BIO FAX!

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# Biofuels

## Flinn STEM Design Challenge™

## Introduction

The search for alternative energy sources to replace fossil fuels has turned attention to the production of *biofuel*—fuel made from living organisms. The fermentation of carbohydrates by yeast cells produces ethanol, which may be blended with gasoline to make "gasahol." One advantage of gasohol over regular gasoline is that it burns cleaner, resulting in fewer harmful emissions. Today, nearly all of the gasoline sold in the U.S. contains some ethanol. Explore the fermentation process of yeast cells and its byproducts.

## Concepts

- Fermentation
- Biomass and biofuel
- Renewable energy
- Engineering design

## Background

Respiration is the process that converts food into usable energy and is carried out by individual cells. Cells may undergo two types of respiration. One requires oxygen and the other, called anaerobic (without air) respiration, does not. *Fermentation* is the word commonly used for anaerobic respiration. As cells consume glucose ($C_6H_{12}O_6$) and produce energy, additional compounds are made. When no oxygen is present, the fermentation reaction may produce carbon dioxide ($CO_2$) and alcohol as seen in Equation 1.

$$C_6H_{12}O_6 \rightarrow 2CO_2 + 2C_2H_5OH + \text{energy} \qquad \textit{Equation 1}$$

Yeast is the most common organism used in the production of ethanol (ethyl alcohol). Yeast can be dried, inducing a dormant state until activated. Warm water and a food source are all that is required to "awaken" or activate the yeast. Carbohydrates from plants, called *biomass*, are used as a food source for yeast during fermentation. Sugar cane and sugar beets are sources often used, as the sugar is easily consumed by the yeast cells. Starches from grain and grasses require enzymes to break down the more complex starch molecules to simpler sugar molecules the yeast can use. Corn and switchgrass are popular crops grown for biomass. An oil refinery in Sweden uses biofuel made from bakery items that are past their sell-by date!

## Experiment Overview

The purpose of this activity is to investigate the products of fermentation. The lab begins with an Introductory Activity to determine which food source yields better results during the yeast fermentation. Since determining the presence of ethanol is a complex process, the amount of carbon dioxide gas produced over time will be used as an indication of fermentation. The results from the Introductory Activity will lead to a design of a procedure to collect and quantify the amount of $CO_2$ produced during fermentation.

## Pre-Lab Questions *(Answer on a separate sheet of paper.)*

1. Why is biofuel considered a renewable energy source?
2. Read through the *Procedure* for the Introductory Activity. Why is it important to remove as much air as possible from each zipper bag (step 5)?
3. What safety precautions need to be taken during the lab activity?



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## Materials for Introductory Activity

Corn starch, 1.5 g

Sucrose, $C_{12}H_{22}O_{11}$, 1.5 g

Yeast, 0.5 g

Water, warm tap, 40–43°C

Bags, zipper top, 3″ × 4″, 3

Balance, 0.1-g precision

Clock or timer

Graduated cylinder, 50-mL

Paper towel

Ruler, Metric

Scoop or spatula

Tape, transparent with matte finish or masking

Weighing dishes, 3

### *Safety Precautions*

*Food sources brought into the lab are considered chemicals and should never be consumed. Although materials in this activity are considered nonhazardous, wear goggles whenever working with chemicals, heat, or glassware in the laboratory. Wash hands thoroughly with soap and water before leaving the laboratory. Please follow all laboratory safety guidelines.*

