



Kansas Corn: Fermentation in a Bag

This lab is made possible with the support and content contributions of the Kansas Corn Commission.



Kansas Corn: Fermentation in a Bag

Grade Level: Middle School

Overview

In this experiment, students investigate the process for fermentation in resealable bags with yeast, warm water, enzymes, and various sources of sugars from plants. Students will observe and measure the inflation of the bag as the reaction produces gas (CO_2) during the production of ethanol. Students will set up several bags to compare how yeast ferments simple sugars, starch, and complex carbohydrates. Students can compare the rates of ethanol productions and the effects of different enzymes using the inflation of the bag, inflation of balloons, water, or breathalyzers. This lab is used to explore and understand the process of ethanol production as a biofuel.

Ethanol is a renewable source of fuel for vehicles that is widely produced from corn. Ethanol production is reliant on anaerobic fermentation of corn sugars by yeast. Scientists and industry professionals are always 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 students to experiment with different variables in the fermentation process to determine their effect.

When a fuel, such as gasoline, is burned, carbon is released into the atmosphere in the form of carbon dioxide. Burning fossil fuels adds extra carbon dioxide to the atmosphere. This extra carbon dioxide traps heat from the sun and is a major contributing factor in climate change.

Ethanol made from corn also releases carbon dioxide into the atmosphere, but unlike burning fossil fuels, corn plants have already absorbed the CO_2 from corn grown last season. This means that by using ethanol we can cycle carbon dioxide between the plants and atmosphere, as opposed to releasing fossilized carbon from oil.

Kansas College and Career Ready Standards

Science Performance Expectation

- **HS-LS2-3.** Construct and revise an explanation based on evidence for the cycling of matter and flow of energy in aerobic and anaerobic conditions.

Clarification Statement: Emphasis is on conceptual understanding of the role of aerobic and anaerobic respiration in different environments.

Science Disciplinary Core Ideas

- **LS2.** Ecosystems: Interactions, Energy, and Dynamics
- **LS2.B.** Cycles of Matter and Energy Transfer in Ecosystems
Photosynthesis and cellular respiration (including anaerobic processes) provide most of the energy for life processes.

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Science Practices

- Constructing explanations and designing solutions in 9–12 builds on K–8 experiences and progresses to explanations and designs that are supported by multiple and independent student-generated sources of evidence consistent with scientific ideas, principles, and theories.
 - Make a quantitative and/or qualitative claim regarding the relationship between dependent and independent variables.
 - Construct and revise an explanation based on valid and reliable evidence obtained from a variety of sources (including students' own investigations, models, theories, simulations, peer review) and the assumption that theories and laws that describe the natural world operate today as they did in the past and will continue to do so in the future.
 - Apply scientific ideas, principles, and/or evidence to provide an explanation of phenomena and solve design problems, taking into account possible unanticipated effects.
 - Apply scientific reasoning, theory, and/or models to link evidence to the claims to assess the extent to which the reasoning and data support the explanation or conclusion.
 - Design, evaluate, and/or refine a solution to a complex real-world problem, based on scientific knowledge, student-generated sources of evidence, prioritized criteria, and trade off considerations.
- Energy drives the cycling of matter within and between systems.

Learning Objectives

- The student will differentiate between aerobic respiration and anaerobic fermentation.
- The student will design and conduct an investigation to determine the effect of enzymes on fermentation and/or glucose release.
- The student will collect and analyze data to determine the rate of fermentation.

Materials

- *Fermentation in a Bag* PowerPoint (available at kansascornstem.com)
- Student Data Sheet A or B (pg. S1-S4, or at kansascornstem.com)
- Feed stock: cracked corn, corn syrup, corn starch, corn meal, dextrose or glucose, fructose, sucrose (table sugar), sweet corn
- Active dry yeast (1 tsp per bag)
- Amylase solution (1 tsp/500 ml water or 1g/125 ml)
- Glucoamylase solution (1 tsp/500 ml water or 1g/125 ml)
- Measuring spoons
- Graduated cylinders (100 ml)
- Resealable bags
- Index cards
- Rulers

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- Dry yeast or yeast solution
- Paper towels
- Breathalyzer (optional)
- 100 ml syringe (optional)

Safety Considerations

This lab contains materials that students might consider edible. Remind students that while working in a lab, all materials should be considered dangerous and not be tasted. There should be no eating or drinking in the laboratory. Students should wear appropriate eye protection.

