BIOLOGY 163 LABORATORY

RESPIRATION AND FERMENTATION BY YEAST

(Revised Fall 2011)

Cells produce and use chemical energy in the form of ATP. Many cells can accomplish this either aerobically (in the presence of oxygen) through aerobic respiration (Figure 1), or anaerobically (without oxygen) through fermentation (Figure 2).

$$\underset{\text{glucose}}{C_6H_{12}O_6} + 6\,O_2 \xRightarrow{\text{(yeast)}} 6\,CO_2 + 6\,H_2O + \text{enough energy to make 38 ATP}$$

Figure 1. Aerobic respiration utilizes oxygen to convert a carbohydrate into 38 ATP. Carbon dioxide and water are produced as byproducts.

$$\underset{\text{glucose}}{C_6H_{12}O_6} \xRightarrow{\text{(yeast)}} 2\,CO_2 + 2\,\underset{\text{ethanol}}{C_2H_5OH} + 2\,\text{ATP}$$

Figure 2. Fermentation does not require oxygen but produces only 2 ATP. Carbon dioxide and ethanol are produced as byproducts.

Both respiration and fermentation begin with the process of glycolysis (Figure 3). In glycolysis, glucose (or some other carbohydrate) is converted through a series of enzymatic steps into pyruvate. Depending upon how much oxygen is available, the pyruvate will enter into respiration or fermentation.

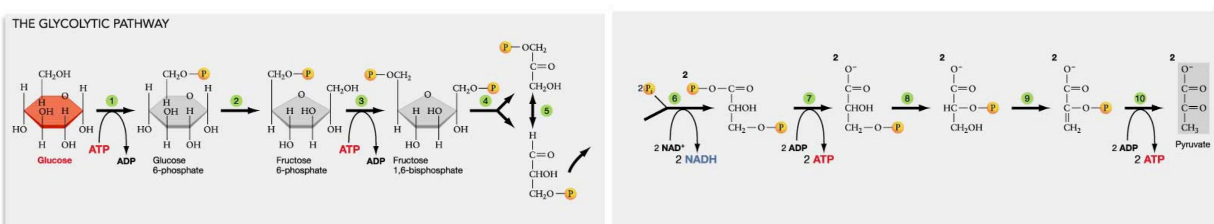
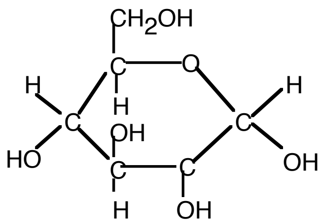


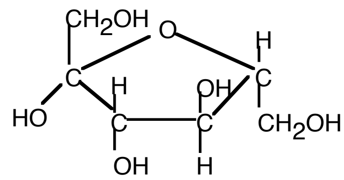
Figure 3. Glycolysis converts glucose to pyruvate for use in respiration or fermentation.

Yeast is a single-celled fungus long used commercially by bakers (CO2 make bread dough “rise”) and brewers and vintners (for ethanol production). Yeast is also an ideal model for studying energy metabolism, as the CO2 produced as a byproduct of both respiration and fermentation may be collected and quantified.

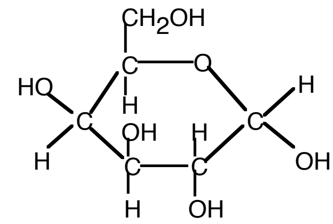
Glucose has been well established as a substrate for yeast energy production—it will serve as a control against which other treatments can be compared. In your investigation, you will determine if yeast can use other sugar substrates (fructose, galactose, lactose, maltose, or sucrose) and, if so, whether those sugars are utilized at a different rate from glucose. Glucose, fructose and galactose are monosaccharides, while the last three are disaccharides (Figure 4). Lactose is found in milk and consists of a galactose molecule attached to one of glucose. Maltose is a breakdown product of starch and is made up of two glucose molecules. Sucrose is table sugar and consists of glucose and fructose.



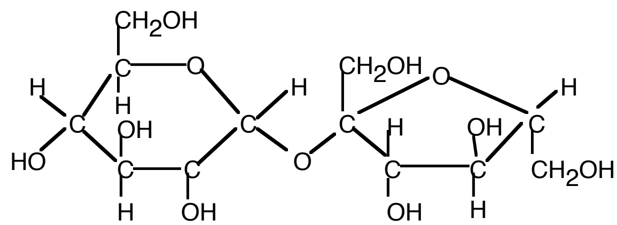
D-glucose



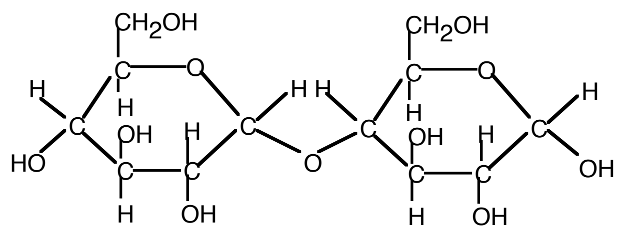
D-fructose



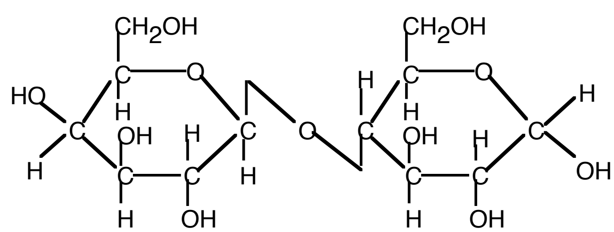
D-galactose



Sucrose



Maltose



Lactose

Figure 4. Chemical structure of six common sugars. Note that the disaccharides are comprised of two joined monosaccharides

Before beginning your experiments, develop a hypothesis regarding the ability of yeast to utilize these sugars. Remember, your hypothesis *does not* necessarily need to be ultimately supported by your results (it's not about getting the "right" answer), but it does need to be based in sound scientific principle. Be sure the reasoning behind your hypothesis is clear.

