Fermentation of Honey

Name _____

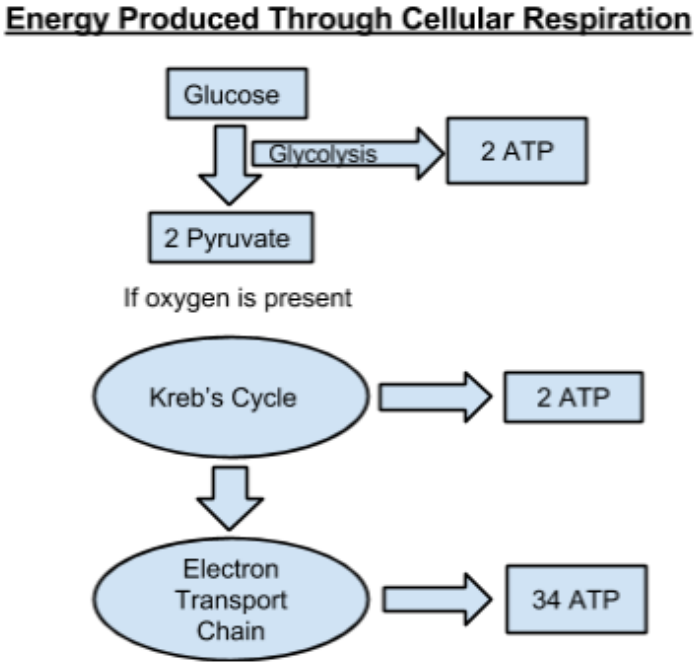
Cellular Respiration (Aerobic Respiration)

All living organisms need energy to support activities necessary to survive. They obtain this energy from food molecules which contain chemical energy that is released when chemical bonds are broken. In the presence of oxygen, aerobic respiration occurs. Glucose is an energy source for the cell but it must be converted into ATP which is the energy source that the cell can use. The equation for cellular respiration is shown.

$C_6H_{12}O_6 + 6\ O_2 \rightarrow 6\ CO_2 + 6\ H_2O + ATP$
Glucose + Oxygen → Carbon Dioxide + Water + ATP

Glycolysis is the first stage where glucose in broken into two pyruvate molecules. The next stage depends on the presence of oxygen. If oxygen is present, the 2 pyruvates enter the Kreb's Cycle where two more ATP are formed. The final step is the electron transport chain where 34 ATP are produced. Each glucose yields a total of 38 ATP through aerobic respiration.

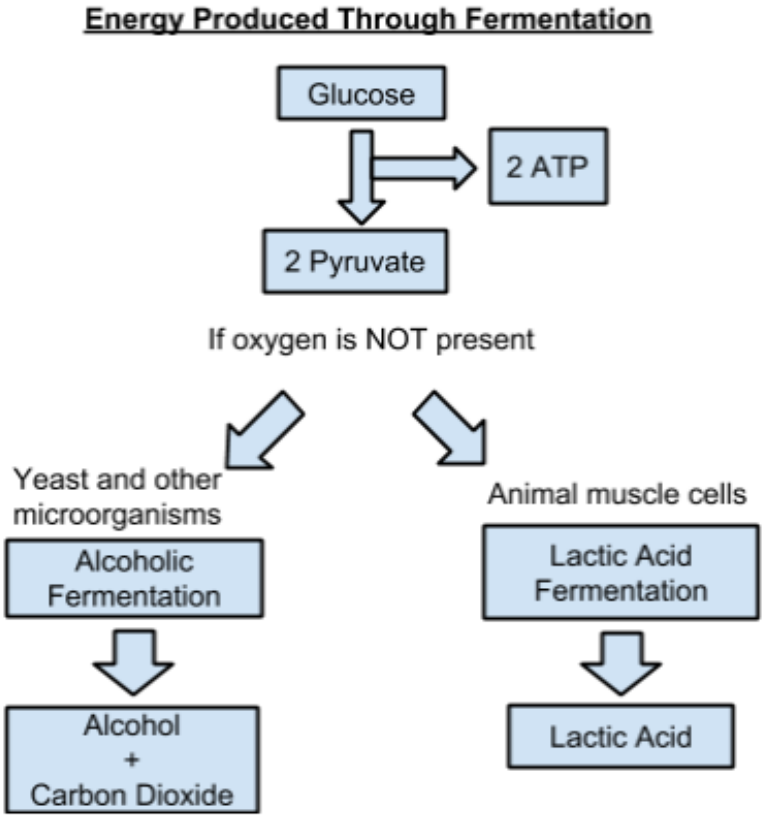
The diagram below summarizes the stages of aerobic respiration. Notice that ATP are produced in 3 different stages.



