Respiration of Sugars by Yeast

Objectives
1. Students will measure the ability of yeast to respire using various sugars present the respiration rate in terms of parts per million (ppm) over time.
2. Students will understand what yeast is, and why it can metabolize some sugars and not others.
3. Students will describe two types of respiration, aerobic and anaerobic, and describe the differences.

Cellular Respiration of Sugars by Yeast

What is cellular respiration? **Cellular respiration** is the process by which cells break down organic molecules, including *carbohydrates*, *protein*, and *lipids*, to obtain the energy from them. In most cells, respiration begins with the oxidation of glucose, creating pyruvate through reactions called glycolysis (-lysis means to break). This process yields some energy (2 ATP). If oxygen is present, then cells can continue onto further oxidation of the pyruvate through the Kreb's cycle (Citric acid cycle). Energy from the Kreb's cycle is then funneled through the Electron Transport Chain by electron carriers to result in the cells capturing energy in an additional 34 ATP. Oxygen must be present to accept the electrons from the electron carriers, or the electron transport chain cannot function. If there was no oxygen present, this reaction would not continue through Kreb's cycle and the ETC.

This overall **aerobic** reaction (in the presence of oxygen) is written:

$$\underset{\text{(sugar)}}{C_6H_{12}O_6} + \underset{\text{(oxygen)}}{6O_2} \longrightarrow \underset{\text{(carbon dioxide)}}{6\,CO_2} + \underset{\text{(water)}}{6\,H_2O} + \textbf{ENERGY}$$

Some organisms can live without oxygen; they are called anaerobes. Organisms conduct glycolysis, and then the pyruvate goes through a process called fermentation. Animals and microorganisms can undergo lactic acid fermentation, where lactic acid is produced. Plants and some unicellular organisms undergo alcoholic fermentation, and produce CO_2 and ethanol.

These two **anaerobic** reactions (in the absence of oxygen) are written:

Lactic acid fermentation

$$\underset{\text{(sugar)}}{C_6H_{12}O_6} \longrightarrow \underset{\text{(lactic acid)}}{2\,CH_3CHOHCOOH} + \textbf{ENERGY}$$

Alcoholic fermentation

$$\underset{\text{(sugar)}}{C_6H_{12}O_6} \longrightarrow \underset{\text{(ethanol)}}{2\,C_2H_5OH} + \underset{\text{(carbon dioxide)}}{2\,CO_2} + \textbf{ENERGY}$$

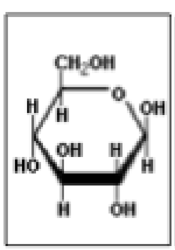
What are yeast? Yeast are single-celled fungi. They exist all around us - in soil, on plants, and even in the air. Yeast can grow with oxygen (using aerobic respiration) or without oxygen (anaerobic respiration). Because it can grow either aerobically or anaerobically, it is known as a "facultative aerobe." Yeast can extract more energy from sugar when oxygen is present in their environment, through the aerobic respiration processes. When oxygen is present, the sugar molecules are broken down aerobically into carbon dioxide and water, plus the energy (36 ATP) that the yeast uses to grow and reproduce. In the absence of oxygen, yeast switch to alcoholic fermentation. Alcoholic fermentation is an anaerobic process, which produces alcohol, carbon dioxide and water. It does not produce nearly as much energy as aerobic respiration processes; it produces a net gain of 2 ATP. With alcoholic fermentation, yeast can still get energy from sugar, but less energy is derived from each sugar molecule. The alcoholic fermentation process is used in baking industry for the rising of cakes and breads. It is also used in the beer and wine industries.

Yeast are able to metabolize some foods, but not others. In order for any organism to make use of a potential source of food, it must be capable of transporting the food into its cells. It must also have the proper **enzymes** capable of breaking the food's chemical bonds in a useful way. Enzymes are proteins that help catalyze a chemical reaction. Are you or someone you know lactose intolerant? This happens when our bodies stop producing the **lactase enzyme** needed to break down lactose sugar into more simple sugars. Remember, sugars are vital to all living organisms. Yeast are capable of using some, but not all sugars as a food source.

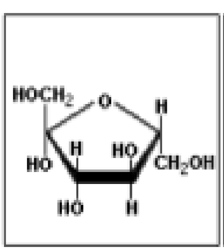
In today's lab, you will try to determine whether yeast are capable of metabolizing a variety of sugars. When yeast respire either aerobically and anaerobically, carbon dioxide (CO_2) is produced. You will use a CO_2 Gas Sensor to monitor the production of carbon dioxide as yeast respire using different sugars. The four sugars that will be tested are glucose (blood sugar), sucrose (table sugar), fructose (fruit sugar) and lactose (milk sugar).

The sugars (and their chemical structures) that we will use today

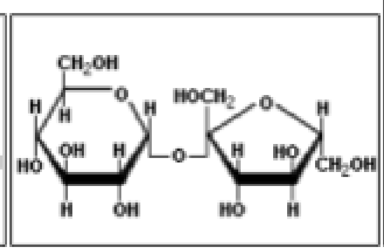
Monosaccharide ----------------------------------- Disaccharide ---



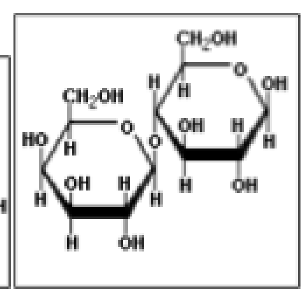
Glucose



Fructose



Sucrose
(glucose + fructose)



Lactose
(glucose + galactose)

PRE-LAB QUESTIONS

1. What is the purpose of today's lab? In 1-2 sentences and in your own words, describe what you are trying to determine by doing this experiment.

