For Teachers - Fermentation Challenge:
Making Ethanol from Cellulose

Objective:

Due to concerns of dwindling supplies of fossil fuels and global climate change, scientists are investigating the use of yeast as one way to produce large quantities of ethanol for transportation fuels. However, due to biological constraints, yeast can only metabolize certain food sources. In this activity, students will investigate the ability of yeast to metabolize a variety of carbohydrates originating from different feedstocks (plant materials). Students are encouraged to think about potential feedstocks and the biochemical processes necessary to convert each type of carbohydrate into fuel.

The lesson is designed to run over the course of two or three 50-minute class periods.

Learning Outcomes: Students will…

1. Identify carbohydrates found in different plant parts.
2. List the products of fermentation.
3. Explain that due to enzyme specificity yeast can only metabolize sucrose, not starch or cellulose.
4. Infer metabolic rates through measurement of carbon dioxide production.
5. Propose methods, such as heat and enzymes, to digest complex carbohydrates into simple sugars.

This lesson assumes prior knowledge in chemical structures including glucose, starch and cellulose, and some discussion of the global energy crisis. It is also recommended that students have been at least briefly introduced to photosynthesis, cellular respiration, and enzyme structure and function.

Sequence:

Part 1. Preview of fermentation rates of different plant materials (feedstocks) (pages 2-5) (30 minutes).
The demonstration is designed as a common exploration to generate discussion and questions amongst the large group. If sucrose, corn and stover all contain carbohydrates, why won't yeast metabolize all of them equally? If the goal for biofuel production is to use all types of carbohydrates to create ethanol, how can we increase the yield of this process? Students should answer Introduction Questions (page 1) and create a hypothesis before viewing the demonstration. Demonstration instructions are provided in the student section (pages 2-4) using a Vernier Gas Pressure Sensor, or alternatively (page 5) using a balloon setup depending on classroom equipment availability. Materials lists, set up instructions and teacher tips for running demonstrations can be found on pages 5T-6T. The demonstration shows that yeast metabolizes sucrose efficiently, but not other feedstocks. Students should be informed that fermentation is one type of metabolism, and the words fermentation and metabolism will be used interchangeably throughout this document. Students should analyze the results of the demonstration using the Analysis Questions (page 6) either individually or in small groups.
Discuss Answers with students. Please see Teacher Answers (pages 3T-4T) for guidance.

Part 2. Provide background information on biofuels and fermentation (time varies by teacher choice).
In order to complete the exercise and design individual experiments, students will need a basic understanding of the fermentation process used to create cellulosic ethanol, including an introduction to the enzymes/chemical changes necessary in this process. This could be presented as a lecture or series of short readings (see Appendix for suggested background materials). Students may want to revisit their answers to the Analysis Questions (page 6) in light of this new information.

Part 3. Designing individual experiments (pages 7-8) (25-50 minutes).

After viewing the demonstration, students will work to design individual experiments to test methods for improving fermentation of alternative feedstocks. In order to brainstorm experiment ideas, students should work to complete Experimental Pre-Design Questions (page 7) individually, perhaps as homework, or in pairs. The Experimental Design Questions (page 8) should be completed by students in small groups or pairs. Below is a list of possible experiments to consider if students have trouble creating ideas:

1) Change pH
2) Change the length of time the sample runs and measure rates over 24 hours.
3) Change feedstock source (fruit juice, flowers, grasses, etc) or concentrations
4) Try using a compost fungus or other fungus as a feedstock source
5) Boil or freeze the sample feedstock before fermenting
6) Change the temperature or concentration of the yeast solution
7) Use enzymes (amylase or cellulase for example). Below are some options:
    a) Cellulase from Flinn Scientific (www.flinnsci.com)
       Order #: C0172, $33.50 for 25g.
    b) Alpha Amylase from Carolina Biological (www.carolina.com)
       Order #: 202350, $27.70 for 100g.

Depending upon your students' comfort level designing experiments you may need to help them through the steps of writing a formal procedure, designing a data table or data collection system, recording observations, analyzing and displaying their results, and drawing conclusions.

Part 4. Run student-designed experiments (minimum of one class period).

Experiments should be run in small groups. Students should use data to draw and record conclusions, analyze the validity of their preliminary hypotheses, and refine these hypotheses if necessary.

Part 5. Share results (minimum 20 minutes).

