

Biology 3A LABORATORY

Cellular Respiration and Fermentation

Objectives

- To study processes of anaerobic and aerobic respiration
- To determine the amount of oxygen consumed during aerobic respiration
- To determine the amount of carbon dioxide produced during aerobic respiration
- To study the effect of substrate difference on anaerobic respiration in yeast
- To investigate the process of fermentation used in food making

PLEASE BRING A USB THUMBDRIVE TO LAB TODAY

Introduction

All living organisms require energy in order to sustain the many processes involved in life. The energy for these processes is provided by **cellular respiration**, a catabolic process that releases energy (exergonic), most often as ATP. It is essential that the chemical reactions involved in cellular respiration occur at a rapid rate and within optimum conditions. Enzymes are critical in this process.

Aerobic respiration in germinating peas

Cellular respiration involves **glycolysis**, the **Krebs cycle** and the **electron transport chain**. As you may recall from lecture, glycolysis is essentially an anaerobic process since it is not dependent upon the presence of oxygen. The fate of pyruvate, the end product of glycolysis, is dependent on the presence of oxygen. If oxygen is not present, the two pyruvates (from the complete oxidation of one glucose molecule) will remain in the cytosol and undergo the anaerobic process called **fermentation**. There is no “extra” energy yield from fermentation. If oxygen is present, the pyruvates will be shuttled to a mitochondrion, altered and enter into a series of reactions involving the Krebs cycle and the Electron Transport Chain (ETC). Both of these processes are dependent on the presence of oxygen and are aerobic in nature. The Krebs cycle only produces 1 ATP molecule directly per cycle. However, it is indirectly responsible for the greatest ATP production by generating coenzymes, both NADH and FADH₂. When these coenzymes are reoxidized in the electron transport chain, many molecules of ATP are generated (a theoretical 36 – 38 ATP per glucose). Many living organisms undergoing aerobic respiration will use oxygen and produce carbon dioxide.

In this lab you will indirectly determine metabolic rate during aerobic respiration in germinating peas placed in a manometer (a closed chamber) by monitoring the amount of CO₂ produced.

Procedure A:

Setup:

1. Obtain 10 – 12 four- to six-day old germinating peas, determine the mass and record the mass on Table 3.
2. Obtain one Pasco Xplorer GLX data logger, CO₂ probe and the CO₂ measurement container.
3. To the container, add a 2 cm ball of **absorbent** cotton to the bottom.
4. Add normal peas to the container.
5. Wrap the container with aluminum foil to inhibit photosynthesis.
6. Place the CO₂ probe onto the container.

Measurement:

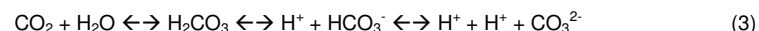
1. Connect the CO₂ probe to the Xplorer GLX **WITHOUT** tipping the container upside down (do not let the wet peas hit the measuring probe) to one of the four PASPORT sensor ports (They look like serial cable ports at the top end of the screen).
2. Turn on the GLX (small green button on the bottom left hand side of the handle)
3. If the Graph is not already displayed on the screen press the Home Screen (🏠) and F1 (📊) at the same time to go to the Graph.
4. If you need to start a new graph, Press F4 then press #7 to start a new graph ready to display data from the PASPORT sensor.
5. Equilibrate the sealed peas in the container for 5 minutes on the table. Do not handle the containers with your warm hands to assure accurate results.
6. After five minutes, Press (▶) to start data recording for 10 minutes.
7. To stop data recording, press (▶) again.
8. Press the Home Screen (🏠) button for the menu display.
9. Use the cursor and select Graph menu and press activate (✔).
10. Return the peas to the original container and discard the used cotton ball. Clean out the container if need be. Do not remove the aluminum foil.
11. Obtain a new 2 cm cotton ball and repeat the procedure with the freeze/thawed peas.

To Download your data to your USB device:

1. Attach your USB drive to the USB port on the right side of the display to save your file to your USB drive.
2. Select Data Files, press (✔).
3. Select the file and press F1. Next to the name, it should say [Open].
4. Press Home (🏠).
5. Cursor down to Table and press activate (✔).
6. Press F4 and cursor down to Export All Data and press (✔).
7. Your data should be downloaded to you drive.
8. Open Excel and select the file (Export file) from your drive. The Text Import Wizard will pop-up.
9. Click Next. Make sure that TAB is selected (that's the default) on the Tab Delimiters and click next.
10. Using Excel, select scatter plot for the entire 10 minute duration. Add a trendline with the equation and r² value.
11. Return the peas to the original container and discard the used cotton ball. Clean out the container for the next group.