Procedures for Instruction

Length of time for preparation: 1 hour

Length of time for classroom teaching: 1 class period for the lab and 1 class period for discussion and wrap up.

Preparation Procedure:

This lab can be used in classrooms without a lot of lab equipment. The entire lab can be completed with items found at a local store. Teachers can choose to teach the lab in two ways.

- Option 1: Teachers Designed Experiment where the teacher assigns the students different feedstock options.
- Option 2: Student Designed Experiment allows more inquiry-based learning.

Students can choose the feedstock and enzymes to try to figure out which produces the best results.

Before the lab, mix the enzymes using the following proportions:

Amylase solution (1 tsp /500 ml water or 1g/125 ml)

Glucoamylase solution (1 tsp /500 ml water or 1g/125 ml)

Using the proportions, you can reduce or increase the amount mixed as need.

Background Information

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, providing 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 two new ATP molecules (cellular fuel) per molecule of glucose broken. If oxygen is not available, the cell needs to recycle the molecules needed in order to break more glucose. In doing this, it converts the broken pieces of glucose (pyruvate) into different molecules. Yeast convert the pyruvate into

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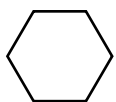
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ethanol, which is a renewable fuel source. This process also produces two carbon dioxide molecules for each glucose molecule fermented. Students can determine the rate and amount of fermentation by measuring the amount of carbon dioxide it produces, as shown in the equation below.



In this activity, students will be using yeast, which needs glucose to break for fuel. Glucose and fructose are both simple sugars, but the ethanol industry feeds yeast corn, in which most of the sugars are contained in long chain molecules, or polymers called “starch”.

Glucose



Starch



Several enzymes have the purpose of breaking 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. Being proteins, enzymes have specific conditions in which they function more effectively, such as pH and temperature.

Classroom Discussion

If you are teaching this as a standalone lesson:

Possible topics for discussions before beginning this lab depending on what has been previously taught:

1. Life cycles
2. Single-celled organisms
3. Asexual reproduction (budding)
4. Enzymes
 - Discuss fermentation with the students. Let the students know that, like you, yeast eat, grow, reproduce, and discard waste. As yeast eat the food products, they give off two waste products, carbon dioxide and ethanol. The more the yeast eats and reproduces, the more carbon dioxide and ethanol they produce.

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- Discuss with your students things that yeast might like to eat and why they might like those foods. Let students discuss their ideas, while you guide the discussion. Tell students that they are going to feed the yeast some different types of food and they can determine the best food for yeast by measuring the amount of carbon dioxide they produce.
- Save the discussion about simple and complex sugars for after the lab. Use the student results to guide that discussion.

If you are teaching this as a larger unit, and using this lab to build understanding for making ethanol, you would want to discuss biofuels and the benefits of producing ethanol.

Option 1: Teacher Designed Experiment

Divide the students into lab groups. Assign each group one of the feedstock options.

1. Provide each student with a copy of the Student Data Sheet.
2. Groups will need 4 labeled, resalable bags (see pg S5, or kansascornstem.com, for printed labels):
 - (Feedstock ex. corn starch) no enzymes
 - (Feedstock) amylase
 - (Feedstock) glucoamylase
 - (Feedstock) both enzymes
3. In a snack-sized resalable bag, combine 1 tsp of feedstock material and one tsp of yeast.
4. Add 1 ml of enzymes into the resalable bags that call for them.
5. Add 50 ml (1/4 cup) of warm water and zip the bag closed, removing as much air as possible.
6. Mix gently. Lay the bag on a flat surface.
7. Lay the fermenting bag flat on a horizontal surface.
8. Place an index card on top of the bag, parallel to the table.
9. Hold the ruler perpendicular to the table and record where the paper intersects the ruler.
10. Record this measurement in 5-minute intervals in the data section. After 20-30 minutes, some of the bags will approach their maximum height. Thus, the recommended time for data collection is 20 minutes. (Note: As the yeast produce carbon dioxide, the expanding bag may pop. Be sure to monitor the bag and release the gas if the bag becomes over inflated.)
11. Compare the final bag heights for different feedstocks and fermentation rates. Fermentation rates can be calculated by plotting height vs. time on a graph, then calculating the slope of the line. The slope of the line is the reaction rate.