According to your preference, ask students to present their findings to you in written form and/or directly to the class. If students do not make formal presentations, at a minimum they should describe their independent and dependent variables and conclusions to the class. Creating a master list of experiments for all students to read will aid in the overall discussion of results and further questions for investigation.

Students should participate in a large group discussion of results including Post-Experiment Questions (page 9). Teachers may also want students to include a formal report of results, including discussion of post-experiment questions.

Extension

Follow up references about pretreatment methods and fermentation are included in the Appendix. Please check the GLBRC Education website for updates to this activity and new bioenergy activities.

ANSWER KEY

Introduction Questions (page 1)

1. **Which parts of a plant contain carbohydrates? Do all these parts contain the same type of carbohydrate? Elaborate.**
   Fruits contain simple sugars, such as fructose – this is the first one we usually think of because we can think of tasting a plant, and what will taste sweet. Plant cell walls also contain carbohydrates – almost 50% in the form of cellulose, which looks like starch but has inverted bonds between the glucose molecules. Starch can be found in plant parts such as endosperm (grains) and tubers.
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?**
   We will measure metabolic activity of the yeast using the CO2 gas sensor. This works as an indicator of metabolic rate because CO2 is a product of yeast metabolism. The greater the amount of CO2 produced per minute, the higher the 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.**
   Sucrose is a disaccharide sugar, corn meal contains starch, and corn stover contains mainly cellulose, but also starch and other sugars. Sucrose is a disaccharide that can be broken into two monosaccharides by an enzyme in yeast called invertase (sucrase). Starch and cellulose are both chains of six-sided glucose molecules, but the bonds between molecules in the cellulose chain are inverted compared to starch.
4. **Which of the feedstocks to be tested in this experiment do you think will be metabolized fastest by the yeast? Why? Make your hypothesis below.**
   Student hypotheses should be supported with rational information about carbohydrates, enzymes, or cellular respiration.

Analysis Questions (page 6)

1. **What is the chemical formula for the metabolism observed in this experiment?**
   $C_6H_{12}O_6$ ---> 2 CH_3CH_2OH + 2 CO_2 + ATP
   Glucose ---> ethanol + carbon dioxide + energy
2. **Which feedstock fermented the most? How do you know?**
   Sucrose fermented the most because that mixture produced the most CO_2. CO_2 is a product of fermentation, so when large quantities are produced in the chamber, the probe detects pressure increases, indicating high rates of fermentation.
3. **Was your hypothesis supported by the experimental results? Use data to support your answer.**
   Answers will vary.
4. **Think about the differences in metabolic rates for the feedstocks you tested. What can you infer about the enzymes in yeast from the different rates you measured?**
   Enzymes in yeast are substrate specific and convert only specific forms of sugars. Yeast must only have enzymes for sucrose, not starch or cellulose (or whatever students think is in the other feedstocks), because that fermented best compared to the other feedstocks. In this experiment, sucrose is a disaccharide, which can be broken up into monosaccharides of glucose and fructose by the yeast enzyme invertase (sucrase). See supporting materials on Enzymes for more information.
5. **Table sugar is pure sucrose, which is fermentable by yeast. What do you think the**

carbohydrate content is for the other feedstocks tested? Are they homogeneous or heterogeneous? What evidence do you have?

Acceptable student answers should explain that other feedstocks (plant materials) contain sugar, but it might not be pure sucrose, so they may not ferment as well.

Teachers may want to explain that corn stover is heterogenous in carbohydrate makeup. It contains cellulose (44%), hemicellulose (30%), and lignin (26%). Cellulose is made of chains of linked glucose molecules. This glucose is fermentable by yeast, but only when cellulose is broken down into glucose. Sucrose ferments best of all the feedstocks (because yeast have enzymes to break down sucrose into glucose and fructose), while corn, stover, and other feedstocks may show some (but not much) fermentation in comparison.

6. **The demonstration is meant to model the fermentation of carbohydrates into ethanol using yeast. What are some of the limitations of this demonstration as a model?**

   The model we use does not actually measure ethanol (fuel) production. We are measuring CO_2 production, which implies ethanol production, as they are both products of fermentation. Experiment is too short, fermentation may occur over much longer periods of time. What we get as a rate result in the first few minutes may not be representative of a longer batch fermentation because there could be a small amount of simple sugar in the mix which is fermented first.

