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Experiment Components

Storage:
Store entire experiment in the refrigerator.

None of the experiment components have been prepared from human sources.

All components of this experiment are intended for educational research only. They are not to be used for diagnostic or drug purposes, nor administered to or consumed by humans or animals.

This experiment is designed for 10 lab groups.

Contents

30 pipets, (1 ml)
Glass beads, 1 lb.
Peas, 0.25 lbs.
Potassium Hydroxide solution (15%), 60 ml
30 Cork stoppers
Absorbent cotton
Non-absorbent cotton
30 vials
Parafilm®

Requirements

- 10 Thermometers
- 10 Trays (at least 14" long)
- Silicon glue
- Small weights, to hold vials in water
- Ice
- Cork borer
- Tape
- Timers

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BACKGROUND INFORMATION

Cell Respiration

All life on earth ultimately depends upon the sun for energy. Photosynthesis in plants traps energy from sun by formation of covalent bonds in complex organic compounds, such as glucose. Organisms release this stored energy by the breakdown of glucose, using a set of enzymatic reactions involving many steps. Breakdown of glucose can occur in the presence of oxygen (aerobically), or in the absence of oxygen (anaerobically). Energy released by the breakdown of glucose is stored in the high energy phosphate bonds of adenosine triphosphate, ATP. Aerobic respiration yields the most energy for organisms, with every mole of glucose producing about 36 - 40 moles of ATP. Under anaerobic conditions, only 2 moles of ATP are produced.

Cellular respiration is the breakdown of organic compounds, resulting in the release of energy. The oxidative breakdown of glucose during cellular respiration produces the energy needed for life in living organisms, as given in equation 1:

1. $C_6O_6H_{12} + 6\,O_2 = 6\,CO_2 + 6\,H_2O + 686\text{ kCal}$

Carbon dioxide, CO_2, a by-product of cellular respiration, is required for trapping the energy of the sun by photosynthesis. Photosynthesis results in the formation of glucose, oxygen, and water, as shown in equation 2:

2. $6\,CO_2 + 12\,H_2O + \text{sunlight} = 6\,O_2 + C_6O_6H_{12} + 6\,H_2O$

The free energy content in the terminal phosphate bond of ATP is 7.3 kCal per mole of ATP. Assuming the synthesis of 36 moles of ATP per mole of glucose broken down during aerobic respiration, a total of 262.8 kCal of energy is stored in the high energy bonds of ATP. This represents about 38.3% of the total free energy released during the breakdown of one mole of glucose.

This experiment is designed to measure oxygen consumed by either germinating or non-germinating pea seeds. To measure consumed oxygen, the ideal gas law (equation 3) will be utilized:

3. $PV = nRT$

P = the pressure of the gas
R = the gas constant (a fixed value)
V = the volume of the gas
T = the temperature of the gas
n = the number of molecules of the gas

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BACKGROUND INFORMATION

Cell Respiration, continued

In equation 3, R is always constant. If the pressure of the system (P), and the temperature of the system (T) remain constant, the volume occupied by the gas is directly proportional to the number of molecules of the gas.

During this experiment, a respirometer will be used to measure the volume of oxygen, and therefore the number of molecules of oxygen, consumed by the pea seeds during cellular respiration. The CO_2 released through respiration (see equation 1) is removed from the system by potassium hydroxide (KOH), as shown in equation 4. Therefore, the amount of CO_2 gas released does not factor into the analysis and measurement of the amount of oxygen consumed.

4. $CO_2 + 2\ KOH = K_2CO_3 + H_2O$

The number of oxygen molecules consumed during respiration by the pea seeds will be directly related to the decrease in volume occupied by gas within the respirometer. The water in the pipet will move toward the region of lower pressure, which is created within the respirometer due to oxygen consumption. This assumes constant volume and pressure of the system. The control vial containing the glass beads will be used to measure any change in water volume due to alterations of temperature and pressure. The data will be corrected to reflect these external influences on the respirometer.

