Corn fermentation in a bag
How can we determine the fastest rate of fermentation?

Background
Fossil fuels are made from the remains of ancient, fossilized plants that lived millions of years ago. Similar to plants today, these microorganisms used the process of photosynthesis to grow. Photosynthesis captures the energy of the sun, water, and carbon dioxide (CO$_2$) to produce energy and generate cell growth. Over time, these microorganisms were buried and developed into oil that is used to produce gasoline and diesel fuel today. All of the CO$_2$ that was used to create those microorganisms is stored in the oil in the ground.

When a fuel like gasoline is burned in a car’s engine, the CO$_2$ that was stored in it from the ancient remains of microorganisms is released back into the atmosphere. When a biofuel such as ethanol is burned in a car’s engine, the CO$_2$ that is released into the atmosphere is from the previous growing season. This means that when ethanol is burned, CO$_2$ is recycled between plants and the atmosphere annually, instead of millions of years later from gasoline.

Commercial production of fuel ethanol in the United States involves breaking down the starch present in corn into simple sugars (glucose), feeding these sugars to yeast (fermentation), and then recovering the main product (ethanol) and byproducts (animal feed and carbon dioxide). Ethanol is an alcohol produced through the process of yeast fermentation of sugars.

\[
\text{C}_6\text{H}_{12}\text{O}_6 \quad \rightarrow \quad 2\text{CH}_3\text{CH}_2\text{OH} \quad + \quad 2\text{CO}_2
\]

Glucose \quad \rightarrow \quad Ethanol \quad + \quad Carbon dioxide

The complex carbohydrates found in corn must be broken down into simple sugars for fermentation to be successful. Heating the feedstock can help to break apart carbohydrate bonds but is not 100% successful. Enzymes are used to efficiently cut carbohydrates into simple sugars. For example, amylase breaks down complex carbohydrates into a two sugar molecule called maltose, and glucoamylase breaks down maltose into the 1 sugar molecule, glucose. Glucose is the simple sugar that is used during fermentation for industrial ethanol production.

Overview
In this simple experiment, students will use the Engineering Design process to investigate the production of industrial ethanol. The Engineering Design process will help the students define the problem, research and identify specific requirements, choose the best solution and build their design for production, then retest and redesign. Students will test the process of fermentation using resealable bags with yeast, warm water, various feedstocks and enzymes. Students will measure the changes in their plastic bags due to the fermentation of the feedstocks with breathalyzers and bag inflation.

Essential questions
• Which feedstock material will produce the largest volume of CO$_2$ gas?
• How can data/evidence be collected to measure CO$_2$ gas?
Possible materials

• Resealable bags
• Dry activated yeast
• Warm water
• Various feedstocks (cracked corn, corn syrup, corn meal, whole # 2 dent corn, corn stover powder, sweet corn, table sugar, fructose, glucose)
• Various Enzymes (amylase, glucoamylase)
• Teaspoon measuring spoon (one per feedstock source)
• 100 mL graduated cylinder
• Labels
• Ruler
• Index cards
• Pocket Breathalyzer (optional)
• 100 cc syringe (optional)
• Ethanol probes (optional)

Producing feedstocks

Using the engineering design process, students will create and test different feedstock variations.

• Students will need to research each possible feedstock in order to create a test to break it down into simple sugars so that fermentation is successful.
• Students will need to research various enzymes to understand their role in carbohydrate breakdown to enable them to release simple sugars from more complex sugars.
• Students will need to test various ways to physically break down complex sugars.

The Engineering Design process will help the students define the problem, research and identify specific requirements, choose the best solution and build their design for production, then retest and redesign their process.

Design

Divide students into research teams, students complete the following steps as they plan and carry out their design.

• Brainstorm several research problems, select one problem to investigate.
• Identify what is needed to solve the problem.
• Brainstorm solutions to the problem. Design a test to create the most productive feedstock source.
• Check with instructor before beginning your test.
• Determine how to measure/collect data.
• Make and test your feedstock source.
• Evaluate the results. Redesign the test to improve the results.
• Share your test design.

Directions

1. In a snack-sized resealable zipper bag combine 1 teaspoon of feedstock material, 1 teaspoon of yeast, and any additional enzymes of your design choice.
2. Add 50 mL (1/4 cup) of warm water and zip the bag closed, removing as much air as possible.
3. Mix gently. Lay the bag on a flat surface and watch for results – collect data at 5-minute intervals when using the Bag Height Measurement Method:
   • Lay the fermenting bag flat on a horizontal surface.
   • Place an index card on top of the bag, parallel to the table.
   • Hold the ruler perpendicular to the table and record where the paper intersects the ruler.
   • Record this measurement with the appropriate time in the data section.
Plot all data on your graph to measure slope. Slope is the fermentation rate for the feedstock.

4. Discuss and interpret your results.
   - Optional: Measure and compare ethanol and/or CO₂ production using a pocket Breathalyzer (BAC) to measure CO₂.
   - Optional: Measure and compare ethanol and/or CO₂ production using ethanol probes.
   - Warning: As the yeast produce CO₂, the bag will expand – it may even pop! Be sure to monitor the bag and release the gas if it becomes too inflated.

Note: Using a feedstock that is a simple sugar will yield the most rapid results. Feedstocks with a starchy composition will not ferment well. Yeast does have the enzymes required to break down starch into glucose, but this happens very slowly so you see little fermentation. Students can also develop their own methods to measure fermentation and extend the investigation by changing variables to increase fermentation rates.

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<th>Time (minutes)</th>
<th>Bag height (mm)</th>
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**Bag height vs. time**

Fermentation rates can be calculated by plotting height vs. time, fitting a line to the points and then calculating the slope of the line. The slope of the line is the fermentation rate (mm of inflation per minute).

1. Are you observing fermentation? How do you know? What is filling the bag up?
2. How did the yeast respond to different kinds of food? What did you do to your food to make it more available to the yeast?
3. How can we change dent corn into simple sugar? What process does commercial ethanol production use to create simple sugars from starch?
4. What is the fermentation rate (slope = Change y/Change x) of your feedstock? Show your calculation below.