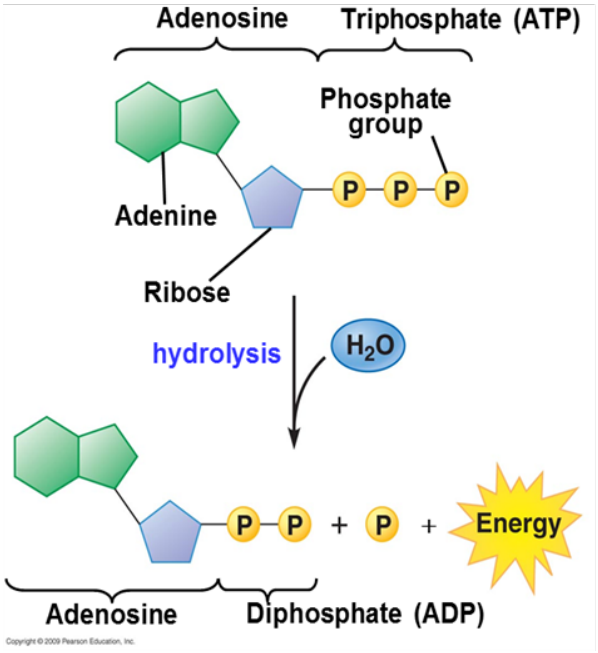
LAB 7 – Fermentation & Cellular Respiration

Objectives

1. Measure fermentation products produced by yeast.
2. Assess citric acid cycle activity in mitochondria.

INTRODUCTION

The cells of all living organisms require energy to keep themselves alive and fulfilling their roles. Where does this energy come from? The answer is energy released from molecules of the nucleotide **adenosine triphosphate** or **ATP**.



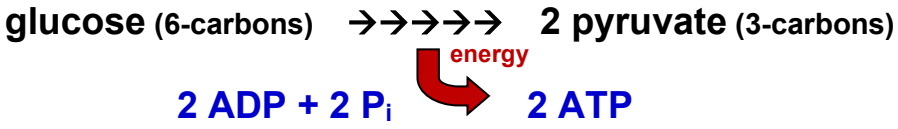
As you can see in the diagram to the left, the hydrolysis of **ATP** to **ADP (adenosine diphosphate)** and **inorganic phosphate (P_i)** is **exergonic** and thus *releases* energy which cells can use to do any number of things. Once hydrolyzed, ATP can be regenerated from ADP and P_i, though this is **endergonic** and thus *requires* energy. The energy needed to regenerate ATP is obtained from "food", whatever that may be.

The food we eat is first digested by enzymes as you learned in the previous lab. Once the polymers in your food (e.g., polysaccharides, triglycerides, protein) have been broken down by enzymes into monomers (e.g., monosaccharides such as glucose, fatty acids, amino acids), they enter the blood circulation and are delivered to the cells of the body. Within cells, the processes of **fermentation** and **cellular respiration** will further **catabolize** (break down) these molecules, harvesting the energy they contain for the synthesis of ATP.

Let us now take a brief look at **fermentation** and **cellular respiration** to see how each process produces ATP using energy released from molecules of **glucose**. Keep in mind that, although we are focusing on glucose, other molecules such as fatty acids can be used for the same purpose, though in slightly different ways.

