Corn fermentation in a bag

What is necessary for fermentation to take place? 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.

 $C_6H_{12}O_6 \rightarrow 2CH_3CH_2OH + 2CO_2$ glucose $\rightarrow 2$ ethanol + 2 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, maltose, and **glucoamylase** breaks down maltose into a one sugar molecule, glucose. Glucose is the simple sugar that is used during fermentation for industrial ethanol production.

Overview

Use the Engineering Design process to investigate the production of industrial ethanol. The Engineering Design process can be used to define the problem, research and identify specific requirements, choose the best solution and build a design for production, then retest and redesign. Test the process of fermentation using resealable bags with yeast, warm water, various feedstocks and enzymes. Measure the changes in plastic bags due to fermentation of the feedstocks with breathalyzers and bag inflation.

Possible materials

- Snack-sized resealable bags
- Dry instant (rapid rise) activated yeast
- Warm water (95° F)
- Corn flour
- glucose
- Various feedstocks for Engineering Design (corn syrup, honey, cornmeal, fructose, sweet corn, dent corn)
- Amylase
- glucoamylase
- ¼ teaspoon measuring spoon (one per Amylase and Glucoamylase)

Instructions

- 1. Create 4 bags, each in a snack-sized resealable zipper bag as follows:
 - a. Combine 1 teaspoon of yeast and 1 teaspoon of crushed glucose material.
 - b. Combine 1 teaspoon of yeast, 1 teaspoon of corn flour and 1/4 teaspoon of amylase.
 - c. Combine 1 teaspoon of yeast, 1 teaspoon of corn flour and ¼ teaspoon of glucoamylase.
 - d. Combine 1 teaspoon of yeast, 1 teaspoon of corn flour, and ¼ teaspoon of both amylase and glucoamylase.

• Teaspoon measuring spoon

• 50 mL graduated cylinder

Optional: Pocket Breathalyzer
Optional: 100 cc syringe and tubing

• Marker to label bags

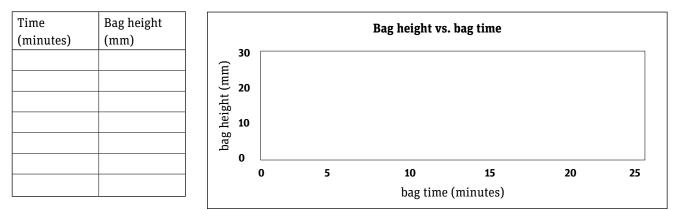
• Mortar and pestle

• Ruler

• Index cards

(one per feedstock source and yeast)

- 2. Add 50 mL (¹/₄ cup) of warm water to each bag, then 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 *explain* your results. (Use the Background to help).
 - Optional: Measure and compare ethanol and/or CO, production using a pocket Breathalyzer (BAC) to measure CO,
 - 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.

Reflection

- How much CO₂ is produced by glucose compared to corn flour?
- What enzymes are required to produce simple sugars?

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Engineering design

Now that students have a better understanding of fermentation and what it takes to break down feedstocks, they can use the Engineering Design process to create and test different feedstock variations.

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

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

- Brainstorm several research problems and 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.

Record the experiment design and data in a lab notebook and reflect on how the chosen feedstocks responded to the fermentation process.

- How did the yeast respond to various feedstocks? What did you do to your feedstock to make it more available to the yeast?
- How can we change dent corn into simple sugar?
 What process does commercial ethanol production use to create simple sugars from starch?
- 3. What is the fermentation rate (slope = Change y/Change x) of your feedstock? Show your calculation below.



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.