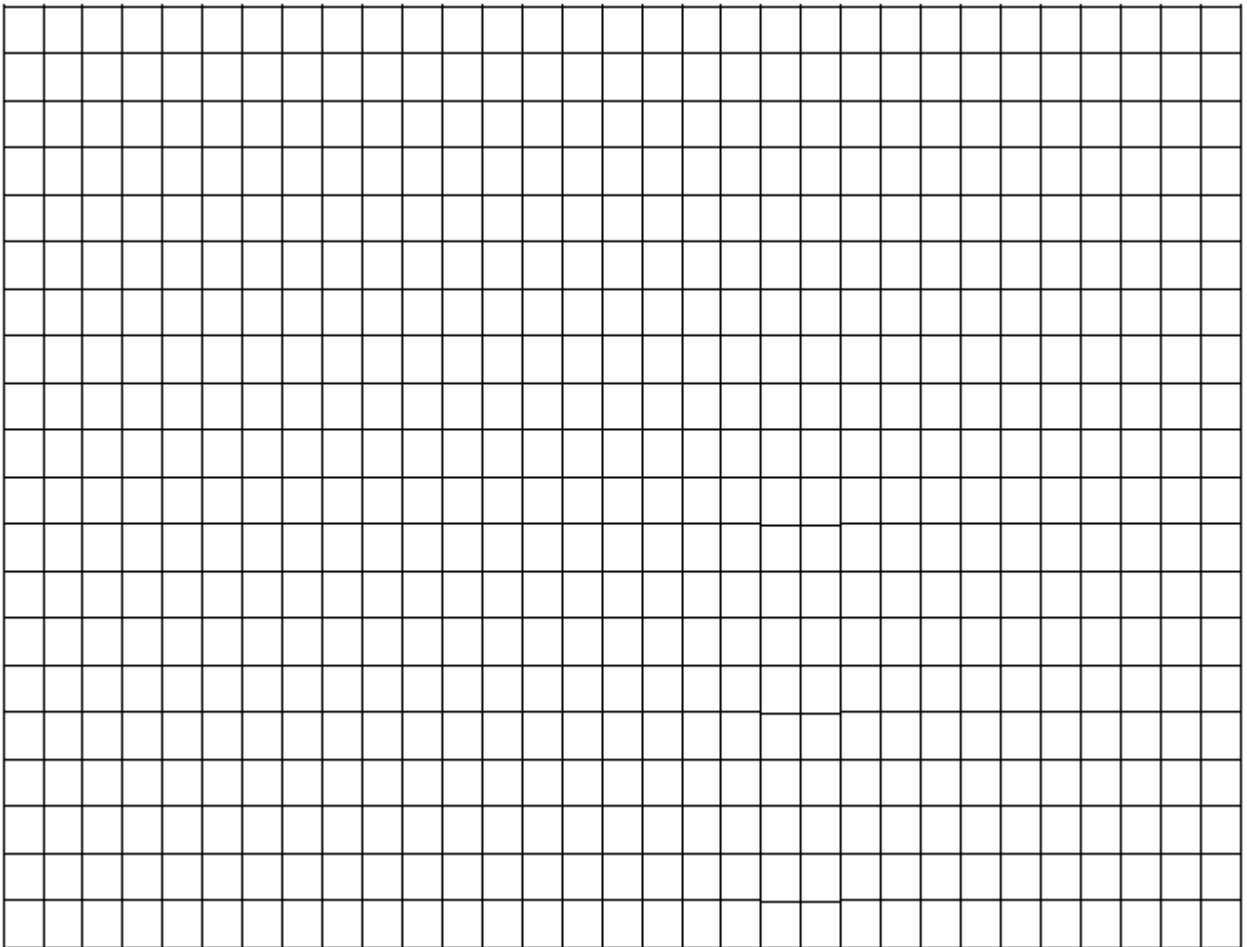
8. Did any leaf disks float in the dark treatment? If so, what may explain this result?

9. What process cannot occur in the dark treatment?

10. Explain the changes that occurred within the leaf tissue that allowed the leaf disks to rise to the surface.

### Question 1 –Graph

In the area below create a double line graph to display the results of the experiment. Provide a title, label the X and Y axis, and label each line appropriately or use a color key.



