

Brewing for Bioenergy

Bioenergy Education Initiative

Levels:

Grades 6-12

Content Areas:

Chemistry; Physical Science

Lesson Time:

45 -60 minutes

Next Generation Science Standards:

LS1.C

HS-LS1-7

Objectives & Outcomes:

Students will compare different starting materials (feedstocks) for yeast fermentation.

Students will measure and observe chemical changes caused by fermentation.

After completing this lesson, students will be able to:

- Understand that biofuels can be created through fermentation.
- Demonstrate differences in the outcome of fermentation between first- and second-generation feedstocks.

Contact:

SMILE Program

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Description:

In this experiment students observe and measure evidence of chemical changes caused by fermentation. They will test multiple feedstocks (starting materials) and compare how yeast ferments sugar, starch and cellulose-based biomass (cellulose is a complex polymer made from many glucose molecules and is found in plant cell walls). This experiment also provides insight into the fermentation process and the challenge of producing ethanol and other biofuels from cellulosic materials.

Using This Lesson:

The experiment in this lesson is done in small groups. Advanced student questions and activities to expand the lesson are available.

The background information has been written so it can also be used as reading material for students. Vocabulary words are highlighted in the text and defined. Additionally, leading questions are included to promote discussion and critical thinking. A link with supporting video material can be found on the Resources page.

Fermenting to Make Fuel:

Organisms use the metabolic process of **fermentation** to convert sugars into acids, gases or alcohol. Fermentation is used for making popular foods like yogurt, cheese, beer and wine. Fermentation by yeast and bacteria is one of the processes used to make ethanol biofuel.

Humans use enormous amounts of energy every day for heating and cooling homes, transportation, powering factories and countless other energy-dependent activities. Because of our energy dependence, it is vital that we discover more efficient and sustainable ways to meet our energy needs. Bioenergy, power from plants and other organic materials and waste, is part of a sustainable future.



Brewing for Bioenergy

Set Up:

To make it easier to manage multiple student groups, we recommend creating stations where students can get their supplies.

Directions:

1. Using a Sharpie marker, have students label one of the bags "CONTROL". It will only have yeast and water in it. Have them label the others bags with the feedstocks.
2. Students will add 15 grams (1 tablespoon) of the appropriate feedstock to each bag.
3. Students will add 5 grams (1 teaspoon) of yeast to each bag. Note: make sure the yeast is fresh and has not expired.
4. Have students add 50 mL (approximately 1/4 cup) of very warm tap water to each bag. Note: The water needs to be between 100 and 110 degrees Fahrenheit (37 to 43 degrees Celsius). If the water is too cold, the yeast will not be active. If the water is too hot, you run the risk of killing the yeast.
5. Students should then zip the bags closed, making sure to remove as much air as possible before they close the bags.
6. Have students mix the contents of each bag gently with their fingers.
7. Students should lay the bags on a flat surface and then start the timer.
8. Depending on the feedstock, signs of fermentation will be visible in the bags after a few minutes. The bags with the simple sugars should begin to expand with CO₂ gas. After five minutes, have students record their initial observation on the worksheet.
9. Students should repeat observations every three minutes until 20 minutes have elapsed.
10. WARNING! As the yeast produce carbon dioxide, the bag will expand. It may even pop. Be sure to monitor the bag and release the gas if it becomes over inflated.
11. Have students compare their results. Did they observe differences between the feedstocks? What happened in the CONTROL bag?

Materials: Per Group

- 8 zippered sandwich or snack-sized bags
- Sharpie marker
- 1/4 measuring cup and tablespoon and teaspoon
- 40 grams (8 teaspoons) dry quick yeast
- 15 grams (1 tablespoon) of the following feedstocks:
 - ~ Cornmeal
 - ~ Corn stems, leaves or wood chips
 - ~ White table sugar
 - ~ High fructose corn syrup
 - ~ Powdered milk
 - ~ Powdered milk with crushed Lactaid (lactase tablet)
 - ~ Artificial sweetener
- Warm tap water
- Timer



The top image shows the beginning of fermentation after 5 minutes.

The bottom image, taken after 20 minutes, shows the bag with the sugar feedstock full of CO₂ gas, while the wood shavings bag is flat.

Expected Outcomes:

You should expect the following results.

The bags with the sugars, including the powdered milk with crushed lactase tablet and the artificial sweetener, should show signs of fermentation (the bags will expand with CO₂ gas). CO₂ is a byproduct of the fermentation reaction. This gas will build up in the plastic bag and should be quite visible. The other byproduct of fermentation is ethanol, a simple alcohol. While they won't be able to see the ethanol, students can sniff the mixture and detect the smell of an alcohol after the reaction has filled the bag with CO₂.

