Sugar Fermentation
(Method 2–Ethanol Sensor)

Yeast are able to metabolize some foods, but not others. In order for an 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. Sugars are vital to all living organisms. Yeast are capable of using some, but not all sugars as a food source. Yeast can metabolize sugar in two ways, aerobically, with the aid of oxygen, or anaerobically, without oxygen. When yeast metabolizes a sugar under anaerobic conditions, ethanol (CH₃CH₂OH) and carbon dioxide (CO₂) gas are produced. This process is called ethanol fermentation. An equation for the fermentation of the simple sugar glucose (C₆H₁₂O₆) is:

\[
\text{C₆H₁₂O₆} \rightarrow 2 \text{CH₃CH₂OH} + 2 \text{CO₂} + \text{energy}
\]

If sugars are readily available, baker’s yeast (Saccharomyces cerevisiae) prefers to metabolize glucose and other sugars anaerobically, through fermentation. This is called the Crabtree effect.¹ The metabolic activity of yeast can be determined by the measuring the rate of ethanol production using an Ethanol Sensor inside a fermentation vessel.

In this lab, you will determine whether yeast are capable of metabolizing a variety of sugars. You will use an Ethanol Sensor to monitor the rate of ethanol production as yeast metabolizes various sugars. Ethanol production is a direct indicator of fermentation. The three sugars that will be tested are glucose (blood sugar), galactose (derivative of milk sugar), and fructose (fruit sugar). Water will be used as a control.

OBJECTIVES

In this experiment, you will

- Use an Ethanol Sensor to measure concentrations of ethanol.
- Determine the rate of fermentation by yeast while metabolizing different sugars.
- Determine which sugars are mostly easily used as a food source by yeast.

MATERIALS

- computer
- Vernier computer interface
- Logger Pro
- Vernier Ethanol Sensor
- plumber’s tape
- 250 mL fermentation chamber
- 5% glucose, galactose, and fructose sugar solutions
- magnetic stir bar
- magnetic stir plate
- Beral pipettes
- four 15 mL conical tubes
- yeast suspension
- #6 split stopper

PROCEDURE

1. Wear goggles.

2. Prepare the Ethanol Sensor for use.
   a. Remove the tip of the cap from the sensor.
   b. Cut a 1 cm piece of plumber’s tape.
   c. Cover the cap entirely with plumber’s tape and inspect the tape for wrinkles or gaps.
   d. Replace the tip on the sensor.
   e. Wrap the split stopper around the Ethanol Sensor and position it so that the stopper is touching the handle.

3. Set up the Ethanol Sensor for data collection.
   a. Connect the Ethanol Sensor to the data-collection interface.
   b. Start Logger Pro and choose New from the File menu.
   c. Set the duration to 10 minutes and the rate to 10 samples/minute.
   d. Choose Change Units CH1: Ethanol from the Experiment menu and choose “ppm.”

4. Obtain the three sugar solutions: glucose, fructose, and galactose.
   a. Place 5 mL of the glucose solution in a conical tube and label Glu.
   b. Place 5 mL of the fructose solution in a conical tube and label Fru.
   c. Place 5 mL of the galactose solution in a conical tube and label Gal.
   d. Place 5 mL of distilled water in conical tube and label W.

5. Pipet 5.0 mL of yeast suspension into the conical tube containing the glucose. Important: The yeast suspension must be removed from the middle of a yeast source that is being stirred by a magnetic stirrer at a constant stirring speed. Mix by inverting the conical tube three times.
6. Add a stir bar to the 250 mL fermentation chamber, and then add the yeast-glucose suspension. Place the fermentation chamber on a stir plate at medium speed.

7. Quickly place the Ethanol Sensor into the fermentation chamber, twisting the stopper snugly into the mouth of the chamber. The Ethanol Sensor will be in the air above the sample. **Important:** The Ethanol Sensor is a gas sensor–do not submerge in liquid.

