

AP Biology Lab - Cell Respiration

Big Idea 2: Biological systems utilize free energy and molecular building blocks to grow, to reproduce and to maintain dynamic homeostasis.

- Enduring understanding 2.A: Growth, reproduction and maintenance of the organization of living systems require free energy and matter.
- Enduring understanding 2.B: Growth, reproduction and dynamic homeostasis require that cells create and maintain internal environments that are different from their external environments.
- Enduring understanding 2.C: Organisms use feedback mechanisms to regulate growth and reproduction, and to maintain dynamic homeostasis.
- Enduring understanding 2.D: Growth and dynamic homeostasis of a biological system are influenced by changes in the system's environment.

Learning Objectives:

LO 2.1 The student is able to explain how biological systems use free energy based on empirical data that all organisms require constant energy input to maintain organization, to grow and to reproduce.

LO 2.2 The student is able to justify a scientific claim that free energy is required for living systems to maintain organization, to grow or to reproduce, but that multiple strategies exist in different living systems.

LO 2.24 The student is able to analyze data to identify possible patterns and relationships between a biotic or abiotic factor and a biological system (cells, organisms, populations, communities or ecosystems).

Science Practices employed by activity:

- Science Practice 2: The student can use mathematics appropriately
- Science Practice 5: The student can perform data analysis and evaluation of evidence
- Science Practice 6: The student can work with scientific explanations and theories

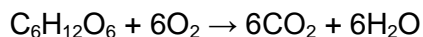
This investigation uses respirometry techniques to calculate the rate of oxygen consumption (cellular respiration) in germinating pea seeds. The effect of temperature and whether a seed has broken dormancy are quantified and graphed. The ideal gas law and its concepts are reviewed and applied.

Objectives

- Understand the relationships between temperature, pressure and volume.
- Study the effects of diffusion through a semipermeable membrane
- Quantify oxygen consumption rates in germinating peas under different conditions
- Predict the effect of temperature and germination state on the rate of cell respiration

Background

Each individual cell is responsible for the energy exchanges necessary to sustain its ordered structure. Cells accomplish this task by breaking down nutrient molecules to generate ATP (adenosine triphosphate), which can then be used to run cellular processes that require energy. This process is called cellular respiration which requires nutrient molecules and oxygen. Carbon dioxide and water are products of the series of reactions involved in cellular respiration.



There are several methods of indirectly measuring the rate of cellular respiration in organisms. One method involves monitoring changes in temperature; since the process of respiration is exergonic (produces heat). Another method is to measure either the oxygen consumption or the carbon dioxide production. Respirometers are devices that measure these types of gas volume changes, and therefore provide information about the rate of cellular respiration.

In order to be able to use a respirometer, you will need to use the ideal gas law, which describes the relationship between temperature, pressure and volume. ($PV = nRT$)

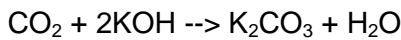
Where P is the pressure of the gas, V is the volume of the gas, n is the number of molecules of gas, R is the gas constant, and T is the temperature of the gas (in K°)

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.

During cellular respiration, two gases are changing in volume. Oxygen gas is being consumed by the respiring cells and carbon dioxide gas is diffusing out of the cells. The respirometer, therefore, has to be able to deal with two simultaneously changing gas volumes. This is accomplished by introducing potassium hydroxide into the device. KOH absorbs carbon dioxide, following this equation:



Potassium carbonate (K_2CO_3) is a solid precipitate. Any CO_2 produced is immediately converted from a gas to a solid and is therefore no longer governed by gas laws. 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.

Two sets of three respirometers will be assembled during this lab exercise. Each set will be incubated at a different temperature. One respirometer will contain germinating seeds, one will contain a mix of nongerminating seeds and glass beads, and a third will contain only glass beads.

