# 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

## 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 $FADH_2$. When these coenzymes are reoxidized in the electron transport chain, many molecules of ATP are generated (a theoretical 32 – 38 ATP per glucose). Many living organisms undergoing aerobic respiration will use oxygen and produce carbon dioxide.

In this lab you will determine aerobic respiration indirectly by measuring the production of carbon dioxide using a Pasco Data Logger system.

## 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, aCO2 probe and the CO2 measurement container.
3. To the container, add a 2 cm ball of **absorbent** cotton to the bottom.
4. Add the peas to the container.
5. Wrap the container with aluminum foil to inhibit photosynthesis.
6. Place the CO2 probe into the container, sealing tightly.

*Measurements*

1. Connect the CO2 probe to one of the four PASPORT sensor ports on the Xplorer GLX **WITHOUT** tipping the container upside down (do not let the wet peas hit the measuring probe. NOTE: They look like serial cable ports on the end above the screen).
2. Turn the GLX "ON" (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. To assure accuracy, do not handle the container with your warm hands (NOTE: We are measuring gas volumes).
6. After five minutes, press the arrow to start data recording. Record for 10 minutes.
7. To stop data recording, press the same button again.
8. Press the Home Screen button for the menu display.
9. Use the cursor and select Graph menu and press activate (the checkmark key).
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.

Obtain a new 2 cm cotton ball and repeat the procedure with the frozen/thawed peas. When you have finished discard the cotton ball, return the peas to the original container and clean out the container for the next group.

*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 activate (the checkmark key).
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 (the checkmark key).
6. Press F4 and cursor down to Export All Data and press activate (the checkmark key).
7. Your data should be downloaded to your drive.
8. On your computer, 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 $r^2$ value.

**$CO_2$ production during aerobic respiration**

From earlier labs you should recall that $CO_2$ can combine with water for form carbonic acid which dissociate as follows:

$$CO_2 + H_2O \leftrightarrow H_2CO_3 \leftrightarrow H^+ + HCO_3^- \leftrightarrow H^+ + H^+ + CO_3^{2-} \qquad (3)$$

In this exercise, you will indirectly determine the amount of $CO_2$ produced during cellular respiration in a plant and an aquatic animal. You will use phenolphthalein to detect changes in pH resulting from $CO_2$ production ($H_2CO_3$). Recall that phenolphthalein is red in basic solutions and colorless in acidic solutions. Since we are not directly measuring $CO_2$ 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. 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 are 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 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*

8. Add four drops of phenolphthalein to the contents of each experimental beaker. The solution should be clear.
9. 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).
10. 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 4.

*Calculations*

11. The relative respiration rate for each organism, for twenty minutes, is the number of ml NaOH added to the organism' water minus ml of NaOH added to the control water.
12. The weight specific metabolic rate is relative respiration rate divided by 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 $CO_2$ 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 5 ml of DI water.
3. To tube #2 add 5 ml of sucrose solution.
4. Test tube #3 add 5 ml of 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 mix gently.
7. Place a cork on each of the tubes.
8. Carefully tip the fermentation tubes to remove air bubbles as directed by your instructor.
9. 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 bacterial 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 worldwide that result from fermentation. Most of these food items were the result of microbial interactions in detoxifying substances. Europeans have long use 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, yogurt, etc.).

*Making Yogurt: Lactose to Lactic Acid Fermentation*

Yogurt originated in the Balkans and the Middle East; it is now quite popular in Europe and America, as well. The microorganisms used in the production of yogurt accomplish two tasks: production of lactic acid and flavor components. The secret to tasty yogurt is in the proper control of the temperature at various stages. If the temperature is too low, the culture grows too slowly to adequately acidify milk and to achieve a good texture. The commercial starter is a mixed culture of *Lactobacillus thermophilus* and *L. bulgaricus*. These cultures are killed if the temperature is too high. In addition, there is a subtle difference in the taste because the formation and secretion of metabolites which contribute to the overall taste are dependent on the growth rate. The window of proper fermentation is quite small, i.e. from 42 ºC to 44 ºC. In general, as the temperature is raised up to 44 ºC, the rate of culture metabolism is higher, and the yogurt is sweeter. Faster growth also prompts the yogurt to set faster. When the desired acidity is reached, yogurt is cooled to halt further fermentation and metabolic activity. This cooling step is quite critical in industrial yogurt production; it must be done quickly to control tightly the acidity of the yogurt, which has a profound effect on the taste.