8. Begin measuring ethanol concentration by clicking **Collect**. Data will be collected for 10 minutes.

9. When data collection has finished, remove the Ethanol Sensor from the fermentation chamber. Fill the fermentation chamber with water and empty it. Repeat this rinsing process two more times. Make sure that all yeast have been removed. Thoroughly dry the inside of the chamber with a paper towel.

10. Determine the rate of fermentation.
    a. Select the region where the ethanol concentration was increasing.
    b. Click on the Linear Fit button, **Linear Fit**. A floating box will appear with the formula for a best fit line.
    c. Record the slope of the line, \( m \), as the fermentation rate in Table 1.
    d. Close the linear regression floating box.
    e. Share your data with the class as directed by your instructor.

11. Store the data by choosing Store Latest Run from the Experiment menu.

12. Allow the Ethanol Sensor to equilibrate in room air for 5 minutes.

13. Repeat Steps 5–12 using fructose, galactose, and distilled water. You do not need to store the final run.

### DATA

<table>
<thead>
<tr>
<th>Sugar</th>
<th>Fermentation Rate (ppm/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>glucose</td>
<td></td>
</tr>
<tr>
<td>fructose</td>
<td></td>
</tr>
<tr>
<td>galactose</td>
<td></td>
</tr>
<tr>
<td>water (control)</td>
<td></td>
</tr>
</tbody>
</table>
Table 2: Class Averages

<table>
<thead>
<tr>
<th>Sugar</th>
<th>Fermentation Rate (ppm/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>glucose</td>
<td></td>
</tr>
<tr>
<td>fructose</td>
<td></td>
</tr>
<tr>
<td>galactose</td>
<td></td>
</tr>
<tr>
<td>water</td>
<td></td>
</tr>
</tbody>
</table>

PROCESSING THE DATA

1. When all other groups have posted their results, calculate the average fermentation rate for each solution tested. Record the average rate values in Table 2.

2. Make a bar graph of Rate of Ethanol Production vs. Sugar Type in Logger Pro.
   a. Disconnect the Ethanol Sensor from the interface
   b. Choose New from the File menu in Logger Pro.
   c. Double-click the x column heading and name it “Sugar.”
   d. Double-click the y column heading and name it “Fermentation Rate,” short name of “rate,” and units of “ppm/min.”
   e. Enter the class data with the name of the sugar type in the “Sugar” column and the class averages in the “Fermentation Rate” column.
   f. Choose Graph Options from the Options menu. Add an appropriate title and select Bar Graph under Appearance.

QUESTIONS

1. Considering the results of this experiment, do yeast equally utilize all sugars? Explain.

2. Hypothesize why some sugars were not metabolized while other sugars were.

3. Yeast live in many different environments. Make a list of some locations where yeast might naturally grow. Estimate the possible food sources at each of these locations.

EXTENSIONS

1. Measure both CO₂ and ethanol concentrations to show all products of alcoholic fermentation.
Sugar Fermentation

a. Prepare and set up the Ethanol Sensor as described in the above experiment.
b. If your CO₂ sensor has a switch, set it to the High (0–100,000 ppm) setting and connect to channel 2 of the interface.
c. Place a BioChamber 2000 on a stir plate with a magnetic stir bar. Add 250 mL of water and turn on medium speed.
d. Add 30 g of the sugar to be tested and allow it to dissolve.
e. Add one package of baker’s yeast (7 g).
f. Adjust the split stopper on the Ethanol Sensor so that the tip of the sensor will be 1 cm above the level of the liquid when inserted into the BioChamber. Using a gentle, twisting motion, place the Ethanol into one of the openings in the BioChamber.
g. Using a gentle, twisting motion, place the CO₂ gas sensor in the second opening in the BioChamber.
h. Begin measuring ethanol and CO₂ concentration by clicking Collect. Data will be collected for 10 minutes.
i. Determine the rate of CO₂ and ethanol production as described in the previous section.