## Procedure

**Part A. Introductory Activity**

1. Obtain three zipper top bags and label with a marker: control, corn starch, and sucrose, respectively.
2. Using a balance and weighing dish, measure 0.5 g of yeast and add to the control bag. Repeat for the cornstarch bag and sucrose bag.
3. Measure 50 mL of warm water using the graduated cylinder and add to the control bag. Repeat for the cornstarch bag and sucrose bag. *Note:* the water is warm and caution should be used when pouring.
4. Using the balance and separate weighing dishes, add the following food source quantities to each bag:
   *a.* Control – none
   *b.* Corn starch – 1.5 g
   *c.* Sucrose – 1.5 g
5. Remove as much air as possible and seal the zipper bags.
6. Use transparent or masking tape around the top corners to prevent leaking; pay particular attention to sealing the edges.
7. Mix the contents of each bag by gently patting your fingers together on both sides of each zipper bag, along the length of the bag.
8. Lay the bags flat on a paper towel on the lab bench and start the timer.
9. Record data record after 10 minutes. Continue data collection every 5 minutes for 25 minutes. *Note:* As gas is produced, the bags will swell and the pressure increase may cause the bags to leak if not sealed completely. Look for any liquid on the paper towel and re-seal with tape if needed.
10. Measure and record the dimensions (width and length) of any gas bubble that forms in Data Table A on the Biofuels Worksheet.
11. Record observations in the data table.

**Part B. Design Challenge**

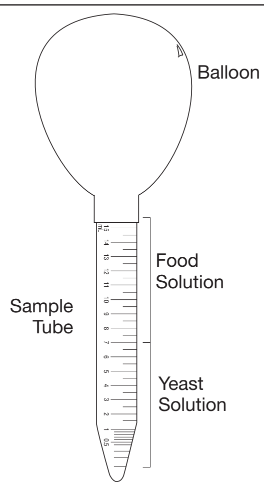
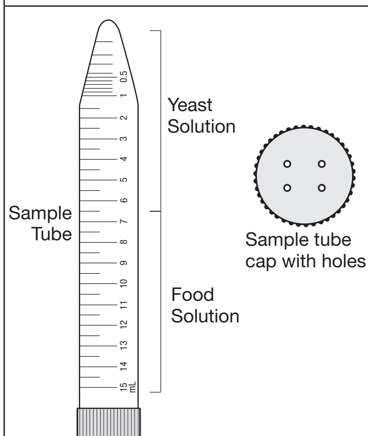
The purpose of this challenge is to design a procedure for collecting and quantifying the amount of carbon dioxide produced during fermentation.

Form a working group with other students and discuss following questions to aid in the experimental design.

1. Based on the results from the *Introductory Activity*, which food source is a better choice for producing large quantities of carbon dioxide?

2. Maintaining an ideal temperature range during the fermentation process can be a difficult task. The optimum temperature range for yeast fermentation is between 32–35 °C. Every degree over this range depresses fermentation. Given this information how will temperature be controlled during carbon dioxide collection?

3. How might the set-ups pictured below be used to capture the carbon dioxide from fermentation? How would the amount of carbon dioxide be measured in each method? What possible sources of error would need to be taken into account for each method? Fill in the table below.

| Set-Up | Method to Collect | Process to measure collected $CO_2$ | Possible Errors |
|---|---|---|---|
|  | | | |
|  | | | |

4. The results of each group's gas-collecting method will be compared. What variables will need to be controlled in each method for a fair comparison? Which variables are not able to be controlled with each method?

5. Explain why the second method requires holes in the cap.

6. Write a step-by-step procedure for collecting the carbon dioxide gas produced from fermentation. Construct a data table that clearly shows the data that will be collected and the measurements that will be made.

## Disposal

Consult your instructor for appropriate disposal procedures.

Name: ____________________

# Biofuel Worksheet

## Data Table A.

| Control | | |
|---|---|---|
| **Time (min)** | **Gas Bubble Dimensions (cm × cm)** | **Observations** |
| 10 | | |
| 15 | | |
| 20 | | |
| 25 | | |
| **Cornstarch** | | |
| **Time (min)** | **Gas Bubble Dimensions (cm × cm)** | **Observations** |
| 10 | | |
| 15 | | |
| 20 | | |
| 25 | | |
| **Sucrose** | | |
| **Time (min)** | **Gas Bubble Dimensions (cm × cm)** | **Observations** |
| 10 | | |
| 15 | | |
| 20 | | |
| 25 | | |

## Part A. Post-Lab Questions

1. Consider the products of fermentation as described in the *Background* section.

   *a.* Which product caused the bags to expand during the experiment?

   *b.* Where was the location of the gas bubble in the bag?

   *c.* Why do you think the gas collected in this location?

