



Kansas Corn: Fermenting Fuel- Designing a Procedure for Fast Fermentation

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



Kansas Corn: Fermenting Fuel

Grade Level: High School

Overview

Ethanol is a renewable source of fuel for vehicles, which is widely produced from corn. Ethanol production is reliant on the 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 in order 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. Students can then use their findings to develop a fermentation procedure that they may use on a larger sample for the distillation lab, Kansas Corn: Corn Mash and Distillation (available online at kansascornstem.com).

Variables teachers and/or students can select to test:

- Feedstock: Suggest using ground 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

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, and 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.

Kansas College and Career Ready Standards

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.

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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.

Practices: High School (9-12)

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

Cross Cutting Concept

- 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.
- Using data students will design a recipe for producing ethanol in a larger scale lab for distillation.

Materials

- Fermenting Fuel PowerPoint (available online at kansascornstem.com)

Materials for Preparing Samples:

- Ground corn (enough for 1 g per sample)

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- Balance
- Rubber stoppers
- Beaker (500 ml)
- Hot Plate
- Test tube holder
- Stirring rod

Materials for Fermentation:

- Fermenting Fuel Student Handout (pg. S1-S7, or at kansascornstem.com)
- 5 test tubes of prepared samples (per group)
- Test tube rack (per group)
- Rubber stoppers with tubing connectors and tubing to collect CO₂ produced (per group)
- Tub for water displacement (per group)
- 3D printed manifold with 5, 50 ml centrifuge tubes (per group)
- Distilled water
- Food coloring for more visibility (recommended especially if time-lapse is being used)
- 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, etc.)

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 are not to be tasted. There should be no eating or drinking in the laboratory.
- Students should wear appropriate eye protection.

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.

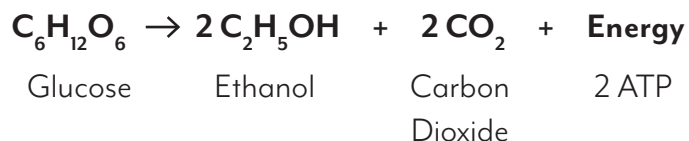
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The difference between fossil fuels, such as diesel and gasoline, and biofuels, such as ethanol and biodiesel, is the source of the carbon. When fossil fuels are refined from crude oil that is pumped from deep underground, the carbon in biofuels comes 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.

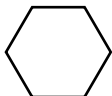
Fermentation is an anaerobic process (without oxygen) carried out by yeast, bacteria, and even muscle cells. This is an alternative pathway for organisms to release energy from food when oxygen is not available. Yeast and muscle cells can carry out both aerobic respiration and anaerobic fermentation, 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. Students can determine the rate and amount of fermentation by measuring the amount of carbon dioxide produced.



In this activity students will be using yeast, which needs the simple sugar 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.

Glucose



Starch



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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. Students may research or experiment to find the most efficient range for the enzymes used.

Procedures for Instruction

Length of time for preparation:

Day 1: 1 hour

Length of time for classroom teaching:

Day 1: 1 hour (Preparing samples and designing experiment)

Day 2: 1 class period (running experiment, 1 hour or block)

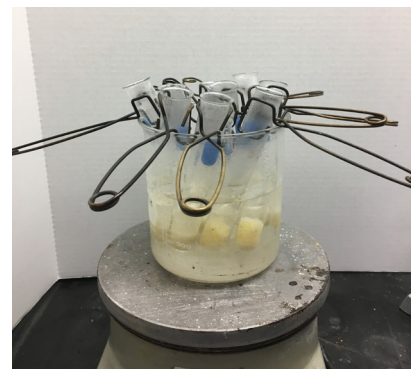
Day 3: 1 hour (reflections, conclusions, discussion and assessment)

Introducing Lab

1. Assign lab groups and present students with the basic procedure for preparing samples and fermenting, and the variables that they may alter in an attempt to improve the procedure.
2. Make sure each student receives a copy of the Fermenting Fuel Student Handout (pg. S1-S7, or available online at kansascornstem.com) so they can reference background information, instructions, and enter data as well as answer reflection and conclusion questions. (Sample data and key are included on pg. T11-T13.)
3. Allow students to design the experiment with up to five samples, reminding them that there must be a comparison sample that is only one variable different from another sample.
4. The design should be written so that each sample has a recipe on the 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. Label 5 test tubes.
3. Add 1 g of ground corn to each sample test tube.
4. Add amylase solution to the test tubes prescribed in the procedure.
5. Add 5-10 ml of distilled water, making sure final levels are equal.
6. Mix the corn and water mixture with a stirring rod and add a test tube holder.