Experimental Pre-Design Questions (page 7)

1. **What feedstocks are used to create biofuels?**

   Biofuels can be created from corn or cellulosic biomass. Cellulosic ethanol is made from sugars in plant cell walls. Cellulosic ethanol can be made from anything that is or ever was plant material, including woodchips, corn stover, switchgrass, straw, hay, yard trimmings, and urban waste.

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

   The challenges when making corn ethanol include efficiency of production, land use changes to plant corn, and massive inputs into the system (fertilizers, fuel for product transport, etc.)

   Challenges in making cellulosic ethanol include the difficulties of breaking down cellulose plant material to release plant sugars, conversion of these sugars into ethanol, and efficiency of production process. See the GLBRC handout “Why is it so difficult to create cellulosic ethanol?” for more information (www.glbrc.org/education).

   In both cases, environmental and economic impacts must be taken into consideration for large-scale production and use. (See http://genomicsgtl.energy.gov/biofuels/benefits.shtml for more information on benefits and challenges of creating 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.**

   Pretreatment of feedstocks (using heat, chemicals, or enzymes) are important steps researchers must take in converting biomass to fuels. These steps break down cellulose, hemicellulose, and lignin complexes in the plant material in order to release sugars for fermentation. Changing concentrations of yeast or feedstock sources may change results, as well as changing water temperature or pH of the system.

   Hypotheses will vary. A list of possible experiments to consider if students struggle with creating ideas can be found on page 2T.

Experimental Design Questions (page 8) - Answers will vary.

Post-Experiment Questions (page 9) - Answers will vary.

Materials Lists

Preview Demonstration with Vernier Setup

Computer or Graphing Calculator or Vernier Lab Quest
Vernier computer interface, Logger Pro software
Vernier gas pressure sensor, rubber stopper, tubing for each set-up
2mL water dropper or small pipets
7% yeast solution – 12mL per set-up
5% feedstock solution of each carbohydrate source – 3mL per solution per set-up
Water bath container (600-1000mL beaker or plastic container)
Hot and cold water
Beral pipet or basters to remove water (and a waste container or sink nearby)
Thermometer
Four 15mL conical tubes or test tubes
Test tube/ conical rack or ring stand set-up seen in Fig. 1
Vegetable oil – 3-5mL per set-up

Preview Demonstration with Balloons

3 Balloons
3 cups very warm tap water
3 packets dry yeast
Sugar
Corn meal or Ground field corn
Corn stover or other plant materials (grass clippings, composting materials, sawdust, etc.)

Teacher Set Up for Demonstration/Student Activity (15-20 minutes)

Prepare 2 solutions before class begins.

1. You will need a 7% yeast solution (7g or one packet of dry yeast for every 100mL of warm tap water). The yeast solution should be incubated in a 37-40°C water bath before students begin the activity. If students complete the demonstration in groups, each student group will require approximately 12mL yeast solution.
2. You will also need a 5% solution of each of the feedstock sources (5g substrate for every 100mL water). Each student group will need approximately 3mL of the feedstock sources they use. Ideally, the class should at least observe the differences between sucrose, corn meal, and a stover-like material. Note: Sucrose is the positive control. If you do not run sucrose, the rest of the results will not make sense. Potential feedstock sources include:
    - sucrose
    - glucose
    - corn meal (make sure to look at ingredient list of the corn meal, note if sugar is an ingredient as it will affect your results).
    - Ground field corn
    - Ground sweet corn
    - Corn stover powder (If corn stover is not available, try using other plant materials like dead leaves, dried grass clippings, brush pile clippings, sawdust, etc. Pulverize samples in a food processor or spice grinder to achieve a powder-like consistency.)

Teacher Tips…

1. Depending on the length of class periods and the number of days to be used for these experiments, adjustments can be made to the suggested timeframes.

2. For the demo or class experiment procedure outlined above – use as many carbohydrate feedstocks you desire or can find. Adjust group sizes or the number of feedstock samples tested by each group so that the experiment can be completed in the time allotted. Allow students to calculate class averages on the data so that they need not test every feedstock available.

3. It may be helpful to name one student per group the Timer. Timing in this experiment is everything – students need to watch the clock and work efficiently in order to complete tests on more than one feedstock in a given class period.

4. Graphing – Vernier 12B should set up a graph with pressure on the Y-axis and time on the X-axis. Make sure to keep the graph scales and labels consistent for easy comparison. Also, creating and using a linear average line (best-fit) from the data may be easier than using raw-data (jagged) lines.

