Cellular Respiration

In this experiment, the relative volume of 0_2 consumed by germinating and nongerminating (dry) peas at two different temperatures will be recorded as a measure of cellular respiration.

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 ^{o}K).

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:

$$CO_2 + 2KOH \rightarrow K_2CO_3 + H_20$$

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 (Figures 1 and 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 are used, as outlined in Table 1.

Table 1: Contents of respirometers

Respirometer	Temperature	Contents
1	Room	25 Germinating Seeds
2	Room	25 Dry Seeds + Beads
3	Room	Beads
4	10ºC	25 Germinating Seeds
5	10ºC	25 Dry Seeds + Beads
6	10ºC	Beads

It is important that each respirometer contain the same volume of materials. Obtain a 100 mL graduated cylinder and fill it with 50 mL of H_20. Drop in 25 germinating peas and determine the amount of water that was displaced (which is equivalent to the volume of peas). Record the volume of 25 germinating peas. They will be used in respirometer 1. Refill the graduated cylinder with 50 mL of H_20. Drop 25 dried peas (not germinating) into the graduated cylinder and then add enough beads to attain a volume equivalent to that of the expanded germinating peas. These peas and beads will be used in respirometer 2. Refill the graduated cylinder with 50 mL of H_20. Determine how many beads would be required to attain a volume equivalent to that of the germinating peas. These beads will be used in respirometer 3.

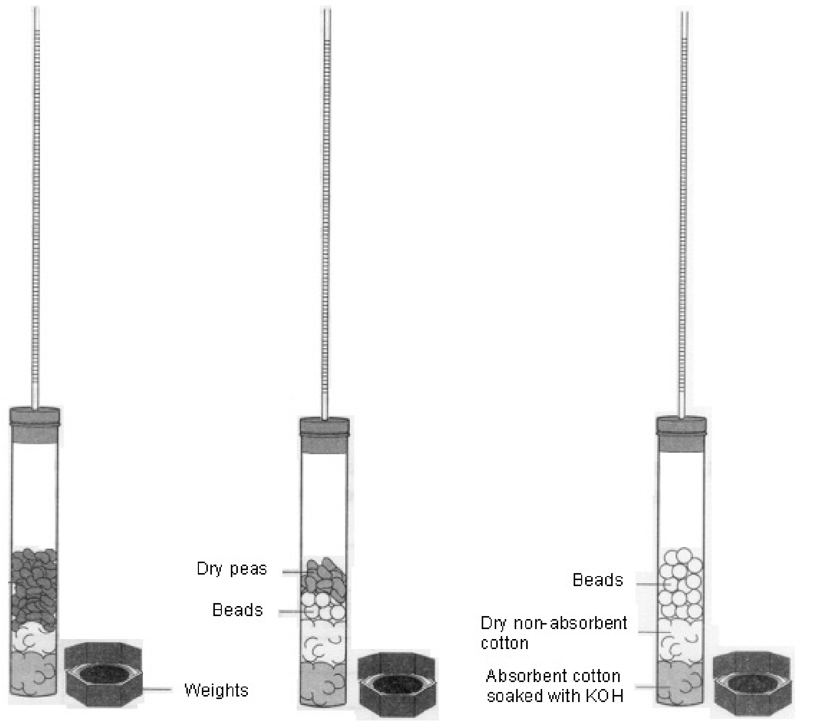

Figure 1: Assembled respirometers

Repeat the procedures above to prepare a second set of germinating peas, dry peas plus beads, and beads for use in respirometers 4, 5, and 6, respectively. Assemble the six respirometers as shown in Figure 1. Place a small wad of absorbent cotton in the bottom of each vial, saturated with 15% KOH. It is important that the amounts of cotton and KOH be the same for each respirometer.

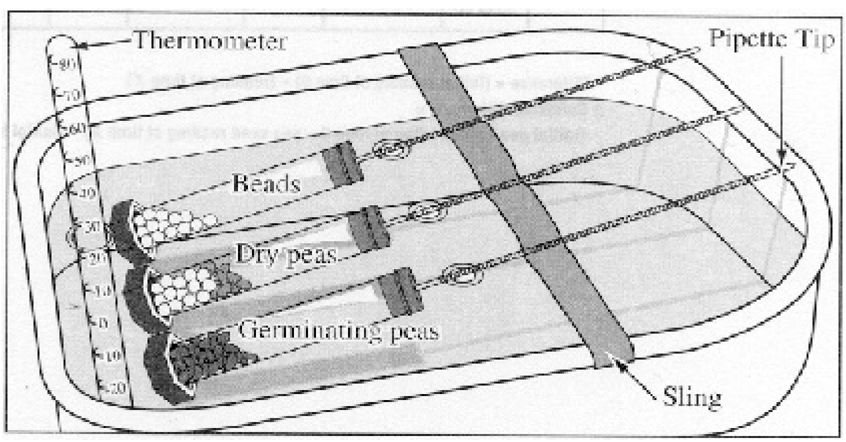

Figure 2: Respirometers in the Water Bath

Vials 1, 2, and 3 should rest in the room temperature water bath (approximately 25ºC) and vials 4, 5, and 6 should rest in the 10ºC water bath as shown in Figure 2. Make sure that a constant temperature is maintained.

Water will enter the pipettes for a short distance and then stop. 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 1. Every 5 minutes for 20 minutes, take readings of the water's position in each pipette, and record the data in Table 1. (sample data are provided)

Graph the results from the corrected difference column for the germinating peas and the dry peas at both temperatures.

Table 1: Measurement of 0_2 Consumption by Soaked and Dry Pea Seeds at Room Temperature (25ºC) and 10ºC Using Volumetric Methods

Temp (ºC)	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.**
25	Initial - 0	0.93		0.91			0.92		
	0 - 5	0.91		0.84			0.89		
	5 - 10	0.90		0.77			0.87		
	10 - 15	0.90		0.71			0.87		
	15 - 20	0.90		0.64			0.85		
10	Initial - 0	0.95		0.92			0.91		
	0 - 5	0.94		0.88			0.90		
	5 - 10	0.92		0.85			0.87		
	10 - 15	0.93		0.83			0.86		
	15 - 20	0.93		0.80			0.85		

* 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)

Questions

1. In this experiment, we are investigating the effects germination versus nongermination and warm temperature versus cold temperature on respiration rate. Identify two hypotheses being tested.
2. This experiment uses a number of controls. Identify at least three of the controls, and describe the purpose of each.
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 O_2 consumption (in mL O_2 /min) of germinating and dry peas during the experiments at both temperatures. Remember from math class that the rate can be found by calculating $\Delta Y/\Delta X$. (I guess math is useful after all)

Condition	Calculation	Rate (mL O_2/minute)
Germinating peas at 10ºC		
Germinating peas at room temp.		
Dry peas at 10ºC		
Dry peas at room temp.		

5. Why is it necessary to correct the readings from the peas with the readings from the beads?
6. Explain the effect of germination (versus nongermination) on pea seed respiration.

7. What do you think would happen to oxygen consumption if the experiment was done at 45ºC?
8. What is the purpose of KOH in this experiment?
9. If you used the same experimental design to compare the rates of respiration of a 25 g mammal and a 25 g reptile, at 10ºC, what results would you expect? Explain.
10. If respiration in a small mammal were studied at both 21ºC and 10ºC, what results would you expect? Explain.
11. Explain why water moved in the respirometers.