The bags with the cornmeal (contains starch), corn stover and wood chips (cellulosic feedstocks) will not expand. This is because the yeast cannot metabolize starch and cellulosic plant material. Before the yeast can digest these materials, enzymes are required to break them down into digestible forms. In a commercial biofuels plant, this breaking down process is called pretreatment.

Powdered milk contains lactose, a **disaccharide** (double sugar) made up of molecules of glucose and galactose joined together. Bread yeast cannot digest this particular disaccharide. In the bag containing powdered milk and lactaid (lactase enzyme), the lactaid enzyme broke the lactose molecules apart into their component molecules, allowing the yeast to ferment them.

Yeast can also ferment certain artificial sweeteners. Even if the sweetener itself can't be digested, many artificial sweeteners are packaged with dextrose to create a granular texture; dextrose is a form of glucose.

Experiment Questions:

Below are basic and advanced level questions and observations students should make from the experiment.

BASIC LEVEL

1. Which bag(s) expanded the fastest? (*White sugar and high fructose corn syrup should be fastest*)
2. Explain what is happening when the bags expand. (*The sugars are being metabolized by the yeast, resulting in the byproducts of CO₂ and ethanol. Yeast can metabolize some of the feedstocks provided, but not others.*)
3. How did the yeast respond to the different kinds of feedstocks? (*It fermented the simple sugars, but not powdered milk with no lactaid, the starch (cornmeal) or cellulose (wood chips).* Which fermented the fastest? (*sugars*) Which didn't ferment? (*powdered milk without lactaid, cornmeal, wood chips, control*) Why do you think there is a difference? (*Even though starch and cellulose are polymers (chains) of glucose, yeast cannot break them down to metabolize them directly.*)
4. What was the purpose of the "control" bag? (*The control demonstrated that fermentation did not occur without some kind of feedstock. Without it, we would not know whether the effect we observed (the bags expanding) was really connected to the presence of specific feedstocks.*)
5. How do you know fermentation is occurring? (*The presence of CO₂ expanding the bag.*) What is the gas being produced? (*CO₂; the liquid is mostly water with some ethanol.*)
6. What types of products does yeast create when it ferments? (*CO₂ and ethanol*)
7. Which bag has the most alcohol? Can you tell? (*You can't tell by looking at the bags, but you could hypothesize based on the bag with the most CO₂.*)
8. In the reading you learned that yeast uses glucose, a simple sugar, as its energy source. So why would

you want to use cellulosic plant material, such as corn stover or wood chips, instead of sugar found in food to make ethanol? (*This would avoid the food vs. fuel debate. This is the problem associated with using farmland or food crops for production of biofuels.*)

ADVANCED LEVEL

1. Have students research the chemical structure and contents of artificial sweeteners and hypothesize which ones could be digested by yeast.
2. Have students research other ways they could ferment feedstocks, besides using yeast. Then have them design an experiment to test their hypothesis.
3. Have students research designs of yeast bioreactors and find photos of bioreactors for various types of processes yeast are involved in (*Alcohol, biofuels, bioproducts*).
4. Have students identify challenges when using food crops to create ethanol. Have them consider if there is enough feedstock available. (*Food vs. fuel debate*)
5. The table below is included in the student worksheet. Have students refer to their experimental data to infer whether the bread yeast used in the experiment possesses the listed enzymes. Then have them fill in the column on the far right. Answers provided below.

Feedstock Type	What it contains	An enzyme required to break it down	Your hypothesis: Does bread yeast contain this enzyme? Y/N
Corn syrup	Monosaccharide: Fructose	Fructokinase	<i>Y, since the fructose was fermented</i>
Table sugar	Disaccharide: sucrose	Invertase	<i>Y, since the sucrose was fermented</i>
Powdered milk	Disaccharide: lactose	Lactase	<i>N, since powdered milk was not fermented unless it had added lactaid enzyme</i>
Corn Meal	Glucose polymer: starch	Amylase	<i>N, since corn meal was not fermented</i>
Wood chips or corn stover	Glucose polymer: cellulose	Cellulase	<i>N, since it was not fermented</i>

Brewing for Bioenergy

Set Up:

Read completely through the experiment directions. Then gather your group's supplies.

Directions:

1. Using a sharpie marker, label one of the bags "CONTROL". It will only have yeast in it. Label the others bags with the names of the feedstocks.
2. Add 15 grams (1 tablespoon) of the appropriate feedstock to each bag.
3. Add 5 grams (1 teaspoon) of yeast to each bag.
4. Add 50 milliliters (approximately $\frac{1}{4}$ cup) of warm tap water to each bag.
5. Remove as much air as possible, then seal the bags closed.
6. Mix the contents of each bag gently with your fingers.
7. Lay the bags on a flat surface and start your timer.
8. A reaction will begin in the bags after a few minutes. After five minutes, record your initial observations of the different bags on the worksheet.
9. Repeat observations every three minutes until 20 minutes have elapsed. Record your observations on the worksheet.
10. Read the background information, *Brewing for Bioenergy*. Then, using your observations from the experiment, complete the worksheet questions.