Procedure for making yogurt

1. Heat ½ gallon (2 quarts) of milk slowly to 85 ºC and maintain at that temperature for 2 minutes. This step kills undesirable contaminant microorganisms. It also denaturizes inhibitory enzymes that retard the subsequent yogurt fermentation.

2. Cool milk in a cold water bath to 42-44 ºC. The cooling process should take about 15 minutes.

3. Add two or three tablespoons of live culture yogurt to the milk and mix well.

4. Cover the container to minimize the possibility of contamination. Incubate at 42ºC for 3 to 6 hours undisturbed until the desired custard consistency is reached. Yogurt is set when the mixture stops flowing as the container is tipped slowly. Fluid yogurt results if the mixture is stirred as the coagulum is being formed.

5. The fresh made yogurt is ready for consumption when it is set. However, you may want to refrigerate it first if you are not accustomed to warm yogurt. Refrigeration also stops the growth of the lactic acid culture, which is thermophilic. (Thermophilic cultures grow best at high temperatures.)

6. For entrepreneurs or simply hungry/thrifty students: You can recycle a small part of the finished product as the starter culture for the next batch. Theoretically, you can multiply or maintain your supply of yogurt indefinitely. However, in actuality, extended recycling is not recommended because the composition of the mixed culture will gradually change, and thus the flavor will not be consistent.

**Biology 3A Laboratory** **Name: _______________________**
**Cellular Respiration Worksheet** **Lab Section: ___________**

## A. Aerobic respiration in germinating peas

1. Using Excel, graph the carbon dioxide procudtion data as a function of time. Calculate the slope of each line. The slope of the line is the **respiration rate** (ml/min or ml $min^{-1}$).

2. For what were we testing in this experiment? State a reasonable hypothesis.

3. Using Excel, graph the mass specific oxygen consumption for the peas that would support your hypothesis.

4. Did freezing and then thawing the peas inhibit respiration? What was the percentage of inhibition?

5. Why was it necessary make the mass specific determination?

6. If you repeated the experiment with half as many seeds, how would the respiration rate (slope of the line) be different?

7. Would the mass-specific respiration rate be different for the sample from question #6? Explain.

**B. $CO_2$ production during aerobic respiration**

8. State a reasonable hypothesis being tested in this experiment.

**Table 4**. Data for $CO_2$ production during aerobic respiration

| Tube | Total mass of Organism (gm) | ml of NaOH | Relative respiration rate (ml NaOH) | Resp. Rate/ gm of organism (ml NaOH/ gm organism) |
|---|---|---|---|---|
| **Goldfish** | | | | |
| **Elodea** | | | | |
| **Control** | ----- | | ----- | ----- |

9. Briefly explain why *Elodea* was placed in the dark.

10. What most likely contributed to the observed differences in respiration rate?

11. Explain why you think snails were chosen for comparison.

## C. Anaerobic respiration

**Table 5**. Anaerobic respiration in yeast

| | | Time (minutes) | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **Tube** | **Contents** | **0** | **5** | **10** | **15** | **20** | **25** | **30** |
| 1 | | | | | | | | |
| 2 | | | | | | | | |
| 3 | | | | | | | | |
| 4 | | | | | | | | |

12. Which is (are) the control tube(s)? Which are the experimental tube(s)?

13. Smell the contents of the tube containing the most $CO_2$. Describe this smell?

14. Consider the results of this experiment. Can yeast utilize all of the sugars equally well? Use the results to support you explanation (i.e. quote specific numerical values from the results).

15. Consider the sugars that *were* metabolized in this experiment. Why do you think they were metabolized at different rates?