Respiration Rates of Storage Tissue in Plants

Effect of "Aging" and Inhibitors on Respiration

Energy obtained from the oxidation of food is used by the cells of plants (and animals) to do work. The synthesis of new cells and tissues for growth, the transport of minerals across membranes and the opening of stomata for gas exchange are all obvious examples of cellular work requiring energy. Essentially all processes in living cells require energy.

Even though plants can make their own food through photosynthesis, they must break down some of that food to supply their own energy needs. When all forms of food are metabolized in the plant, oxygen is consumed and energy is released, along with water and carbon dioxide, as seen in the equation below:

$$\textbf{Food} + O_2 \longrightarrow CO_2 + \textbf{energy}$$

If the starting material for respiratory breakdown of food is a sugar such as glucose a great deal of energy can be produced. The complete oxidation of a mole of glucose releases 673 kilocalories of energy:

$$C_6H_{12}O_6 \text{ (glucose)} + 6O_2 \longrightarrow 6CO_2 + 6H_2O + 673 \text{ kcal}$$

Notice that the amount of O_2 consumed is equal to the amount of CO_2 produced. Thus the rate of respiration of a plant oxidizing carbohydrate could be determined by measuring either the rate of O_2 consumption or the rate of CO_2 production.

Measuring Respiratory Rate of Potato Discs

You will be measuring respiration with an O_2 sensor that measures the amount of O_2 in the atmosphere of a chamber containing your plant material. You will be able to determine the rate at which O_2 is being removed by determining the slope of the line recorded as the O_2 concentration changes. You must read the file “Measurement of Plant Respiration Using an O_2 Sensor” on the Web at **www.grinnell.edu/courses/bio/qubitmanual** before proceeding with this experiment.

Materials

60 "aged" or fresh discs (8 mm in diameter by 2 mm thick) cut from potato tuber per treatment
Phosphate buffer, pH 5.0
CCCP, NaCN or KCN, NaN_3 (sodium azide), or potassium malonate inhibitor
Qubit O_2 sensor apparatus

Procedures

For this experiment potato tubers provide very suitable plant material. For "aging," all tissues should be sliced into discs 8 mm in diameter by 2 mm thick and washed for about 24 hours in aerated tap water, with changes in water at least every 12 hours. They may also be aged by covering with water and shaking continuously for about 24 hours, again with occasional changes of the water.

Each student will be assigned the responsibility for one or more of the treatments in the table. Weigh the tissue and determine its volume before placing it in each treatment. Determine the volume of the tissue by placing it in a graduated cylinder containing a known volume of water and determining the volume of displacement of the tissue. Subtract this volume from 47 ml, the total volume of the leaf chamber. Use this difference as the volume of the leaf chamber in your calculations of respiratory rate.

Prepare the appropriate solutions and allow tissues to soak in them for at least 15 min. Remove the discs you are about to measure from the solution and blot with a paper towel. Then place the discs in one or two layers in the leaf chamber of the O_2 sensor and measure respiration rates, as directed. Begin your measurements with the control tissue, if that is one of your assigned treatments.

Calculate average microliters of O_2 absorbed per gram of fresh weight per hour for each treatment following the instructions provided. Pool your results with those of the other students so that everyone has all the class data. List the results in the report sheet.

Treatment Description	Treatment solutions
Fresh tissue, control	12.0 ml of 0.05 M Pi buffer
"Aged" tissue, control	12.0 ml of 0.05 M Pi buffer
Fresh + 2 x 10^{-5} M CCCP	6.0 ml of 0.1 M Pi buffer + 6.0 ml of 4 x 10^{-5} M CCCP
"Aged" + 2 x 10^{-5} M CCCP	6.0 ml of 0.1 M Pi buffer + 6.0 ml of 4 x 10^{-5} M CCCP
Fresh + 10^{-3} M Cyanide	6.0 ml of 0.1 M Pi buffer + 6.0 ml of 2 mM KCN
"Aged" + 10^{-3} M Cyanide	6.0 ml of 0.1 M Pi buffer + 6.0 ml of 2 mM KCN

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Treatment Description	Treatment solutions (continued)
Fresh + 10^{-3} M Azide	6.0 ml of 0.1 M Pi buffer + 6.0 ml of 2 mM NaN_3
"Aged" + 10^{-3} M Azide	6.0 ml of 0.1 M Pi buffer + 6.0 ml of 2 mM NaN_3
Fresh + 0.05 M Malonate	6.0 ml of 0.1 M Pi buffer + 6.0 ml of 0.1 M potassium malonate, pH 5
"Aged" + 0.05 M Malonate	6.0 ml of 0.1 M Pi buffer + 6.0 ml of 0.1 M potassium malonate, pH 5

1. Before starting the experiment adjust the time axis on both graphs to a maximum of 20 minutes by highlighting the maximum value present and then typing 20. Hit **return**. Set the maximum oxygen concentration on the upper graph to 22% and the minimum at 19% using the same technique.

2. Remove the potato discs from one treatment solution and blot with a paper towel to remove excess liquid. Don't dry them excessively. Distribute the discs evenly in the lower part of the leaf chamber, in one or two layers. Ensure that a **very thin** coating of vacuum grease has been applied to the gaskets of the chamber. If any grease is visible it will **not** be necessary to apply more.