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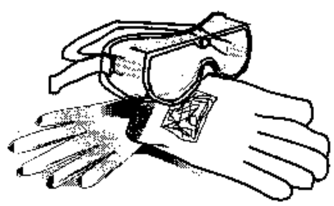
EXPERIMENTAL PROCEDURES



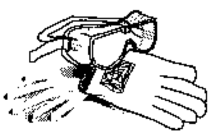
EXPERIMENT OBJECTIVE:

The objective of this experiment is to apply the gas laws to the function of the respirometer. Students will observe cell respiration of germinating and non-germinating seeds and describe the effects of temperature on the rate of cell respiration.

LABORATORY SAFETY

Gloves and goggles should be worn routinely as good laboratory practice.

Student Experimental Procedures



Wear safety goggles and gloves

1. Set up an ice bath at 10° C or room temperature bath as assigned by the instructor.

2. Label the 3 vials A, B, and C.
   - Place a circle of absorbent cotton (approximately the size of a nickel) into the bottom of each vial.
   - Carefully soak with 1- 2 ml of 15% KOH solution, not allowing any KOH solution to touch side walls of vials.
   - Place a circle of non-absorbent cotton into vial directly on top of KOH/cotton circle in the vial. This will keep the KOH solution from touching peas during the experiment.

3. Determine the volume of germinating peas.
   - Fill a 100 ml graduated cylinder with 50 ml of water.
   - Add 25 germinating peas to the cylinder and measure the increase in water volume. The difference represents the volume of the peas.
   - Place the peas on a paper towel. These germinating peas will be used in respirometer A.

4. Determine the volume of non-germinating peas.
   - Fill 100 ml graduated cylinder with 50 ml water.
   - Add 25 non-germinating peas.
   - Add glass beads to raise volume to equal that obtained with swollen germinating peas.
   - Remove peas and beads. Place them on a paper towel. Non-germinating peas and beads will be used in respirometer B.

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EXPERIMENTAL PROCEDURES

Experimental Procedures, continued

5. Determine the volume of glass beads.
    - Fill the 100 ml graduated cylinder with 50 ml water.
    - Add glass beads to raise volume so it equals volume of germinating peas as determined previously in Step 3.
    - Place glass beads on a paper towel, to be used in Vial C.

