Laboratory 5: Cell Respiration

adapted from *AP Biology Lab Manual*

Overview

In this experiment, you will work with seeds that are living but **dormant.** A seed contains an embryo plant and a food supply surrounded by a seed coat. When the necessary conditions are met, germination occurs, and the rate of cellular respiration greatly increases. In this experiment you will measure oxygen consumption during germination. You will measure the change in gas volume in respirometers containing either germinating or nongerminating pea seeds. In addition, you will measure the rate of respiration of these peas at two different temperatures.

Introduction

Cellular respiration is the release of energy from organic compounds by metabolic chemical oxidation in the mitochondria within each cell. Cellular respiration involves a series of enzyme-mediated reactions. The equation below shows the complete oxidation of glucose. Oxygen is required for this energy-releasing process to occur.

$C_6H_{12}O_6 + 6O_2 \text{ -----> } 6\ CO_2 + 6\ H_2O$ + 686 kilocalories of energy / mole of glucose oxidized

By studying the equation above, you will notice there are three ways cellular respiration could be measured. One could measure the:

1. Consumption of O_2 (How many moles of oxygen are consumed in cellular respiration?)
2. Production of CO_2 (How many moles of carbon dioxide are produced by cellular respiration?)
3. Release of energy during cellular respiration.

In this experiment, the relative volume of O_2 consumed by germinating and nongerminating (dry) peas at two different temperatures will be measured.

Background Information

A number of physical laws relating to gases are important to the understanding of how the apparatus that you will use in this exercise works. The laws are summarized in the general gas law that states: **$PV = nRT$**

where

- P is the pressure of the gas,
- V is the volume of the gas,
- n is the number of molecules of gas
- R is the gas constant (its value is fixed)
- T is the temperature of the gas (in K^0)

This law implies the following important concepts about gases:

1. If temperature and pressure are kept constant, then the volume of the gas is directly proportional to the number of molecules of gas.
2. If the temperature and volume remain constant, then the pressure of the gas changes in direct proportion to the number of molecules of gas present.
3. If the number of gas molecules and the temperature remain constant, then the pressure is inversely proportional to the volume.
4. If the temperature changes and the number of gas molecules are kept constant, then either pressure or volume (or both) will change in direct proportion to the temperature.
5. It is also important to remember that gases and fluids flow from regions of high pressure to regions of low pressure.

In this experiment, the CO_2 produced during cellular respiration will be removed by potassium hydroxide (KOH) and will form solid potassium carbonate (K_2CO_3) according to the following reaction.

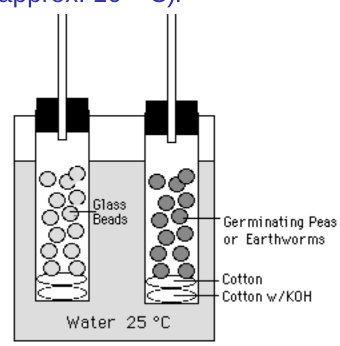
$$CO_2 + 2\ KOH \text{ ----> } K_2CO_3 + H_2O$$

Since the carbon dioxide is being removed, the change in the volume of gas in the respirometer will be directly related to the amount of oxygen consumed. In the experimental apparatus if water temperature and volume remain constant, the water will move toward the region of lower pressure. During respiration, oxygen will be consumed. Its volume will be reduced, because the carbon dioxide produced is being converted to a solid. The net result is a decrease in gas volume within the tube, and a related decrease in pressure in the tube. The vial with glass beads alone will permit detection of any changes in volume due to atmospheric pressure changes or temperature changes. The amount of oxygen consumed will be measured over a period of time. Six respirometers should be set up as follows:

Respirometer	**Temperature**	**Contents**
1	Room	Germinating seeds
2	Room	Dry Seeds
3	Room	Beads
4	10^{0}C	Germinating Seeds
5	10^{0}C	Dry Seeds
6	10^{0}C	Beads

Procedure

1. Prepare a room-temperature bath (approx. 25^{o} C) and a cold-water bath (approx. 10^{o} C).
2. Find the volume of 25 germinating peas by filling a 100mL graduated cylinder 50mL and measuring the displaced water.
3. Fill the graduated cylinder with 50mL water, 25 nongerminating peas, and add enough glass beads to attain an equal volume to the germinating peas.
4. Using the same procedure as in the previous two steps, find out how many glass beads are required to attain the same volume as the 25 germinating peas.
5. Repeat steps 2-4. These will go into the 10^{o} C bath.
6. To assemble 6 respirometers, obtain 6 vials, each with an attached stopper and pipette. Number the vials. Place a small wad of absorbent cotton in the bottom of each vial and, using a dropper, saturate the cotton with 15% KOH (potassium hydroxide). It is important that the same amount of KOH be used for each respirometer.



