

Energy & Ethanol Unit

HS: Matter and Energy in Organisms and Ecosystems		
<p>Students who demonstrate understanding can:</p> <p>HS-LS1.6: Construct and revise an explanation based on evidence for how carbon, hydrogen, and oxygen from sugar molecules may combine with other elements to form amino acids, and/or other large carbon-based molecules. <i>Clarification Statement: Emphasis is on using evidence from models and simulations to support explanations.</i> <i>Assessment Boundary: Assessment does not include the details of the specific chemical reactions or identification of macromolecules.</i></p> <p>HS-LS2-3: Construct and revise an explanation based on evidence for the cycling of matter and energy flow of energy in aerobic and anaerobic conditions. <i>Clarification Statement: Emphasis is on conceptual understanding of the role of aerobic and anaerobic respiration in different environments.</i> <i>Assessment Boundary: Assessment does not include the specific chemical processes of either aerobic or anaerobic respiration.</i></p>		
Science and Engineering Practices	Disciplinary Core Ideas	Crosscutting Concepts
<p>Constructing explanations and Designing solutions.</p> <ul style="list-style-type: none"> 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 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. Construct and revise an explanation based on valid and reliable evidence obtained from a variety of sources (including student's own investigations, models, theories, simulations, and peer review) and the assumption that theories and that describe the natural world 	<p>LS1.C: Organization for Matter and Energy Flow in Organisms</p> <ul style="list-style-type: none"> As matter and energy flow through different organizational levels of living systems, chemical elements are recombined in different ways to form different products. The sugar molecules thus formed contain C, H, and O; their H backbones are used to make amino acids and other C-based molecules that can be assembled into larger molecules. <p>LS2.B: Cycles of Matter and Energy Transfer in Ecosystems</p> <ul style="list-style-type: none"> Photosynthesis and cellular respiration (including anaerobic processes) provide most of the energy for life processes. 	<p>Energy & Matter:</p> <ul style="list-style-type: none"> Energy drives the cycling of matter within and between systems. Changes of energy and matter in a system can be described in terms of energy and matter flows into, out of, and within that system.

Feeding the World: Energy & Ethanol Overview

This high school unit focuses on the practice of constructing explanations and designing solutions to understand the processes of fermentation and ethanol production. Constructing explanations and designing solutions this unit's focus because the fermentation process and ethanol production involve many variables that are constantly evaluated to make the processes efficient and profitable. Students will investigate and evaluate the many competing variables used in these processes to create an efficient design solution for fermentation and ethanol production.

Lesson 1, *Corn Fermentation in a Bag*, utilizes the engineering design process as a model that engages the students in a race to create ethanol (a result of fermentation). In the process of designing fermentation models, the students investigate how different reactants drive the fermentation process to create different amounts of product (carbon dioxide and ethanol) dependent upon system conditions. Lesson 2, *Ticketase*, builds upon Lesson 1 by predicting the action of enzymes on polysaccharides for breakdown into glucose. Students evaluate enzymatic evidence to explain their importance in commercial ethanol production. Lesson 3, *Biomass to Sugars*, builds upon the Lesson 2 as students investigate the feedstocks currently used in commercial ethanol production and explain why dent corn is the current feedstock of choice for commercial production. Lesson 4, *Ethanol: Nutrient Testing*, builds upon Lesson 3 as students investigate the food versus fuel myth and identify weaknesses in explanatory accounts that ethanol production takes away from our current food system. Lesson 5, *Ethanol: Corn Mash and Distillation*, builds upon the Lessons 1, 2, 3 and 4 as students explain their design process and predict the efficiency of their design based upon previous evidence and research. Students test their design as they distill their fermented feedstocks into ethanol and redesign.

The *Next Generation of Science Standards (2013)* suggest the goal of science is to construct explanations of the world in which we live and the goal of engineering is to design solutions. In high school classrooms, students are encouraged to use evidence to construct explanations that specify variables as they describe and predict phenomena in the design of multiple solutions to a problem. In this unit, students investigate ethanol production using the engineering design process to explain industrial ethanol production. They will make a quantitative or qualitative claim regarding the relationship between

dependent and independent variables in their design. They will then construct and revise their explanation based on valid and reliable evidence obtained from a variety of sources. In this process, they will apply scientific reasoning and models to link evidence to the claims to assess the extent to which the reasoning and data support their explanation. Constructing Explanations (science) and designing solutions (engineering) helps us to think about the connection between science and engineering. Three simple questions help tie science (constructing explanations) and engineering (designing solutions) (1) asking a question about an occurrence *What is occurring?* (2) determining the *why of the how or why of the occurrence*, and (3) determining the basis for the evidence in other words, *how do you know?*

The assessment for this unit will focus on Constructing Explanations and Designing Solutions to construct and revise explanations for the current practices employed in ethanol production based on valid and reliable evidence obtained from a variety of sources. Students will be assessed as they generate data to investigate the components of ethanol production and evaluate the limitations of the fermentation bag model. Assessment will continue as they apply evidence to provide explanations for the unit phenomena and solve design problems such as feedstock choice and improvement in process efficiency in current commercial ethanol production. Rubrics will be used to assess their mastery level from “Does not Meet” to “Advanced Understanding” and students will self-assess to determine their knowledge gained on their understanding of both the fermentation process and ethanol production.

