Fermenting Fuel:

Name: ____________________

Class: ____________________

Lab Group: ________________

Overview

Ethanol is a renewable source of fuel for vehicles, which is widely produced from corn. Ethanol production is reliant on anaerobic fermentation of corn sugars by yeast. Scientists and industry professionals are constantly working to make the fermentation procedure more efficient. Different enzymes are added to the corn to break the starch into simple sugars that the yeast can process into ethanol. This lab allows experimentation with different variables in the fermentation process to determine their effect. These findings can then be used to develop a fermentation procedure that may be used on a larger sample for the distillation lab.

Variables that may be tested:

- Feedstock: suggest using cracked corn if students are designing procedure for distillation
- Enzymes, none, alone, or in combination
- Effect of pH on yeast and enzymes
- Amount of yeast solution added
- Starting yeast in a 2% glucose solution
- Temperature

Students will design and conduct their own investigations to answer one or more of the following driving questions or develop their own. (Teacher may choose the questions, it is recommended that all students test enzymes separately, mixture and control as one investigation.)

- What is the effect of each enzyme or combination on glucose concentration and rate of fermentation?
- How much of an effect does starting yeast in glucose solution have on fermentation rate?
- What is the optimal amount of yeast solution per gram of feedstock to produce the most ethanol in the allotted time frame?
- Do the enzymes work more effectively at releasing glucose at a certain pH?
- How does baker's yeast compare to brewer's yeast in ability to produce ethanol from corn?

Students will measure the amount of CO2 produced in the time frame that the teacher selects. This can be one class period or overnight. If allowed to run overnight, time-lapse video of the fermentation allows students to plot data points from the times they were not able to directly observe the fermentation.

Background Information

The chemical reaction that powers most vehicles is a combustion reaction that uses gasoline, diesel, or ethanol as fuel. Each of these fuels has a carbon chain and burning these fuels releases carbon dioxide. Carbon dioxide is a greenhouse gas. Its levels are rising in the atmosphere as a result of burning fossil fuels. This increase is thought to be a major component of climate change.

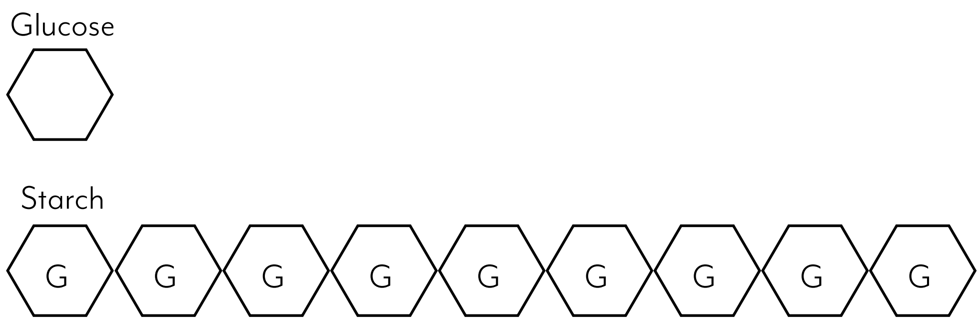
The difference between fossil fuels, such as diesel and gasoline, and biofuels, such as ethanol and biodiesel, is the source of the carbon. When fossil fuels are refined from crude oil that is pumped from deep underground, the carbon in biofuels comes from carbon dioxide, which is fixed from the atmosphere during photosynthesis. The carbon dioxide produced from burning fossil fuels is "old" carbon, not having been in the atmosphere for millions of years, whereas the carbon dioxide produced from burning biofuels is "new" carbon that was in the atmosphere during the lifetime of the plant used to produce the fuel. Burning biofuels requires the removal of a carbon dioxide molecule from the atmosphere for every molecule produced during combustion of the fuels. Producing and burning fossil fuels produces no increase in atmospheric carbon dioxide.

Fermentation is an anaerobic process (without oxygen) carried out by yeast, bacteria, and even muscle cells. This is an alternative pathway for organisms to release energy from food when oxygen is not available. Yeast and muscle cells can carry out both aerobic respiration and anaerobic fermentation allowing an energy source when oxygen is scarce. The first step in both aerobic and anaerobic respiration is the breaking of a glucose molecule called "glycolysis". This yields 2 new ATP molecules (cellular fuel) per molecule of glucose broken. If oxygen is not available, the cell needs to recycle the molecules needed to break more glucose. In doing this it converts the broken pieces of glucose (pyruvate) into different molecules. Most bacteria, and our muscle cells, convert pyruvate into lactic acid, which is important in the production of cheese and yogurt, while yeast and some other bacteria convert the pyruvate into ethanol, which is a renewable fuel source. As shown in the equation below, this process also produces two carbon dioxide molecules for each glucose molecule fermented. The rate and amount of fermentation can be determined by measuring the amount of carbon dioxide produced.

$$C_6H_{12}O_6 \rightarrow 2\,C_2H_5OH + 2\,CO_2 + \text{Energy}$$

Glucose	Ethanol	Carbon Dioxide	2 ATP

In this activity yeast will be used, which needs glucose to break for fuel. The ethanol industry feeds yeast ground corn, in which most of these sugars are contained in long chain-like molecules called "starch". The differences in these food sources are shown in the image below.