2. Why is it necessary to use warm water to mix the yeast and food source?

3. Examine the gas bubble dimensions from the data table.
   *a.* Which food source yielded the greatest quantity of gas?

   *b.* Explain why cornstarch and sucrose did not produce the same amount of gas.

4. Why do you think a small gas bubble was visible in the control bag?

5. What is the purpose of the control in this experiment?

## Part B. Post-Lab Questions

1 How did your group determine the amount of carbon dioxide gas collected during your experiment?

2. Compare your results with a group that used a similar method of $CO_2$ collection.
   *a.* Explain the similarities and differences in the results.

   *b.* Describe possible errors involved and their effect on the results.

3. Compare your results with a group that used a different method of $CO_2$ collection.
   *a.* Which group was able to collect more carbon dioxide gas? (*Reminder:* 1 $cm^3$ = 1 mL)

   *b.* Which method do you think is a more accurate way to measure the amount of $CO_2$ gas produced?

4. Although not visible or tested for, what other compound was present inside the tube?

5. The release of gases from burning fossil fuels is a factor in the rise of the Earth's average surface temperature, known as global warming. How might using more biofuels impact global warming?

# Teacher's Notes

## Biofuels—Flinn STEM Design Challenge™

### Materials Included in Kit (for 10 groups of students)

Cornstarch, 50 g
Sucrose, $C_{12}H_{22}O_{11}$, 100 g
Yeast, 10 packages
Bags, zipper top, 3″ × 4″, 40
Balloons, 50
Push pins, 10
Rubber bands, 20
Sample tubes with caps, 10
Weighing dishes, 30

### Additional Materials Required (for each lab group)

Balance, 0.1-g
Beaker, 250-mL
Graduated cylinders, 10- and 50-mL
Hot water bath
Scoop or spatula
Paper towel
Permanent marker
Ruler*
String*
Tape, transparent with matte finish or masking
Thermometer
Timer or clock
Water

*May be needed, depending on gas-collecting design option chosen

### Additional Material Required (for Pre-Lab Preparation)

Water
Balance, 1-g
Beaker, 250-mL
Graduated cylinder, 100-mL

### Pre-Lab Preparation

1. To prepare the yeast solution for the Design Challenge, mix one package of yeast in 150 mL of warm water (40–43°C) using a 250-mL beaker. Keep the yeast solution on a hot plate on a low setting, maintaining a temperature near 40°C. *Note:* If the water temperature rises above 43 °C, remove the beaker for a few minutes.
2. Prepare the sucrose solution using the following ratio: 2 g sucrose per 10 mL of water. Each group will need a minimum of 10-mL of the solution.

### *Safety Precautions*

*Food sources brought into the lab are considered chemicals and should never be consumed. Although materials in this activity are considered nonhazardous, wear goggles whenever working with chemicals, heat, or glassware in the laboratory. Remind students to wash hands thoroughly with soap and water before leaving the laboratory. Please follow all laboratory safety guidelines.*

### Disposal

Please consult your current *Flinn Scientific Catalog/Reference Manual* for general guidelines and specific procedures, and review all federal, state and local regulations that may apply, before proceeding. All used solutions from this lab, i.e., yeast/sucrose and yeast/cornstarch mixtures, may be disposed of down the drain with large amounts of water according to Flinn Suggested Disposal Method #26b.



## NGSS Alignment

This laboratory activity relates to the following Next Generation Science Standards (2013):

**Disciplinary Core Ideas: Middle School**

- MS-LS1 From Molecules to Organisms: Structures and Processes
  - LS1.A: Structure and Function
  - LS1.B: Growth and Development of Organisms
  - LS1C: Organization for Matter and Energy Flow in Organisms
  - PS3.D: Energy in Chemical Processes and Life
- MS-ESS2 Earth's Systems
  - ESS2.A: Earth's Materials and Systems
- MS-ESS3 Earth and Human Activity
  - ESS3.A: Natural Resources
  - ESS3.C: Human Impacts on Earth Systems
- MS-ETS1 Engineering Design
  - ETS1.A: Defining and Delimiting Engineering Problems
  - ETS1.B: Developing Possible Solutions
  - ETS1.C: Optimizing the Design Solution