The purpose of the beads is to ensure that each respirometer is uniform in volume. The vial with glass beads alone will permit detection of any changes in volume due to atmospheric pressure changes or temperature changes. The respirometers will also contain a layer of cotton that has been saturated with KOH so that carbon dioxide will be absorbed. The respirometers will be submerged in a pan of water; water will flow from an area of high pressure to an area of low pressure. As oxygen is used up by the respiring seeds, the gas pressure inside the respirometer will decrease and the water will flow into the pipet down its pressure gradient.

Lab Materials: 50 germinating pea seeds, 50 dry seeds, 100 plastic beads, 3 respirometer vials, Weights for vials, 3 stoppers, 1 ml graduated pipets, sealant (Vaseline), absorbent cotton, nonabsorbent cotton, 1 round wood stick, 3 pieces of paper towel, marking pen, water bath, ice, 100 ml graduated cylinder, thermometer, masking tape, stopwatch or clock, water. Dropper Bottle of 15% KOH



The amount of O_2 consumed will be measured over a time course. The experiment requires six respirometers to 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

Past Student Hints:

Hint 1: Check the respirometers for leaks before putting the absorbent cotton and KOH in them. (i.e.: before completing step 6) This will prevent you from redoing the experiment because of water leaks. Also, wrap parafilm around each stopper after assembling the respirometer. This will help prevent leaks.

Hint 1a: One way to check for leaks would be to submerge the respirometers completely (except for pipette tips) in room temperature water, and see if water enters the respirometers.

Hint 2: Make certain that the respirometers can be read easily when you place them in the water baths.

Hint 3: Keep checking the temperatures, and be sure to keep the temperatures stable. This should decrease variations in the $PV = nRT$ equation.

Safety – wear safety goggles. KOH is caustic, avoid direct skin contact. If contact occurs, flush affected area with running water for 10 minutes.

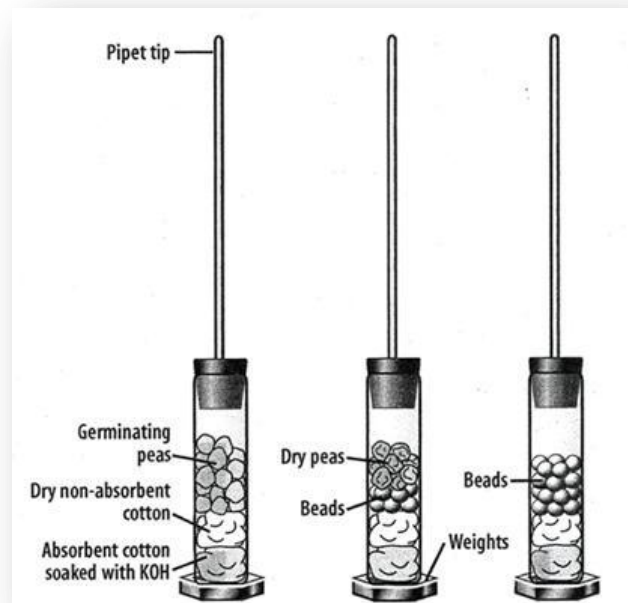
Procedure: Day 1

1. Both a room-temperature bath (approximately 25°C) and a 10°C bath should be set up immediately to allow for time to adjust the temperature of each. (Add ice to attain the 10°C bath.)
2. **Respirometer 1:** Obtain a 100-mL graduated cylinder and fill it with 50 mL of water. Place 25 germinating peas into the graduated cylinder. Measure the amount of water that was displaced (which is equivalent to the volume of the peas) and record this number.

$$\text{PEA VOLUME} = \text{_____ mL}$$

This number is the volume of the peas, and will be used to determine the volume of peas and beads in the other respirometers (all volumes should be equal). Remove the peas and place them on a paper towel. These peas will be used in Respirometer 1.