3. Before attaching the lower part of the leaf chamber containing the potato discs, click on the **Start** button on the bottom left hand side of the computer screen. The button will change to a **Stop** button and data will begin to appear on the two graphs on the screen, and as numerals on the bottom of the screen. The initial O_2 concentration should be close to 20.7% O_2 and the light intensity readings should be close to zero since you will not be using the light source for this experiment.

4. Attach the lower chamber to the upper chamber with the thumb-screws, turning them finger tight only. Seal the gas ports with the plugs provided.

5. Follow the decline in O_2 concentration in the leaf chamber for the next 5-10 minutes or until you have a fairly linear rate of decline. Click on the **Stop** button.

6. Save your data by selecting **Save As** . . in the File menu. Give your data an appropriate file name and save it to your data folder. Remove the discs from the leaf chamber and, if you have not previously done so, measure their total volume by submerging them in a pre-measured amount of water in a graduated cylinder. Record this volume in your data table along with the previously measured fresh weight of the discs.

7. Repeat these measurements with the discs from the remaining treatments.

Data Analysis

The O_2 sensor measures only the partial pressure of O_2 present in the leaf chamber; it does not measure the rate at which this O_2 is produced or consumed. To measure the rate of oxygen consumption in your experiment, you will need to measure the decrease in O_2 concentration within the leaf chamber as a function of time. This is achieved by measuring the rate of the O_2 response which, when the *x* axis of your graphs is presented in minutes, will give a rate in $\%O_2$ per min. The procedure for analyzing your data is as follows:

1. Open the file containing data from one of your experiments. A command box will appear asking you whether or not you wish to load the calibration stored with your data file. Answer **Yes**. Your data will appear on the screen exactly as it appeared when you saved it at the end of the experiment.

2. Select **Examine** from the Analyze menu at the top of the screen. A vertical line will appear on your graphs which can be moved along the data points on the graphs by moving the mouse. Note that as you move the vertical line, the numerical display in the box on the screen will change to show you the exact O_2 concentration and time value at the point on the graph where the line is situated.

3. Measure respiration rate during the linear part of the decrease in chamber O_2 concentration. To do this, move the vertical line to the point on your O_2 data where you wish to start the measurement, click on the mouse button and hold it down. Move the mouse over the part of the data you wish to analyze, and then release the mouse button capturing the portion to be analyzed inside the box that appears.

4. Select **Linear Fit** from the Analyze menu. In the command box that is on the screen you will see the equation for a straight line, $y = mx + b$, along with values for m and b. The value for m is the slope of the line, which is the rate of O_2 utilization. Record this in your data table. Close the box on the screen by clicking in the upper right hand corner.

5. Measure respiration rate for each treatment by moving the vertical line to the linear part of the next set of data. Select the next area of data to be analyzed by clicking and dragging with the mouse. Again select **Linear Fit** from the Analyze menu. The equation on the screen will reflect the new m value for the range of data that you have selected. Record the new value of m in the Results section.

6. Repeat this procedure for all of the treatments that you tested.

Calculations

Each *m* value from each linear fit that you performed represents the rate of decrease of O_2 concentration in the chamber with time. As such, each of these *m* values are rates of respiration expressed as $\%O_2$ per min. However, respiration is usually expressed in terms of µl O_2/ gfw/hr. To make this conversion the following procedure is required:

Let us assume that the *m* value was ***x***, i.e. the O_2 concentration of the chamber decreased by ***x*** $\%O_2$/min. ***x*** $\%O_2$ is equivalent to 10,000***x*** parts per million (ppm) O_2 which, in turn, is equivalent to 10,000***x*** µl of O_2 per liter of gas in the chamber.

x $\%O_2$/min = 10,000***x*** µl O_2/min/liter of gas in the chamber

To obtain the respiratory rate we must now multiply by the volume of the chamber expressed in liters. The chamber is designed so that when closed it has a fixed internal volume of 0.047 liters. However the potato discs occupied some of this volume; thus the total volume of the chamber must be reduced by the volume of the potato discs

µliters O_2/min/liter X [0.047 liter – volume of leaf discs (liter)] = µliters O_2/min

However we would like to express the respiratory rate in µl O_2/gfw/hr. To do this we must perform the following calculations:

µliters O_2 /min X 60 min/hour = µliters O_2 /hour

µliters O_2 /hour ÷ fresh weight (gm) = µliters O_2 /gfw/hour

Results

Treatment	Fresh Weight (gm)	Volume (liters)	*m* $\%O_2/min$	$\mu l\ O_2/gfw/hr$

Report Sheet

Effect of "Aging" and Inhibitors on Respiration

Treatment	$\mu l\ O_2$ Absorbed/g fr wt/hr
Fresh tissue, control	
"Aged tissue, control	
Fresh + 0.02 mM CCCP	
"Aged"+ 0.02 mM CCCP	
Fresh + 1 mM CN^-	
"Aged" + 1 mM CN^-	
Fresh + 1 mM N_3-	
"Aged" + 1 mM N_3-	
Fresh + 50 mM malonate	
"Aged" + 50 mM malonate	

Show how you calculated these rates.

Questions:

1. Did "aged" discs utilize oxygen at the same rate as fresh discs? Discuss some of the reasons these rates may differ.

2. Did CCCP inhibit or stimulate oxygen utilization, or both? How could this substance increase rates?

3. Did malonate influence the oxygen utilization of "aged" discs any differently than of fresh discs? Explain.

4. How do azide and cyanide inhibit respiration? Did you see any differences between aged and fresh tissue? What might these be due to?