Name____________________________________ Date________________ Hour_______

Introduction Questions

Yeast metabolize carbohydrates for energy for their own growth and reproduction. In anaerobic conditions they produce ethanol as a by-product, which can be used as a transportation fuel. Due to concerns of dwindling supplies of fossil fuels and global climate change, scientists are investigating using yeast to produce large quantities of ethanol for transportation fuels. However, yeast can only metabolize certain food sources due to biological constraints. In this activity, you will investigate the ability of yeast to metabolize a variety of carbohydrates originating from different feedstocks (plant materials).

In order to create ethanol, yeast must have a good source of digestible sugars. Plants contain a tremendous amount of carbohydrates (about 50% of a plant is carbohydrates) but not all of this is digestible for yeast. The chemical formula for fermentation is:

$$\mathbf{C_6H_{12}O_6 \dashrightarrow 2\ CH_3CH_2OH + 2\ CO_2 + ATP}$$

Glucose ---> ethanol + carbon dioxide + energy

1. Which parts of a plant contain carbohydrates? Do all these parts contain the same type of carbohydrate?

2. Look at the materials list provided by your teacher for this activity. How will we measure the metabolic activity of the yeast? Why is this measurement an indication of metabolic rate?

3. What are the plant materials (also known as feedstocks) to be tested in this experiment? Compare the carbohydrate composition of table sugar (sucrose) with the other feedstocks and hypothesize what some of the differences may be.

4. Which of the feedstocks to be tested in this experiment do you think will be metabolized the fastest by the yeast? Why? Make your hypothesis below.

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Simple Demonstration to Compare Metabolic Rates of Different Feedstocks: Method A (Vernier Gas Pressure Sensor)

This introductory activity demonstrates that yeast can digest some sugars but not others. Your job is to determine why this happens. After the demonstration, discuss why the results varied for different feedstocks (plant materials) and join other scientists in the field of biofuel production to develop your own experimental methods to increase CO_2, and therefore ethanol, production rates from cellulosic biomass.

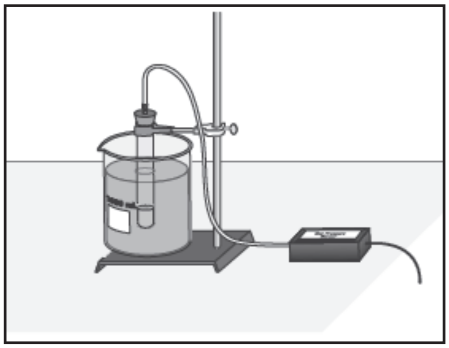

Figure 1. Vernier Setup

Procedure - Anaerobic Respiration with a Vernier Gas Pressure Sensor

Wear goggles or safety glasses when working with glassware or rubber stoppers

1. Set up a water bath for the yeast solution in a large glass beaker that will fit the test tubes. To maintain the water-bath at 37°-40°C, place the beaker on a hot plate and set it to a low temperature. The temperature that maintains the water bath will vary depending on the air temperature and rate of heat loss from the beaker. Monitor the water bath with a thermometer during the experiment. If you don't have access to a hot plate, combine warm and cool tap water until a temperature in this range is achieved. Use a beral pipet or baster to maintain the water bath by removing the cooling water and adding warm water.
2. Label 4 test tubes – sucrose (S), cornmeal (CM), stover (CS), and a negative control (water).
3. Obtain 2mL of 5% sucrose solution. In addition, obtain .5g of CM and place it in the corresponding test tube and add 2mL of water. Repeat this process for the CS test tube. Be sure to also add 2mL of warm tap water to the negative control test tube.
4. Test only one feedstock source at a time. Add 2mL of the yeast solution to the first of the four test tubes feedstock solution for a total volume of 4mL per test tube (2mL feedstock + 2mL yeast). Use the same procedure with the control test tube as with the ones containing feedstock solutions.

5. Gently swirl the test tube to mix the contents.
6. In the test tube, add enough vegetable oil to cover the surface of the mixture. Be careful not to get oil on the inside walls of the test tube. The addition of vegetable oil is to create an anerobic environment. Place the test tubes in the water bath.

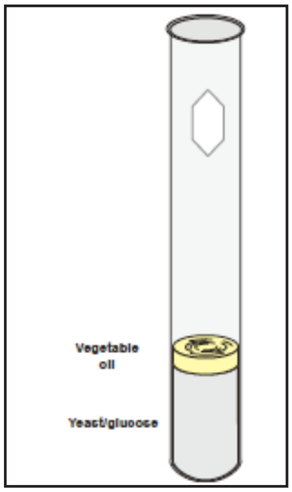

Figure 2. Cover yeast solution with oil (step 6).

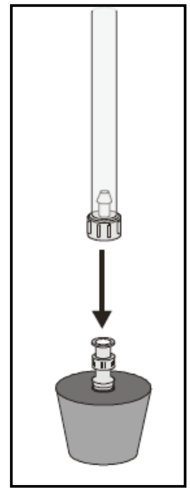

Figure 3. Connect tubing to stopper (step 11).