References:

POET. (2016). The Ethanol Process (Infographic), Retrieved January 1, 2019, from:
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(Driving Ethanol). (2007, November 28). *How Ethanol Is Made Animated Feature (Video Feature)*. Retrieved from: <https://youtu.be/59R-NqykoXs>

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HS: Energy & Ethanol Storyline

Lesson/Routine	Questions	Phenomena/Problems	What We Figured Out
Anchoring Phenomena		4 fermentation bags <ul style="list-style-type: none"> Set up 20 minutes earlier by teacher. 	Each of the fermentation bags reacted differently to the contents inside.
Lesson 1 Corn Fermentation in a Bag	<ul style="list-style-type: none"> Why is happening inside of each bag? What is the purpose/function of each ingredient? 	Students analyze the 4 fermentation bags to determine what is occurring and how the reactants drive the reaction.	Each component of the fermentation bag has an impact on the efficiency of the fermentation process.
Lesson 2 Ticketase	<ul style="list-style-type: none"> What do enzymes (Ticketase and glucoticketase) do to starch? 	Enzymes catalyze reactions to make them occur more rapidly than they would without them. How can amylase and glucoamylase help to break starch down into glucose?	Enzymes break molecules like starch into smaller molecules that can be used for fermentation.
Lesson 3 Biomass to Sugars	<ul style="list-style-type: none"> How much glucose is available in sweet corn and dent corn for ethanol production? 	How much sugar is available in each feedstock? Why are we currently using dent corn as a feedstock for ethanol production?	Dent corn contains more glucose after starch break down than sweet corn.
Lesson 4 Ethanol: Nutrient Testing	<ul style="list-style-type: none"> What is the nutrient profile of dent corn, before and after fermentation. 	Food versus fuel? How can the co-products of ethanol help feed us?	The nutrient profile of corn changes as the carbohydrates are removed for fermentation into alcohol.
Lesson 5 Ethanol: Corn Mash &	<ul style="list-style-type: none"> What are the steps in ethanol production? 	How are we able to create ethanol so quickly? What	Corn starch is broken down by heat and enzymes into glucose molecules which are

Distillation		can the coproducts of ethanol be used for?	consumed by yeast to produce carbon dioxide and ethanol.
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Teacher: Lesson 1: Corn Fermentation in a Bag

DCI (Standard)	
HS-LS2: Matter and Energy in Organisms and Ecosystems	
Performance Expectations	
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. <ul style="list-style-type: none"> <i>Clarification Statement: Emphasis is on conceptual understanding of the role of aerobic and anaerobic respiration in different environments.</i> 	
Science & Engineering Practices	
Constructing explanations and Designing solutions. <ul style="list-style-type: none"> 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 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. 	Students make a quantitative and/or qualitative claim regarding the relationships between the dependant and independent variables in the fermentation process.
Disciplinary Core Idea	
LS2.B: Cycles of Matter and Energy Transfer in Ecosystems <ul style="list-style-type: none"> Photosynthesis and cellular respiration (including anaerobic processes) provide most of the energy for life processes. 	Students apply scientific reasoning or models to link evidence to explanations describing the anaerobic process of fermentation.
Crosscutting Concept	
Energy & Matter <ul style="list-style-type: none"> Energy drives the cycling of matter within and between systems. 	Students examine energy and matter relationships to design solutions for how matter may create renewable energy for human use

This lesson focuses on Constructing Explanations and Designing Solutions as a means to make sense of the phenomena below. Students will develop experimental models to generate data in order to construct explanations about the relationships between the components of the fermentation process and predict how those relationships can be manipulated to produce carbon dioxide. The students will design solutions to make the fermentation process as efficient as possible and generate the maximum amount of ethanol in a small bag environment. Prepare the 4 fermentation bags below to use as the phenomena for the Energy & Ethanol unit.

Phenomena:

Create the following bags 25-30 minutes prior to class. If possible use warm water (95 °F/35 °C) to hydrate the fermentation bags. Remove all of the air from the bags, seal and incubate the bags in a warm location (98.6 °F/37 °C) for optimum fermentation. Remove the bags from the incubator and ask the

students what they are observing. Allow the students to generate discussion with their observations. Do not confirm or deny ideas as you lead the conversation with your students.