## Further exploration:

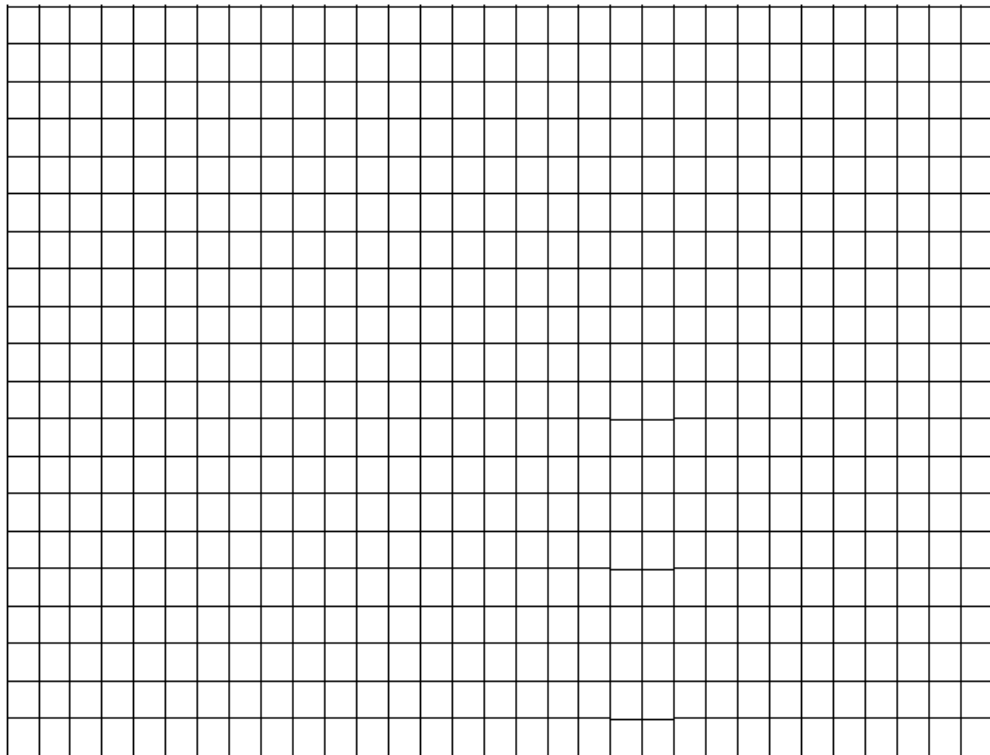
### Rate of photosynthesis and CO<sub>2</sub> concentration-

The data below shows how the rate of photosynthesis depends on CO<sub>2</sub> concentration. The rate of photosynthesis is measured here by the amount of CO<sub>2</sub> uptake, in units of milli-mol per square meter per second [mmol/(m<sup>2</sup>s)], where the unit mol is used in chemistry to measure the amount of a substance. Concentration of CO<sub>2</sub> is measured in parts per million (ppm).

| Ambient CO <sub>2</sub> concentration (ppm) | Rate of photosynthesis: CO <sub>2</sub> uptake [mmol/(m <sup>2</sup> s)] |
|---|--|
| 0   | 0.0  |
| 200   | 11.5   |
| 400   | 13.0   |
| 600   | 13.9   |
| 800   | 14.5   |
| 1000  | 15.0   |

**Graph the data (be sure to LABEL your graph/axes) and answer the analysis questions**

1. Identify the independent and dependent variable.
2. Describe how CO<sub>2</sub> concentration affects the rate of photosynthesis.
3. Give an explanation for the shape of the curve.