Materials: Per Group

- 8 zippered sandwich or snack-sized bags
- Sharpie marker
- $\frac{1}{4}$ measuring cup and tablespoon and teaspoon
- 40 grams (8 teaspoons) dry quick yeast
- 15 grams (1 tablespoon) of the following feedstocks:
 - ~ Cornmeal
 - ~ Wood chips
 - ~ White table sugar
 - ~ High fructose corn syrup
 - ~ Powdered milk
 - ~ Powdered milk with crushed Lactaid (lactase tablet)
 - ~ Artificial sweetener
- Warm tap water
- Timer

Before sealing the bags, make sure to press out as much air as possible. The method shown in the far right photo works well.



Experiment Worksheet

OBSERVATIONS	CONTROL	WHITE SUGAR	CORN SYRUP	CORN MEAL	WOOD CHIPS	POWDERED MILK	POWDERED MILK W/ LACTAID
5 MINUTES							
8 MINUTES							
11 MINUTES							
14 MINUTES							
17 MINUTES							
20 MINUTES							

Experiment Questions:

1. Which bag(s) expanded the fastest? How do you know?
2. Explain what is happening when the bags expand.
3. How did the yeast respond to different kinds of feedstocks? Which fermented the fastest? Which didn't ferment? Why do you think there is a difference?
4. What was the purpose of the "control" bag?
5. How do you know fermentation was occurring? What is the gas being produced? Which bag has the most alcohol?
6. What types of byproducts does yeast create when it ferments?
7. In the reading you learn that glucose, a simple sugar, is the food yeast use to obtain energy. So why would you want to use cellulosic plant material, such as corn stover or wood chips, instead of sugar found in food to make ethanol?

ADVANCED:

Directions: Carefully read the table below. Then, refer to your experimental data to infer whether the bread yeast used in the experiment possesses the listed enzymes. Fill in the column on the right.

Feedstock Type	What it contains	An enzyme required to break it down	Your hypothesis: Does bread yeast contain this enzyme? Y/N
Corn syrup	Monosaccharide: Fructose	Fructokinase	
Table sugar	Disaccharide: sucrose	Invertase	
Powdered milk	Disaccharide: lactose	Lactase	
Corn Meal	Glucose polymer: starch	Amylase	
Wood chips or corn stover	Glucose polymer: cellulose	Cellulase	



Brewing for Bioenergy

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Fermentation for Fuels

Fermentation is a metabolic process that converts sugars into acids, gasses or alcohol. A fermentation process is used for making popular foods like yogurt, cheese, beer and wine. For this to occur, yeast or bacteria must be present along with sugar or other **feedstocks** (starting material).

Alcohol fermentation breaks down sugars to produce CO₂ and ethanol. It is one of the processes used to make ethanol biofuel.

Yeast can be one of several species of single-cell fungi commonly used in alcohol fermentation. Yeast secretes enzymes that allow it to break down the food sources in its surroundings and recover energy from them. Yeast prefers simple sugars (**monosaccharides**), such as glucose and fructose. Yeast can break down other substrates, including some larger sugar molecules (**disaccharides**), but not others, such as **lactose**. The fermentation process is slower with larger sugars due to the greater effort it takes to break the sugar into its different components. Breaking down more complex

compounds, such as starch and cellulose, requires yet more enzymes. Starch and cellulose are polymers (long chains of repeating molecules) made of glucose.

After breaking down the food source, yeast produces CO₂ and alcohol. The gas is the most visible sign that fermentation is occurring. An example of this is seen when bread rises, due to the release of gas given off by the yeast fermenting the sugars in the dough.