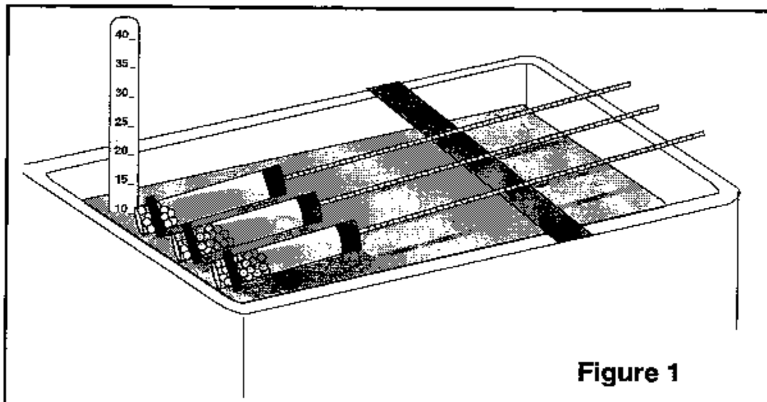


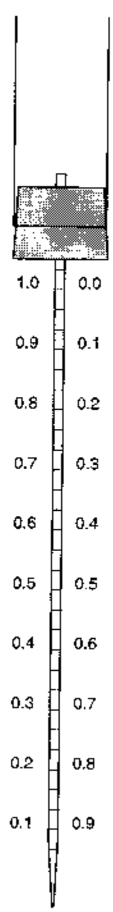
Figure 1

6. Place germinating seeds into vial A. Insert cork/pipet assembly into vial.
7. Place non-germinated seeds + glass beads into vial B. Insert cork/pipet assembly into vial.
8. Place glass beads into vial C. Insert cork/pipet assembly into vial.
9. Wrap Parafilm® or plastic wrap tightly around the seams (cork and tube) to seal <u>any</u> potential leak.
10. Make sure ice water bath has equilibrated to 10°C, or record temperature of room temperature bath.
11. Place a piece of masking tape over water bath to suspend pipet tips out of water during equilibration phase.
13. Place each respirometer into water bath with calibrated side of pipet facing up since measurements will be taken. See Figure 1.
14. Allow respirometers to equilibrate in water bath for 8 minutes.
15. After equilibration phase, immediately submerge each respirometer. Pipets will fill slightly with water. Make sure vial does not fill up with water. If it does, there is a leak which must be corrected. Reassemble faulty respirometer.
16. Arrange vials so you can read volume markings on each pipet. Place lead donuts or other weighted objects on vials to keep vials submerged.
17. Record starting temperature. Maintain temperature by adding ice or water as necessary during the experiment.
18. Record starting point ("Time 0") volume of each pipet. Take readings of the volume of water in each pipet every 5 minutes for 20 minutes. Record these values in the table on Page 9.

Useful Hint!

When reading the pipets, use the inverse markings on the pipet (i.e. starting with 0.1ml rather than 0.9ml) at the pipet tip.

Use these markings (on the left.) Start Here



EXPERIMENTAL PROCEDURES

Experimental Procedures, continued

17. Collect Vial A, B, and C class data for other temperature bath.

18. Correct volumes measured for changes in environmental variables.

    - Vial C measures volume changes due to environmental variables. For Vial C, the environmental effects are calculated by subtracting the volume at given time (T_x) from the starting volume at starting time (T_0). This can be written as:

      Vial C volume at T_x - Vial C volume at T_0.

    - Environmental effects must be applied to data collected for vial A as shown below:

      [Vial A volume at T_x - Vial A volume at T_0] -
      [Vial C volume at T_x - Vial C volume at T_0]

    - Environmental effects must be applied to data collected for vial B as shown below:

      [Vial B volume at T_x - Vial B volume at T_0] -
      [Vial C volume at T_x - Vial C volume at T_0].

    - Sample calculations are shown in Expected Results section on page 13.

Experimental Procedures, continued

Bath Temp 21°C	Volume Readings (ml)			(ml O_2 consumed) Vial C vol at T_x - Vial C vol at T_o	(ml O_2 consumed) [Vial A vol T_x-Vial A vol T_o] -[Vial C vol T_x-Vial C vol T_o]	(ml O_2 consumed) [Vial B vol T_x-Vial B vol T_o] -[Vial C vol T_x-Vial C vol T_o]
Time (T) min.	Vial A	Vial B	Vial C			

Bath Temp 11°C	Volume Readings (ml)			(ml O_2 consumed) Vial C vol at T_x - Vial C vol at T_o	(ml O_2 consumed) [Vial A vol T_x-Vial A vol T_o] -[Vial C vol T_x-Vial C vol T_o]	(ml O_2 consumed) [Vial B vol T_x-Vial B vol T_o] -[Vial C vol T_x-Vial C vol T_o]
Time (T) min.	Vial A	Vial B	Vial C			

T_o = Reading at Time 0

T_x = Reading at Time X

e.g., The reading volume (ml) of Vial A at Time X minus the reading volume of Vial A at Time 0

Study Questions

1. Graph your corrected data (difference) for Vials A, B, and C. Place time (in minutes) on the x-axis and volume (ml O_2 consumed) on the y-axis. Data from both temperatures should be plotted on the same graph. Draw the best straight line through the data points.

2. What accounts for the difference in oxygen consumption seen between the germinating and non-germinating seeds?

3. Why do the glass beads seem to be using oxygen?

4. Why are the readings corrected using the glass bead values?

5. From the slope of the lines, determine the rate of oxygen consumption at 10°C and room temperature for the germinating and non-germinating pea seeds. Determine the slope of the lines over a middle section of each line by dividing the difference in volume reading by the difference in time. Volume (ml O_2 consumed) values are determined from the line.

6. Compare the rate of oxygen consumption at 10°C and room temperature. Why are they different?

7. What basic cellular process is responsible for the oxygen consumption?