Fermentation (Anaerobic Respiration)

In the absence of oxygen, organisms can still obtain the energy they need to survive through the process of fermentation. One type of fermentation, lactic acid fermentation, occurs in the muscle of animals. You may experience lactic acid fermentation when you do short bursts of hard exercise such as lifting weights or running up stairs. A second type of fermentation called alcoholic fermentation occurs in yeast and other microorganisms. This type of fermentation is important in the food science industry for baking bread or brewing beer and wine.

It is also important in the biofuels industry as the alcohol ethanol is produced as a byproduct. Although fermentation is necessary when energy is needed and oxygen is not available, it has disadvantages. Fermentation only produces 2 ATP per glucose while aerobic respiration produces a total of 38 ATP per glucose. Also, the byproducts of anaerobic respiration are toxic to cells.



Teacher Prep for Part 1: How does the concentration of sugar affect the rate yeast produces CO_2?

Materials needed for teacher prep:

- Sugar
- Warm water
- Four 100mL beakers labeled 1, 5, 30, and 50% sugar solution
- 'Rapid rise' activated dry yeast
- Bowl and spoon to prepare yeast mixture
- Weighing scale
- Weighing boats or portion cups
- Materials to make Respirometers, 4 per group
    - 5cc syringes (non-luerlock)
    - 1mL pipets (glass disposable with 0.01 gradations)
    - Plastic tubing (I.D. = 1/8"; O.D. =1/4"; wall = 1/16") cut into 1 inch sections

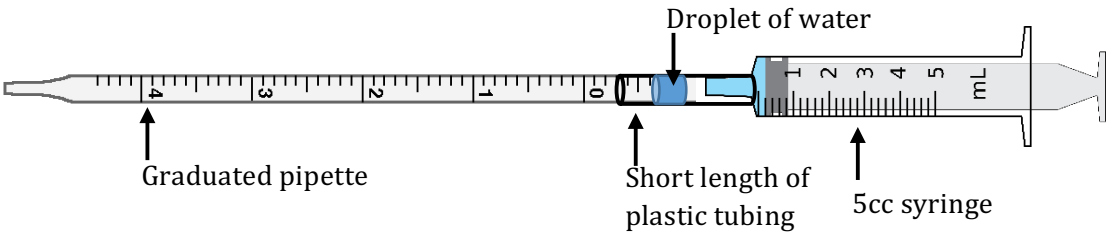
Teacher Prep Instructions:

1. **Approximately one hour before lab**, suspend the yeast (80 g per liter or 8 g per 100mL) in water on a magnetic stirrer. Each lab group needs access to ~40 mL of yeast suspension.
2. **Prior to lab**, mix 100mL of 1, 5, 30, and 50% sugar solutions in 4, 100mL beakers. Note: Sugar solution concentrations are weight: volume ratios. To calculate, use the following equation:

$$\%\frac{w}{v} = \frac{grams\ of\ substance\ added}{final\ volume\ (mL) of\ solution} \times 100$$

    a. 1 g of sugar in 100 mL of water = 1% sugar solution
    b. 5g of sugar in 100mL of water =5% sugar solution
    c. 30g of sugar in 100mL of water =30% sugar solution
    d. 50g of sugar in 100mL of water =50% sugar solution

Assembly of respirometer:



It may be beneficial to show students how to assemble the respirometer prior to starting the lab activity. Respirometers can stand upright on their own, however, if there are concerns about tipping over, the respirometers can be placed within a ring stand for stability.