Phenomena: Fermentation Bags					
A	1 tsp. yeast	50 ml water	1 tsp. glucose		
B	1 tsp. yeast	50 ml water	1 tsp. Corn flour	¼ tsp. amylase	
C	1 tsp. yeast	50 ml water	1 tsp. Corn flour	¼ tsp. glucoamylase	
D	1 tsp. yeast	50 ml water	1 tsp. Corn flour	¼ tsp. amylase	¼ tsp. glucoamylase

What is occurring in each of the four bags? Write the ingredients of each bag on the board and have students brainstorm observations or questions surrounding the function of each ingredient individually for 1 minute. Have them record both the bag contents and their observations on their charts for later use. Next, have the students share their observations in a small group for three minutes. Generate class discussion by asking groups to share their observations with the class.

Possible observations or questions about the Corn Fermentation in a Bag ingredients.

- *Glucose is a simple sugar (monosaccharide).*
- *Yeast are organisms/decomposers that eat sugars.*
- *Starch is a complex sugar (polysaccharide).*
- *Fermentation occurs when yeast consume sugar (glucose) and produce alcohol (ethanol) and carbon dioxide.*
- *Bag A produced the most CO₂ in 20 minutes (glucose).*
- *Bags B and C did produced very little CO₂ in 20 minutes.*
- *Bag D produced the second largest amount of CO₂ in 20 minutes.*
- *What do amylase and glucoamylase do? How do they function with sugars or yeast?*

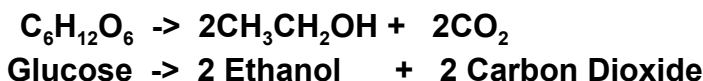
The Problem: *Read the problem to the students and generate discussion with some of the following possible prompts:*

Human consumption of fuels is on the rise as both population and affluence steadily increase.

Renewable fuels, such as ethanol, can help to decrease the need for **non-renewable fuel** sources such as crude oil. In addition, ethanol has replaced methyl tertiary-butyl ether (MTBE) as the major octane source in gasoline which has resulted in gasohol blends of up to 10% in almost every pump in the United States. Ethanol is a renewable fuel source that is both **energy positive**, which means it generates more potential energy than it consumes, and helps to reduce greenhouse gas emissions. More cars are on the road than ever before, so we need to be able to produce high quality ethanol quickly and efficiently to fuel the increase of active automobiles.

Fermentation is an anaerobic process where yeast consume sugars to produce alcohol and carbon dioxide. Ethanol is created when yeast consume **glucose** (simple sugar). Ethanol in the United States is produced by breaking down corn flour to create glucose, which is then consumed by yeast to produce CO₂, ethanol, and distillers grains. **Distillers grains** are the leftover corn fiber, protein and oil that result from the breakdown of starch in corn.

Here is the equation for the fermentation of glucose into ethanol and carbon dioxide.



Possible Student Prompts:

- What is ethanol?
- How is ethanol used in gasoline to power cars?
- What is a gasohol blend?
- What is the feedstock for ethanol?
- How does ethanol help to decrease greenhouse gas emissions?
- Are there any co-products of ethanol production?

The Challenge: Read the challenge to the students.

Create the greatest volume of ethanol (measured by the volume of carbon dioxide generated) in the fastest time possible.

Students should work in groups of 2-3 individuals for this challenge. Review the criteria and constraints for the challenge.

Criteria and Constraints

- Plan an (several) experiment(s) to produce ethanol in a small bag environment.
- You can only use the following materials/amounts provided by your teacher.
- You have 1 or more class period(s) to experiment on your initial design(s) based on your plan.
- Data must be collected and analyzed to provide evidence for your explanation and future design solution.
- Report back to the class and provide future design solutions as a result of your current explanation.

Provide some, all, or additional material items to the students that are listed below.

Possible materials:

- | | |
|---|---|
| • snack sized bags | • Ruler to measure gas volume |
| • 50 ml water | • Index card or clipboard to measure gas volume |
| • 1 tsp. Yeast | |
| • ¼ tsp. enzymes (amylase, glucoamylase) | |
| • 1 tsp. sugars (simple & complex) as feedstocks: corn flour, corn starch, corn syrup, honey, and glucose | |

How will you investigate your challenge?

Discuss the engineering design process with your students. Encourage the student groups to create 2 or more experimental designs based upon their knowledge of what occurs in the phenomena bags. Why are they investigating their design? What is their reasoning for their materials? What patterns do they expect to see? They will also need to create a method for measuring their CO₂ gas. We suggest that they measure volume by height displacement using a clipboard and ruler to demonstrate their volume change in CO₂ gas.

Prediction

Students should be able to predict the outcome of some of their experimental designs based upon previous background knowledge and their observations of the anchoring phenomena.

Observations related to your investigation.

Encourage the students to create charts and graphs to show the volume change within their bags over time. Students should create their own experimental procedure to collect and record data.

- *What are some physical ways your group can measure carbon dioxide production and/or ethanol production?*
- *How can your group predict the amount of carbon dioxide and ethanol that is generated from your fermentation bags?*

Reflection: Students should be able to reflect on the following questions to construct their explanation below.

1. What is the purpose/role of each component in your group's fermentation bag design(s)? How did each component act upon another? Write/draw your most efficient design below.
Possible Answers: Students answers will vary.

