OAK RIDGE INSTITUTE FOR SCIENCE AND EDUCATION

OAK RIDGE National Laboratory

Title of Lesson: Bioconversion of Sugars to Ethanol

Target Grades: High School Chemistry

Time Required: 4 days (partial periods on days 1, 2, and 3)

Alignment to Standards:
CHEM1.PS.1.3. Perform stoichiometric calculations involving the following relationship: mole-mole; mass-mass; mole-mass; and mass-particle. Show a qualitative understanding of the phenomenon of percent yield, limiting and excess reagents in a chemical reaction through pictorial and conceptual examples.
CHEM1.PS.1.6. Use the ideal gas law PV=nRT, to algebraically evaluate the relationship among the number of moles, volume, pressure, and temperature for ideal gases.

Materials Needed:
Biomass samples
Cellulase enzyme
Test tubes
Yeast
Balloons
Mortar and Pestle
Measuring Tape (sewing)
Alcohol detection strips (for milk)
Benedicts Reagent
Hot Water Bath

Background Information:
Plant biomass is used to produce fuels in many different forms, and one of these forms is cellulosic ethanol. The process for creating cellulosic ethanol is different from traditional corn-based ethanol because it seeks to harness energy in the entire plant by breaking down the lignocellulosic structures and using enzymes or microorganisms to convert the substances to ethanol. The process to create ethanol from corn is relatively simple because the sugars are already in a usable state, and we have been doing it for centuries. The process of breaking down the complex molecules in lignocellulosic biomass and utilizing it for ethanol is relatively new.

This activity tests the process of simultaneous saccharification and fermentation by using the enzyme cellulase and yeast simultaneously. Cellulase is a common enzyme that can be purchased from science supply companies, such as Carolina Biological (item 853630, $35.10).

Lesson Objectives:
Students will use stoichiometry to predict how much of a desired product is made based on how much byproduct is produced.
Students will be able to explain the process of converting sugars to ethanol.
Students will be able to compare and contrast biofuels with petroleum-based fuels.

Instructional Process:

Introduce Problem to Students
Ethanol is a biofuel that can be produced when yeast ferments glucose. Ethanol has traditionally been made from the simples sugars found in corn, but it can also be made from more complex sugars if we use enzymes or other processes to break them into simple sugars. In this experiment, you will observe the process of converting biomass to ethanol and measure the ethanol output using stoichiometry.

Special note:
This activity will also work with small water bottles, glucose, and yeast. Students can still use the same procedure of measuring the inflating balloon and calculate the amount of ethanol produced using stoichiometry.

Laboratory Activity:

Assign each group a biomass sample. Examples include: cellulose paper (filter paper), dried grass clippings, cotton, algae, duckweed, etc.

Testing for Ethanol Production via CO_2 by-product
Day 1:

1. Prepare your biomass sample by mashing it with a mortar and pestle. (It is best if your biomass is dry when you begin.)
2. Add approximately 1 gram each in 4 different test tubes. Add 10 ml of water to each test tube.
3. Add .05 g cellulase enzyme and .05 g yeast to 2 of the test tubes, .05 g cellulase enzyme to one of the test tubes, and leave the other as a control (no enzyme or yeast). Label these tubes: No Enzyme/Yeast, Enzyme Only, Enzyme + Yeast A, Enzyme + Yeast B.
4. Place a deflated balloon over the mouth of each test tube and secure by tying a string around it or wrapping a strip of parafilm tightly around the mouth of the balloon.

Day 2, 3, and 4:

5. Observe your balloon and measure the circumference. Record your data in the data table.

Additional Tests on Day 4:
Test for sugar (optional):

1. Using 4 new test tubes, add .5 mL water to one, .5 ml of the control sample to the second, .5 ml of cellulase only sample to the third, and .5 ml of one of the

cellulase/yeast samples to the third. Add 5 drops of Benedicts Reagent to each tube and place in a hot water bath for approximately 5 minutes.

2. Record the color change on your data table.

Test for alcohol (optional):

1. Use 4 alcohol test strips (used for testing milk) to test each of the test tubes for the presences of alcohol. Dip each strip in the test tube, remove it, and wait 2 minutes.
2. Record any color change on your data table.

Quantify the alcohol production:

1. Calculate the volume of gas produced and captured in the balloons using the formula for volume of a sphere. The following formulas may be helpful: $C = \pi d$, $d = 2r$, $V = 4/3\pi r^3$
2. Use the following equation to calculate the amount of alcohol produced when the calculated amount of gas was produced. $C_6H_{12}O_6 \rightarrow C_2H_5OH + 2CO_2\,(g)$
   We will use the molar volume of gas at STP: 1 mole = 22.4 L.