7. Place a small wad of dry, nonabsorbent cotton on top of the saturated cotton.
8. Place the first set of germinating peas, dry peas & beads, and glass beads in the first three vials, respectively. Place the next set of germinating peas, dry peas & beads, and glass beads in vials 4, 4, and 6, respectively. Insert the stopper with the calibrated pipette. Seal the set-up with silicone or petroleum jelly. Place a weighted collar on each end of the vial. Several washers around the pipette make good weights.
9. Make a sling of masking tape attached to each side of the water baths. This will hold the ends of the pipettes out of the water during an equilibration period of 7 minutes. Vials 1, 2, and 3 should be in the room temperature bath, and the other three should be in the 10 degree bath.
10. After 7 min, put all six set-ups entirely into the water. A little water should enter the pipettes and then stop. If the water continues to enter the pipette, check for leaks in the respirometer.
11. Allow the respirometers to equilibrate for 3 more minutes and then record the initial position of the water in each pipette to the nearest 0.01mL (time 0). Check the temperature in both baths and record. Record the water level in the six pipettes every 5 minutes for 20 minutes.

Table 5.1: Measurement of O_2 Consumption by Soaked and Dry Pea Seeds at Room Temperature (25^0C) and 10^0C Using Volumetric Methods. (Data generated previously)

		Beads Alone		Germinating Peas			Dry Peas and Beans		
Temp (^oC)	**Time (min)**	Reading at time X	Diff*	Reading at time X	Diff*	Corrected Diff. ^	Reading at time X	Diff*	Corrected diff ^
25^o	**Initial - 0**	0.93		0.91			0.92		
	0-5	0.91	0.02	0.84	0.07	0.05	0.89	0.03	0.01
	5- 10	0.90	0.03	0.77	0.14	0.11	0.87	0.05	0.02
	10 -15	0.90	0.03	0.71	0.20	0.17	0.87	0.05	0.02
	15-20	0.90	0.03	0.64	0.27	0.24	0.85	0.07	0.04
10^o	**Initial - 0**	0.95		0.92			0.91		
	0-5	0.94	0.01	0.88	0.04	0.03	0.90	0.01	0.00
	5- 10	0,92	0.03	0.85	0.07	0.04	0.87	0.04	0.01
	10 -15	0.93	0.02	0.83	0.09	0.07	0.86	0.05	0.03
	15-20	0.93	0.02	0.80	0.12	0.10	0.85	0.06	0.04

* difference = (initial reading at time 0) - (reading at time X)
^ corrected difference = (initial pea seed reading at time 0 - pea seed reading at time X) - (initial bead reading at time X).

Analysis of Results

1. Identify the hypotheses being tested in this activity.
2. This activity uses a number of controls. Identify at least three of the controls, and describe the purpose of each control.
3. **Graph the results from the corrected difference column for the germinating peas and dry peas at both room temperature and 10^0C. (There will be 4 lines on the graph).**
4. Describe and explain the relationship between the oxygen consumption and time.
5. From the slope of the four lines on the graph, determine the rate of oxygen consumption of germinating and dry peas during the experiments at 25^o C and 10^0C.
   Recall that:

$$\textbf{slope} = \textbf{rate} = \frac{?Y}{?X}$$

Table 5.2

Condition	Show Slope Calculations Here	Rate in $ml.O_2$ / min
GerminatingPeas/10^0C		
Germinating peas /25 ^oC		
Dry peas/10^0C		
Dry Peas /25 ^{o} C		

6. Why is it necessary to correct the readings from the peas with the readings from the beads?
7. Explain the effect of germination (versus nongerminating) on pea seed respiration.
8. What is the purpose of KOH in this experiment?
9. If you used the same experimental design to compare the rates of respiration of a 25 g reptile and a 25 g mammal, at 10^0C, what results would you expect? Explain your reasoning.
10. If respiration in a small mammal were studied at both room temperature (21^0C) and 10^0C, what results would you predict? Explain your reasoning.

DO NOT FORGET CONCLUSION AND REFLECTION