**Disciplinary Core Ideas: High School**

- HS-LS1 From Molecules to Organisms: Structures and Processes
  - LS1.A: Structure and Function
  - LS1C: Organization for Matter and Energy Flow in Organisms
- HS-LS2 Ecosystems: Interactions, Energy, and Dynamics
  - LS2.B: Cycles of Matter and Energy Transfer in Ecosystems
- HS-ETS1 Engineering Design
  - ETS1.A: Defining and Delimiting Engineering Problems
  - ETS1.C: Optimizing the Design Solution

**Science and Engineering Practices**

- Asking questions and defining problems
- Developing and using models
- Planning and carrying out investigations
- Analyzing and interpreting data

**Crosscutting Concepts**

- Patterns
- Cause and effect
- Energy and matter
- Systems and system models

## Lab Hints

- Enough materials are provided in this kit for 30 students working in groups of three or for 10 groups of students. Both parts of this laboratory activity can reasonably be completed in two 50-minute class periods. The *Pre-Laboratory Assignment* may be completed before coming to lab. Students may use the wait time between measurements in the *Introductory Activity* to read through the *Design Challenge* and begin brainstorming ideas.
- Yeast cells die at temperatures above 55 °C.
- The ideal amount of yeast solution used in the water sample tubes is 7 mL with the rest of the tube filled with the sucrose solution. It is important to fill the tubes completely to remove any air.
- Keeping the sample tubes in a water bath at a temperature between 34 °C and 37 °C will yield the fastest results. Students may share water baths. Up to four 250-mL beakers may fit on one 7″ × 7″ hot plate.
- If students are using the balloon method, collecting data for two balloons is preferred to reduce the risk of a faulty balloon. If time allows, groups may repeat their experiment. Otherwise, teams may pair up and combine their data.
- Stretching the balloons before the activity will allow the balloon to fill easier and faster with $CO_2$ (for example, a balloon circumference of about 11 cm may be reached about 10 minutes faster).
- If students are capping the water collection tube and allowing the gas to rise within the tube, make sure they have poked holes in the cap.



## Teaching Tips

- Determine whether students may use an alternative method other than the ones described to collect and quantify the carbon dioxide gas. Keep in mind that fewer design constraints make analyzing the results more difficult.
- As an extension to this activity, have students research the pros and cons of using ethanol as a fuel product.

## Answers to Pre-Lab Questions *(Student answers will vary.)*

1. Why is biofuel considered a renewable energy source?

   *Biofuel comes from plant material. New crops may be planted each year.*

2. Read through the Procedure for the Introductory Activity. Why is it important to remove as much air as possible from each zipper bag (step 5)?

   *Fermentation is an anaerobic process. It occurs when oxygen is not present. If air is left in the bag, then the yeast may undergo aerobic respiration instead until the oxygen is depleted.*

3. What safety precautions need to be taken during the lab activity?

   *Food products brought into the lab are considered chemical and should not be ingested. Goggles must be worn whenever chemicals, heat or glassware are used.*

## Sample Data Table. *(Student data will vary.)*

**Data Table A.**

| **Control** | | |
|---|---|---|
| **Time (min)** | **Gas Bubble Dimensions (cm × cm)** | **Observations** |
| **10** | *1 × 1* | *A very small gas bubble appeared at the top of the bag in the center. Bag leaked a little. Sealed with more tape. No other bubbles visible. No change in size of bubble over time.* |
| **15** | *1 × 1* | |
| **20** | *1 × 1* | |
| **25** | *1 × 1* | |
| **Cornstarch** | | |
| **Time (min)** | **Gas Bubble Dimensions (cm × cm)** | **Observations** |
| **10** | *1.2 × 1.3* | *One gas bubble visible at the top of the bag in the center. Grew slightly larger over time. Bag leaked after 10 minutes. Taped all around the edges. Bag swelled slightly.* |
| **15** | *1.4 × 1.6* | |
| **20** | *1.4 × 1.7* | |
| **25** | *1.6 × 1.7* | |
| **Sucrose** | | |
| **Time (min)** | **Gas Bubble Dimensions (cm × cm)** | **Observations** |
| **10** | *3.0 × 2.9* | *Largest gas bubble of the three that grew over time. Many tiny bubbles are visible throughout the bag. Bag very swollen.* |
| **15** | *3.5 × 3.5* | |
| **20** | *3.4 × 4.0* | |
| **25** | *3.7 × 4.2* | |