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7. Place test tubes in boiling water for 10 minutes, as shown in the image above, to break down corn. (Note: This time can be shortened or step skipped if students want to test the importance of heating time.)
8. Place a stopper in the samples and allow them to cool. The samples may be placed in cold water bath or allowed to cool overnight. Do not add enzymes until sample has cooled.

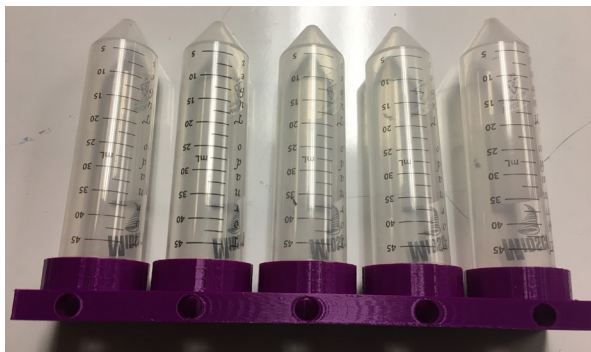
Procedure for Lab

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 3-5 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.



3D Printed manifold for gas collection. The .stl file for this manifold is available on the kansascornstem.com to allow teachers to print more if needed.



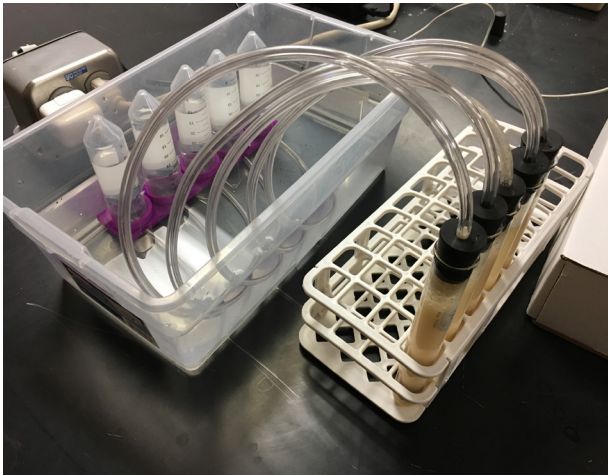
Manifold with centrifuge tubes inserted. The manifold will be submerged in water in this position to fill with water.

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Filled manifold with tubing from samples inserted. Ready to collect any CO₂ gas produced.



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

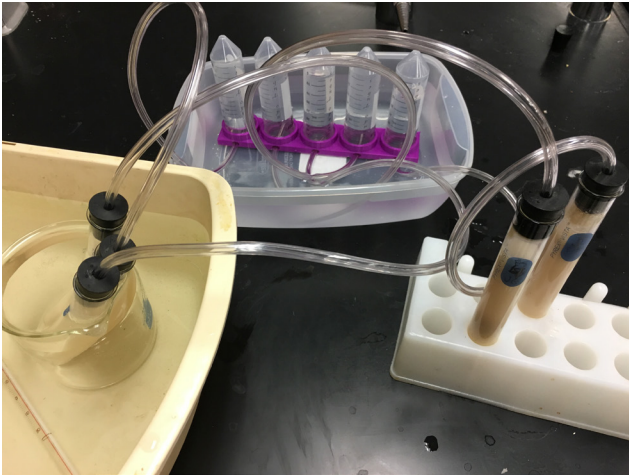


Student set up above with iPad included for time lapse recording of data.

Video recorded during this experiment is available at kansascornstem.com.

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Student set up to test the effect of a warm water bath (38C-40C) on the rate fermentation.

Reflection and Conclusion

Multiple documents are available for the teachers and students to use during this lab. Use the Fermenting Fuel Student Handout (pg. S1-S7, or at kansascornstem.com) to guide students through the lab, but to also conclude and reflect on what is was learned. Sample data, assessments, and answer keys are provided for teacher guidance.

Assessment

Available at kansascornstem.com (T10-T16).