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Optional:

Students can use an ethanol probe or breathalyzer to compare relative differences in ethanol concentrations. Breathalyzers are designed for measuring blood alcohol content in people; therefore, the readings will not tell you directly how much ethanol in each sample. Still, the readings can be used to determine if ethanol was produced and to make comparisons in ethanol production between the various feedstocks.

1. After 20-30 minutes and determining the height of bags, open a small part of the bag and insert the syringe while being careful not to release too much air.
2. Extract 100 ml of air from the space above the fermenting mixture, without sucking in any of the liquid.
3. Turn on the breathalyzer and follow the directions for the device. Substitute the syringe of air from the sample for the “breath.” Slowly expel the air over a 5-second period.
4. Record the readings on the breathalyzer. The higher the number on the display, the higher the ethanol concentration in the sample. Use these values to compare the ethanol concentrations from each sample.

Option 2: Student Designed Experiment

Before the lab, mix the enzymes using the following proportions:

Amylase solution (1 tsp. /500 mL water or 1g/125 ml)

Glucoamylase solution (1 tsp. /500 ml water or 1g/125 ml)

Using the proportions, you can reduce or increase the amount mixed as need.

Identify the different variables in this experiment. Students choose a variable that they would like to investigate.

1. Provide each student with Student Sheet B.
2. In a snack-sized resealable zipper bag combine 1 tsp of feedstock material, 1 tsp of yeast, and any additional enzyme solutions of your design choice.
3. Add 50 ml (1/4 cup) of warm water and zip the bag closed, removing as much air as possible.
4. Mix gently. Lay the bag on a flat surface and watch for results – collect data in 5-minute intervals.
5. Lay the bag flat on a horizontal surface.
6. Place an index card on top of the bag, parallel to the table.
7. Hold the ruler perpendicular to the table and record where the paper intersects the ruler. Record this measurement in 5-minute intervals in the data section.
8. See “Optional” section for directions on how to use the ethanol probes and breathalyzers.

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Teachers Tips

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.

Visit kansascornstem.com to access video tips for this lab.

Reflection and Conclusion

This can be done as a standalone lab or it can be done as part of a larger unit covering ethanol production. This lab is designed to introduce the fermentation process using yeast and a combination of enzymes. The next labs in sequence are *Ethanol-Corn Mash* and *Distillation and Nutrient Testing*.

The following questions will allow the students to review what they have learned and apply this new knowledge in the fermenting of ethanol lab.

- How could you tell when fermentation was occurring?
- What was filling the bag up?
- 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?
- What effect did the enzymes have on the fermentation?
- Was one enzyme or combination more effective?
- How can we change corn into simple sugar?

Science and Agriculture Careers

To learn more about agriculture careers visit www.agexplorer.com. You can also find career profiles at kansascornstem.com.

Sources

Ohio Corn and Wheat curriculum – <http://ohiocorneducation.org/>

Any educator electing to perform demonstrations is expected to follow *NSTA Minimum Safety Practices and Regulations for Demonstrations, Experiments, and Workshops*, which are available at <http://static.nsta.org/pdfs/MinimumSafetyPracticesAndRegulations.pdf>, as well as all school policies and rules and all state and federal laws, regulations, codes and professional standards. Educators are under a duty of care to make laboratories and demonstrations in and out of the classroom as safe as possible. If in doubt, do not perform the demonstrations.

Name _____

Date _____ Hour _____

Fermentation in a Bag

Student Sheet A

Materials:

- Feedstock: ground corn, corn syrup, cornstarch, corn meal, dextrose or glucose, table sugar, flour, dried honey.
- Active dry yeast (1 tsp per bag)
- Amylase solution 1 mL
- Glucoamylase solution 1mL
- Resealable bags
- Index cards
- Rulers
- Graduated cylinders

Procedures:

1. Feedstock assigned to group _____.
2. Label 4 zip lock bags with the following description:
 - Bag 1 - (Feedstock assigned) no enzymes
 - Bag 2 - (Feedstock assigned) with amylase
 - Bag 3 - (Feedstock assigned) with glucoamylase
 - Bag 4 - (Feedstock assigned) with both amylase and glucoamylase
3. In each bag combine 1 tsp of feedstock material and 1 tsp of yeast.
4. Add 1 ml of each enzyme to the correct bag.
5. Add 50 ml of warm water to the bag with no enzyme and zip the bag closed, removing as much air as possible. Repeat for the remaining bags.
6. Mix each bag gently and lay the bag flat on a horizontal surface. Start your timer.
7. Place an index card on top of the bag parallel to the table.
8. After 5 minutes, hold a ruler perpendicular to the table and record where the index card intersects the ruler. Record the data in the table below every 5 min.