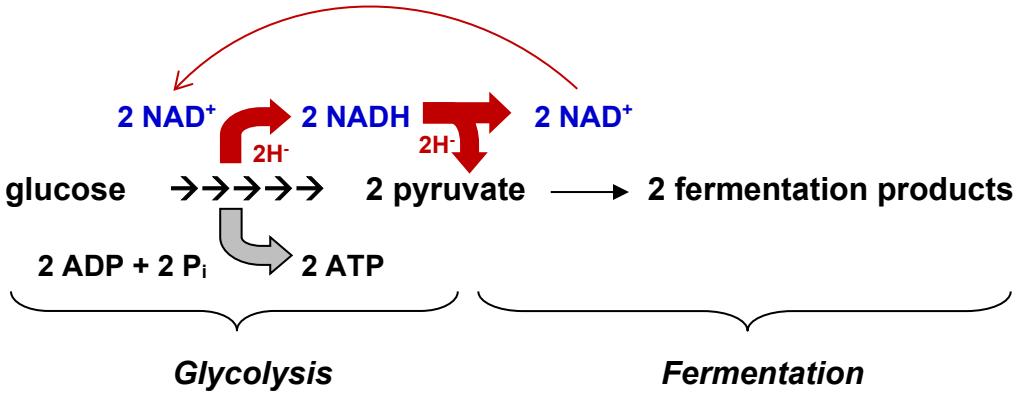
Part 1: FERMENTATION

To produce ATP from glucose, whether by fermentation or cellular respiration, cells must first partially break it down by **glycolysis** ("sugar" "separation"). The enzymes involved in glycolysis are located in the cell cytoplasm and sequentially break down each 6-carbon molecule of glucose to two 3-carbon molecules of **pyruvate**. In the process, enough energy is extracted to produce 2 molecules of ATP.



In conjunction with glycolysis, cells will carry out fermentation if there is no oxygen (O_2) available. When you overexert yourself for example, your muscles do not receive enough oxygen and temporarily ferment glucose. In another familiar example, yeast will ferment when placed in an enclosed environment with a source of carbohydrate such as grapes (for making wine) or hops and barley (for making beer).

Interestingly, fermentation does not produce any additional ATP. What it does do is regenerate an important molecule needed for a particular step in glycolysis. This molecule is the electron carrier NAD^+, which if depleted will bring a halt to glycolysis and ATP production, resulting in cell death. Fermentation therefore contributes to ATP production indirectly by allowing glycolysis, and the production of 2 ATP per glucose, to continue unhindered.



As shown above, NAD^+, an *empty* **electron carrier**, is converted to NADH, a *full* electron carrier (the electrons being "carried" are associated with the hydrogen atom) during glycolysis. Fermentation is simply one or more biochemical steps that transfer the H in NADH and an extra electron to a molecule of pyruvate. As a result, NADH is restored to NAD^+, which is needed for glycolysis, and pyruvate is converted to a "fermentation product" which can be a variety of things depending on the organism.

Animals, including human beings, produce **lactic acid** when their cells ferment. In organisms from other kingdoms the fermentation products can be quite different. Some bacterial species produce acetic acid (vinegar) when they ferment, whereas others produce acetone (the main ingredient in nail polish) or other organic molecules. In the Kingdom Fungi, single-celled yeasts

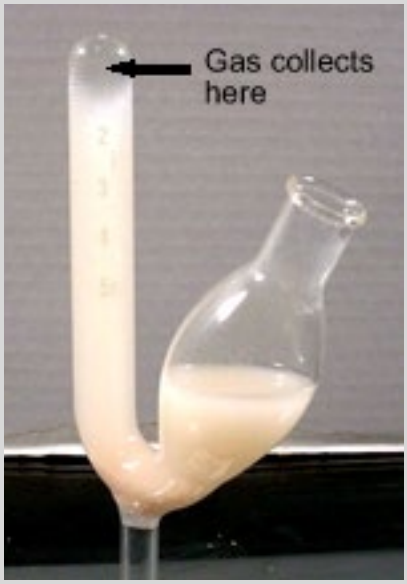
when fermenting will produce **CO_2** and **ethanol** instead. This process, known as **alcohol fermentation**, is the basis for beer and wine production. Regardless of the fermentation products, the purpose of fermentation is always the same – to regenerate NAD^+ so that glycolysis can continue to produce 2 ATP per glucose without interruption.

In the following exercise you will investigate alcohol fermentation in yeast under different conditions and measure the production of one fermentation product – CO_2.

Exercise 1 – Observing and Measuring Alcohol Fermentation in Yeast

1. You will use the following table to mix the proper amounts of water, yeast solution and corn syrup (a source of sugar) in small beakers. *Be sure to add the yeast last.* This will allow the reactions to begin at approximately the same time. Before you begin, review the experiment, write your hypothesis on your worksheet, and identify the independent and dependent variables as well as the control.