PROCEDURE

Practice Set-Up

Fill a small (15 x 125) test tube completely with water. Now hold a large (18 x 150) test tube upside down and slide it over the water-filled small tube. Let the rounded bottom of the large tube come to rest against the open rim of the small tube. Using your fingers to hold the tubes in this position, quickly invert the entire system. The large tube will now be right side up and, in the small tube, an air space will be trapped over the water column. Your goal is to make this air space as small as possible—continue to practice until you are confident in your technique.

Experimental Design and Set-Up

Plan your experiment by completing Table 1. The contents of tube #1 are provided as a starting point—it contains glucose and will serve as your *positive* control. Tube #7 is indicated as a *negative* control—design this trial to rule out volume changes that occur as the result of something *other* than sugar utilization. Keep in mind that good experimental design requires that you do not manipulate multiple variables simultaneously. Note that the yeast should NOT be added until you are ready to begin incubation of the tubes.

Before you begin your experiments, label your respirometers carefully. (Since tape labels tend to fall off as well as obscure your ability to make measurements, it is recommended you write directly on the tubes with a Sharpie marker.)

Table 1. Initial Preparation of Tubes for Yeast Respiration Experiment.

Tube #	Buffer	Sugar	Temperature
1 (+ control)	5 ml	5 ml glucose	37^o
2			
3			
4			
5			
6			
7 (- control)			

1. Add the solutions to the small numbered test tubes according to your experimental design.

2. After preparing all the tubes as indicated in Table 1, gently <u>resuspend the yeast culture</u> by swirling the bottle it is in. Rapidly, but carefully, add 5ml of yeast suspension to each small tube.

3. Stopper each tube with PARAFILM and your thumb and invert the tube to mix the contents.

4. Fill each tube to the top by adding buffer, and assemble the respirometers as previously practiced.

5. Record the initial gas reading of each tube, in millliters. To do this, measure the height of the air space in each tube with your millimeter ruler, and convert this reading into milliliters, on the assumption that *every 8 mm length of the air column in the test tube corresponds to an air volume of 1 ml.*

6. Place your respirometers into a 37^{o}C water bath. **Note and record the time**. During incubation, monitor the evolution of CO2 in your respirometers. *Remove all tubes and make your final measurements when one or more respirometers are nearly filled with gas*. **DO NOT LET ANY RESPIROMETERS OVERFILL**--gas will be lost to the atmosphere, confounding your results!

7. At the end of the incubation, **again note the time**. Record the terminal gas reading for each tube in milliliters as above. Subtract the initial from the terminal readings and calculate the net CO2 evolution in milliliters per hour. Record your results on the class data sheet.

8. Clean all respirometers thoroughly and leave them to drain at your lab bench.

ASSIGNMENT

You will present this study in the form of a complete scientific paper. Consult the "Guide to Writing Scientific Papers" and "Working with Statistics" for general ideas and assistance. Use the following guidelines to focus your efforts:

Introduction (two or three well-developed paragraphs)
Provide some relevant background information, but stay focused on the key point of your study. (HINT: Think about what needs to happen for these sugars to enter the glycolytic pathway!) Be sure to clearly state the *specific* question you are investigation and include the hypothesis you developed in lab.

Materials and Methods (one paragraph)
It is not necessary to re-write detailed procedures that have been previously published. Summarize the general protocol in a few concise sentences, and then reference the laboratory handout for the details. *Be sure to cite the handout as you would any other source!*

Results (figure and one or two short paragraphs)
Using group data provided by your instructor, present the results of the study so that the different treatments can be clearly compared. The *text* of the results should indicate whether the observed differences between each sugar treatment and the glucose (i.e., positive control) treatment are significantly different or not (based on a comparison of standard error).

Discussion (three or four well-developed paragraphs)
Address each of the following questions thoroughly but concisely. (HINT: Again, what needs to happen for each of these sugars to enter the glycolytic pathway!)

- Does the type of sugar have any effect on yeast energy metabolism? Can all sugars tested be utilized? Of those that can be utilized, how to they compare to glucose as an energy substrate?
- Did your results support or refute your hypothesis?
- Explain your observations. What factors might influence the yeast's ability to utilize a particular sugar? Draw upon what you have learned in lecture this semester to consider such factors as membrane transport, enzymatic activity, genetic transcription and translation, and glycolysis.

Your paper should also include a proper *title*, as well as *acknowledgements* and *literature cited* sections.

Your instructor may provide additional detailed instructions.