Pre-lab Cell Respiration (# 5)

1. Read the overview. What is the difference between germinating and non-germinating seeds?

2. Why do seeds need oxygen? And, what would measuring the oxygen consumption of germinating and non-germinating seeds tell you?

3. What other ways besides measuring oxygen could be utilized to find out how germinating and non germinating seeds respire differently? Why is measuring the oxygen the best for this lab?

4. Explain the formula $PV= nRT$

5. Describe the role of KOH in this experiment. Why is KOH so important to the success of this experiment? What do you need to be careful of in the experimental set up when using KOH?

6. What is the purpose of using two different temperatures?

7. You will be using beads, dry peas and beads and germinating peas? What is the role of each of these in the experiment? (controls, variables)

8. How are you going to insure that you have the same volume of beads, and peas in your respirometers?

9. What are the dependent and independent variables in this experiment?

10. Read the pipettes in the 2 respirometers in the diagrams below. What is causing the water to move up the respirometer?

LAB FIVE CELL RESPIRATION

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 lab you will:

1. measure oxygen consumption during germination,
2. measure the change in gas volume in respirometers containing either germinating or nongerminating pea seeds, and
3. measure the rate of respiration of these peas at two different temperatures.

OBJECTIVES

Before doing this lab you should understand:

- respiration, dormancy, and germination;
- how a respirometer works in terms of the gas laws;
- the general processes of metabolism in living organisms; and
- how the rate of cellular respiration relates to the amount of activity in a cell.

After doing this lab you should be able to:

- calculate the rate of cell respiration from experimental data;
- relate gas production to respiration rate;
- test the rate of cellular respiration in germinating versus nongerminated seeds in a controlled experiment; and
- test the effect of temperature on the rate of cell respiration in germinating versus nongerminated seeds in a controlled experiment.

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.

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

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 O_2 are consumed in cellular respiration?)
2. **Production of CO_2** (How many moles of CO_2 are produced in 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), and

T is the **temperature** of the gas (in °K).

This law implies the following important concepts about gases:

1. If the temperature and pressure are kept constant, then the volume of the gas is directly proportional to the number of molecules of the 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 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:

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

Since the CO_2 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 shown in Figures 5.1 and 5.2, 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.

The amount of O_2 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 + Beads
3	Room	Beads
4	10°C	Germinating Seeds
5	10°C	Dry Seeds + Beads
6	10°C	Beads

Procedure

1. Both a room-temperature bath (by convention, 25°C) and a 10°C bath should be set up immediately to allow time for the temperature of each to adjust. Add ice to attain 10°C.

2. **Respirometer 1:** Obtain a 100-mL graduated cylinder and fill it with 50 mL of H_2O. Drop 25 germinating peas in the gradulated cylinder and determine the amount of water that was displaced (which is equivalent to the volume of the peas). Record the volume of the 25 germinating peas. Remove these peas and place them on a paper towel. They will be used in respirometer 1.

   Pea Volume ______________ mL

3. **Respirometer 2:** Refill the graduated cylinder with 50 mL of H_2O. Drop 25 dried peas (not germinating) into the graduated cylinder and then add enough glass beads to attain a volume equivalent to that of the expanded germinating peas. Remove these peas and beads and place them on a paper towel. They will be used in respirometer 2.