	Tube #1	Tube #2	Tube #3
Water	8 ml	6 ml	4 ml
Corn syrup	4 ml	4 ml	4 ml
Yeast	0 ml	2 ml	4 ml
TOTAL	12 ml	12 ml	12 ml



2. Once complete, transfer each mixture to a *labeled* saccharometer (do not overfill), gently tilting until no air is trapped inside the top of the tube. At this point you will begin the timing of your experiment (record on your worksheet).

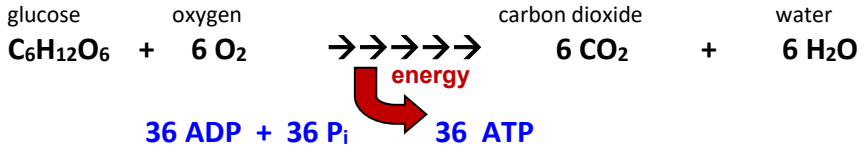
3. At 5 minute intervals, record the volume of gas collected in each saccharometer on your worksheet. (*NOTE: the saccharometers are graduated in **ml***)

4. Record the data on your worksheet, graph the data **(*be sure your X-axis is <u>time</u> and draw a separate curve for each tube*)**, and answer any associated questions.

<u>Part 2: CELLULAR RESPIRATION</u>

While 2 ATP per glucose molecule is clearly better than nothing, it is not nearly enough to meet the energy needs of complex multicellular organisms such as plants and animals. To get the maximum ATP yield from molecules of glucose requires cellular respiration, which and produce up to 36 ATP per glucose molecule. In **aerobic** organisms, cellular respiration requires O_2 (which is why we breathe!), hence the term **aerobic respiration**.

The overall process of cellular respiration can be summarized in the following equation:



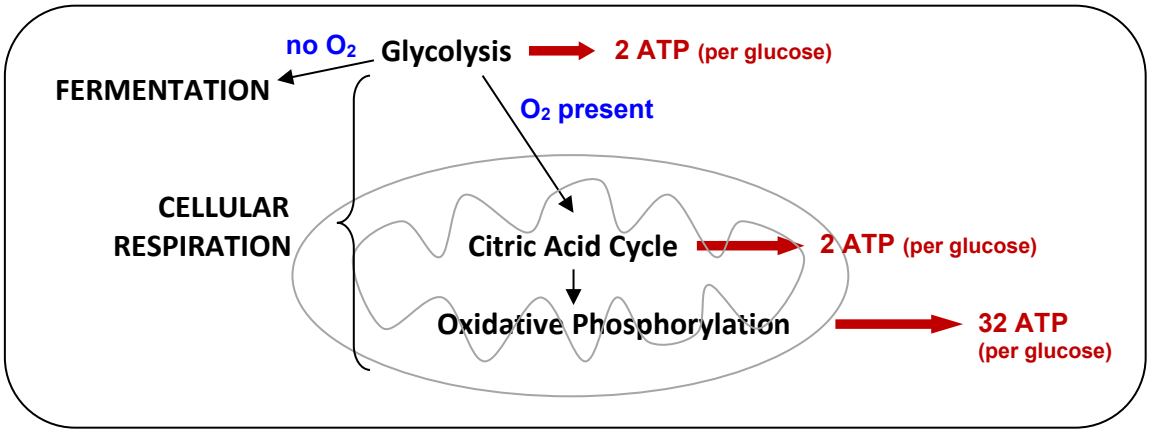
In eukaryotic cells, cellular respiration begins with glycolysis in the cytoplasm and continues in the mitochondria as outlined below:

The Citric Acid Cycle – This is a biochemical pathway involved in breaking pyruvate down to CO_2. In the process, energy rich electrons in hydrogen atoms are transferred to NAD^+ and FAD producing NADH and $FADH_2$. In addition, 2 ATP per original glucose are also produced.

