Lab: Cell Respiration (Modified AP Lab 5) **Name _____**

College Biology **Hour ___ Date _____**

Paula Donham, Olathe East High School pdonhamoe@olatheschools.com
Adapted from AP Biology Labs

OVERVIEW:

In this lab you will work with seeds that are living but **dormant** and seeds that are **germinating**. A seed contains an embryo plant and a food supply (endosperm) surrounded by a seed coat. When the necessary conditions are met, germination occurs and the rate of cellular respiration greatly increases. In this lab you will:

1. measure oxygen consumption during germination by measuring the change in gas volume in respirometers (the carbon dioxide produced is captured as a solid so the change in volume is solely due to oxygen consumption),
2. measure this change in volume/oxygen consumption in germinating and nongerminating (dormant) seeds, and
3. measure the rate of change of the same seeds at two different temperatures.

OBJECTIVES:

Before doing this lab you should understand:

- respiration, dormancy, and germination;
- how a respirometers works in terms of the gas laws; and
- how the rate of cellular respiration relates to the amount of activity in a cell.

After doing this lab you should be able to:

- calculate the rate of cell respiration from experimental data;
- relate oxygen consumption to respiration rate;
- test the rate of cellular respiration in germinating versus nongerminated seeds in a controlled experiment; and
- test the effect of temperature on the rate of cell respiration in germinating versus nongerminated seeds in a controlled experiment.

INTRODUCTION:

Aerobic 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.

$C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + 6H_2O + 686\text{kcal/mole of glucose oxidized}$

From this equation you should notice there are three ways cellular respiration could be measured. One could measure:

1. **Consumption of O_2** – how many moles of O_2 are consumed
2. **Production of CO_2** – how many moles of CO_2 are produced
3. **Release of energy during cellular respiration** – how much ATP is produced or how much glucose is oxidized

In this experiment the relative volume of O_2 consumed by germinating and nongerminating (dry) seeds at two different temperatures will be measured (the third option would be the most difficult to do accurately).

SAFETY:

You'll be working with some glass, and using KOH. Use caution, especially when you're putting the KOH in the respirometer and WEAR GOGGLES.

BACKGROUND:

In this experiment the CO_2 (a gas) produced during cellular respiration will be removed by exposing it to potassium hydroxide (KOH) which will result in a double replacement reaction with solid and liquid products. [$CO_2 + 2KOH \rightarrow K_2CO_3 + H_2O$] Since this reaction removes the CO_2 gas (by

turning it into the solid potassium carbonate), the change in the volume of gas in the respirometers 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. As a result of this consumption the total gas volume will be reduced (since 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 – which will be visible in measurable increments on the attached pipette. The vial with glass beads alone will permit detection of any changes in volume due to atmospheric pressure changes or temperature changes.

PRE-LAB QUESTIONS:

1. What is the purpose of the water bath? _____

   _____

   Why is **water** used for this purpose? (relate it to the specific heat of water) _____

   _____

2. Why do you let the respirometers equilibrate for 7 minutes BEFORE immersing them in the water? _____

   _____

3. What is the purpose of the pipette on the end of your respirometers? _____

   _____

4. Why are you using 5.00g dry mass of total material in each vial (think about keeping something "constant")? _____

5. What is the purpose of the KOH (this is a **very important** concept – make sure you understand what's going on with the KOH)? _____

   _____

   What would happen to your measurements if KOH wasn't used: _____

   _____

6. In this activity you are investigating both the effect of germination versus nongermination and warm temperature versus cold temperature on the rate of cellular respiration. Complete the following two hypotheses/predictions related to **oxygen consumption** for this activity by choosing "more" or "less" for each of the pairs of words.

   Hypothesis 1: *Germinating seeds will be doing* {more / less} (choose one) *cellular respiration than dormant/non-germinating seeds so they will be using* {more / less} (choose one) *oxygen over 20 minutes.*

   Hypothesis 2: *Seeds in a warm environment (room temperature) will be doing* {more / less} (choose one) *cellular respiration than seeds in a cold (below room temperature) environment so they will be using* {more / less} (choose one) *oxygen over 20 minutes.*

PROCEDURE: 3 respirometers should be set up for *each* group to put in *each* tray at *each* temperature –

Respirometer 1: germinating/moist grass seeds (5.00 g dry mass)
Respirometer 2: dormant/dry grass seeds (5.00 g dry mass)
Respirometer 3: 20 medium glass beads (5.00 g dry mass)

1. The two temperatures for the water baths should be room temperature and 10 degrees (C) ***below*** room temperature – use ice to lower the temperature of the second set of water baths. **Be sure to record** these temperatures in the titles for your data tables.