Science and Agriculture Careers

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

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.

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Fermenting Fuel: Student Data

Name Sample Data

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)	2.0 ml	0 ml	2.0 ml	0 ml	2.0 ml	2.0 ml
Glucoamylase (ml)	2.0 ml	0 ml	0 ml	2.0 ml	2.0 ml	2.0 ml
pH buffer	2.0 ml	2.0 ml	2.0 ml	2.0 ml	2.0 ml	2.0 ml
Yeast (g)	1.0 g	1.0 g	1.0 g	1.0 g	1.0 g	0.5 g
Heated	No	10 min	10 min	10 min	10 min	10 min

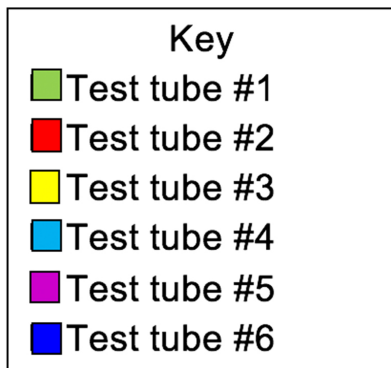
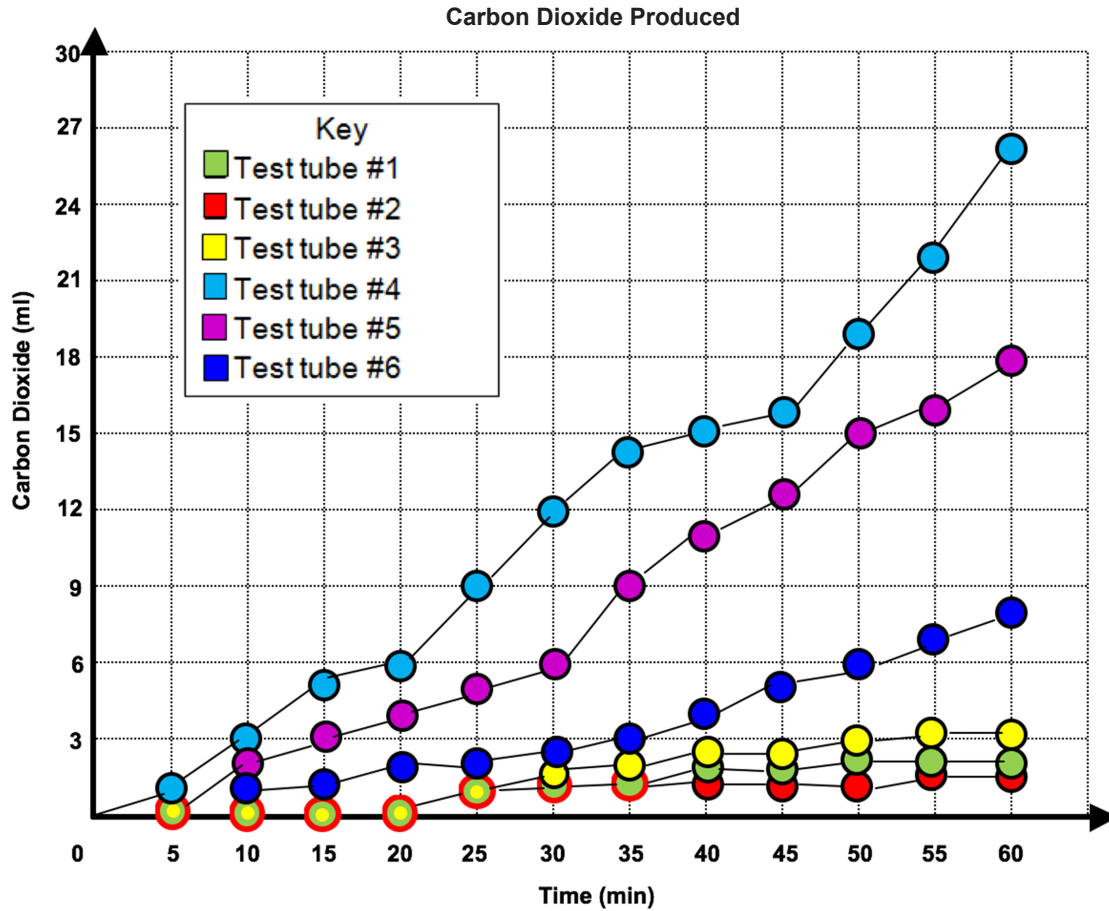
Data Collection: CO₂ produced (ml)