3. **Respirometer 2:** Refill the graduated cylinder until it has 50 mL of water. Place 25 dried peas (they are dormant, and not germinating) into the graduated cylinder and then add enough glass beads to reach a volume equivalent to that of the germinating peas in Respirometer One. Remove the peas and beads and place them on a paper towel. These peas and beads will be used in Respirometer 2.
4. **Respirometer 3:** Refill the graduated cylinder until it has 50 mL of water. Fill it with glass beads alone until the volume is equivalent to the volume of the germinating peas in Respirometer 1. Remove these beads and place them on a paper towel. These beads will be used in Respirometer 3.
5. Repeat the above procedures (2-4) to prepare a second set of germinating peas, dry peas + beads, and beads for use in Respirometers 4, 5, and 6, respectively.
6. Your group will be assigned to do one of the temperature sets.
7. To assemble the respirometers, obtain 3 vials, each with an attached stopper and pipette. Make sure that the vials are dry on the inside. **It is important that the amounts of cotton and KOH be the same for each respirometer.** Place a small wad of absorbent cotton in the bottom of each vial and, using a dropper, saturate the cotton with 15% KOH. Do not get KOH on the sides of the respirometer. Place a small wad of dry nonabsorbent cotton on top of the KOH-soaked absorbent cotton.
8. Place the first set of germinating peas, dry peas + beads, and beads in vials one, two, and three, respectively.
9. Place the second set of germinating peas, dry peas + beads, and beads in vials four, five, and six, respectively.
10. Insert the nontapered end of one pipet into the upper surface of one of the rubber stoppers. It should fit tightly. Place a layer of sealant around the junction between the pipet and the stopper so that no air can escape. (*The pointy end of the pipet should be outside, not inside)
11. Wrap parafilm tightly around each stopper (where it enters the vial and where the pipette is inserted). This is necessary to ensure against any leaks. Any leaks will cause the experiment to fail. Set your apparatus aside for day 2.



Day 2

1. Place a strip of masking tape across the narrow width of the water bath, approximately 2/3 of the way from one end (see diagram).
2. Place respirometers 1, 2, and 3 into the room temperature water bath and respirometers 4, 5, and 6 into the cold water so that the pipets rest on the masking tape prop. Begin time for a total of 7 minutes – this is the equilibration period, where your respirometers will become the same temperature as the water.
Use a thermometer to determine the water temperature of each bath and record the measurement.
3. At the end of 7 minutes, submerge each of the tubes entirely in the water bath. Some water will enter the tip of the pipet, but the influx of water should stop fairly quickly. If it does not stop, check the respirometer for leaks.
4. At this point, check to make sure you can read the pipets. The air bubble should extend from the main chamber up the tube of the pipet. The pipet may need to be rotated so that you can see the numbers.
If your respirometers float, you may need to weight them. Some come with weights inside and some do not. You can improvise here, stainless steel dissection scissors; for instance, can serve to weight the tubes.
5. Record the water level in each pipet onto the data table at the Time Interval 0.
6. Record the position of the water in each pipet at the end of 5, 10, and 15, 20 min on Data Table 2.

Possible Extension: Remove your peas from the chamber and replace with a living organism. You could use a cricket or a mealworm for instance. Determine the respiration rate of your living organism in both cold and warm water. *You may be assigned to a particular temperature*

DATA & ANALYSIS

Table1: Calculation of Volume in Respirometers

	Respirometer ____ (germinating seeds)	Respirometer ____ (non germinating)	Respirometer ____ (beads only)
Initial Volume (mL)	50	50	50
Final Volume (mL)			
Volume of beads/seeds			

