Big Idea 2

Cellular Processes: Energy and Communication

Baker's Dozen Lab 6: Cellular Respiration

Objectives

Before doing this lab you should understand respiration, dormancy, and germination.
After doing this lab you should be able to relate gas production to respiration rate.

Introduction

Aerobic 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.

$$C_6H_{12}O_6 + 6O_2 \rightarrow 6H_20 + 6CO_2 + 686 \text{ kilocalories of energy/mole of glucose}$$

By studying the equation above, you will notice there are three ways the rate of cellular respiration can be measured.

1) **Consumption of O_2:** How many moles of O_2 are consumed during cellular respiration?
2) **Production of CO_2:** How many moles of CO_2 are produced during cellular respiration?
3) **Release of energy during cellular respiration**

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

A number of physical laws relating to gases are important to the understanding of how the respirometers work. The general gas law states **PV=nRT. P** represents **pressure of gas**, **V** is the **volume of gas**, **n** is the **number of molecules of gas**, **R** is the **gas constant**, and **T** is **temperature in Kelvin**. This law implies the following concepts about gases:

1) If the temperature and pressure are kept constant, then the volume of 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 is kept constant, then either the pressure or volume (or both) will change in direct proportion to the temperature.

It is also important to remember that gases (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:

$$CO_2 + 2KOH \rightarrow K_2CO_3 + H_2O$$

Since the CO2 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 CO_2 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.

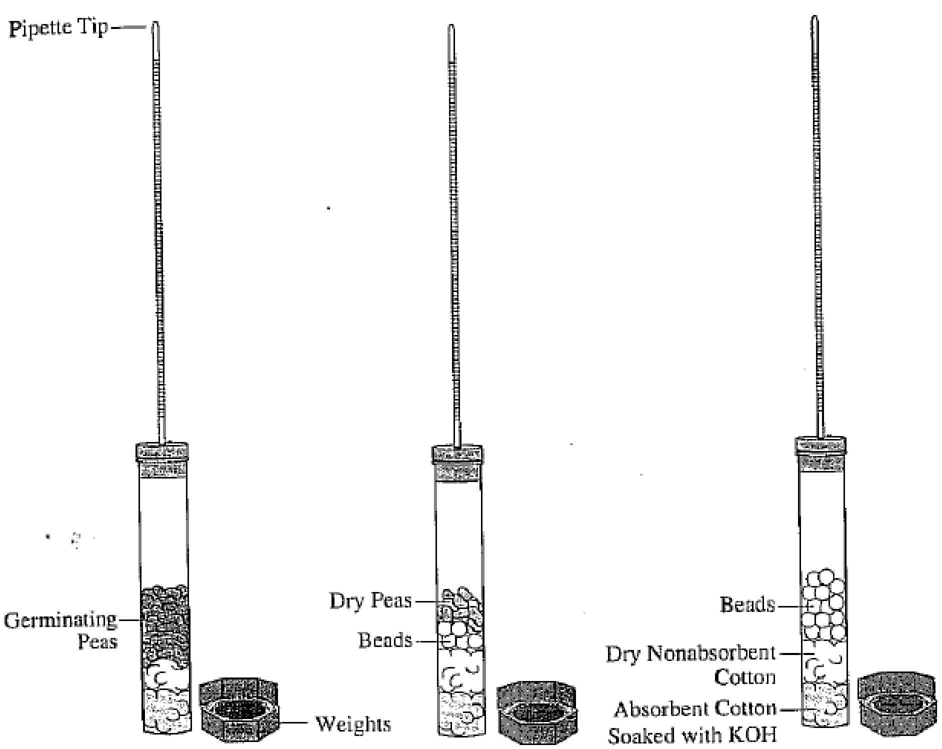
Materials

- Germinating peas
- Nongerminating peas
- Nonabsorbent cotton
- Potassium hydroxide
- vial
- beads
- weights
- pipette
- graduated pipette
- absorbent cotton
- timer
- graduated cylinder

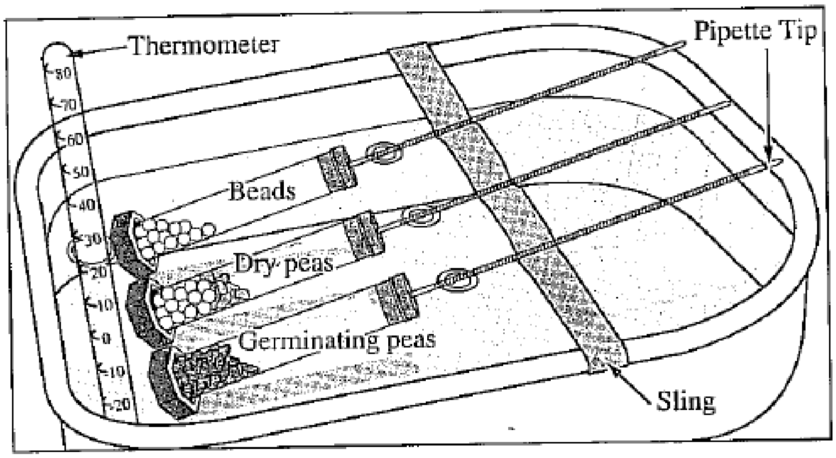
Procedure:

1. Place a small piece of absorbent cotton in the bottom of each of the three vials.
2. Using a pipette, moisten the cotton with 15% KOH solution. **Do NOT get KOH on the sides of the vial.**
3. Place a small piece of nonabsorbent cotton on top of the KOH-soaked cotton. **It is important that the amounts of cotton and KOH be the same in each vial.**
4. **Respirometer 1:** Fill a 100-mL graduated cylinder with 50 mL of water. Drop 25 germinating peas in the graduated cylinder and record the amount of water that was displaced below. This is equivalent to the volume of the peas. Remove the peas from the graduated cylinder and place them into one vial.