2. What evidence did your group generate to clarify the role of each component in your group's design?
Possible Answers: Students answers will vary.

3. What are the reactants and products of your fermentation reaction?
Possible Answers: Students answers will vary.

Constructing Explanations:

Construct an explanation of the fermentation process of corn into ethanol.

Possible Answers: Students answers will vary.

Redesign: Create a future design solution to make ethanol production more efficient in a small bag environment based upon your explanation of the fermentation process and additional research.

Create a discussion with your students to determine if they could improve upon their experimental design based upon the evidence presented. What could they improve upon? Materials used? Experimental conditions? What research could they do to make their design as efficient as possible?

- *Can you create a more efficient design using different materials?*
- *Can you predict the outcome of other experimental designs?*
- *How can you change you original design to become more efficient by changing the experimental conditions?*
- *Make predictions using all of the available feedstocks in separate designs to determine which one will generate the most carbon dioxide and ethanol over time.*

Differentiation

Other ways to connect with students with various needs:

- **Local community:** students may investigate the use of ethanol in their community. (i.e. Do any fuel stations offer gasohol blends? What gasohol blend %'s are available? Which type of gas/fuel do your parent use and why)?
- **Students with special needs (auditory/visual/language/reading):** See the extra support below.
- **Extra Support:** Video: How ethanol is made (<https://youtu.be/59R-NqykoXs>) This video helps demonstrate relationships between the components of the ethanol fermentation ecosystem.
- **Extensions:** Students can research the use of ethanol as a fuel alternative and current gasohol blends available to the public.

Student Self-Assessment Rubric

Content	Self-Check	YES	NO
Constructing Explanations	Did we construct an explanation for the fermentation process based on evidence?		
Designing Solutions	Did we provide possible future solutions for a more efficient fermentation design?		

Teacher Assessment Rubric: Corn Fermentation in a Bag

Does Not Meet 1	Approaching 2	Meets the Standard 3	Advanced Understanding 4
The student is not able to use the data generated to create an explanation for the fermentation process.	The student is able to use the data generated to create an explanation for the fermentation process.	The student is able to: <ul style="list-style-type: none"> • Independently develop explanations based on data to describe the fermentation process. • Analyze explanations of the fermentation process to determine the validity of their findings. 	The student is able to: <ul style="list-style-type: none"> • Independently develop explanations based on data to describe the fermentation process. • Analyze explanations of the fermentation process to determine the validity of their findings with increased depth and complexity. • Design future solutions to create a more efficient fermentation process.

Lesson 1: Corn Fermentation in a Bag

Essential Questions: *What is fermentation? How can we speed up and measure the fermentation of starch?*

Phenomena - What is in each bag? Describe the reaction. Fill in the boxes below.

Bag Ingredients					Reaction
A	yeast	water			
B	yeast	water			
C	yeast	water			
D	yeast	water			

The Challenge

Create the greatest volume of ethanol (measured by the volume of carbon dioxide generated) in the fastest time possible.

Criteria and Constraints

- Work together in groups of 2-3 students.
- Plan an (several) experiment(s) to produce ethanol in a small bag environment.
- You can only use the following materials/amounts provided by your teacher.
- You have 1 or more class period(s) to experiment on your initial design(s) based on your plan.
- Data must be collected and analyzed to provide evidence for your explanation and future design solution.
- Report back to the class and provide future design solutions as a result of your current explanation.

How will you investigate your challenge? How could your group plan an investigation to test the challenge? What two (or more) experiments can your group design to create ethanol as quickly as possible? How will your group measure which one of your experiments changes the most? Write your experimental groups below. Be sure to include your explanations for your group's experimental choices.

Prediction: What do you think will happen to each experimental group and why?

Group	Reactants in Fermentation Bag	Prediction/Why
Group 1:		
Group 2:		
Group 3:		

Measurement of Data:

How will your group measure the carbon dioxide that is generated? Write your plan to measure CO₂ below.

Observations:

Collect data from the group's experimental designs below. Include charts here to show your results.

Include graphs here to show your results.

Reflection: Create an explanation of the fermentation process of corn into ethanol. Reflect on the following questions while creating your explanation.

1. What is the purpose/role of each component in your group's fermentation bag design(s)? How did each component act upon another? Write/draw your most efficient design below.
2. What evidence did your group generate to clarify the role of each component in your group's design?
3. What are the reactants and products of your fermentation reaction?

Constructing Explanations:

Construct an explanation of the fermentation process of corn into ethanol.

Redesign: Create a future design solution to make ethanol production more efficient in a small bag environment based upon your explanation of the fermentation process and additional research.

Rubric for Self-Assessment

Content	Self-Check	YES	NO
Constructing Explanations	Did we construct an explanation for the fermentation process based on evidence?		
Designing Solutions	Did we provide possible future solutions for a more efficient fermentation design?		