Time (min)	Test Tube 1	Test Tube 2	Test Tube 3	Test Tube 4	Test Tube 5	Test Tube 6
5	0	0	0	1	1	0
10	0	0	0	3	2	1
15	0	0	0	5	3	1
20	0	0	0	6	4	2
25	1	1	1	9	5	2
30	1	1	1.5	12	6	2.5
35	1	1	2	14	9	3
40	1.5	1	2.5	15	11	4
45	1.5	1	2.5	16	12.5	5
50	2	1	3	19	15	6
55	2	1.5	3.5	22	16	7
60	2	1.5	3.5	26	18	8

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Graph of sample data



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Reflection and Conclusion

How could you tell when fermentation was occurring?

Bubbles were forming in the sample.

What effect did the enzymes have on the fermentation?

From Sample Data: The enzymes increased the rate of the fermentation when comparing samples 3-6 with sample 2 that did not contain enzymes.

Was one enzyme or combination more effective?

Answers may vary, from Sample Data: Sample 4, only containing glucoamylase, produced the fastest rate of fermentation.

What evidence supports your claim?

Sample 4 produced much more carbon dioxide in the time measured.

Based on your data, write a step-by-step procedure to produce the highest rate of fermentation of ethanol from corn?

- 1.
- 2.
- 3.
- 4.
- 5.
- 6.

Fermenting Fuel from Corn: Assessment

Lisa

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)	2.0 ml	0 ml	2.0 ml	0 ml	2.0 ml	2.0 ml
Glucosylase (ml)	2.0 ml	0 ml	0ml	2.0 ml	2.0 ml	2.0 ml
pH buffer	2.0 ml	2.0 ml	2.0 ml	2.0 ml	2.0 ml	2.0 ml
Yeast (g)	1.0 g	1.0 g	1.0 g	1.0 g	1.0 g	0.5 g
Heated	No	10 min	10 min	10 min	10 min	10 min

1. In Lisa's experiment, which two samples should she compare to determine the effect of heating the sample?

Test Tube # _____ and _____

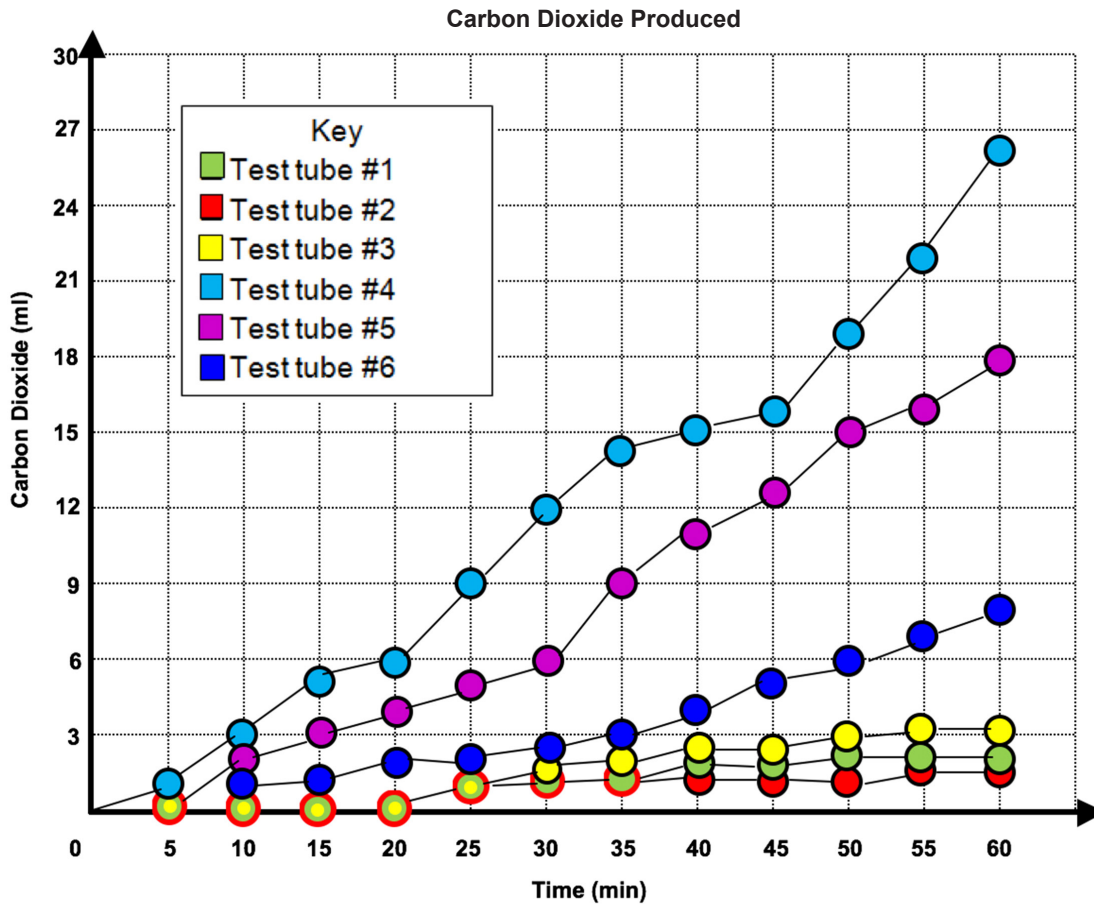
Why did you select those two samples?

