

Cell Respiration

PRE-LAB QUESTIONS:

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. What is the purpose of KOH in this experiment?
4. Explain the purpose of the vial with plastic beads.
5. In this experiment, what are we going to measure?

OVERVIEW

In this lab you will work with seeds that are living but **dormant** and seeds that have been stimulated to **germinate** because they have been soaked in water. A seed contains an embryo plant and a food supply surrounded by a seed coat. When the necessary conditions (moisture etc...) are met, germination occurs, and the rate of cellular respiration greatly increases. A dormant seed still has a plant embryo, but the embryo is not undergoing mitosis and is therefore **not** needing to produce large quantities of ATP. In this experiment you will measure oxygen consumption during germination. You will measure the change in gas volume in respirometers containing either germinating or non-germinating pea seeds. In addition, you will measure the rate of respiration of these peas at three 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.

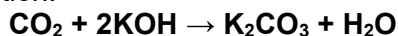


By studying the equation above you will notice there are three ways cellular respiration could be measured. One could measure the:

1. **Consumption of oxygen** --how many moles of oxygen are consumed in cellular respiration?
2. **Production of carbon dioxide**--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 oxygen consumed by germinating and non-germinating (dry) peas at two different temperatures will be measured.

In this experiment, the CO₂ produced during cellular respiration will be removed by KOH and will form solid K₂CO₃ according to the following reaction.



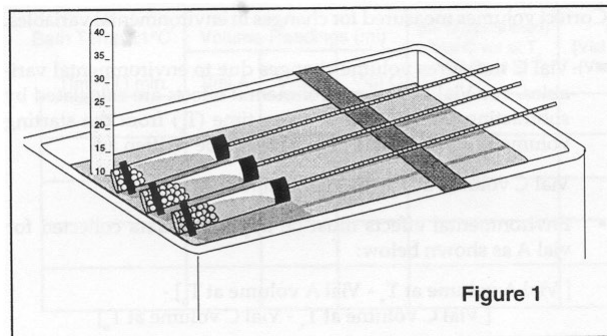
Since the carbon dioxide is being removed, the change in the volume of gas in the respirometer will be directly related to the amount of oxygen consumed. In the experimental apparatus if water temperature and volume remain constant, the water will move toward the region of lower pressure. During respiration, 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.

Each group will set up 3 respirometers. Each group will have a DIFFERENT temperature of water (warm, room, cold).

RESPIROMETER	TEMPERATURE	CONTENTS
1	Room	Germinating Seeds
2	Room	Nongerminating (Dry) seeds
3	Room	Beads
4	Cold	Germinating Seeds
5	Cold	Nongerminating (Dry) seeds
6	Cold	Beads
7	Hot	Germinating Seeds
8	Hot	Nongerminating (Dry) seeds
9	Hot	Beads

PROCEDURE:

1. Prepare a water bath at the assigned temperature: _____
2. Assemble 3 respirometers:
 - a. Label 3 vials A, B and C
 - b. Place circle of **absorbent cotton** (*approximately the size of a nickel*) into bottom of each vial.
 - c. Carefully soak with **1-2 ml of 15% KOH solution**--Do not allow KOH to touch sides of vials
 - d. Place circle of **non absorbent cotton** into vial directly on top of KOH/cotton circle. This will keep KOH solution from touching peas during experiment
3. Find the **volume of 25 germinating peas**.
 - Fill a 50 ml graduated cylinder to 25 ml with water
 - Place the peas in the cylinder and measure the increase in water volume _____
 - Place peas on paper towel
 - These are germinating peas to be used in respirometer A
4. Determine **volume of 25 non-germinating peas**
 - Fill 50 ml graduated cylinder with 25 ml water
 - add 25 non-germinating peas
 - Add **plastic beads** to raise volume to equal that obtained with germinating peas
 - Remove peas and beads. Place them on paper towel—dry them off!
 - Non-germinating peas and beads will be used in respirometer B
5. Determine the **volume of plastic beads**
 - fill the 50 ml graduated cylinder with 25 ml water
 - add plastic beads to raise volume so it equals volume of germinating peas
 - place plastic beads on paper towel
 - Plastic beads will be used in respirometer C
6. Fill each vial with the appropriate **seed/bead combination**. Insert Cork/pipet assembly into vial
7. Wrap parafilm tightly around seams (*cork and tube*) to seal any potential leak
8. Place piece of masking tape over water bath to suspend pipet tips out of water



9. Place each respirometer into water bath with calibrated side of pipet facing up so that measurements can be taken and tips out of the water.
10. Allow respirometers to equilibrate in water bath for **3 minutes** as shown in the diagram above.
11. Place one drop of food coloring in the tips of the respirometers so that you can see the movement of water into the respirometers.
12. Immediately submerge each respirometer (*put them all the way into the water*). Pipettes will take on some water. Make sure vial does not fill up with water. If it does, there is a leak which must be corrected. Make sure they stay level—or at least don't change how they are lying in the water bath during the experiment. Allow the respirometers to equilibrate for 2 more minutes.