CO₂ production during aerobic respiration

From the acid, bases and buffers lab, you should recall that CO₂ can combine with water to form carbonic acid, which dissociate as follows:



In this exercise, you will indirectly determine the amount of CO₂ produced during cellular respiration in a plant and an aquatic animal. You will use phenolphthalein to detect changes in pH resulting from CO₂ production (H₂CO₃). Recall that phenolphthalein is red in basic solutions and colorless in acidic solutions. Since we are not directly measuring CO₂ production, calculate a relative measure of respiration by measuring the volume of NaOH required to neutralize carbonic acid.

Procedure B

Setup & Volume determination

1. Place 75 ml of the **dechlorinated** water in each of the three labeled 150 ml beakers ("control", "fish", and "plant"). The solution has been made slightly acidic.
2. Obtain one goldfish and a 6 cm piece of *Elodea*. Rinse the *Elodea* to remove any other organisms (snails, worms, algae, etc.)
3. Place 75 ml of dechlorinated water in a 150 ml beaker.
4. Place the beaker on a top loading balance and tare the balance. Carefully remove the goldfish with a net, and remove as much water as possible. Place the goldfish into the tared beaker and record its weight. Record the weight on your worksheet. Place the goldfish into the experimental beaker. Weight the *Elodea* in the same fashion and place it into the other experimental beaker.
5. Cover each beaker with Parafilm.
6. Place the beaker with the *Elodea* in the **dark**.
7. Allow the organisms to respire for 20 minutes.
8. Carefully remove the organisms from the beaker and return them to the original containers.

Do not lose the water in the experimental beakers!

Titration

9. Add four drops of phenolphthalein to the contents of each experimental beaker. The solution should be clear.
10. Obtain an eyedropper of 0.2M NaOH. Add the NaOH drop by drop to the contents of the control beaker. Mix thoroughly after each drop. Continue adding drops until the solution is pink. Convert the number of drops to ml (there's approximately 20 drops/ml).
11. Repeat the step for the remaining two experimental beakers until the solutions are the same shade of pink as the control beaker. Record your data on Table 2.

Calculations

12. The relative respiration rate for each organism, for twenty minutes, is the number of ml NaOH added to the organism's water minus ml of NaOH added to the control water.
13. The mass-specific metabolic rate is the relative respiration rate divided by the weight of the organism.

Anaerobic Fermentation

Fermentation involves the oxidation of NADH by the removal of electrons (or hydrogen ions) from the NADH + H⁺ and their acceptance by pyruvate, forming either **lactic acid** or **ethyl alcohol**. The products, which result from the reduction of pyruvate, depend upon the presence of the specific enzymes of the organisms involved. Many cells are capable of fermentation, but animal cells can produce only lactic acid. Prokaryotic cells can produce not only lactic acid, but also many other products, including ethyl alcohol. Yeast and certain other fungi are known for their fermentation abilities, producing ethyl alcohol and CO₂ in the process. In this exercise, you will study anaerobic respiration in yeast.

Procedure C

1. Obtain three fermentation tubes.
2. To tube #1 add 10 ml of DI water.
3. To tube #2 add 10 ml of the warm sucrose solution.
4. Test tube #3 add 10 ml of the corn syrup solution.
5. Obtain more fermentation tubes if there are other possible substrates available.
6. Add 5 ml of activated yeast solution to each tube and carefully mix the solutions.
7. Carefully tip the fermentation tubes to remove air bubbles as directed by your instructor.
8. Place a cork on each of the tubes.
9. Place the fermentation tubes in the 40°C waterbath.
10. Record and measure any gas production with a millimeter ruler every 5 minutes for 30 minutes. Note any changes in the appearance of the tubes.

Anaerobic respiration and food items

Microbes have adapted to live virtually anywhere on Earth. Given time, microbes can evolve to the given set of environmental conditions and thrive. Since we do not live in a sterile environment, we have learned to cope with microbes. We often ingest microbes when we eat. For the most part, these microbes do not affect us. However, some microbes can cause illness, infections and diseases. We have developed a symbiotic relationship with certain microbes. Take for instance the various types of bacteria we have living on us and with our intestines. We provide the microbes within our intestines with a "home" and food. In return, they assist us in fighting off pathogens and provide some nutrients.

About a thousand years ago, our ancestors began utilizing beneficial strains of microbes in preserving food. There are literally hundreds of food items world wide that result from fermentation. Most of these food items were the result of microbial interactions in detoxifying substances. Europeans have long used microbes to produce wine as a source of "clean" water. Bulgarians were one of the first to preserve milk in the form of yogurt and cheese. Many of the items that we find at our grocery stores were developed from fermentation (sauerkraut, pickles, kim chi, kefir, etc.).

In this section, you may use the microbes found in various grocery items to examine the natural fermentation process. We will use the ancient art of pickling to examine anaerobic fermentation of sugars to lactic acid. The acid will lower the pH, therefore creating an environment in which food-spoiling organisms can not grow. After the fermentation process, we'll try the products, as long as there is not an overabundance of fermentation bacteria present and there has not been any spoilage (which should not occur, but could).