Teacher: Lesson 2: Ticketase

DCI (Standard)	
HS-LS1: Matter and Energy in Organisms and Ecosystems	
Performance Expectations	
<p>HS-LS1.6: Construct and revise an explanation based on evidence for how carbon, hydrogen, and oxygen from sugar molecules may combine with other elements to form amino acids, and/or other large carbon-based molecules.</p> <ul style="list-style-type: none"> • <i>Clarification Statement: Emphasis is on using evidence from models and simulations to support explanations.</i> • <i>Assessment Boundary: Assessment does not include the details of the specific chemical reactions or identification of macromolecules.</i> 	
Science & Engineering Practices	
<p>Constructing Explanations and Designing Solutions</p> <ul style="list-style-type: none"> • Construct and revise an explanation based on valid and reliable evidence obtained from a variety of sources (including student's own investigations, models, theories, simulations, and peer review) and the assumption that theories that describe the natural world operate today as they did in the past and will continue to do so in the future. 	Students deconstruct a model of starch in order to provide reliable evidence for their explanation on how enzymes work synergistically to produce glucose for ethanol production.
Disciplinary Core Idea	
<p>LS1.C: Organization for Matter and Energy Flow in Organisms</p> <ul style="list-style-type: none"> • As matter and energy flow through different organizational levels of living systems, chemical elements are recombined in different ways to form different products. 	Students construct explanations for enzymatic action on complex sugar molecules to create simple sugars for fermentation.
Crosscutting Concept	
<p>Energy and Matter</p> <ul style="list-style-type: none"> • Changes of energy and matter in a system can be described in terms of energy and matter flows into, out of, and within that system. 	Students design new solutions for enzyme action on starch to make the fermentation process more efficient.

This lesson is designed to follow Lesson 1, *Corn Fermentation in a Bag*, where students utilize different components (enzymes, yeast, feedstocks, and water) to produce ethanol and carbon dioxide through the process of fermentation. This lesson focuses on Constructing Explanations and Designing Solutions as a way to make a qualitative or quantitative explanation regarding the relationship between feedstock and glucose availability for ethanol production. Students will deconstruct a model of starch to examine enzyme and sugar reactions and determine how starch, is changed into smaller molecules for yeast

consumption. Students will construct an explanation for enzyme action and design solutions for future enzyme use in ethanol production.

Remind the students of the 4 fermentation bags used as the phenomena in lesson 1. What was occurring in each of the four bags? You can help guide the students' discussion by asking questions as you record their observations/questions.

- *How did the amylase and/or glucoamylase impact the fermentation reaction?*
- *What are the roles of amylase and glucoamylase?*
- *Did amylase/glucoamylase help the fermentation process occur more slowly, rapidly, or have no net effect?*

To begin investigating these questions/observations and learn more about enzymes, have the students work through the enzyme lab, Ticketase.

Materials

- 4 sets of 50 single entrance tickets (all connected) per student group
- *Optional: timer*
- *Optional: blindfold*

Procedure

Students should work together in groups of 5. Provide each group with 4 strings of 50 connected tickets.

Reflection:

Construct an explanation and design future solutions for the current use of enzymes in commercial ethanol production in the United States. Reflect on the following questions while creating your explanation.

1. What happened to the first polysaccharide as Ticketase was introduced? What happened to the second polysaccharide when Glucoticketase was introduced? How are they similar and different?

Possible answer: The first polysaccharide was broken into small ticket groups of 2-3 tickets per group. The second polysaccharide was broken into single tickets. They are similar in that they break the polysaccharide into smaller pieces. They are different in the size that they break off of the polysaccharide.

2. What happened to the reaction rate when the **Enzyme Concentration** and **substrate concentration** increased? Why did this happen?

Possible answer: The reaction rate increased due to the additional substrate and activation sites that were available to both of the enzymes.

3. Why does industry use a combination of enzymes such as Ticketase and Glucoticketase for the fermentation process in ethanol production?

Possible answer: The synergy of the enzymes working together help to produce more products that are available to yeast for fermentation.

Constructing Explanations and Designing Solutions:

Construct an explanation and design future solutions for the current use of enzymes in commercial ethanol production in the United States. How can enzyme use make ethanol production more efficient in the future?

Possible answers: *Student answers will vary.*

Differentiation:

Other ways to connect with students with various needs:

- **Local community:** Students may investigate the use of enzymes in digestion. How can the amylase found in human saliva help to break apart food molecules in preparation for digestion? Students can communicate with their findings with their local community.
Video: <https://youtu.be/Lmma9Fwx7KY>
- **Students with special needs (auditory/visual/language/reading):** See the extra support below.
- **Extra Support:** Video: How ethanol is made (<https://youtu.be/59R-NqykoXs>) This video helps demonstrate relationships between the components of the ethanol fermentation ecosystem. Infographic: <http://vitalbypoet.com/infographics/ethanol-process-2> This infographic represents the process of corn flour breakdown into glucose for fermentation.
- **Extensions:** Students can research the current use of enzymes in corn ethanol production. Students can also research additional feedstocks for ethanol such as cellulosic switchgrass and the enzymes that would be necessary for carbohydrate breakdown.