Oxidative Phosphorylation – This is the process by which the remaining 32 ATP molecules are produced involving two distinct stages:

- **Electron Transport** - electrons gathered by NADH and $FADH_2$ during glycolysis and the citric acid cycle are used to produce an H^+ gradient within mitochondria in a process that requires O_2
- **Chemiosmosis** – the H^+ gradient produced by electron transport provides energy for ATP synthase to make 32 ATP per original glucose

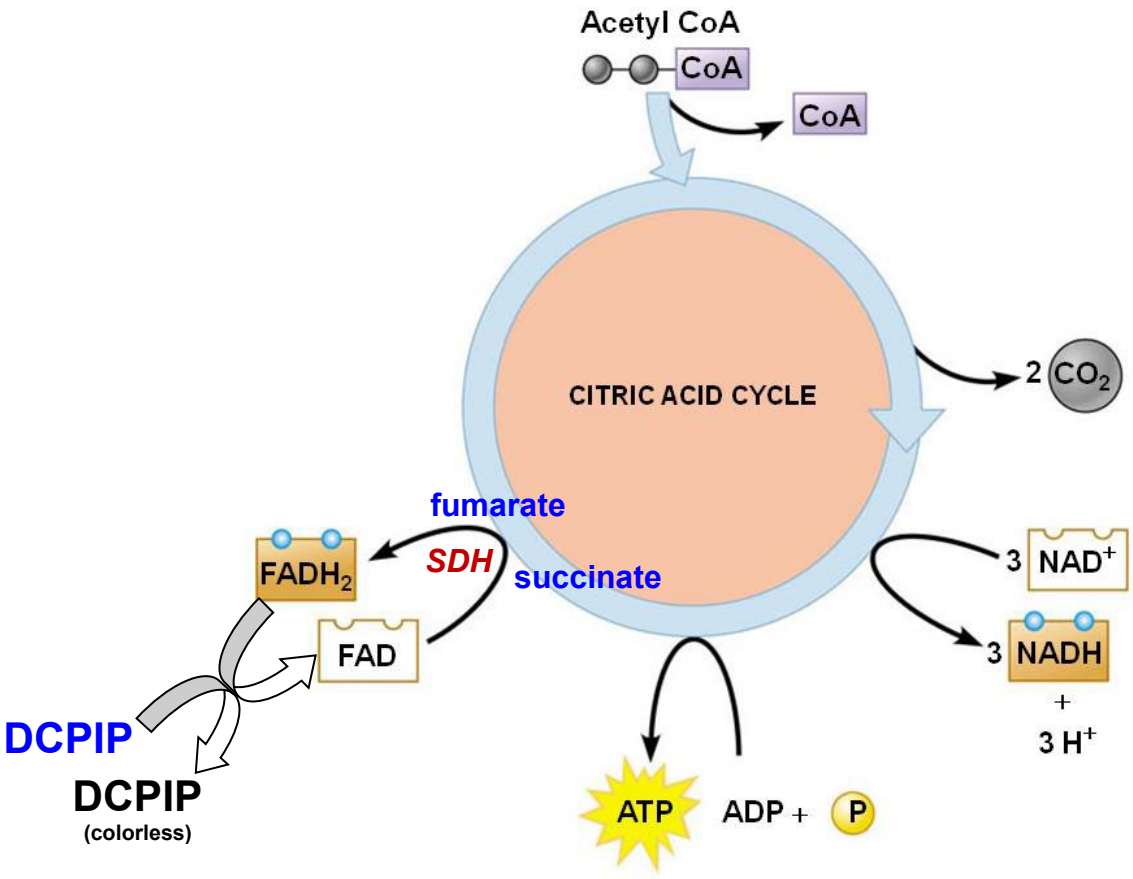
The importance of O_2 for cellular respiration cannot be overemphasized. O_2 is the final electron acceptor in the electron transport chain. Without O_2 electron transport does not occur, bringing cellular respiration to a halt, and the only option for ATP production is fermentation. This means 2 ATP per glucose instead of 36. The cell diagram below summarizes fermentation and cellular respiration in relation to O_2 and where each process occurs in eukaryotic cells, and the number of ATP molecules produced.