5. Timing – It is extremely important to stagger the starting incubation times in both setups. The yeast and feedstock sources should incubate mixed together for the same amount of time for all samples before measurements are conducted. For example, combine yeast and feedstock #1 and allow to incubate for 10 minutes, take measurements for 4 minutes. Do not start incubating yeast and feedstock #2 until the first sample is at least half way complete. The goal is to allow fermentation to occur for the same amount of time in each test tube before taking measurements of the rates.

6. Feedstocks – use what you can find. Sugar and Corn Meal are available in grocery stores. Note: Sucrose is the positive control. If you do not run sucrose, the rest of the results will not make sense.

7. When purchasing corn meal, note the ingredients. If the corn meal contains sugar, results will be much more similar to the table sugar tested. If it contains lime or other ingredients, make a note, as these ingredients may act as catalysts to change metabolic rates.

8. Ground field corn produces similar results to corn meal. Sweet corn is another option, and will produce different results due to the genetic differences between field corn and sweet corn (which contains more sugar).

9. Corn stover powder (the ground up plant material from corn fields) can be difficult to find. You may be able to contact a local farmer and ask for this material, but there are many substitutes (listed above).

10. Working with the gas pressure sensor: An airtight fit is extremely important and can be achieved by gently twisting the rubber stopper into the test tube or conical tube. Make sure all plastic tubing is securely fit as well.

11. Additional materials providing in-depth explanations of biofuels research at GLBRC can be found on the GLBRC website under Educational Materials. Consider the handout "Why is it so difficult to create cellulosic ethanol?" and other materials on the website to provide students with more information to complete the Discussion and Conclusion questions.

Appendix

Video Resources:

NREL video—Excellent 5-minute summary of difference between corn and cellulosic ethanol and process currently used to make cellulosic ethanol. Be aware the process discussed here is evolving and may vary from other information sources you read.
http://www.nrel.gov/learning/re_biofuels.html

Fields of Energy video: From the Minnesota Department of Agriculture, a free DVD with student hosts. Two short segments show how corn ethanol is made and the research into cellulosic ethanol. These two segments are currently available online as well.
http://www.mda.state.mn.us/kids/

Text Resources:

Why is it so difficult to make cellulosic ethanol? GLBRC. 2008.
http://www.greatlakesbioenergy.org/wp-content/uploads/2009/02/cellulosic_ethanol.pdf

A four-page handout that discusses the difficulties in creating cellulosic ethanol. Appropriate for high school and college students.

US Department of Energy Office of Science (2007) Biofuels for Transportation.
http://genomicsgtl.energy.gov/biofuels/index.shtml

FAQ-style pages with overview material such as "What is biomass?", "How is ethanol produced from cellulosic biomass", "Can one gallon of ethanol displace one gallon of gasoline?" Links to many other quality resources available from the Department of Energy.

US Department of Energy: ABC's of Biofuels (2009).
http://www1.eere.energy.gov/biomass/abcs_biofuels.html#prod

Information aimed towards high school students about the production steps involved in making bioethanol and other biofuels. Also includes an appendix of additional teacher lesson plans on biofuels for middle and high school students.

Redding, K., D. Masterman (2007). Biology with Vernier. Beaverton, OR: Vernier Software & Technology.
http://www2.vernier.com/sample_labs/BWV-12B-COMP-sugar_fermentation.pdf

Biology with Vernier Lab 12B is the basis for the preview activity procedures used in this activity guide.

Follow-Up Resources:

These are primary source articles by authors who study various parts of the fermentation process in creating cellulosic ethanol, especially the pretreatment process. University Library permissions may be necessary to access full text articles.

Yang, B. and E. Wyman, (2007). "Pretreatment: the key to unlocking low-cost cellulosic ethanol." Wiley Interscience: Biofuels, Bioproducts, and Biorefining 2:26-40.

Gives a nice background of the use of petroleum and biofuels in the introduction. Also provides a comparison each pretreatment method, including pros and cons, on page 31. Figure 1 (page 29) provides an outline of the biological conversion of cellulosic biomass into ethanol, including effects of pretreatment processes on other operations. Best suited for high school students or teachers who want to know more about pretreatment methods.

Mosier, N., Wyman, C., Dale, B., et al. (2005). "Features of promising technologies for pretreatment of lignocellulosic biomass." Bioresource Technology 96 (2005) 673-686.