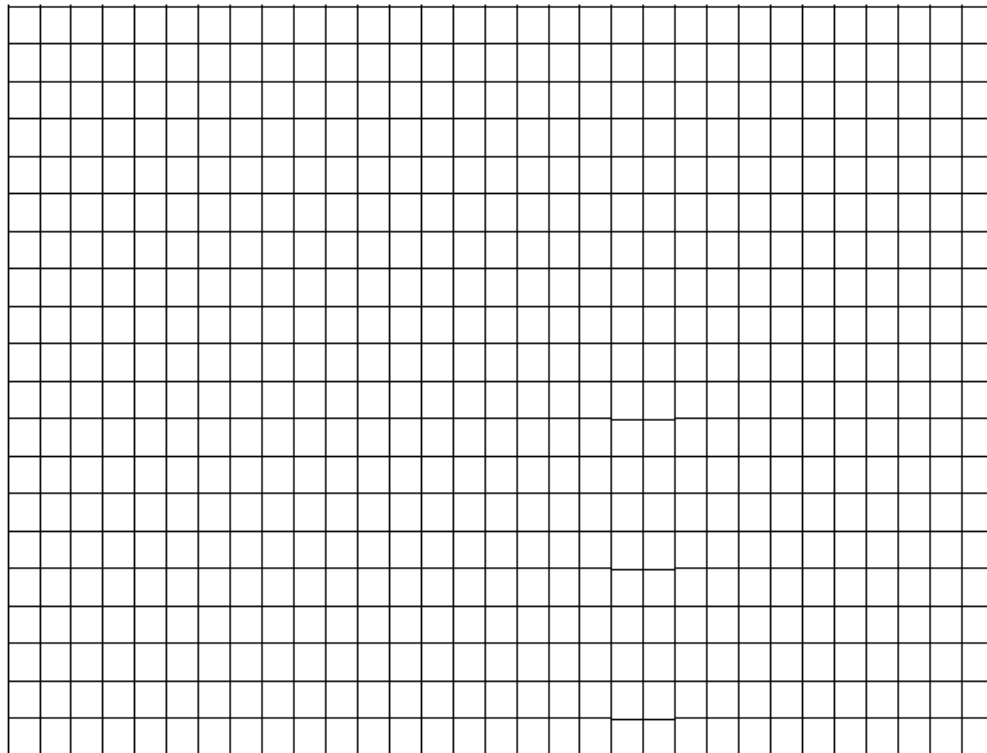
## Rate of Photosynthesis and light intensity-

Below is data showing the rate of photosynthesis as a function of the intensity of light on the plant (same units as above). Irradiance is measured as light intensity per unit of area of leaf (light intensity is measured in candelas, abbreviated as cd).

| Irradiance (cd/m <sup>2</sup> ) | Rate of CO <sub>2</sub> uptake [mmol/(m <sup>2</sup> s)] |
|---------------------------------|--|
| 0                               | -5.0   |
| 1                               | 0  |
| 2                               | 5.0  |
| 3                               | 10.0   |
| 4                               | 15.0   |
| 5                               | 17.0   |
| 6                               | 18.5   |
| 7                               | 19.2   |
| 8                               | 19.8   |
| 9                               | 20.0   |
| 10                              | 20.0   |
| 11                              | 20.0   |

**Graph the data (be sure to LABEL your graph/axes) and answer the analysis questions**

1. Identify the independent and dependent variable.
2. Describe the effect of light intensity on the rate of photosynthesis.
3. Label the light saturation point on the graph. What is the significance of this point?
4. Why do you think the net rate of CO<sub>2</sub> uptake has a negative value at zero irradiance?



## Temperature and Rate of Photosynthesis-

Below is data showing the rate of photosynthesis as a function of temperature.

| Temperature (°C) | Rate of CO <sub>2</sub> uptake [mmol/(m <sup>2</sup> s)] |
|------------------|--|
| 10               | 11.0   |
| 15               | 15.0   |
| 20               | 18.0   |
| 25               | 18.5   |
| 30               | 20.2   |
| 35               | 19.0   |
| 40               | 12.0   |
| 45               | 7.0  |
| 50               | 0.0  |

**Graph the data (be sure to LABEL your graph/axes) and answer the analysis questions**

1. Identify the independent and dependent variable.
2. Describe the dependence of the rate of photosynthesis on temperature.
3. Is there an optimum temperature that maximizes the photosynthesis rate? What is it?
4. Give an explanation for the shape of the curve. How can you explain the existence of an optimum temperature for photosynthesis?