Making Biofuels

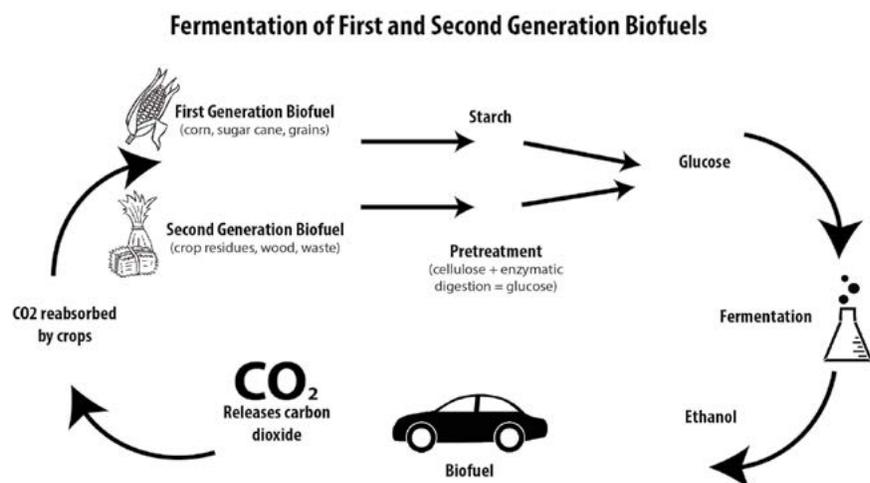
Biofuels are fuels produced from biomass. **Biomass** is organic material, including plant material, animal byproducts, microbes and waste material. Biofuel can be produced from forestry, agricultural or fishery products. It can also be produced from agricultural, food industry and municipal waste. Although biofuel typically refers to liquid fuels for transportation, it can be produced in a solid or gas form.

First generation biofuels are produced directly from food crops. The biofuel is produced from the raw starch, sugar, animal fats and vegetable oils these crops provide. Examples include corn, wheat, sugar cane, soybeans and vegetable oils.

Second generation biofuels are produced from non-food crops that are cellulose-based, or material not suitable for human consumption. Second generation feed stocks must be processed differently. This is particularly true of **lignocellulose feedstock** such as agricultural residues (corn stover, straw and bagasse), herbaceous crops (alfalfa, switchgrass), short rotation woody crops (poplars), forestry residues, waste paper and other municipal and industrial waste.

These feedstocks require several processing steps (pretreatment) before they can be fermented into ethanol and other biofuels. Second generation biofuels can be processed in a number of ways including fermentation, **gasification** (conversion into gas) and **pyrolysis** (chemical change brought about by the action of heat).

One of the main reasons second generation biofuels are attractive is because their feedstock is not food humans eat. This is sometimes referred to as the **food versus fuel debate**. It is a long running debate over the potential risks of diverting farmland or crops to produce biofuels, and the possible harm this could have on the food supply.



Next Generation Science Standards

DISCIPLINARY CORE IDEAS:

LS1.C: Organization for Matter and Energy Flow in Organisms

PERFORMANCE EXPECTATIONS:

HS-LS1-7: Use a model to illustrate that cellular respiration is a chemical process whereby the bonds of food molecules and oxygen molecules are broken and the bonds in the new compounds are formed resulting in a new transfer of energy.

PRACTICES:

- Developing/using models
- Constructing explanations/design solutions
- Obtaining/evaluate/communicate

CROSSCUTTING CONCEPTS

- Systems and system models
- Energy/matter: Flows, cycles, conservation
- Structure and function

VIDEO RESOURCES

[Lecture 17: Fermentations](#),

Time: 1:56 – 4:30; 9:19 – 10:45

GRANT SUPPORT

This work is part of the Advanced Hardwood Biofuel Northwest project (hardwoodbiofuels.org) and is supported by Agriculture and Food Research Initiative Competitive Grant no. 2011-68005-30407 from the USDA National Institute of Food and Agriculture.

Resources:

[Engineer Converts Yeast Cells into Sweet Crude Biofuel](#), Science Daily (January 22, 2014). Retrieved from <http://www.sciencedaily.com/releases/2014/01/140122091950.htm>

[Fermentation in a Bag](#), Great Lakes Bioenergy (2011). Retrieved from <https://www.glbrc.org/education/classroom-materials/fermentation-bag>

[Fun Facts About Fungi](#), Intermountain Herbarium, Utah State University (2006). Retrieved from <http://herbarium.usu.edu/fungi/funfacts/Fermentation.htm>

Additional Resources:

[Genetically Modified Yeast Turns Crop Wastes into Liquid Fuel](#), BBC News (October 11, 2013). Retrieved from <http://www.bbc.com/news/science-environment-24489800>

[Liquid Biofuels for Transport Prospects, Risks and Opportunities](#), GreenFacts. Refer to <http://www.greenfacts.org/en/biofuels/1-2/1-definition.htm>

[Second Generation Biofuels](#), Biofuel.Org.UK (2010). Refer to <http://biofuel.org.uk/second-generation-biofuels.html>

[What's That Smell? Exotic Scents Made from Re-engineered Yeast](#), The New York Times (October 20, 2013). Retrieved from http://www.nytimes.com/2013/10/21/business/whats-that-smell-exotic-scents-made-from-re-engineered-yeast.html?_r=1