## Answers to Design Challenge Guiding Questions

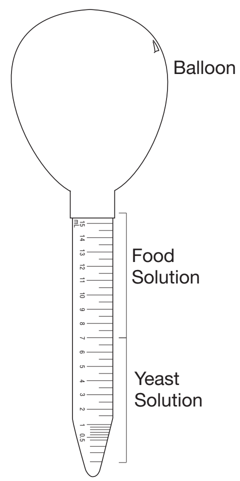
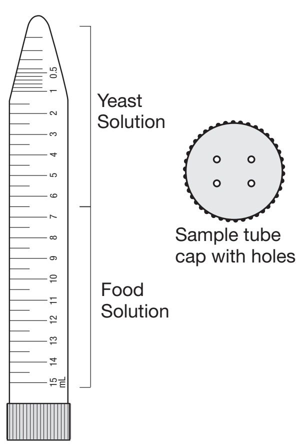
1. Based on the results from the *Introductory Activity*, which food source is the better choice for producing large quantities of carbon dioxide?

   *Sucrose is the better food source. Much more carbon dioxide gas was produced in the bag containing sucrose than the one containing cornstarch.*

2. Maintaining an ideal temperature range during the fermentation process can be a difficult task. The optimum temperature range for yeast fermentation is between 32–35 °C. Every degree over this range depresses fermentation. Given this information, how will temperature be controlled during carbon dioxide collection?

   *Using a water bath, consisting of a beaker of water on a hot plate with a thermometer, will allow the optimum temperature range to be controlled.*

3. How might the set-ups pictured below be used to capture the carbon dioxide from fermentation? How would the amount of carbon dioxide be measured in each method? What errors would need to be taken into account for method? Fill in the table below.

| Set Up | Method to Collect | Process to measure collected $CO_2$ | Possible Errors |
|---|---|---|---|
|  | *Place 7 mL of yeast solution into sample tube, fill to very top with sucrose solution, cap and invert to mix contents. Remove cap, stretch balloon mouth over tube and secure with rubber band. Place tube into water bath (34–37°C).* | *- Collect $CO_2$ in balloon and measure circumference of balloon with string and ruler, calculate volume of sphere ($V=4/3 \cdot \pi r^3$)*<br>*- Collect $CO_2$ in the balloon, tie off and use water displacement to determine volume* | *- Balloon malfunction*<br>*- Water bath temperature fluctuation*<br>*- Loss of gas when removing balloon from sample tube*<br>*- Balloon not perfect sphere*<br>*- Water displacement method will include balloon*<br>*- A small amount of air may be present in the balloon* |
|  | *Using the push pin, put 4 holes into the cap. Place 7 mL of yeast solution into sample tube, fill to very top with sucrose solution. Cap and place thumb over holes and invert tube to mix contents. Place inverted tube into a water bath (34–37°C).* | *- As fermentation occurs $CO_2$ will rise up the sample tube and push the yeast/sucrose solution out through the holes in the cap into the water bath. The sample tubes are marked with volume graduations and the volume of $CO_2$ can be read directly.* | *- Water bath temperature fluctuation*<br>*- Spillage* |

4. The results of each group's method will be compared. What variables will need to be controlled in each set up for a fair comparison? Which variables are not able to be controlled in each method?

