

LAB 11:

Fermentation

I. Objectives:

Upon completion of this topic you should be able to describe:

- the role of glucose and ATP in the powering of cellular reactions
- the different types of fermentation in metabolism
- the products of fermentation in yeast
- how different sugars, temperature, and pH affect the rate of fermentation

II. Safety Considerations:

- Parts A to C (Yeast Fermentation) note that the water bath is at 70°C (hot). Also be careful not to leave spilled ice on the floor for others to slip on.

III. Introduction:

All cells must acquire and then use energy to carry out their necessary functions (moving, growing, dividing, etc). Cells acquire their energy by either using sunlight to make the high-energy molecule glucose, or by consuming organisms that have glucose (or other high-energy molecules). Regardless of how the glucose is obtained, all organisms (even plants) must then break down glucose in order to harness the stored energy and do something useful. It may be surprising that no cellular process is powered directly by the breakdown of glucose. Glucose stores so much energy that if it were all released at once, it could damage the cell. In much the same way your car burns its gasoline a small amount at a time (instead of in one big fiery explosion), cells harvest the chemical energy of glucose a small amount at a time over several steps. Collectively, the set of chemical reactions used to harvest the chemical energy of glucose as it is broken down into carbon dioxide (CO₂) and water is called **cellular respiration**. Through cellular respiration, the chemical energy in glucose is used to create a large number of high-energy molecules of adenosine triphosphate--**ATP**.

For most cells, cellular respiration can be divided into two general steps; glycolysis and oxidative phosphorylation. **Glycolysis** is the set of chemical reactions that starts the breakdown of glucose. These reactions take place in the cytosol and do not require oxygen (O₂) and are therefore **anaerobic**. If oxygen is available, cells are able to use the pyruvate from glycolysis in the oxidative phosphorylation phase of **aerobic respiration**. If oxygen is not available, cells are only able to carry out fermentation, which yields far less ATP than does aerobic respiration. See Figure 1 below (this is figure 9.19 from your text).

Fig. 9-19

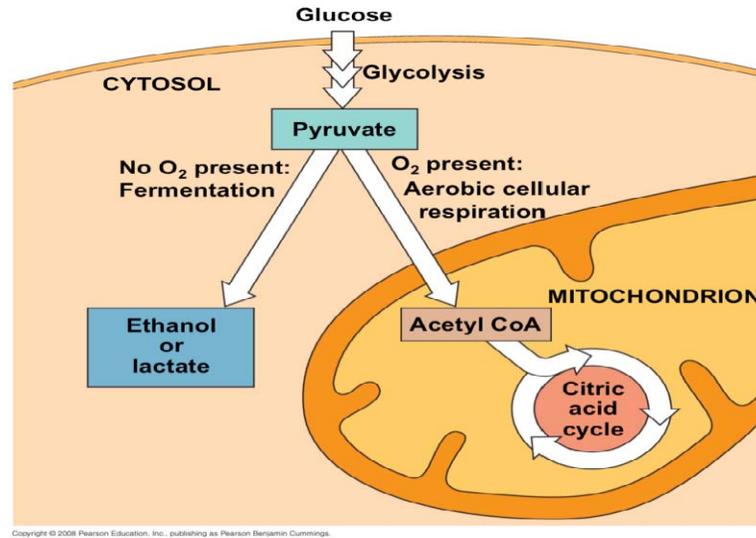


Figure 1. Energy conversion in cells under aerobic vs. anaerobic conditions. (From Campbell et al., 2009)

Some cells can capture energy from glucose in oxygen-deficient environments by carrying out glycolysis followed by fermentation. Some cells, such as yeast, even prefer fermentation to oxidative phosphorylation, even if oxygen is present. Fermentation is also a way for cells to regenerate NAD^+ , which is used in glycolysis when it is converted to NADH . In yeast, NAD^+ is regenerated in a two-step process called **alcoholic fermentation**. In the first step, CO_2 is released when pyruvate is converted to acetaldehyde. In the second step, acetaldehyde is reduced to ethanol by NADH , which regenerates NAD^+ . When oxygen is not present in our cells, our cells carry out fermentation also, but in this case, lactic acid is produced in the regeneration of ATP and no CO_2 is released (Figure 2). This process is called lactic acid fermentation.