In the next exercise you will detect the oxidation of **succinate**, a metabolic intermediate in the Citric Acid Cycle, as evidence of cellular respiration. **Succinate dehydrogenase (SDH)** is an enzyme in the Citric Acid Cycle which catalyzes the removal of 2 hydrogens from succinate (i.e., the oxidation of succinate) which are transferred to the electron carrier **FAD**. This yields the products **fumarate** and **$FADH_2$** as shown below:

$$\textbf{FAD + succinate} \xrightarrow{\textit{succinate dehydrogenase}} \textbf{FADH}_2 \textbf{ + fumarate}$$

$FADH_2$ in turn will donate the electrons from these 2 hydrogens to **coenzyme Q** in the electron transport chain. The compound **DCPIP** (di-chlorophenol-indophenol) is not normally found in cells, however when added to mitochondria it will substitute for coenzyme Q and receive electrons from $FADH_2$. Before receiving the electrons (in its *oxidized* state) DCPIP is a blue color, however after receiving the electrons (being *reduced* by $FADH_2$) DCPIP is colorless. Because of this color change, DCPIP is a good indicator of respiration as illustrated below.



In the next exercise you will add DCPIP to a mitochondrial suspension made from lima beans (yes, plants carry out cellular respiration too!) and detect the citric acid cycle step illustrated above by the loss of blue color in DCPIP.

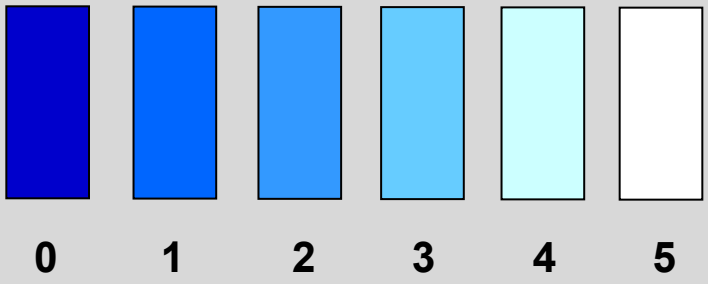
Exercise 2 – Detecting cellular respiration in a mitochondrial suspension

1. Review the experiment below, write your hypothesis on your worksheet and identify the independent and dependent variables as well as the control.

2. Label 3 test tubes and add the components indicated in the chart below, in order:

	Tube #1	Tube #2	Tube #3
Phosphate Buffer Solution	4.2 ml	3.9 ml	3.6 ml
DCPIP	0.3 ml	0.3 ml	0.3 ml
Succinate Solution	0 ml	0.3 ml	0.6 ml
Lima Bean Extract	0.5 ml	0.5 ml	0.5 ml
TOTAL	5.0 ml	5.0 ml	5.0 ml

3. Make sure each tube is mixed and score the color of each tube every 5 minutes for a total of 30 minutes using the scale shown below:



4. Graph color score vs time for each tube and answer the associated questions.

Part 3: DESIGNING AN EXPERIMENT

Having investigated alcohol fermentation in yeast and cellular respiration in a mitochondrial suspension, you and your group will design and carry out a new experiment to expand on what you have already learned.

Exercise 3 – Design an experiment

1. Decide as a group to further investigate yeast fermentation or cellular respiration in lima bean mitochondrial suspension.

2. Identify an independent variable you have not already investigated (e.g., amount of corn syrup or mitochondrial suspension) and come up with a hypothesis with regard to this variable. Write the hypothesis on your worksheet.

3. Design an experiment to test this hypothesis. On your worksheet, briefly describe your experimental plan, and identify the independent variable, dependent variable and control.

4. Carry out your experiment, record and graph the results on your worksheet, and write your conclusion.

Before you leave, please make sure your table is clean, organized, and contains all supplies listed below so that the next lab will be ready to begin. Thank you!