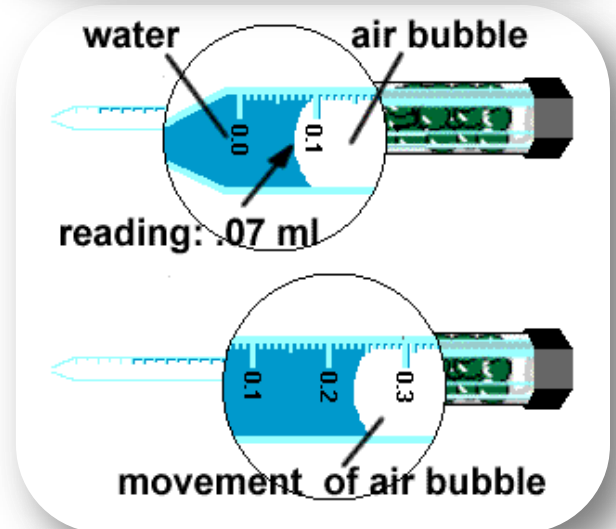
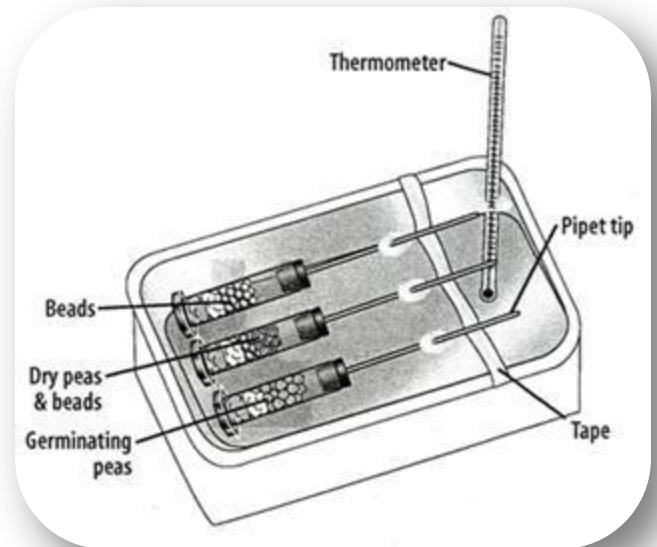


Table 2. Measurement of O₂ Consumption by Germinating and NonGerminating Seeds at Room Temperature and 10°C using Volumetric Methods.

Temp (°C)	Time (min)	Beads Alone		Germinating Seeds			Nongerminating Seeds and Beads		
		Reading at Time X	Diff.*	Reading at Time X	Diff.*	Corrected diff. Δ	Reading at Time X	Diff.*	Corrected diff. Δ
Room	0								
	5								
	10								
	15								
	20								
Cold	0								
	5								
	10								
	15								
	20								

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

ΔCorrected Difference = (initial seed reading at time 0 – seed reading at time X) - (initial bead reading at time 0 – bead reading at time X)

When graphing your data create best fit lines in addition to plotted data to calculate the rate of cellular respiration.

Post-Lab Questions

1. Identify the hypotheses being tested in this activity. Indicate the variable factor(s), the control(s), and the purpose of each control.
2. In this lab exercise, what is the purpose of the
 - a. Beads
 - b. KOH
 - c. Making sure the respirometer is completely sealed
3. Explain why the water moved into the respirometers' pipets.
4. Describe and explain the relationship between the amount of O₂ consumed and time.
5. Calculate the rate of O₂ consumption of the germinating and nongerminating seeds during the experiments at room temperature and at 10°C. Show your calculations for each. Reproduce table in lab book.

Condition	Show Calculations (show all work)	Rate (mL O ₂ /min)
Germinating Seeds/10°C		
Germinating Seeds/Room Temp		
Nongerminating Seeds/10°C		
Nongerminating Seeds/Room Temp		

6. Explain the effect of germination (versus nongermination) on pea seed respiration.
7. If you used the same experimental design to compare the rates of respiration of a 25g reptile and a 25g mammal at 25°C and 10°C, what results would you expect? Be sure to fully explain your reasoning. (It may be helpful to think of a specific reptile/mammal and compare them.)
8. To the right is a graph of possible data obtained for oxygen consumption by germinating peas. Assuming that peas begin to dehydrate at a temperature of 45°C, draw in predicted results of respiration through 70°C. Explain your results.