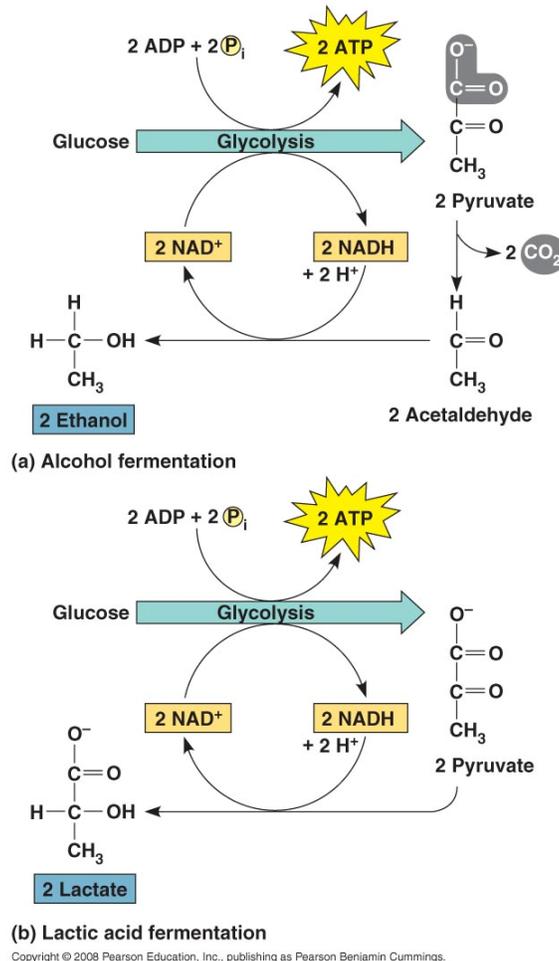


Figure 2. Alcohol and Lactic Acid Fermentation (From Campbell et al., 2009)

Introduction for Part A – Yeast Fermentation Of Different Sugars:

In this experiment, we will test the ability of yeast to ferment different sugars. Two of the sugars (glucose and fructose) are monosaccharides, or simple sugars. The other two sugars (sucrose and lactose) are disaccharides--they are each made up of two simple sugars. Sucrose is composed of linked glucose and fructose monosaccharides and lactose is composed of one glucose monosaccharide and one galactose monosaccharide. Only monosaccharides can be used directly by yeast in fermentation. This means that sucrose and lactose must be broken down to their component monosaccharides by the yeast before fermentation can occur.

Because CO₂ is released during fermentation of sugars by yeast, it provides a convenient way of measuring how much fermentation (glycolysis) has taken place. The rate of fermentation can be measured by placing a small amount of yeast and sugar solution in a fermentation tube. As CO₂ is produced, the bubbles collect at the top of the tube. The fermentation rate of the yeast can be calculated by measuring the volume of CO₂ at the top of the tube and dividing it by the amount of time it took for that volume to form.

In this exercise, you will be testing and comparing the fermentation rates of yeast cells that are using different sugars. It is important that you label your tubes (with a grease pencil) so that you

can identify what sugars are in each tube. After the solutions are mixed in the tubes, you will be tipping the tubes, placing them in a water bath, and recording the time. As soon as the gas in ONE of the tubes reaches the halfway mark, you will record the time and remove ALL the tubes, including those that haven't yet reached the halfway mark. You will take the tubes back to your table and IMMEDIATELY measure the volume of gas in each, recording the volumes and time in the appropriate table.