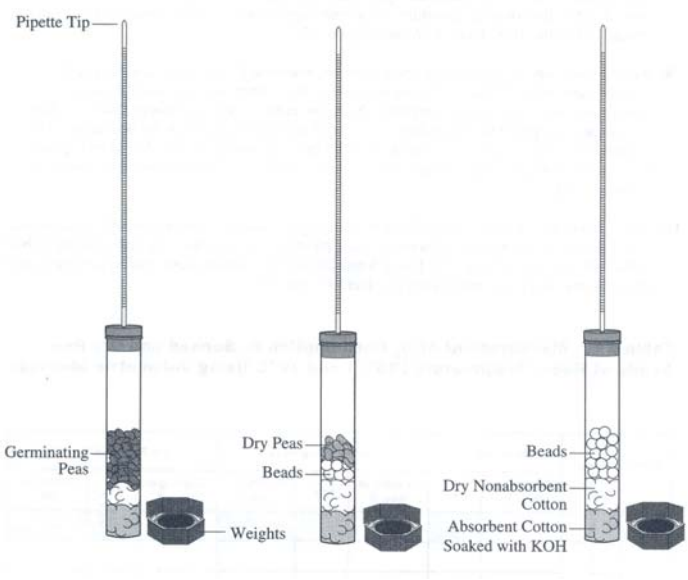
2. Another lab group will set up the same 3 respirometers as replicates at the same temp, other lab groups will do the same at the second temperature then the data will be pooled so class means for all the respirometers with the seeds can be graphed (the data from the respirometers containing only glass beads are to be used for volume adjustments only).

3. **To assemble the respirometers** obtain 3 vials, each with stopper and attached pipette – **be careful** not to dislodge the pipette or break the seal between the pipette and the stopper since any leaks will result in data inaccuracies. Make sure your vials are dry on the inside.

   Place a small piece of absorbent cotton in the bottom of each vial (take care to use the same amount in all 3 vials). **Taking care not to get any KOH on the sides of the vial**, moisten the cotton with 3 drops of 15% KOH using a thin stem pipet. ***Be sure to replace the lid on your KOH*** so it doesn't absorb CO_2 from the air.

   Now add an equally small piece of **non**absorbent cotton (to protect your seeds from the KOH).

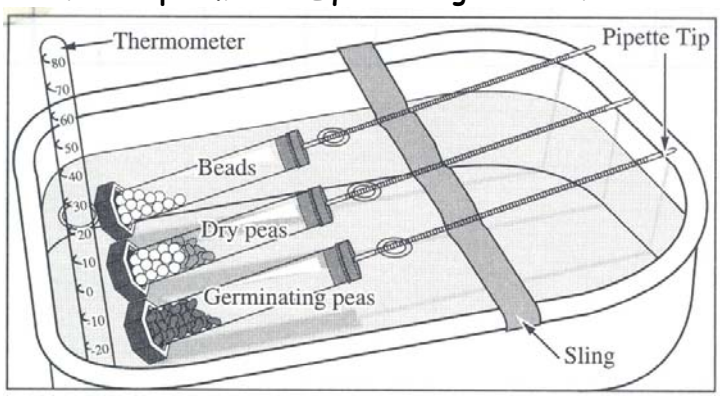
Figure 5.1: Assembled Respirometers



4. Place the germinating seeds, dry seeds, and beads in vials 1, 2 (**don't use beads** in #2 even though they're in the picture), and 3, respectively as pictured in Figure 5.1 (**note**: you're using ***grass seed*** instead of ***peas***). Insert the stopper fitted with the calibrated pipette.

5. See Figure 5.2. Make a sling of masking tape attached to each side of the water bath to hold the pipettes out of the water during an equilibration period of 7 minutes. Place the respirometers as illustrated in Figure 5.2 (**note**: you're using grass seed instead of peas); use a weight at the junction of the pipettes and stoppers to hold the respirometers under water.

Figure 5.2: Respirometers Equilibrating in the Water Bath



6. **AFTER** an **equilibration period of seven minutes**, place a drop of water with red dye in each respirometer tip then remove the masking tape and lower the tips of the respirometers into the water bath – ***take care NOT to*** lift the vials out of the water – you don't want to change the temperature of the respirometers when lowering them. ***Make sure*** respirometers are fully submersed in the water baths.

   Water will enter the pipettes for a short distance and then should appear to stop – if water continues to move in check for leaks in the respirometer.

   Work swiftly and arrange respirometers so that the pipettes can be read through the water. Keep your hands out of the water after the experiment has begun and strive to maintain a constant temperature in the water.

7. ***Allow the respirometers to equilibrate for three more minutes*** and then record, to the nearest 0.01mL, the initial position of water in each pipette (= time 0 value). Check the temperature and **record it** in title for Table 5.1. Columns A, C, and F are for recording your raw data – the other columns are calculated values.