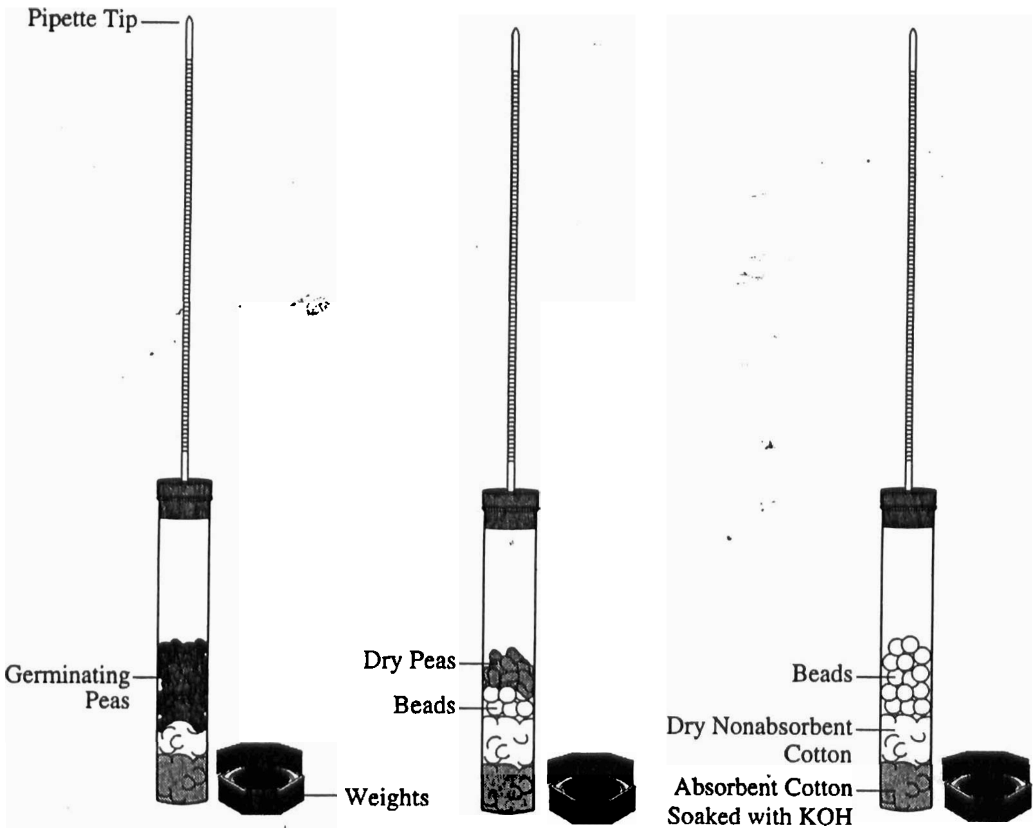
4. **Respirometer 3:** Refill the graduated cylinder with 50 mL of H_2O. Determine how many glass beads would be required to attain a volume equivalent to that of the germinating peas. Remove these beads and place them on a paper towel. They will be used in respirometer 3.

5. Repeat Steps 1–4 to prepare a second set of germinating peas, dry peas plus beads, and beads for use in respirometers 4, 5, and 6, respectively.

6. To assemble the six respirometers, obtain six vials, each with an attached stopper and pipette. Place a small piece of cotton in the bottom of each vial and, using a dropper, moisten the cotton with 15% KOH.* Make sure that the respirometer vials are dry on the inside. Do not get KOH on the sides of the respirometer. Place a small wad of nonabsorbent cotton on top of the KOH-soaked absorbent cotton (see Figure 5.1). *It is important that the amounts of cotton and KOH be the same for each respirometer.*

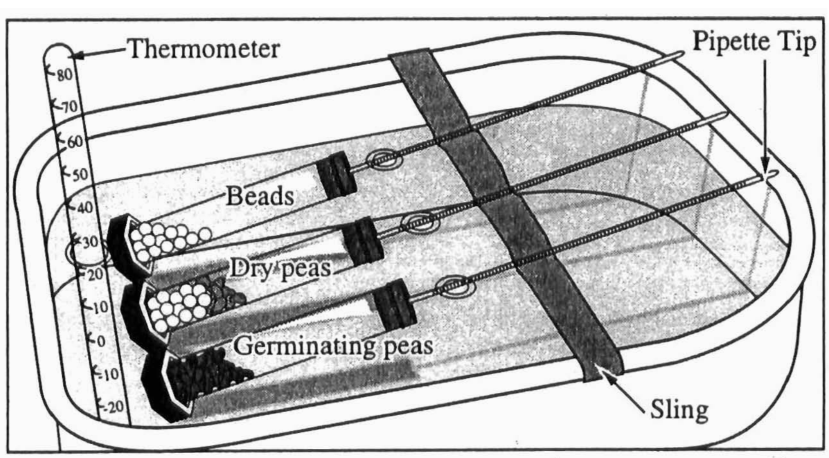
* Your teacher may ask you to use soda-lime pellets instead of KOH Solution.