Overview of plant structure and challenges of accessing cellulose provided on pages 673-676. Also provides detailed descriptions of pretreatment options. Useful for high school students and teachers, especially chemistry teachers who may be interested in mimicking procedures outlined for pretreatment.

Name________________________________ Date_________________ Hour__________

Fermentation Challenge: Making Ethanol from Cellulose

Introduction Questions

Yeast metabolize carbohydrates for energy for their own growth and reproduction. In anaerobic conditions they produce ethanol as a byproduct, 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, due to certain biological limitations, yeast can only metabolize certain food sources. 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:

$$C_6H_{12}O_6 \text{ ---> } 2\ CH_3CH_2OH + 2\ CO_2 + ATP$$

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.

Name________________________________ Date_________________ Hour__________

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.

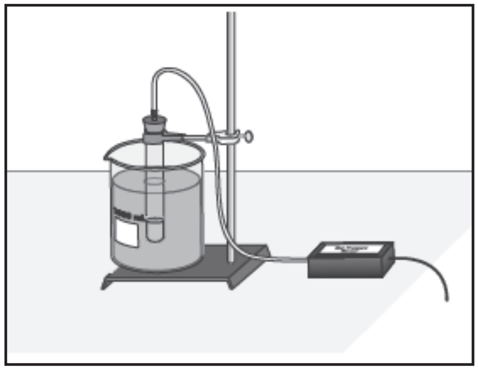

Figure 1. Vernier Setup.

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 plastic container or beaker that will fit the test tube rack inside. The desired water temperature throughout the experiment is between 37-40 °C . Combine warm and cool tap water until a temperature in this range is achieved. The beaker should contain enough water to cover the test tube rack and most of the testing tubes once in the rack. Leave a thermometer in the water bath during the experiment to monitor the temperature and add warm water to adjust if the temperature falls too low.

2. Label four test tubes – sucrose, Corn Meal (CM), Stover, and a negative control (water).

3. Obtain the three 5% feedstock solutions and place 2mL of each solution into its corresponding labeled tube (e.g. 2mL of the 5% sucrose solution in the tube labeled sucrose, 2mL of the 5% CM solution into the tube labeled CM, etc.). 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 containing the first 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. Place the test tubes in the water bath.

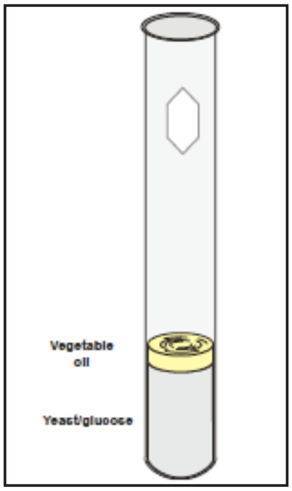

Figure 2. Cover yeast solution with oil (step 6).

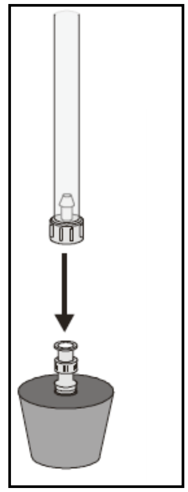

Figure 3. Connect tubing to stopper (step 10).

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. If it gets too cold (below 37 °C), remove half of the water with a beral pipet (make sure to have a large waste container or sink nearby to dispose of old water) and refill with warm tap water until the correct temperature is reached. The water should still surround the test tube when correct temperature is reached. While the test tube is incubating, begin computer setup for data recording.

9. Open the Vernier file "12B Fermentation (Pressure)" from the Biology with Vernier file in Logger Pro. 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. When incubation is complete, connect the free end of the plastic tubing to the rubber stopper.

11. Click the collect data button to begin recording data. Make sure to keep the water bath temperature constant during the course of data collection. Data will be collected for 15 minutes.

12. 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.

13. 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.

14. 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.

15. 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.

16. 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	
Corn Meal	
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	
Corn Meal	
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 corn meal 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.

Record data in the table below.

Feedstock Tested	Balloon Circumference
Water (control)	
Sucrose	
Corn Meal	
Stover	

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

Feedstock Tested	Class Average Balloon Circumference
Water (control)	
Sucrose	
Corn Meal	
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?

Name________________________________ Date_________________ Hour__________

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 (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 variable.

3. 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)?

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

Name_______________________________ Date________________ Hour__________

Post-Experiment Questions

1. Rate the success of the techniques you attempted in your experimentation. 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?