7. Insert the single holed rubber stopper into the test tube, firmly twisting it in for an airtight fit.
8. Incubate the test tube in the water bath for 10 minutes. Carefully monitor the temperature of the water bath. The water should surround the solutions inside the test tubes. While the test tube is incubating, begin computer setup for data recording.
9. Open the Vernier Logger Pro or Logger Lite software on the computer. Connect the gas pressure sensor to the computer interface. Connect the plastic tubing to the valve on the gas pressure sensor, leaving the other end to be connected to the rubber stopper once incubation is complete.
10. Select 'Data Collection' from the *Experiment* menu. Set the experiment length to be 15 minutes. Set the sampling rate at 12 samples/minute (a sample every 5 seconds).
11. When incubation is complete, connect the free end of the plastic tubing to the rubber stopper.
12. Click the green collect data button to begin recording data. Make sure to keep the water bath temperature constant during the course of data collection.
13. Monitor the pressure readings displayed on the computer. If the pressure exceeds 130kPa, the pressure inside the tube may cause the rubber stopper to pop off. Disconnect the plastic tubing from the gas pressure sensor if it exceeds 130kPa.

14. When data collection has finished, disconnect the plastic tubing from the rubber stopper. Remove the rubber stopper from the test tube and discard the contents in a waste beaker or sink.
15. Determine the rate of fermentation.
    a. Move the mouse to the point on the graph where values begin to increase. Drag the mouse to the point on the graph where data values end and release.
    b. Click the Linear Fit button to perform a linear regression of the data. A box will appear with the formula for the line of best fit.
    c. Record the slope of the line, m, as the fermentation rate in the table below.
16. Store this data run by choosing Store Latest Run from the Experiment drop down menu. Your teacher may ask you to print your final results or save your data for further analysis, but be sure to also record results in the table below.
17. Repeat steps 4-15 for the each of the other test tubes, remembering to always monitor and adjust the temperature of the water bath. If each group only does a few samples, average class data to obtain the missing values.

Record data in the table below.

Table 1.

Feedstock Tested	Fermentation Rate (kPa/min)
Water (control)	
Sucrose	
Cornmeal	
Stover	

Calculate class average respiration rates and record results in the table below.

Table 2.

Feedstock Tested	Class Average Fermentation Rate (kPa/min)
Water (control)	
Sucrose	
Cornmeal	
Stover	

Name__________________________________ Date_______________ Hour_______

Simple Demonstration to Compare Metabolic Rates of Different Feedstocks: Method B (Balloons)

This introductory activity demonstrates that yeast can digest some sugars but not others. Your job is to determine why this happens. After the demonstration, discuss why the results varied for different feedstocks (plant materials) and join other scientists in the field of biofuel production to develop your own experimental methods to increase CO_2, and therefore ethanol, production rates from cellulosic biomass.

Procedure – Anaerobic Respiration with Balloons

1. Stretch out 3 balloons by blowing them up a few times and then lay them aside.
2. Add one packet of dry yeast to one cup of very warm tap water and stir. Repeat this twice so that 3 cups of warm water are activating yeast. Allow yeast to activate for about 5 minutes.
3. Add 2 tablespoons of sugar to the 1st bottle, 2 tablespoons of ground corn or cornmeal to the 2nd bottle, and add 2 tablespoons of corn stover or other plant material to the 3rd bottle.
4. Add the one cup of the yeast water mixture to each bottle and gently swirl until the sugar/corn/plant is as dissolved as possible.
5. Attach a stretched out balloon to the mouth of each bottle, securing with a rubber band if necessary.
6. After 10-20 minutes, the balloons may stand upright. Eventually the balloons may begin to inflate. Allow experiment to run for a minimum of 1 hour, and for as long as desired afterwards.
7. Record visual results at 20-minute intervals. Measurements of balloon circumference maybe taking for quantitative analysis.

Name___________________________________ Date_______________ Hour_______

Record data in the table below.

Feedstock Tested	Balloon Circumference
Water (control)	
Sucrose	
Cornmeal	
Stover	

Calculate class average respiration rates and record results in the table below.

Feedstock Tested	Class Average Balloon Circumference
Water (control)	
Sucrose	
Cornmeal	
Stover	

Name__________________________________ Date_______________ Hour_______

Analysis Questions

1. What is the chemical formula for the metabolism observed in this experiment?

2. Which feedstock fermented the most? How do you know?

3. Was your hypothesis supported by the experimental results? Use data to support your answer.

4. Think about the differences in metabolic rates for the feedstocks you observed. What can you infer about the enzymes in yeast from the different results you observed?

5. Table sugar is pure sucrose, which is fermentable by yeast. What do you think the carbohydrate content is for the other feedstocks you tested? Are they homogeneous or heterogeneous? What evidence do you have?

6. What are some of the limitations of this demonstration as a model of fermentation?

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Experimental Pre-Design Questions

Use data from this experiment and the information your teacher provided to answer the following questions and begin thinking about experiments to test your ideas.

1. What feedstock sources are used to create biofuels?

2. What are the challenges associated with making biofuels from corn grain (corn ethanol) or cellulosic material (cellulosic ethanol)?

3. What could be done to improve fermentation rates seen in the demonstration? Make 2 hypotheses below, and explain why you think each idea would work.

Name________________________________ Date______________ Hour_______

Experimental Design Questions

Design a new experiment that could be done to test one of your hypotheses from the questions in the Pre-Design section.

1. Which hypothesis will you test?

2. Write a paragraph or draw a picture of your experimental setup including your control.

What measurements will you need to make when you record your data? Will you measure metabolic rate or another variable? What equipment will you need to make quantitative measurements (Vernier probes or other)?

3. How will you measure the success of your experiment?

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Post-Experiment Questions

1. Rate the success of the techniques you attempted in your experiment. Use data to support your claims.

2. Using evidence from the experiments to support your answer, discuss which variables or techniques should be investigated further.

3. Using your experiment results and what you have read or learned about biofuels, speculate on why certain techniques worked better than others.

4. If you could speak with an expert in the field of biofuels, what would you want to ask them about this experiment or about biofuels in general? Why?