2. Which samples should she compare to determine the effect of the amount of yeast in a sample?

Test Tube # _____ and _____

Why did you select those two samples?

Graph of data



Looking at Lisa's graph above of the data her group collected; answer the following questions.

3. ____ Which enzyme or combination produced the greatest rate of fermentation?
- A) Amylase
 - B) Glucoamylase
 - C) Both Amylase and Glucoamylase
 - D) She did not conduct a test that would answer this question.

What evidence supports your answer?

4. ____ How did the reduced amount of yeast affect the rate of fermentation?
- A) The sample with half as much yeast produced twice the CO₂.
 - B) The sample with half as much yeast produced half as much CO₂.
 - C) The sample with half as much yeast produced roughly the same amount of CO₂.
 - D) Lisa's group did not isolate the amount of yeast as a variable so we don't know.

Fermenting Fuel from Corn: Assessment (KEY)

Name Lisa

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)	2.0 ml	0 ml	2.0 ml	0 ml	2.0 ml	2.0 ml
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pH buffer	2.0 ml	2.0 ml	2.0 ml	2.0 ml	2.0 ml	2.0 ml
Yeast (g)	1.0 g	1.0 g	1.0 g	1.0 g	1.0 g	0.5 g
Heated	No	10 min	10 min	10 min	10 min	10 min

1. In Lisa's experiment, which two samples should she compare to determine the effect of heating the sample?

Test Tube # 1 and 5

Why did you select those two samples?

The only difference between these samples is that test tube 5 was heated for 10 minutes while test tube 1 was not