Supply List

- Small bottle of water
- Small bottle of corn syrup 50%
- Pipettes for water, yeast, and corn syrup (rinsed out with hot water)
- Pipette pump
- 3 Saccharometers (**carefully** rinsed with hot water)
- Marker pen or China marker
- 3 beakers (50 ml)
- 3 stirring rods
- 3 test tubes in rack

Also PLEASE be sure to do the following before you leave:

- *Dispose of yeast solution down the drain and rinse out saccharometers with hot water*
- *Dispose of all DCPIP waste in the jug in the fume hood.*
- *Wash test tubes with soap and water using brush and leave upside down in test tube rack in sink to drain.*

Laboratory 7 worksheet – Fermentation & Cellular Respiration

Name: _____ **Group:** _____ **Date:** _____

Exercise 1 – Yeast fermentation

Start time: _____End Time: _____

State your hypothesis below and identify the indicated components of this experiment:

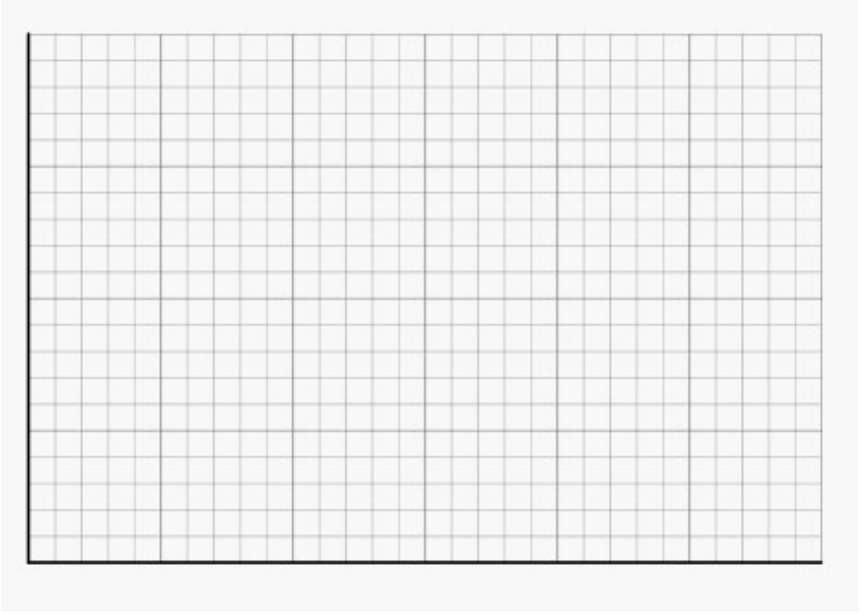
- **Hypothesis:**

- Independent variable:
- Dependent variable:
- Control:

Results:

	0 min	5 min	10 min	15 min	20 min	25 min	30 min
Tube 1							
Tube 2							
Tube 3							

On the grid below, graph the results for each tube by plotting the amount of gas produced vs time.



- Did these results support your hypothesis? Explain.

Exercise 2 – Cellular respiration

Start time: _____End Time: _____

Indicate the roles of each of the following components in your experiment:

Lima bean extract:

Succinate:

DCPIP:

Buffer:

State your hypothesis below and identify the indicated components of this experiment:

- **Hypothesis:**

- Independent variable:
- Dependent variable:
- Control:

Results:

	0 min	5 min	10 min	15 min	20 min	25 min	30 min
Tube 1							
Tube 2							
Tube 3							

On the grid below, graph the results for each tube by plotting the color score vs time.

- Did these results support your hypothesis? Explain.

- Why was it important for this and the previous experiment to keep the total volume of each tube constant? (*HINT: Consider the final concentrations of the components in each reaction*)

<u>Exercise 3</u> – Design an experiment

- State your **hypothesis**:

- Provide the details of your experiment below:

Identify the indicated components of your experiment:

- Independent variable:
- Dependent variable:
- Control:

Draw a chart or table and record the results of your experiment below:

Graph your results on the grid below:

- Did these results support your hypothesis? Explain.