There are several enzymes whose purpose is to break starch up into smaller pieces, which are more useful for the yeast. Amylase is an enzyme that breaks starch into smaller pieces, while glucoamylase is more specialized in removing individual glucose molecules from the ends of the starch. Enzymes have specific conditions in which they function more effectively, such as pH and temperature. The optimal conditions for fermentation may be researched or tested in this lab.

Materials Needed

Materials for Preparing Samples:

- Ground corn (enough for 1 g for each sample)
- Dry activated yeast (may want to provide different varieties if students want to test)
- Amylase solution (1 tsp/500 ml water or 1g/125 ml)
- Glucoamylase solution (1 tsp/500 ml water or 1g/125 ml)
- Buffer solution (pH5; pH4 optional if pH is being tested)
- Balance
- 5 test tubes for each group
- Rubber stoppers
- Beaker (500 ml)
- Hot plate
- Test tube holders
- Stirring rod

Materials for Fermentation:

- 5 test tubes of prepared samples
- Test tube rack
- Rubber stoppers with tubing connectors and tubing to collect CO2 produced
- Tub for water displacement
- 3D printed manifold with 5, 50 ml centrifuge tubes
- Distilled water

Materials for Fermentation (Continued)

- Food coloring for more visibility (recommended especially if time-lapse is being used)
- Beaker (500 ml)
- Hot plate
- Balance
- Disposable pipettes
- Dry activated yeast, may want to provide different varieties if students want to test
- Amylase solution (1 tsp./500 ml water or 1g/125 ml)
- Glucoamylase solution (1 tsp./500 ml water or 1g/125 ml)
- Buffer solution (pH5, pH4 optional if pH is being tested)
- Other materials students may need (beaker for warm water bath, etc.)

Procedure for Lab

1. Design the experiment with up to five samples, as in any controlled experiment there must be a comparison sample that is only one variable different from another sample.
2. The design should be written so that each sample has a recipe in your Fermenting Fuel Student Handout.

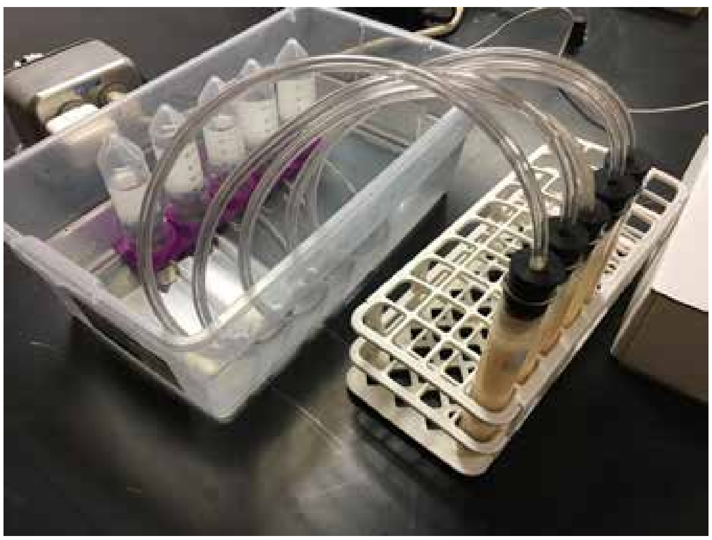
Preparing Samples (May be student or teacher prepared)

1. Add approximately 250 ml of water to a 500 ml beaker and heat to boiling.
2. Add 1 g of ground corn to each sample test tube and add 5 ml distilled water.
3. Mix the corn and water mixture with a stirring rod and add a test tube holder.
4. Place test tubes in boiling water for 10 minutes to break down corn. (Note: This time can be shortened or step skipped if students want to test the importance of heating time.)
5. Place a stopper in the samples and allow to them cool. The samples may be placed in cold water bath or allowed to cool overnight. Do not add enzymes until sample has cooled.