   Pea Volume = _______ mL
5. **Respirometer 2:** Repeat step 4 with 25 nongerminating (dry peas). Add enough glass beads to the graduated cylinder so that the volume of the peas and beads equals the volume of the germinating peas recorded above. Remove the peas and beads and place them into a second vial.
6. **Respirometer 3:** Repeat step 4 with only beads, making sure their volume equals the volume of the germinating peas recorded above. Remove the beads and place them into the third vial.
7. Stopper each vial and slide the pipette through the hole in the each stopper with the pointed end sticking out of the vial. Be sure to leave a gap between the end of the pipette and the peas/beads in each vial.



8. Slide the weights over the pipette and down to the stopper.
9. Each group will be responsible for one water bath, either room temperature or ice bath. If testing the ice bath, ice should be added during the experiment to ensure a temperature as close to 10°C as possible.
10. Make a sling of masking tape attached to each side of the water bath to hold the pipettes out of the water during an equilibration period of five minutes.



11. After the equilibration period, place one drop of food coloring in the pointed end of each pipette.
12. Immerse all three respirometers entirely in the water baths. Water will enter the pipettes for a short distance and stop. Some coloring may float out, but a small amount will aid in reading the volume of gas/water in pipette.
13. Arrange the pipettes so they can be read through the water and will not have to be disturbed until the end of the experiment.
14. Allow the respirometers to equilibrate for two more minutes and then record, the nearest 0.01 mL, the initial position of water in each pipette for time 0 in the data table below.
15. Check the temperature and record it in the data table below, as well.
16. Every 5 minutes for 20 minutes record the water position in each pipette.

DATA TABLE: Rate of respiration of germinating and nongerminating peas in room temperature and cold water.

Temp	Time (min)	Beads Alone		Germinating Peas			Dry Peas and Beads		
		Reading at time X	Diff	Reading at time X	Diff	Corrected diff	Reading at time X	Diff	Corrected diff
	0								
	5								
	10								
	15								
	20								
	0								
	5								
	10								
	15								
	20								

To calculate the effect of air pressure on the volume of gas inside each respirometer, use the following equations.

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 0 – bead reading at time X) OR

Corrected difference = (pea seed difference) – (bead reading difference)

Identify the TWO independent variables: ____

Identify the dependent variable: ____

Graph your data below.

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Analysis of Results:

1. Identify two possible hypotheses being tested in this experiment.

2. This experiment uses a number of controls. What conditions must remain constant? Why?

3. Describe and explain the relationship between the amount of O_2 consumed and time.

4. From the slope of the four lines on the graph, determine the rate of O2 consumption of germinating and nongerminating peas at room temperature and $10^{o}C$. Recall that rate = $\Delta y/\Delta x$.

Condition	Calculation of Slope	Rate (mL O_2/minute)
Germinating Peas at $10^{o}C$		
Germinating Peas at Room Temp		
Nongerminating Peas at $10^{o}C$		
Nongerminating Peas at Room Temp		

5. Why was it necessary to correct the readings from the peas with the readings from the beads?

6. Explain the effect of germination on pea seed respiration.

7. What is the purpose of the KOH?

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8. Explain why water moved into the respirometers' pipettes.

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9. If you were to compare the rates of respiration of a reptile and a mammal at 10°C, which animal's respiration rate would you expect to be higher? Explain your reasoning.

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10. If respiration in a small mammal were compared at both room temperature and 10°C, what results would you expect? Explain your reasoning.

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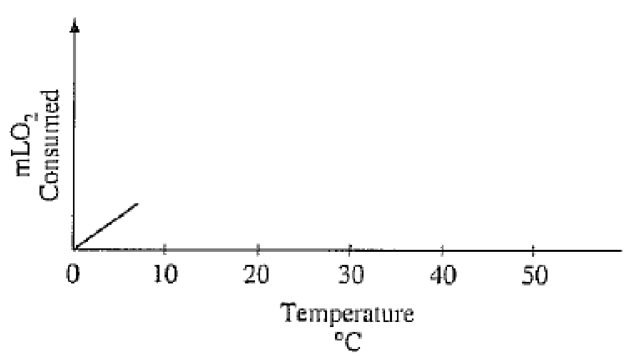
11. The graph below is a sample graph of possible data obtained for oxygen consumption by germinating peas up to about 8oC. Draw in your predicted results through 45oC. Explain your prediction.



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