LAB EXERCISE: Fermentation

Laboratory Objectives
After completing this lab topic, you should be able to:
1. Describe alcoholic fermentation, naming reactants and products.
2. Propose hypotheses and make predictions based on them.
3. Design and execute an experiment testing factors that influence fermentation.
4. Practice scientific communication by analyzing and interpreting experimental results.

Introduction
This lab topic investigates fermentation, a cellular process that transfers the energy in glucose bonds to bonds in adenosine triphosphate (ATP). The energy in ATP can then be used to perform cellular work. Fermentation is an anaerobic (without oxygen) process; cellular respiration is aerobic (utilizing oxygen). All living organisms, including bacteria, protists, plants, and animals, produce ATP in fermentation or cellular respiration and then use ATP in their metabolism.

Cellular respiration is a sequence of three metabolic stages: glycolysis in the cytoplasm and the Krebs cycle and the electron transport chain in mitochondria (Figure 1). Fermentation involves glycolysis but does not involve the Krebs cycle and the electron transport chain, which are inhibited at low oxygen levels. Two common types of fermentation are alcoholic fermentation and lactic acid fermentation. Alcoholic fermentation begins with glycolysis, a series of reactions breaking glucose into two molecules of pyruvate with a net yield of 2 ATP and 2 NADH molecules. In anaerobic environments, in two steps the pyruvate (a 3-carbon molecule) is converted to ethyl alcohol (ethanol, a 2-carbon molecule) and CO2. In this process the 2 NADH molecules are oxidized, replenishing the NAD+ used in glycolysis (Figure 1).

Figure 1. Stages of cellular respiration and fermentation. Cellular respiration consists of glycolysis, the Krebs cycle, and the electron transport chain. Glycolysis is also a stage in fermentation.
In this lab exercise, you will investigate alcoholic fermentation in a yeast (a single-celled fungus), *Saccharomyces cerevisiae*, or baker’s yeast. When oxygen is low, some fungi, including yeast and most plants, switch from cellular respiration to alcoholic fermentation. In this laboratory experiment, the carbon dioxide (CO₂) produced can be used as an indication of the relative rate of fermentation taking place. The rate of fermentation, a series of enzymatic reactions, can be affected by several factors, for example, concentration of yeast, concentration of glucose, or temperature. In this exercise you will investigate the effects of yeast concentration. Your team will then design and carry out an independent investigation based on examining other independent variables (performed during weeks 6-7).

**EXERCISE: Alcoholic Fermentation**

**Materials**
- Vernier Laq Quest
- Vernier Gas Pressure Sensor
- 4 test tubes
- test tube rack
- rubber-stopper assembly
- 10% yeast solution
- Pipette pump
- 3X 5 mL or 10 mL glass pipettes
- vegetable oil in dropper bottle
- sharpie
- 5% glucose solution
- DI water

**Hypothesis**
Hypothesize about the effect of different concentrations of yeast on the rate of fermentation.

**Prediction**
Predict the results of the experiment based on your hypothesis (if/then).

**Procedure**
1. Obtain four test tubes and label them 1-4.
2. Add 5 mL of the glucose solution into test tubes 1, 3 and 4.
3. Add 5 mL DI water to test tube 1, mix the water and glucose solution gently, by pipeting the solutions back into and out of the pipette, then place enough vegetable oil to completely cover the surface of the fermentation solution as shown in Figure 2. Be careful to not get oil on the inside wall of the test tube.

![Figure 2.](image-url)
4. Incubate the test tube for 10 minutes at room temperature.

5. When the incubation has finished, Insert the single-holed rubber-stopper into the test tube. **Note:** *Firmly* twist the stopper for an *airtight* fit.

6. Plug in Vernier LabQuest.

7. We are using a gas sensor, it is set to kPa, it will record for 900s which equals 15 minutes. If it is not reading kPa, go to sensor button and change the units of measure.

8. At the bottom left is a half-moon button, this is the start and stop. When you press the half-moon the collection will start. It will be listed as run 1. After data collection stops, click on graph to see your graph.

9. On analyze, click on curve fit. Under choose fit, pick linear view. Record the graph equation in the form of \( y = mx + b \), where \( m \) is the slope of the fermentation rate. Write the slope on your table.

10. While you are collecting your data for test tube 1, prepare test tube 2. Gently swirl the yeast suspension to mix the yeast that settles to the bottom. Using a pipette, transfer 5 mL of yeast into test tube 2. Be gentle with the yeast—they are living organisms! Add 5 mL DI water to the yeast in test tube 2. Add oil. Allow the tube to incubate at room temperature for approximately 10 minutes.

11. Repeat the data collection steps for test tube 2.

12. While you are collecting your data for test tube 2, prepare test tube 3. Gently swirl the yeast suspension to mix the yeast that settles to the bottom. Using a pipette transfer 2 mL of yeast and 3 mL of DI water into test tube 3. Add oil. Allow the tube to incubate at room temperature for approximately 10 minutes.

13. Repeat the data collection steps for test tube 3.

14. While you are collecting your data for test tube 3, prepare test tube 4. Gently swirl the yeast suspension to mix the yeast that settles to the bottom. Using a pipette transfer 5 mL of yeast into test tube 4. Add oil. Allow the tube to incubate at room temperature for approximately 10 minutes.

15. Repeat the data collection steps for test tube 4.
<table>
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<th>Yeast alone</th>
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Research Proposal
As we discussed at the start of the quarter, you will be required to write a scientific research report on an independent investigation performed during weeks 6-7. Your independent investigation will be based on the Fermentation lab performed in week 3 (April 17/18). The purpose of going to the library and learning about effective literature search techniques was to assist you in the process of developing a well designed experiment.

Your group objective is to design a simple experiment to investigate some factor that affects alcoholic fermentation (based on the fermentation experiment performed today).

Below is a list of questions that will help you to prepare the proposal.

As a group you will turn in one typed proposal. Be sure to include the following:

1. Question being asked.
2. Explanation of the rationale behind your choice of question.
   For example, if you choose to investigate starch as a substrate, you should be able to explain that the yeast must first digest starch before the glucose can be used in alcoholic fermentation and the impact this might have on the experiment.
3. Hypothesis
4. Prediction
5. Complete list of materials
6. Detailed protocol

Possible Questions (you may come up with your own!):

1. Would other substrates be as effective as glucose in alcoholic fermentation?
   For example:
   - Sucrose (table sugar)
   - Starch
   - Saccharin
   - Fructose
   - Pyruvate

2. What pH is optimum for alcoholic fermentation?
3. Sodium fluoride, commonly used to prevent tooth decay, inhibits an enzyme in glycolysis. At what concentration is it most effective?
4. Would adding MgSO$_4$ enhance glycolysis? MgSO$_4$ provides Mg$^{++}$, a cofactor necessary to activate some enzyme in glycolysis.
5. Does a high concentration of glucose inhibit fermentation?
6. Salt is often used as a food preservative to prevent bacterial and fungal growth. But salt is also important to enhance the flavor of bread when added in small amounts. At what concentration does salt begin to inhibit yeast fermentation?

You will have three lab periods to conduct your experiment. You will be present and use all three lab periods so keep that in mind when designing your experiment, number of repeats etc.