Fermentation Procedure

1. Fill tub with enough water to cover the 35 ml mark on the tubes.
2. Tip the manifold over on its back and tap the tubes to remove bubbles.
3. Students will add the ingredients to each sample as they have prescribed in their procedure.
4. Mix each sample with a stirring rod, rinsing between samples and place in test tube rack.
5. Stopper each sample with a one-hole stopper and insert tubing into opening in manifold below the gas collection tube for that sample. Repeat for each sample.
6. Tip manifold up so that gas is now being collected.
7. If time lapse recording is to be used, set up camera.
8. Measure and record volume of CO2 collected every 5 minutes.
9. Graph data and analyze results.
10. Teacher may allow students to design another experiment or procedure for fermentation to use in the follow up ethanol lab.

Filled manifold with tubing from samples inserted. Ready to collect any CO_2 gas produced.



Student set up testing rate of fermentation with different varieties of yeast.

Fermenting Fuel: Student Data

Name ________________________________

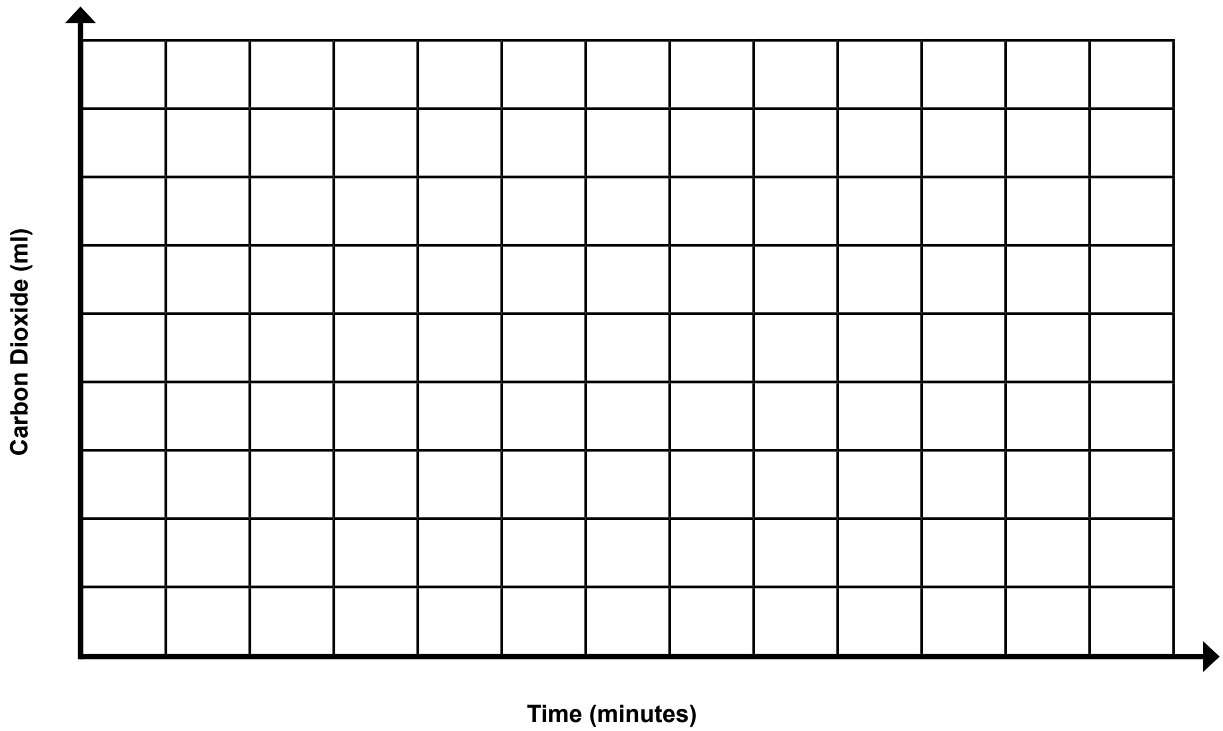
Design an investigation that tests the effect of each enzyme as well as a combination of both on the fermentation rate of corn.

When you have decided what to include in each of your samples; record in the table below.
Record

	Test Tube 1	Test Tube 2	Test Tube 3	Test Tube 4	Test Tube 5	Test Tube 6
Amylase (ml)						
Glucoamylase (ml)						
pH buffer						
Yeast (g)						
Heated						

Data Collection: CO_2 produced (ml)

Time (min)	Test Tube 1	Test Tube 2	Test Tube 3	Test Tube 4	Test Tube 5	Test Tube 6
5						
10						
15						
20						
25						
30						
35						
40						
45						
50						
55						
60						



Key

- ☐ Test Tube #1
- ☐ Test Tube #2
- ☐ Test Tube #3
- ☐ Test Tube #4
- ☐ Test Tube #5

Reflection and Conclusion

How could you tell when fermentation was occurring?

What effect did the enzymes have on the fermentation?