Materials for Part A, B, and C:

- | | | |
|----------------------|-------------------------------|------------|
| 2 - 100 ml beakers | 2 - 10 ml graduated cylinders | 2 pipettes |
| 4 fermentation tubes | 1 grease pencil | |

Procedure for Part A:

- Label 4 clean fermentation tubes (1- 4). Take one of your beakers to the side of the room and obtain 50 ml of stock yeast suspension. Be sure to mix the suspension before dispensing. Using the graduated cylinder in your tray, measure and pour 5 ml of the yeast suspension into each of your fermentation tubes. You may need to use a pipette to accurately bring the volume of the graduated cylinder to exactly 5 ml.
- To each of the tubes, add 7 ml of a single sugar. Use the graduated cylinder (rinsing it and the pipette after each use so as not to cross-contaminate your solutions) to measure and pour:
 - 7 ml of glucose solution to Tube 1
 - 7 ml of fructose solution to Tube 2
 - 7 ml of sucrose solution to Tube 3
 - 7 ml of lactose solution to Tube 4
- Tip the fermentation tubes so that the vertical column of each tube fills with the liquid.
- Place ALL of the tubes in a 37° C (body temperature) water bath and record the time.
- Monitor the amount of CO₂ produced. This may take some time. When ONE of the tubes is half filled with CO₂ (3 ml) record the time, and remove ALL tubes from the water bath.
- Measure the volume (in ml) of gas in each tube and record it in Table 1 below. Calculate the fermentation time by subtracting the starting time from the ending time, and record it in the table. Calculate the fermentation rate of each of the sugars from these data.
- WASH AND RINSE ALL OF YOUR TUBES (contents can be poured down the sink). Tip the tubes as you wash them to thoroughly clean them.

Table 1. Results from Part A.

Tube #	Sugar	Start Time	End Time	Duration (min)	Volume of CO ₂ (ml)	Fermentation Rate (ml CO ₂ /min)
1	Glucose					
2	Fructose					
3	Sucrose					
4	Lactose					

Introduction for Part B – Effect of Temperature on Fermentation

In this experiment you will be investigating the effects of temperature on the fermentation rate of yeast. As in Part A, CO₂ production will be used as a measure of fermentation rate. However, instead of manipulating the sugar types, only one sugar (glucose) will be used in all the fermentation tubes. The fermentation tubes will be placed in water baths of different temperatures (0°C, ~22°C, 37°C, and 70°C) to see how the temperatures affect the fermentation rates.

Procedure for Part B:

1. Label **4 clean** fermentation tubes (1- 4). Using the graduated cylinder in your tray, measure and pour 5 ml of the yeast suspension into each of your four fermentation tubes. Be sure to mix the suspension before dispensing. You may need to use a pipette to accurately bring the volume of the graduated cylinder to exactly 5 ml.
2. Using the graduated cylinder, add 7 ml of glucose solution to each of the fermentation tubes.
3. Tip the fermentation tubes so that the vertical column of each tube fills with the liquid.
4. Place tube #1 in the 0° C ice-water bath; tube #2 in the room temperature bath (record the exact temperature in Table 2); tube #3 in the 37° C water bath; and tube #4 in the 70° C water bath. Record the time in Table 2.
5. Monitor the amount of CO₂ produced. This may take some time. When **ONE** of the tubes is half filled with CO₂, note the time, and remove **ALL** tubes from the water bath.
6. Measure the volumes (in ml) of gas in each tube and record them in the table below. Calculate the fermentation time by subtracting the starting time from the ending time, and record it in the table. Calculate the fermentation rates at each of the temperatures from these data.
7. **BEFORE YOU THROW ANYTHING AWAY!!!...**Take tubes #1 (0° C) and #4 (70° C) and place them both in the 37° C water bath for 5 minutes to let them both equilibrate to that temperature. After 5 minutes, tip the tubes to mix and remove the air, and leave them in the bath to incubate. Record the time.
8. Monitor the amount of CO₂ produced. This may take some time. When **ONE** of the tubes is half filled with CO₂, note the time, and remove **BOTH** tubes from the water bath.
9. Measure the volume (in ml) of gas in each tube and record them in Table 2 below. Calculate the fermentation time by subtracting the starting time from the ending time, and record it in the table. Calculate the fermentation rate at each of the temperatures from these data.
10. **WASH AND RINSE ALL OF YOUR TUBES** (contents can be poured down the sink). Tip the tubes as you wash them to thoroughly clean them.