Additional Resources for student research:

- <http://vitalbypoet.com/infographics/ethanol-process-2>
- Video: <https://youtu.be/59R-NqykoXs>

Self-Assessment Rubric Ticketase

	Yes	No	Maybe/Unsure
Did we explain the function that <i>Ticketase</i> performs?			
Did we explain the function that <i>Glucoticketase</i> performs?			
Did we use reasoning to connect the evidence of enzyme function on starch to construct the explanation to demonstrate the use of enzymes in commercial ethanol production?			

Teacher Assessment Rubric: Ticketase

Does Not Meet	Approaching	Meets the Standard	Advanced
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1	2	3	Understanding 4
<p>The student is not able to use the data generated to create an explanation for the use of enzymes in commercial ethanol production.</p>	<p>The student is able to use the data generated to create an explanation for the use of enzymes in commercial ethanol production.</p>	<p>The student is able to:</p> <ul style="list-style-type: none"> •Independently develop explanations based on data to describe the use of enzymes in commercial ethanol production. •Construct explanations for the use of enzymes in commercial ethanol production to determine the validity of their findings. 	<p>The student is able to:</p> <ul style="list-style-type: none"> •Independently develop explanations based on data to describe the use of enzymes in commercial ethanol production. • Analyze explanations of the use of enzymes in commercial ethanol production to determine the validity of their findings with increased depth and complexity. • Design future solutions to create a more efficient use of enzymes in commercial ethanol production.

Lesson 2: Ticketase

Essential Questions: *How do enzymes act upon complex sugars like starch? What is the rate of enzyme activity for Ticketase and Glucoticketase? Does enzyme or substrate concentration affect the rate of enzymatic activity?*

Background: Enzymes work to speed up biological reactions by lowering their activation energy. There are certain conditions that must be met for enzymes to work efficiently. One of these conditions is **substrate concentration**. Students use tickets to model how various enzymes interact with starch to produce smaller sugar molecules. The tickets represent the **substrate**. Your thumbs and index fingers represent the **active sites** of the enzyme “Ticketase” and “Glucoticketase.” To **catalyze** the reaction (tearing groups or single tickets off of the string of tickets), you may only tear a single ticket off at a time (Glucoticketase) or a group of 2 or 3 tickets off at a time (Ticketase). Next, drop everything back into the pile and repeat the process. A single ticket is the desired product for fermentation and must be torn cleanly off on both sides to count as **product**.

- **Ticketase** represents the enzyme Amylase that acts on starch (polysaccharide) to break off a disaccharide (2-sugar molecule) or a trisaccharide (3-sugar molecule)
- **Glucoticketase** represents the enzyme Glucoamylase that acts on polysaccharides to break off a single sugar molecule.

Procedure:

Part A

1. Organize yourself in groups of 5.
 2. Choose one of the following parts:
 - a. **Ticketase** (person who will tear the 2 or 3 ticket groups)
 - b. **Glucoticketase** (person who will tear the single tickets)
 - c. **Timer** (will tell Ticketase and/or Glucoticketase when to start/stop)
 - d. **Counter** (counts the # of individual tickets)
 - e. **Data Recorder** (record all #s in the data tables)
 3. Place a string of 50 tickets in front of Ticketase.
 4. When told to do so by the Timer, Ticketase will begin by picking up the string of tickets and folding/tearing off a group of either 2 or 3 tickets at a time while **not looking at the desk and pile of tickets at a constant pace** (it is not a race). Ticketase must then drop both the group of tickets and string of tickets into the pile before being allowed to pick up the string of tickets to tear off another group.
 5. As Ticketase tears off the tickets, the Counter will count the number of ticket groups and make sure that the group of tickets is put back in the pile with the chain of tickets (because products and reactants mix during the reaction).
 6. Put 10 seconds on the stopwatch. Count the number of ticket groups torn off in 10 seconds. Record the data in Table 1.
 7. Then, keeping the same pile of ticket groups and the remaining string of tickets, count the number of ticket groups in 20 seconds. Record the data in Table 1.
 8. Repeat #6, but for 30 seconds. Record the data in Table 1.
- * If you run out of tickets, record the amount of time it took Ticketase to do so.

Part B

1. Repeat everything in Part A, but only use Glucoticketase instead of Ticketase.
* Remember that Glucoticketase can only tear a single ticket off of a group of tickets at a time.

Part C

1. Repeat everything in Part A, but now have 2 sets of 50 ticket strings (double substrate concentration) and both Ticketase and Glucoticketase tear off either groups of tickets (2 or 3) or single tickets accordingly (double enzyme concentration). Record all data in Table 3.