Part 1: How does the concentration of sugar affect the rate yeast produces CO_2?

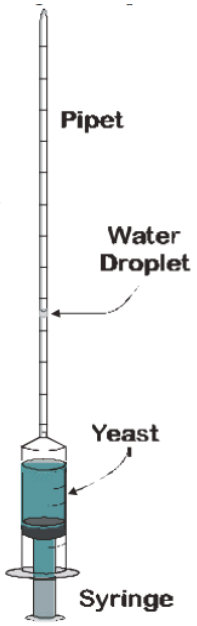


Image source: http://www.cur.org/assets/1/7/ystferm.pdf

Materials needed for each lab group:

- Access to 10mL of each sugar solution
- Access to 40 mL of yeast suspension
- Four 100mL beakers
- Four 50 mL beakers
- Timer or clock
- Respirometers, 4 per group

Lab Procedures:

1. Gather the materials needed for this lab.
2. Measure out 10 ml of the 1% sugar solution and place the solution into a 50 mL beaker.
3. Measure out 10 ml of the yeast solution and add it to the 50 mL beaker with the sugar solution.
4. Allow the yeast and sugar mixture to incubate for 5 minutes occasionally swirling the beaker.
5. Repeat the procedure with the other concentrations of sugar.
6. Draw 3 mL of the yeast and sugar mixture into the syringe.
7. Continue drawing the syringe until it has 1 mL of air on top of the sugar-yeast mixture.
8. Add a drop of water into the bottom of the pipette and attach the pipette to the top of the syringe with plastic tubing. Stand the respirometer upright.
9. Begin timing when the drop of water reaches 0 on the graduated pipette.
10. Record the amount of CO_2 produced every 2 minutes in the data table.
11. Repeat with the other concentrations of sugar.

Record the data: Note how far the water droplet has moved up the pipette every 2 minutes.

	1% sugar	5% sugar	30% sugar	50% sugar
2 minutes				
4 minutes				
6 minutes				
8 minutes				
10 minutes				

1. **After 10 minutes, which concentration of sugar produced the <u>greatest amount</u> of carbon dioxide?**

   Answers will vary. Generally, 30% sugar concentration will produce optimum carbon dioxide.

2. **If you were trying to maintain a yeast population, which concentration of sugar would you want to feed the yeast? Why?**

   30% sugar concentration because this concentration produced the greatest amount of carbon dioxide meaning the yeast consumed the greatest amount of sugar. The yeast will be well-fed.

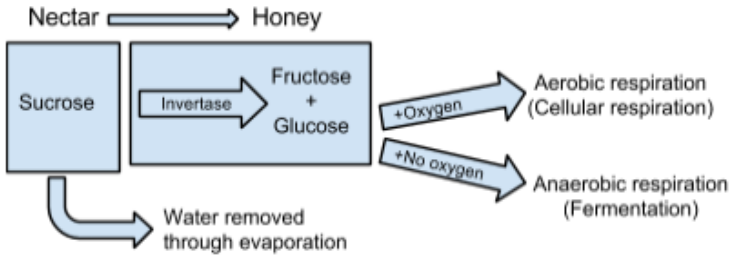
3. **After 10 minutes, which concentration of sugar produced carbon dioxide at the <u>fastest rate</u>?**

   Answers will vary.

Part 2: Predicting fermentation in honey

In addition to table sugar, other foods may provide organisms with the glucose needed for energy. The glucose in honey can be transformed into usable energy (ATP) for living organisms. Let's review the process of how honey is made.