2. What is your hypothesis for the lab experiment? A hypothesis describes what you expect the outcome of the experiment to be, based on previous experience, observations, research, etc. State your hypothesis.

3. **What reasoning did you use to arrive at your hypothesis?** Explain your hypothesis using the scientific concepts of this lab to show the reasoning behind your prediction.

Procedure for today's lab

Students may work in groups of 2 – 4 people today. Each GROUP should start with:

- ➔ one test tube rack
- ➔ five test tubes
- ➔ one medium ~400mL glass beaker to act as a warm water bath
- ➔ one smaller (~300mL) plastic beaker to re-fill the warm water bath
- ➔ one thermometer
- ➔ 250mL respiration chamber (square shaped bottle) that will hold CO_2 gas sensor
- ➔ one LabQuest with a CO_2 gas sensor with a rubber stopper

Students groups will share the

- ➔ four sugar solution bottles
- ➔ yeast solution bottle
- ➔ lactaid solution bottle

1) Prepare a warm water bath for the yeast. A water bath is simply a large beaker of water maintained at a certain temperature. This ensures that the yeast will remain at a constant and controlled temperature. Add warm tap water to obtain a temperature of 38 – 40°C. The beaker should be ¾ full of warm water. Keep the thermometer in the water bath throughout the lab; monitor and maintain this constant temperature.

If the yeast reaches a temperature above 40°C, the cells could begin to cease respiration and die.

2) Obtain five test tubes and label them Glucose, Sucrose, Fructose, Lactose, and Lactose + Lactaid (or G, S, F, L, LL).

3) Obtain the four sugar solutions. Place 2 mL of each of the four sugar solutions in the correctly labeled test tube. You will put 2 mL of Lactose in both the "Lactose" and "Lactose + Lactaid" test tubes.

4) Obtain 2mL of the Lactaid (lactase enzyme) solution. Carefully Lactaid solution to your "Lactose + Lactaid" test tube. You should have 2 mL of Lactaid solution AND 2 mL of lactose solution.

5) Obtain 2mL of the yeast suspension. MIX THE YEAST SUSPENSION before you add it to the sugar solution, as the yeast will settle to the bottom of the container. Add 2mL of the yeast suspension into ONLY ONE of your four sugar test tubes. Swirl the test tube to mix the sugar and the yeast.

Do not add yeast to all the sugars at the same time, as you only have one CO_2 *sensor to detect change!*

6) Incubate your combined yeast and one sugar test tube in your water bath for 10 minutes. Keep the temperature of the bath constant. Add more warm water as necessary, but be sure to not overflow your water bath beaker.

To speed up today's lab, you may stagger your sugar + yeast tube incubation. While a sugar + yeast sample is in the respiration chamber, you can begin to incubate another sugar mixture in your water bath!

7) Check that the CO_2 gas sensor is plugged into Channel 1 of the LabQuest; follow the procedure on the Cellular Respiration by Yeasts instruction sheet. Be sure your LabQuest is ready before you need to collect data.

8) When your 10 minutes of incubation is completed, pour ALL the sugar/ yeast solution from the test tube into the 250mL respiration chamber.

9) GENTLY, holding ONLY THE STOPPER (with the CO_2 gas sensor in the stopper), twist the stopper into the chamber opening. Try not to twist the shaft of the CO_2 gas sensor, as it will break. Twist only the stopper.

10) Immediately tap the **Graph** tab, and then press the **Collect** button to begin data collection. Using the LabQuest, you will collect data for 5 minutes, and will stop

automatically after the time has elapsed.

11) At the end of a sample while under the Graph tab, tap the **Analyze** button.
· Choose “**Curve Fit**” from the list of options.
· Tap the “**CO_2**” box to check it.
· Next, under “**Fit Equation**”, to Choose **Fit**, tap the ▼
· Choose “**Linear**” from the drop-down menu.
· Your results will appear to the right of the graph.
The linear equation is y = **m**x + b. We want **m**, the slope of the line. *This is your respiration rate — record it in Table 1.*
 Tap “**OK**” to return to the graph for your next data collection.

12) Gently remove the CO_2 gas sensor from the respiration chamber.

13) Rinse the respiration chamber with warm tap water. Make sure all the yeast is removed. Thoroughly dry the inside of the respiration chamber, especially the opening, with a paper towel to prevent cross-contamination between sugar samples.

14) Fan fresh air onto the respiration chamber and the CO_2 gas sensor for ~2 minutes. BE SURE TO ALLOW THE CO_2 GAS SENSOR TO RETURN TO A NORMAL (~300-500 ppm) READING before starting your next sample.

15) Repeat steps 4-13 for each of the sugar solutions. When you have tested all five sugar solutions, turn off your LabQuest. Clean up your area: wash all your test tubes, empty your water bath, and wash your respiration chamber.

Data

Table 1. Respiration Rates

SUGAR TESTED:	Respiration Rate (ppm/min)
Glucose	
Sucrose	
Fructose	
Lactose	
Lactose + crushed Lactaid tablet	

Table 2. Class Averages

GROUP NUMBER:	Glucose	Sucrose	Fructose	Lactose	Lactose + crushed Lactaid tablet
1					
2					
3					
4					
5					
6					
7					
8					
Average Respiration Rate (ppm/min)					

Processing the Data

Pool your data with the data of the class to calculate average respiration rates for the class.

ANALYSIS and CONCLUSIONS

1. Does the data support your *original hypothesis*? Why or why not?

2. Considering the results of this experiment, do yeast equally utilize all sugars? Discuss the class data, including which sugars were metabolized and which were not.

3. Why do you think some sugars were metabolized while other sugars were not?

4. What were the differences in respiration rate of yeast and lactose versus yeast and lactose with Lactaid? Why did you get these results?