Table 2. Results from Part B.

Tube #	Temp. (° C)	Temp. (° F)	Start Time	End Time	Duration (min)	Volume of CO ₂ (ml)	Fermentation Rate (ml CO ₂ /min)
1	0						
2							
3	37						
4	70						
1	0 → 37						
4	70 → 37						

Introduction for Part C – Effect of pH on Fermentation

In this experiment you will investigate the effect of pH on the fermentation rate of yeast. Remember, pH is a measure of the H⁺ (acid) levels of a solution, and lower pH values indicate a more acidic solution. As in parts A and B, CO₂ production will be used as a measure of fermentation. In this experiment, the sugar type (glucose) and the temperature (37°C) will remain constant. However, each glucose/yeast suspension will be incubated at a different pH (3.4, 4.5, 5.5, and 6.5), to see how pH affects the fermentation rate.

Procedure for Part C:

1. Label **4 clean** fermentation tubes (1- 4). Take your graduated cylinder to the side table where you will find four small flasks of yeast, each labeled with a different pH (3.5, 4.5, 5.5, and 6.5). Swirl each of the yeast suspensions before you pour them. Pour 5 ml of the buffered yeast into the tubes (as shown below):

Tube #1 – pH 3.5; Tube #2 – pH 4.5; Tube #3 – pH 5.5; Tube #4 – pH 6.5

You may need to use a pipette to accurately bring the volume of the graduated cylinder to exactly 5 ml.

2. Using the graduated cylinder, add 7 ml of glucose solution to each of the fermentation tubes.
3. Tip the fermentation tubes so that the vertical column of each tube fills with the liquid.
4. Place ALL four fermentation tubes into the 37°C water bath. Record the time in Table 3.
5. Monitor the amount of CO₂ produced. This may take some time. When **ONE** of the tubes is half filled with CO₂, note the time, and remove **ALL** tubes from the water bath.
6. Measure the volume (in ml) of gas in each tube and record it in Table 3 below. Calculate the fermentation time by subtracting the starting time from the ending time, and record it in the table. Calculate the fermentation rates of yeast in each of the pHs from these data.

- WASH AND RINSE ALL OF YOUR TUBES (contents can be poured down the sink). Tip the tubes as you wash them to thoroughly clean them. Make sure to remove all grease pencil marks.

Table 3. Results from Part C.

Tube #	Yeast. pH	Start Time	End Time	Duration (min)	Volume of CO ₂ (ml)	Fermentation Rate (ml CO ₂ /min)
1	3.5					
2	4.5					
3	5.5					
4	6.5					

References:

Campbell NA, Reece JB, Urry LA, Cain ML, Wasserman SA, Minorsky PV, Jackson RB. (2009) Biology. 8th ed. San Francisco (CA): Pearson, Benjamin Cummings.

Post-lab questions:

- Which sugar was fermented by the yeast at the highest rate?
- What do the fermentation rates of sucrose and lactose suggest about yeast's ability to break down specific disaccharides?
- Describe the relationship between temperature and fermentation rate in yeast. Is it linear?
- At what temperature was the yeast fermentation rate the highest? Why might yeast have adapted to ferment best at this temperature?
- What happened to the yeast fermentation in the tubes placed in the 0° C and 70° C water baths after they were both allowed to incubate in the 37° C water bath?
Provide an explanation for the results described in question 5 above.
- Describe the relationship between pH and fermentation rate in yeast.