Table 1 (Ticketase - 2 or 3 sugar groups)

Interval Time (sec)	Cumulative time (sec)	# Ticket Groups	# Single tickets	# Total Ticket Groups	Total Single Tickets
10	10				
20	30				
30	60				

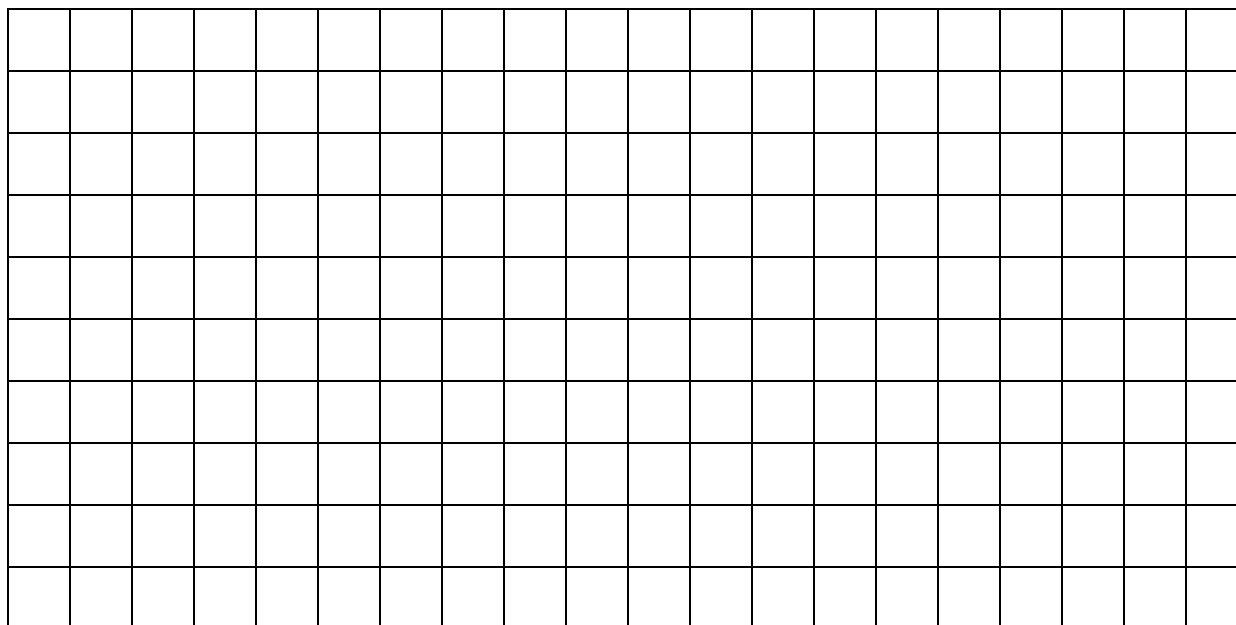
Table 2 (Glucoticketase - single tickets)

Interval Time (sec)	Cumulative time (sec)	# Single tickets	Total Single Tickets
10	10		
20	30		
30	60		

Table 3 (Double Enzyme Concentration)

Interval Time (sec)	Cumulative time (sec)	# Ticket Groups	# Single tickets	# Total Ticket Groups	Total Single Tickets
10	10				
20	30				
30	60				

Graph the number of single tickets (total) over time (cumulative). Put all 3 sets of data on the same graph and include a key to distinguish them.



Reflection:

Create an explanation for the current use of enzymes in commercial ethanol production in the United States. Reflect on the following questions while creating your explanation.

1. What happened to the first polysaccharide as Ticketase was introduced? What happened to the second polysaccharide when Glucoticketase was introduced? How are they similar and different?
2. What happened to the reaction rate when the **Enzyme Concentration** and **substrate concentration** increased? Why did this happen?
3. Why does industry use a combination of enzymes such as Ticketase and Glucoticketase for the fermentation process?

Constructing Explanations and Designing Solutions:

Construct an explanation and design future solutions for the current use of enzymes in commercial ethanol production in the United States. How can enzyme use make ethanol production more efficient in the future?

Self-Assessment Rubric: Ticketase

	Yes	No	Maybe/Unsure
Did we explain the function that <i>Ticketase</i> performs?			
Did we explain the function that <i>Glucoticketase</i> performs?			
Did we use reasoning to connect the evidence of enzyme function on starch to construct the explanation to demonstrate the use of enzymes in commercial ethanol production?			