   *Controlled variables for both methods include the amount and concentration of yeast and sucrose solutions, and the amount of time allowed to collect the $CO_2$. The water bath temperature and yeast/sucrose solution temperature may fluctuate slightly, but will be controlled within a few degrees using a water bath and thermometer. Controlled variable for balloon method include type/size balloon and method of measurement. Each group should inflate the balloon once prior to use.*

   *Variables that are not controllable include temperature fluctuation if allowed to get too hot or too cool. The activity of yeast from one solution to the next may vary.*

5. Explain why the second method requires holes in the cap.

   *As fermentation occurs, the $CO_2$ produced will rise toward the top of the sample tube. The expanding gas will push the liquid down. The liquid must be allowed to escape in order reduce pressure build up, allowing fermentation to continue.*

6. Write a step-by-step procedure for collecting the carbon dioxide gas produced from fermentation. Construct a data table that clearly shows the data that will be collected and the measurements that will be made.

   *Student Answers will vary.*

## Answers to Part A Lab Questions

1. Consider the products of fermentation as described in the *Background* section.

   *a.* Which product caused the bags to expand during the experiment?

   *Carbon dioxide gas caused the bags to expand.*

   *b.* Where was the location of the gas bubble in the bag?

   *The gas bubble was at the top of the bag, near the center.*

   *c.* Why do you think the gas collected in this location?

   *The $CO_2$ gas is less dense than the surrounding liquid mixture and rises to the top of the bag.*

2. Why is it necessary to use warm water to mix the yeast and food source?

   *Yeast requires two conditions to be activated from the dried state, one of which is warm water and the other a food source.*

3. Examine the gas bubble dimensions from the data table.

   *a.* Which food source yielded the greatest quantity of gas?

   *Sucrose produced the greatest quantity of gas. The bubble that formed was much larger than the bubble in the cornstarch or control bags.*

   *b.* Explain why cornstarch and sucrose did not produce the same amount of gas.

   *The yeast is not able to use the cornstarch as a food source because it is a complex carbohydrate that requires enzymes to break it down into simpler sugars the yeast can metabolize.*

4. Why do you think a small gas bubble was visible in the control bag?

   *Student answers will vary. A small amount of sugar is in the dry yeast mixture, allowing for a minimal amount of fermentation. The bag may have still contained some air after being sealed shut.*

5. What is the purpose of the control in this experiment?

   *A control is used to compare results of the experimental group(s) to ensure that any difference is the result of the manipulated variable and not some other factor. In this case, the differences in the amount of gas produced, as indicated by the size of the bubbles, was a result of the food source in the bag, as all other variables remained the same.*

## Answers to Part B Post-Lab Questions

1. How did your group determine the amount of carbon dioxide gas collected during your experiment?

   *Student answers will vary.*

2 Compare your results with a group that used a similar method of $CO_2$ collection.

   *a.* Explain the similarities and differences in the results.

   *Student answers will vary.*

   *b.* Describe possible errors involved and their effect on the results.

   *Student answers will vary. See Design Challenge Guiding Question 3 for possible sources of error. In addition, some foam may be present at the interface of the liquid and gas in the tube, making the exact volume of gas from the gas displacement method difficult to measure.*

## Teacher's Notes *continued*

3. Compare your results with a group that used a different method of $CO_2$ collection.

   *a.* Which group was able to collect more carbon dioxide gas? (*Reminder:* 1 $cm^3$ = 1 mL)

   *Student answers will vary. Differences may result from amount of time the experiment was allowed to run, as well as possible sources of error with either method. The balloon is capable of collecting a greater volume of gas than the sample tube.*

   *b.* Which method do you think is a more accurate way to measure the amount of $CO_2$ gas produced?

   *The gas displacement method is likely to be more accurate. More errors are likely with the balloon method.*

4. Although not visible or tested for, what other compound was present inside the tube?

   *Ethanol (ethyl alcohol) is also produced during fermentation.*

5. The release of gases from burning fossil fuels is a factor in the rise of the Earth's average surface temperature, known as global warming. How might using more biofuels impact global warming?

   *Biofuels burn cleaner that regular gasoline made from fossil fuels, reducing the amount of gas emissions that contribute to global warming.*

## References


"Fermentation in a Bag." Great Lakes Bioenergy Research Center. www.glbrc.org/educaton (Accessed January 2016).

"Stale Swedish Bread Makes Fresh Ethanol." Chemical & Engineering News 15 June 2015: p. 16.


## The *Biofuels—Flinn STEM Design Challenge*™ is available from Flinn Scientific, Inc.

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