TABLE 5.1: Measurement of O_2 Consumption (mL) by Germinating and Dry (Nongerminating) Seeds at ___ °C Temperature

Time (min)	BEADS ALONE		GERMINATING SEEDS			DRY SEEDS		
	A. Pipette reading at time x	B. Cum dif due to air pressure ($A_0 - A_x$)	C. Pipette reading at time x	D. Cum gas volume dif ($C_0 - C_x$)	E. Cum O_2 Consump w/correct'n (D_x-B_x)	F. Pipette reading at time x	G. Cum gas volume dif ($F_0 - F_x$)	H. Cum O_2 Consump w/correct'n (G_x-B_x)
0								
5								
10								
15								
20								

Report your data from Columns E and H to your instructor

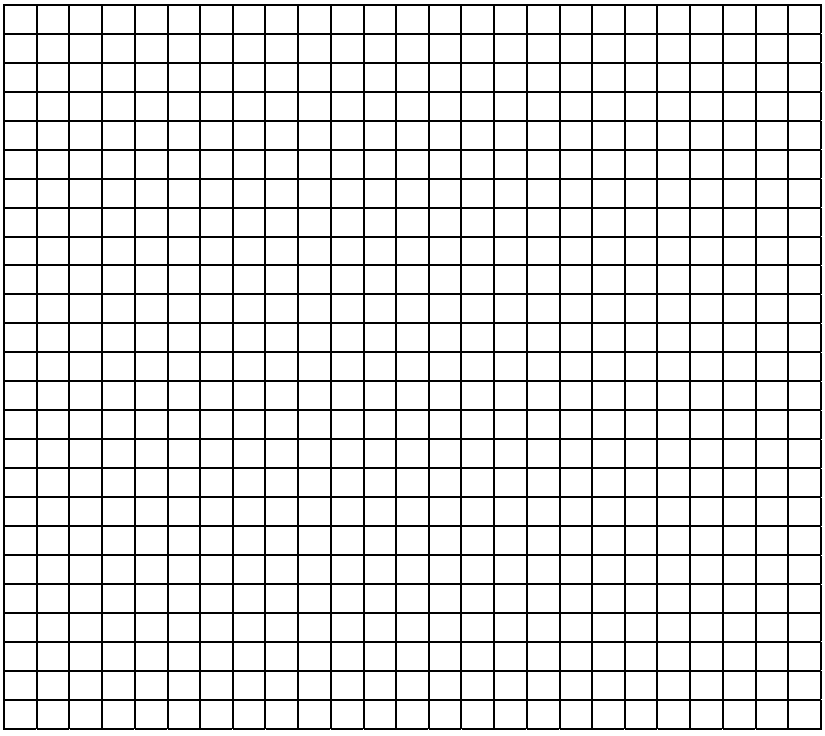
TABLE 5.2: ***See Attached Printout of Class Data (Instructor will provide this all groups have reported their data.)***

ANALYSIS OF RESULTS:

1. Graph the **<u>class</u> means** for the germinating and dry seeds at the two temperatures on the graph below. Use a different color for each type of seed at each temperature (4 colors), **be sure to include** a title and a legend for your graph and label your axes (your *independent variable* should be on the x-axis). **Once your points** (for times 0, 5, 10, 15, and 20) **are plotted**, draw an approximate "*line of best fit*" for **each** set of data (you'll have 4 lines – you may find it easier/better to use your calculator or Excel to find the lines of best fit).

Graph 5.1: (Descriptive) Title: _____

_____



<u>Legend</u>

2. **Based on your graph** describe and explain the relationship between the amount of oxygen consumed and time: _____

_____

In Table 5.3 below, record the rate of O_2 consumption of the seeds for all 4 lines from the above graph. (**The rate of consumption should be the slope of each of the lines ($\Delta y/\Delta x$)**. Show your calculations in the first column and record the final rate value in the last column.

Table 5.3 - The Rate of O_2 Consumption of Germinating and Dry Seeds at Room Temperature and 10°C below Room Temperature

Condition	Rate Calculations (Rate = slope = $\Delta Y/\Delta X$)	Rate (mL O_2/min)
Germinating Seeds/10°C below Room Temperature		
Germinating Seeds/Room Temperature		
Dry Seeds/10°C below Room Temperature		
Dry Seeds/Room Temperature		

4. Discuss whether these results support your original predictions/hypotheses:

My results { *support* / *don't support* } my first hypothesis _____

choose one of the above

_____

_____

My results { *support* / *don't support* } my second hypothesis _____

choose one of the above

_____

_____

5. Summarize your conclusions from this lab: _____

_____

_____

_____