Honey bees gather nectar from flowers as a source of sugar. However, nectar has a high water content. Bees evaporate water from nectar by fanning their wings thus increasing the concentration of sugar in the honey. In addition to removing water, bees add enzymes to the nectar. The enzyme invertase is responsible for breaking down sucrose, a disaccharide into its component monosaccharides, glucose and fructose. Glucose can be used in the cellular respiration process of bees to produce ATP for energy.

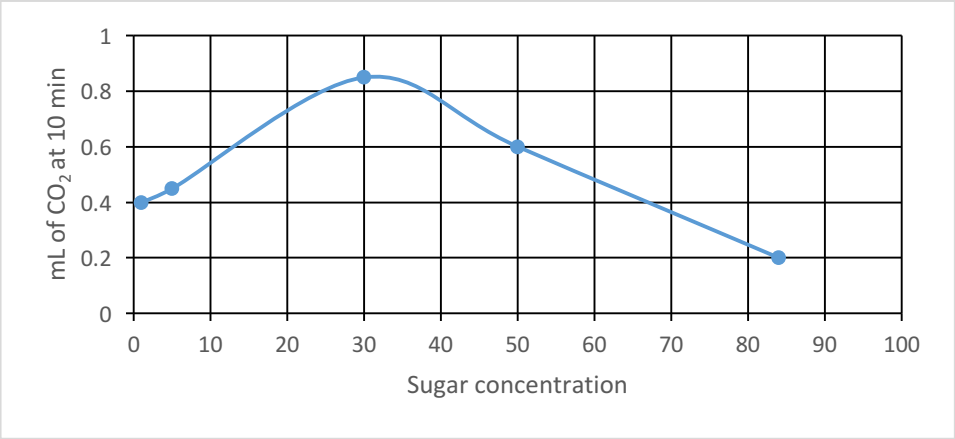


However, microorganisms such as bacteria and yeast compete with other living organisms such as bees for the same food source. They may hijack the carbohydrates for their own energy needs through fermentation or aerobic respiration sometimes producing unwanted results. Using what you learned from Part 1, answer the following questions to predict how successful yeast would be at using the glucose in honey as a source of carbohydrates for energy production.

4. Honey is a rich source of carbohydrates including glucose. If the water content of ripened honey is 16%, what is the percent of sugar found in ripened honey?

   84% is sugar.

5. Graph the data for all sugar concentrations from Part 1 at 10 minutes on the graph below. Now add the sugar concentration of ripened honey to your graph. Predict the amount of CO_2 produced. *Sample data plotted below.*



6. **Based on the trend of CO_2 production, would you predict yeast to be successful in producing energy in ripened honey?**

   No, the trend from Part 1 indicated that at higher sugar concentrations (above 30%) yeast does not produce as much CO_2. and therefore does not produce as much energy.

Part 3: Honey gone haywire!

Image source: http://www.honeybeesuite.com/uncapped-honey-fermenting-in-the-comb/

Honey is a good source of energy for bees. It's also a valuable food product for humans. In 2014, U.S. beekeepers sold over 178 million pounds of honey worth nearly $400 million. In order to produce a high quality honey crop worth the highest market value possible, beekeepers are always on the lookout for contaminants that might compromise the safety or taste of their honey.

Suppose you are a beekeeper. Looking at the honey in the honeycomb, answer the following questions on your own.

7. Where did the bubbles come from in this honey?
   The bubbles are likely CO_2 , a byproduct of fermentation.

8. What undesirable components would be found in the honey?

   CO_2 and alcohol. The CO_2 would give the honey an undesirable texture. The honey would also contain alcohol, an unwanted component in honey.

9. **What process formed these two products?**

   Fermentation

10. **What are the risks of selling this honey product as it is?**

    Accept reasonable answers. The honey may have a detectable alcohol content. As a supplier, your customers may no longer wish to purchase honey from you.