

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

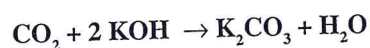
T is the **temperature** of the gas (in °K).

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:



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 O₂ 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

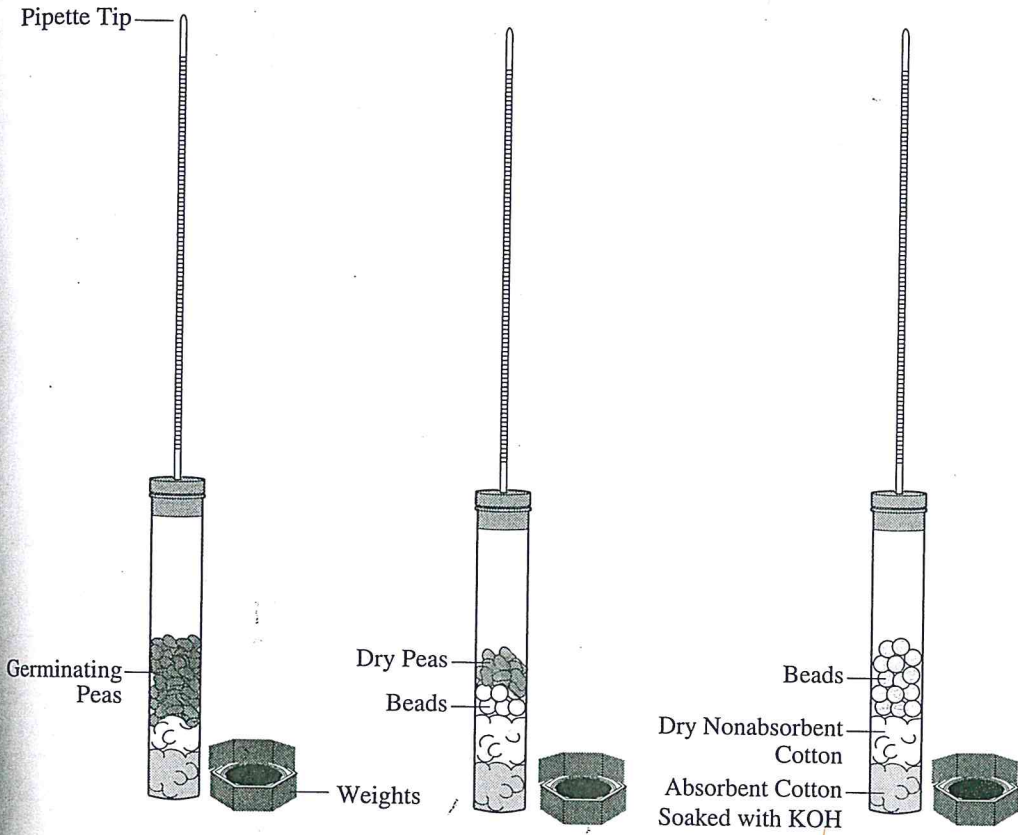
- Both a room-temperature bath (by convention, 25°C) and a 10°C bath should be set up immediately to allow time for the temperature of each to adjust. Add ice to attain 10°C.
- Respirometer 1:** Obtain a 100-mL graduated cylinder and fill it with 50 mL of H₂O. Drop 25 germinating peas in the graduated 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

- Respirometer 2:** Refill the graduated cylinder with 50 mL of H₂O. 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.
- Respirometer 3:** Refill the graduated cylinder with 50 mL of H₂O. 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.
- 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.
- 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.*

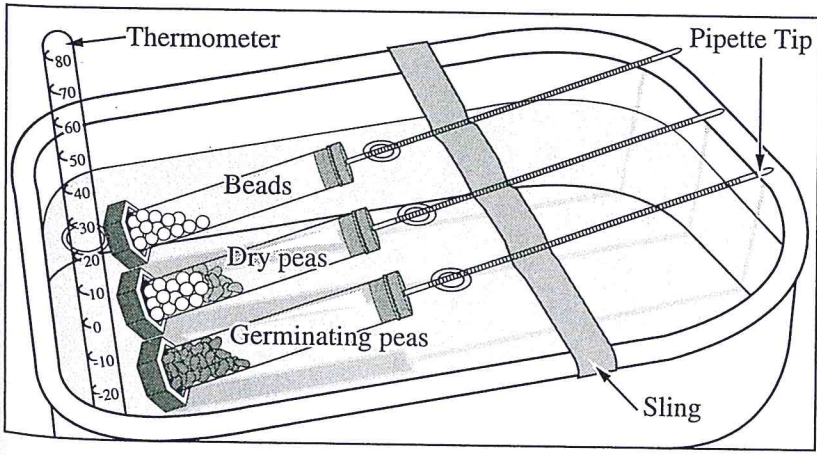
* Your teacher may ask you to use soda-lime pellets instead of KOH Solution.

Figure 5.1: Assembled Respirometers



- 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 O₂ 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)