Figure 5.1: Assembled Respirometers



7. Place the first set of germinating peas, dry peas plus beads, and beads in vials 1, 2, and 3, respectively. Place the second set of germinating peas, dry peas plus beads, and beads in vials 4, 5, and 6, respectively. Insert the stopper fitted with the calibrated pipette. Place a weighted collar on each end of the vial (see Figure 5.2).

Figure 5.2: Respirometers Equilibrating in the Water Bath



8. Make a sling of masking tape attached to each side of each of the water baths to hold the pipettes out of the water during an equilibration period of seven minutes. Vials 1, 2, and 3 should rest in the room-temperature water bath (approximately 25°C) and vials 4, 5, and 6 should rest in the 10°C water bath (see Figure 5.2).

9. After the equilibration period of seven minutes, immerse all six respirometers *entirely* in their water baths. Water will enter the pipettes for a short distance and then stop. If the water continues to move into a pipette, check for leaks in the respirometer. Work swiftly and arrange the pipettes so that they can be read through the water at the beginning of the experiment. They should not be shifted during the experiment. Hands should be kept out of the water bath after the experiment has started. Make sure that a constant temperature is maintained.

10. Allow the respirometers to equilibrate for three more minutes and then record, to the nearest 0.01 mL, the initial position of water in each pipette (time 0). Check the temperature in both baths and record it in Table 5.1. Every 5 minutes for 20 minutes, take readings of the water's position in each pipette and record the data in Table 5.1.

Table 5.1: Measurement of O_2 Consumption by Soaked and Dry Pea Seeds at Room Temperature (25°C) and 10°C Using Volumetric Methods

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

* Difference = (initial reading at time 0) – (reading at time *X*)

Δ 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*)

Analysis of Results

1. In this activity you are investigating both the effect of germination versus nongermination and warm temperature versus cold temperature on respiration rate. Identify two hypotheses being tested in this activity.

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

3. Graph the results from the corrected difference column for the germinating peas and the dry peas at both room temperature and at 10°C.

   For this graph you will need to determine the following:

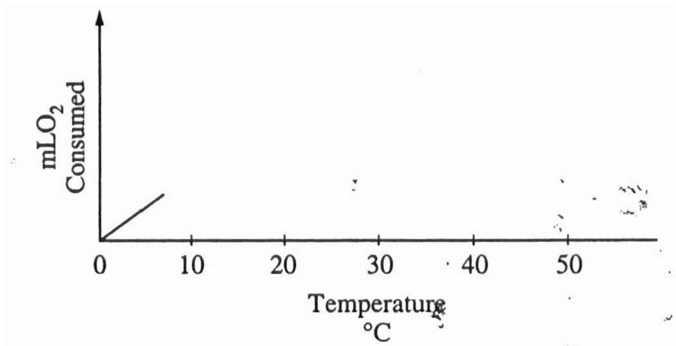
   a. The *independent* variable: ________________________________
      Use this to label the horizontal (x) axis.

   b. The *dependent* variable: ________________________________
      Use this to label the vertical (y) axis.

Graph 5.1 **Title:** ________________________________

8. Graph 5.2 is a sample graph of possible data obtained for oxygen consumption by germinating peas up to about 8°C. Draw in predicted results through 45°C. Explain your prediction.

Graph 5.2 **Title:** ______________________________



9. What is the purpose of KOH in this experiment?

10. Why did the vial have to be completely sealed around the stopper?

11. 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°C, what results would you expect? Explain your reasoning.

12. If respiration in a small mammal were studied at both room temperature (21°C) and 10°C, what results would you predict? Explain your reasoning.

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

14. Design an experiment to examine the rates of cellular respiration with peas that have been germinating for different lengths of time: 0, 24, 48, and 72 hours. What results would you expect? Why?

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

5. From the slope of the four lines on the graph, determine the rate of O_2 consumption of germinating and dry peas during the experiments at room temperature and at 10°C. Recall that rate = $\frac{\Delta y}{\Delta x}$. Record the rates in Table 5.2.

Table 5.2

Condition	Show Calculations Here	Rate (mL O_2/minute)
Germinating Peas/10°C		
Germinating Peas/ Room Temperature		
Dry Peas/10°C		
Dry Peas/Room Temperature		

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 nongermination) on pea seed respiration.