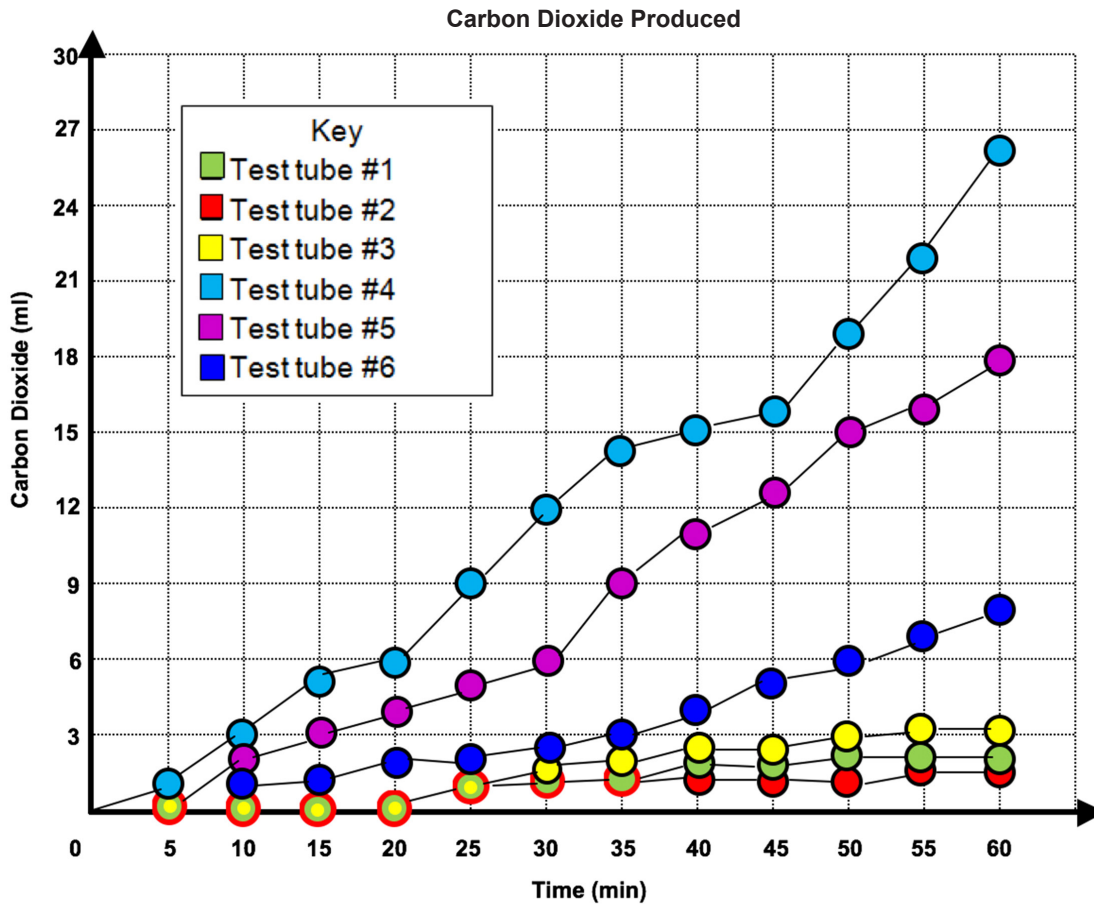
2. Which samples should she compare to determine the effect of the amount of yeast in a sample?

Test Tube # 5 and 6

Why did you select those two samples?

These two samples are the same except that number 6 has half of the yeast as number 5.

Graph of data



Looking at Lisa's graph above of the data her group collected; answer the following questions.

3. B Which enzyme or combination produced the greatest rate of fermentation?
- A) Amylase
 - B) Glucoamylase
 - C) Both Amylase and Glucoamylase
 - D) She did not conduct a test that would answer this question.

What evidence supports your answer?

Test tube #4 had the greatest rate of fermentation and it only contained Glucoamylase.

4. B How did the reduced amount of yeast affect the rate of fermentation?
- A) The sample with half as much yeast produced twice the CO₂.
 - B) The sample with half as much yeast produced half as much CO₂.
 - C) The sample with half as much yeast produced roughly the same amount of CO₂.
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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?

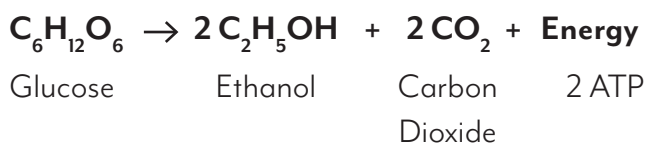
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Background Information

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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)

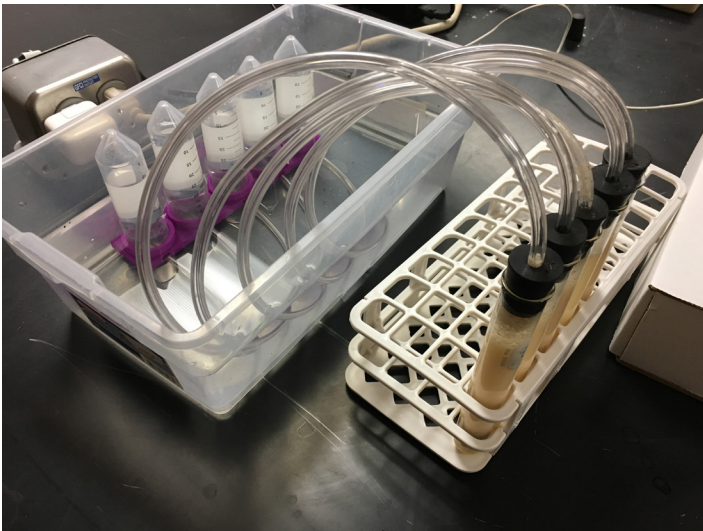
1. Add approximately 250 ml of water to a 500 ml beaker and heat to boiling.
2. Add 1 g of ground corn to each sample test tube and add 5 ml distilled water.
3. Mix the corn and water mixture with a stirring rod and add a test tube holder.
4. Place test tubes in boiling water for 10 minutes to break 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 CO₂ 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.