Name _____

Date _____ Hour _____

Time	Bag 1	Bag 2	Bag 3	Bag 4
5				
10				
15				
20				

Reflection and conclusion:

1. How could you tell when fermentation was occurring?
2. What effect did the enzymes have on the fermentation of the feedstock material?
3. Was there a more effective combination? Why do you think that was?

Name _____

Date _____ Hour _____

Fermentation in a Bag

Student Sheet B

Materials:

- Feedstock: ground corn, corn syrup, cornstarch, corn meal, dextrose or glucose, table sugar, flour, dried honey.
- Active dry yeast (1 tsp per bag)
- Amylase solution 1 mL
- Glucoamylase solution 1mL
- Resealable bags
- Index cards
- Rulers
- Graduated cylinders

Procedures:

1. Variable to test _____.
2. Label 4 zip lock bags with the following description:
 - Bag 1 - Your Feedstock \ Control
 - Bag 2 - Your Feedstock \ Your variable
 - Bag 3 - Your Feedstock \ Your variable
 - Bag 4 - Your Feedstock \ Your variable
3. Fill each bag with the same amount of materials (feed stock, water, enzyme), making sure to only change the amount for the variable you chose.
4. Add warm water to the bag and zip the bag closed, removing as much air as possible.
5. Mix each bag gently and lay the bag flat on a horizontal surface. Start your timer.
6. Place an index card on top of the bag parallel to the table.
7. After 5 minutes, hold a ruler perpendicular to the table and record where the index card intersects the ruler. Record the data in the table below every 5 min.

Name _____

Date _____ Hour _____

Time	Bag 1	Bag 2	Bag 3	Bag 4
5				
10				
15				
20				

Reflection and conclusion:

1. How could you tell when fermentation was occurring?
2. What effect did the enzymes have on the fermentation of the feedstock material?
3. Was there a more effective combination? Why do you think that was?

Fermentation in a Bag

Name _____

Food Source _____

Enzyme _____

Start _____ End _____

Mix tsp of yeast with 1 tsp of food source.
Add 50 ml of warm water and enzyme.



Fermentation in a Bag

Name _____

Food Source _____

Enzyme _____

Start _____ End _____

Mix tsp of yeast with 1 tsp of food source.
Add 50 ml of warm water and enzyme.



Fermentation in a Bag

Name _____

Food Source _____

Enzyme _____

Start _____ End _____

Mix tsp of yeast with 1 tsp of food source.
Add 50 ml of warm water and enzyme.



Fermentation in a Bag

Name _____

Food Source _____

Enzyme _____

Start _____ End _____

Mix tsp of yeast with 1 tsp of food source.
Add 50 ml of warm water and enzyme.



Fermentation in a Bag

Name _____

Food Source _____

Enzyme _____

Start _____ End _____

Mix tsp of yeast with 1 tsp of food source.
Add 50 ml of warm water and enzyme.



Fermentation in a Bag

Name _____

Food Source _____

Enzyme _____

Start _____ End _____

Mix tsp of yeast with 1 tsp of food source.
Add 50 ml of warm water and enzyme.



Fermentation in a Bag

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Food Source _____

Enzyme _____

Start _____ End _____

Mix tsp of yeast with 1 tsp of food source.
Add 50 ml of warm water and enzyme.



Fermentation in a Bag

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Food Source _____

Enzyme _____

Start _____ End _____

Mix tsp of yeast with 1 tsp of food source.
Add 50 ml of warm water and enzyme.



Fermentation in a Bag

Name _____

Food Source _____

Enzyme _____

Start _____ End _____

Mix tsp of yeast with 1 tsp of food source.
Add 50 ml of warm water and enzyme.



Fermentation in a Bag

Name _____

Food Source _____

Enzyme _____

Start _____ End _____

Mix tsp of yeast with 1 tsp of food source.
Add 50 ml of warm water and enzyme.