13. Record starting point (*time 0*) volume of each pipet after the 2 minutes..
14. Take readings of volume of water in each pipet every 5 min. for 20 min.
15. Record these values in your data table.
16. Collect Vial A, B and C class data for the other temperatures. Record in data tables.
17. **Correct volumes measured for changes in environmental variables:**

$$\Delta V = V \text{ at Time } 0 - V \text{ at time of current reading}$$

$$\text{corrected } \Delta V = \Delta V \text{ (for Respirometer A or Respirometer B) - } \Delta V \text{ of Respirometer C}$$

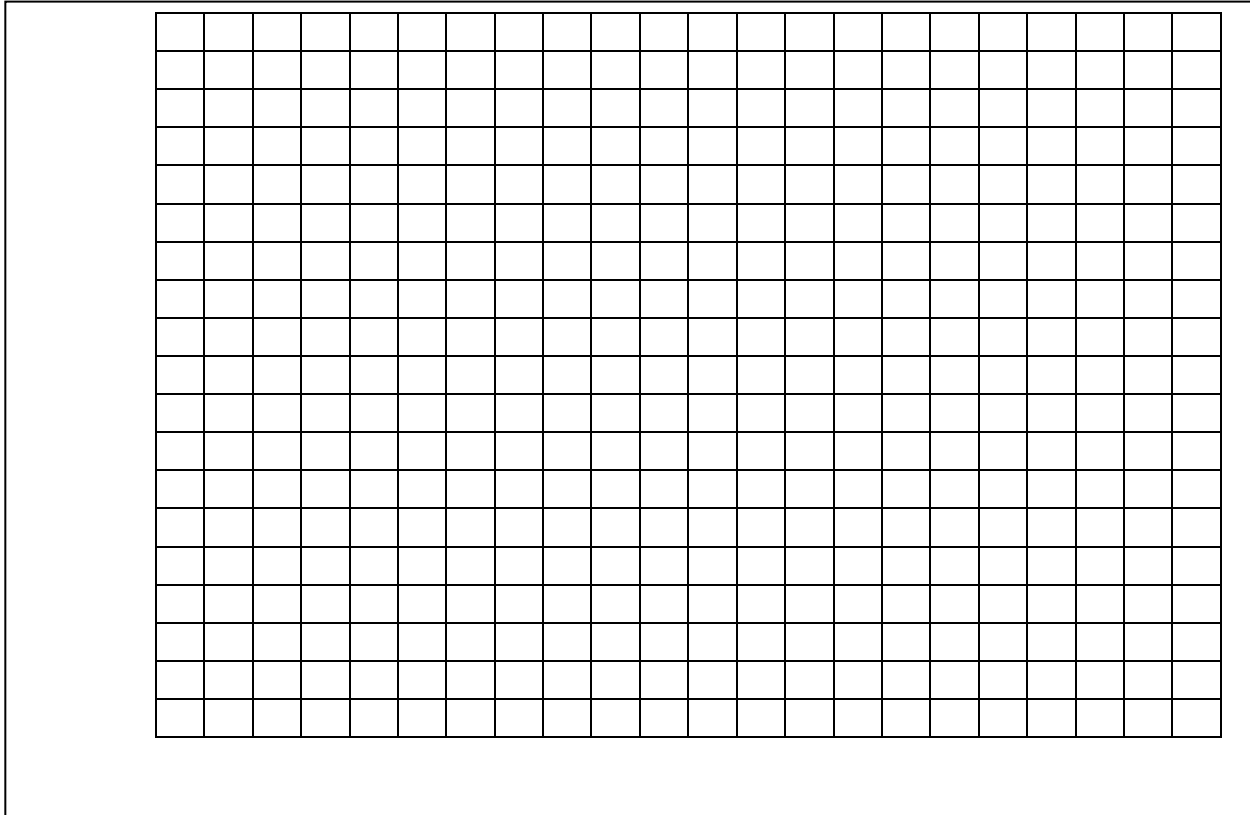
		Respirometer A Germinating Peas			Respirometer B Nongerminating Peas + Beads			Respirometer C Beads only	
°C	Time (min)	Volume of Pipet	*Δ Volume	^Corrected Δ Volume	Volume of Pipet	*Δ Volume	^Corrected Δ Volume	Volume of Pipet	*Δ Volume
	0								
	5								
	10								
	15								
	20								
		Respirometer A Germinating Peas			Respirometer B Nongerminating Peas + Beads			Respirometer C Beads only	
°C	Time (min)	Volume of Pipet	*Δ Volume	^Corrected Δ Volume	Volume of Pipet	*Δ Volume	^Corrected Δ Volume	Volume of Pipet	*Δ Volume
	0								
	5								
	10								
	15								
	20								
		Respirometer A Germinating Peas			Respirometer B Nongerminating Peas + Beads			Respirometer C Beads only	
°C	Time (min)	Volume of Pipet	*Δ Volume	^Corrected Δ Volume	Volume of Pipet	*Δ Volume	^Corrected Δ Volume	Volume of Pipet	*Δ Volume
	0								
	5								
	10								
	15								
	20								

*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)

DATA EVALUATION:

1. Graph the results from the corrected difference column for the germinating peas and dry peas at all three temperatures. (There will be 6 lines on the graph; make a key).



2. Describe and explain the relationship between oxygen consumption and time.
3. From the slope of the six lines on the graph, determine the rate of oxygen consumption of germinating and dry peas during the experiments at all temperatures.
Recall that:

$$\text{Slope} = \text{rate} \frac{?Y}{?X}$$

Show your work!!!!

Temperature	CALCULATIONS	RATE: ml O ₂ /minute
Germinating seeds at _____		
Germinating seeds at _____		
Germinating seeds at _____		
Non Germinating seeds at _____		
Non Germinating seeds at _____		
Non Germinating seeds at _____		

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