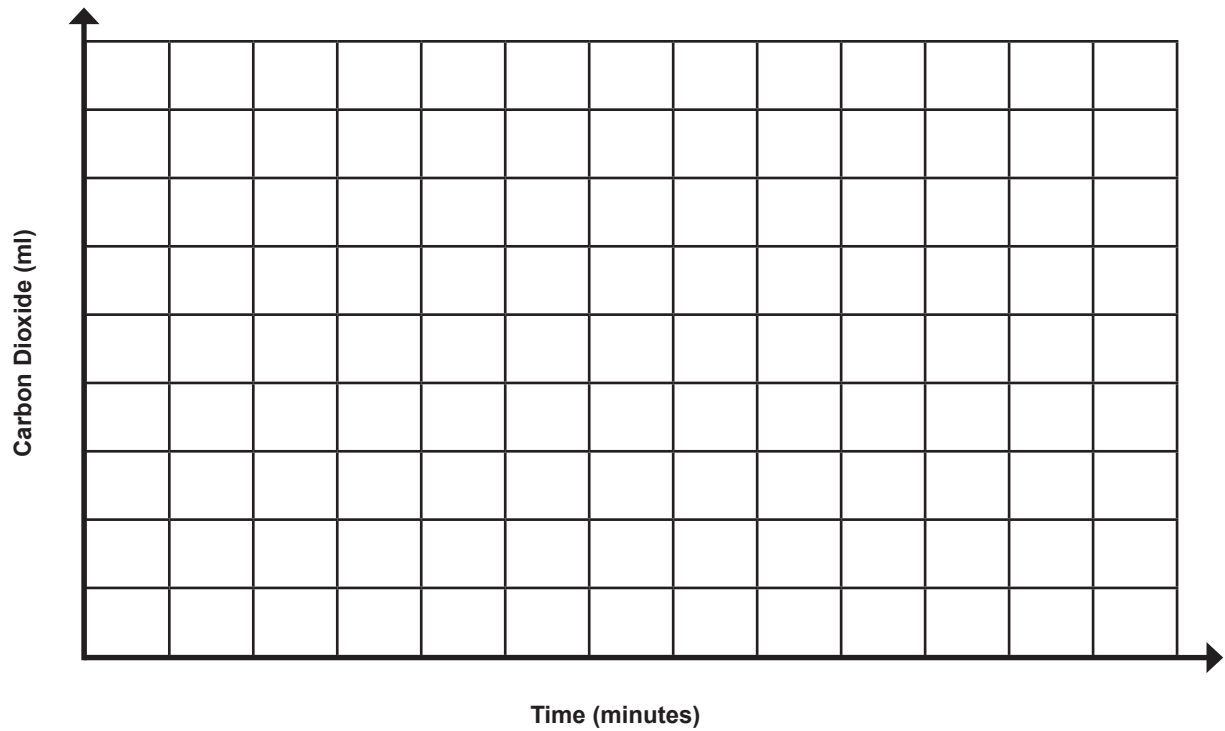
When you have decided what to include in each of your samples; record in the table below.

Record

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

Data Collection: CO₂ produced (ml)

Time (min)	Test Tube 1	Test Tube 2	Test Tube 3	Test Tube 4	Test Tube 5
3					
6					
12					
15					
18					
21					
24					
27					
30					
33					
36					
39					
42					
45					



Key	
<input type="checkbox"/>	Test Tube #1
<input type="checkbox"/>	Test Tube #2
<input type="checkbox"/>	Test Tube #3
<input type="checkbox"/>	Test Tube #4
<input type="checkbox"/>	Test Tube #5

Reflection and Conclusion

How could you tell when fermentation was occurring?

What effect did the enzymes have on the fermentation?