Safety and other concerns:

1. You can kill yeast with bleach before disposing, but yeast is considered a "GRAS" (generally recognized as safe) organism.
2. The balloon needs to be flexible enough that it can easily be expanded. You can stretch out the balloon by blowing it up once and letting it empty to stretch it out.
3. In addition to measuring the balloon volume, you could also measure the before and after dry mass of the samples to get a rough estimate of how much of the sample was converted to sugar and subsequently to alcohol.
4. The reaction will work better and faster if the temperature is 30-35 degrees Celsius. If it is too hot the yeast will die and the enzyme could become unstable.
5. Benedicts Reagent is a hazardous chemical and should be disposed of properly.
6. In a research setting, it is likely that alcohol production would be quantified by a method such as gas chromatography, and alcohol would be purified industrially by distillation. By measuring the gas produced, we can infer the amount of alcohol produced using stoichiometric calculations. It is not advisable to actually separate out the alcohol due to safety and legal concerns.
7. The volume of your balloon can be calculated with the formula $V = C^3/(6\ Pi^2)$. This is simpler than asking students to use multiple steps.

Name:_________________________________ Date:________________ Period:_________

Ethanol Production

Gas production:

Materials

Play dough measured out in 5 gram pieces

Larger container for water, such as an aquarium

Stopwatch

Meter Stick

Procedure

Day 1:

1. Prepare your biomass sample by mashing it with a mortar and pestle. (It is best if your biomass is dry when you begin.)
2. Add approximately 1 gram each in 4 different test tubes. Add 10 ml of water to each test tube.
3. Add .05 g cellulase enzyme and .05 g yeast to 2 of the test tubes, .05 g cellulase enzyme to one of the test tubes, and leave the other as a control (no enzyme or yeast). Label these tubes: No Enzyme/Yeast, Enzyme Only, Enzyme + Yeast A, Enzyme + Yeast B.
4. Place a deflated balloon over the mouth of each test tube and secure by tying a string around it or wrapping a strip of parafilm tightly around the mouth of the balloon.

Day 2, 3, and 4:

5. Observe your balloon and measure the circumference. Record your data in the data table.

Data Table

Balloon Expansion:

	Day 1 (initial)	**Day 2**	**Day 3**	**Day 4**
No Enzyme/Yeast				
Enzyme Only				
Enzyme + Yeast A				
Enzyme + Yeast B				

Benedict's Test:

Materials

Benedict's Reagent
Test Tubes
Hot Water Bath

Procedure

1. Using 4 new test tubes, add .5 mL water to one, .5 ml of the control sample to the second, .5 ml of cellulase only sample to the third, and .5 ml of one of the cellulase/yeast samples to the third. Add 5 drops of Benedicts Reagent to each tube and place in a hot water bath for approximately 5 minutes.
2. Record the color change on your data table.

Data Table

Sample	Color Change
Water	
Control (No Enzyme/Yeast)	
Enzyme Only	
Enzyme+Yeast (A or B)	

Alcohol Test:

Materials

Alcohol Test Strips

Procedure

1. Use 4 alcohol test strips (used for testing milk) to test each of the test tubes for the presences of alcohol. Dip each strip in the test tube, remove it, and wait 2 minutes.
2. Record any color change on your data table.

Data Table

Sample	Color Change
No Enzyme/Yeast	
Enzyme Only	
Enzyme + Yeast A	
Enzyme + Yeast B	

Quantify the Alcohol Production:

1. Calculate the volume of gas produced and captured in the balloons using the formula for volume of a sphere. The following formulas may be helpful: $C = \pi d$, $d = 2r$, $V = 4/3\pi r^3$
2. Use the following equation to calculate the amount of alcohol produced when the calculated amount of gas was produced. $C_6H_{12}O_6 \rightarrow C_2H_5OH + 2CO_2\ (g)$
   We will use the molar volume of gas at STP: 1 mole = 22.4 L.

Stoichiometry Calculations: Show your work to calculate the amount of ethanol (C_2H_5OH) produced.

$$C_6H_{12}O_6 \rightarrow C_2H_5OH + 2CO_2\ (g)$$

Questions

1. Which of the tubes produced gas? Why was the gas produced?

2. For the Benedict's Reagent Test, which test tubes changed color? What does this tell you about the contents?

3. Which test tubes changed the color on the Alcohol strip? What does this tell you?

4. How much alcohol was produced in the fermentation reaction? How do you know?

5. When you consider all the observations – Benedict's Reagent, Alcohol Test Strips, and gas production – were your results consistent? Why or why not?