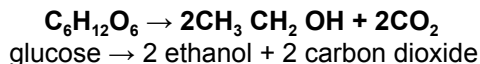
Teacher: Lesson 3: Biomass to Sugars

DCI	
HS-LS1: Matter and Energy in Organisms and Ecosystems	
Performance Expectation	
HS-LS1-6: Construct and revise an explanation based on evidence for how C, H, and O from sugar molecules may combine with other elements to form amino acids and/or other large C-based molecules. <ul style="list-style-type: none"> <i>Clarification Statement: Emphasis is on using evidence from models and simulations to support explanations.</i> <i>Assessment Boundary: Assessment does not include the details of the specific chemical reactions or identification of macromolecules.</i> 	
Science & Engineering Practice	
Constructing Explanations and Designing Solutions <ul style="list-style-type: none"> Construct and revise an explanation based on valid and reliable evidence obtained from a variety of sources (including student's own investigations, models, theories, simulations, and peer review) and the assumption that theories that describe the natural world operate today as they did in the past and will continue to do so in the future. 	Students will construct and revise an explanations to determine which feedstock will provide the most glucose for fermentation over time and design a solution for the most logical feedstock in the United States.
Disciplinary Core Idea	
HS-LS1.C: Organization of Matter and energy Flow in Organisms <ul style="list-style-type: none"> As matter and energy flow through different organizational levels of living systems, chemical elements are recombined in different ways to form different products. 	Students investigate how Carbon, Hydrogen, and Oxygen in starch are broken down to create smaller glucose molecules.
Cross Cutting Concept	
Energy and Matter <ul style="list-style-type: none"> Changes in energy and matter in a systems can be described in terms of energy and matter flows into, out of, and within that system. 	Students observe and research the availability of energy in feedstocks that are used in ethanol production.

This lesson is designed to follow Lesson 1, *Corn Fermentation in a Bag*, and Lesson 2, *Ticketase*, where students utilize different components (enzymes, yeast, feedstocks, and water) to produce ethanol and carbon dioxide through the process of fermentation and model enzyme action on starch to produce simple sugars. This lesson focuses on Constructing Explanations and Designing Solutions to explain the current feedstock choice for commercial ethanol production in the United States. Students will generate data in order to construct explanations for the current feedstock choice and explain the glucose availability of each feedstock. The students will construct an explanation for the current use of dent corn in commercial ethanol production in the United States and design possible future solutions to improve the efficiency of ethanol production.

Background:

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



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.

Investigation:

Students will investigate the glucose content in both sweet and dent corn over a 3 day period. During this process they will compare data and analyze the treatment methods employed to extract glucose from the feedstocks. Students will then construct and revise their explanations as they determine which feedstock will provide the most glucose for fermentation over time. Students will continue to research ethanol production and propose future solutions to improve the efficiency of ethanol production.

Materials

- | | |
|--|--|
| <ul style="list-style-type: none"> • Glucose, ground up (3.0 g per station) • Sweet corn, fresh or frozen, ground up (3.0 g per station) • Cracked kernel corn, ground up (3.0 g per station) • Test tube holder/hot pads • 500 ml beakers • Amylase enzyme solution (1 tsp amylase / 100 ml water) • Glucoamylase enzyme solution (1 tsp glucoamylase / 100 ml water) • Mortar and pestle • Hot plates | <ul style="list-style-type: none"> • Stirring rods • Water • 15 ml centrifuge tubes (10 per group) • Test tube racks • Glucose test strips • Optional: Glucose monitor and test strips • Scale and weigh boats • .5 ml disposable pipettes • Marker/tape • Scale • <i>Optional: Stopwatch, Ice bath, Pipette pump with 10 ml serological pipettes</i> |
|--|--|

Procedure: Students should work together in groups of 4 for the investigation. Prepare the following materials for each group to use.

Each group needs:

- | | |
|---|---|
| <ul style="list-style-type: none"> • 1 test tube rack • 10 test tubes • Marker • Stir rod • Glucose, ground up • 2 types of corn, ground up (sweet, dent) • weigh boat | <ul style="list-style-type: none"> • Hot plate • 500 ml beaker that is half full of water • .5 ml disposable pipette. • Access to: water, glucose test strips or glucose monitor and test strips, enzyme complexes, and a scale are needed. |
|---|---|

Reflection

Students will construct and revise an explanation to determine which feedstock will provide the most glucose for fermentation over time and design a solution for the most logical feedstock in the United States. Reflect on the following questions while creating your explanation.

1. Where does the glucose come from in sweet corn or dent corn?

Possible answers: Starch (polysaccharide) and maltose (disaccharide) are cut into glucose molecules by the enzymes amylase and glucoamylase.

2. How much glucose is present in each feedstock for ethanol production?

Possible answers: The students numbers will range based upon lab technique and measuring ability. Starch should contain the most glucose over time.

3. Which type(s) of pretreatment were most effective at cutting glucose? How does each pretreatment manipulate the feedstock?

Possible answers: Heat treatment and enzyme treatment combined

4. What additional pretreatments for dent corn can be employed to improve the efficiency of ethanol production?

Possible answers: The students will need to research ethanol production for more advanced techniques. Building on information from this lesson storyline, students should make suggestions such as heat treatment, grinding, temperature control, aeration, enzyme addition, etc.

5. What other feedstocks can be used in ethanol production?

Possible answers: Students should conduct research to determine what other feedstocks are available for ethanol production, such as tulip trees, switchgrass, corn stover, etc.

6. What possible alternative feedstock options are available that can help to make ethanol production more efficient and sustainable in the future?

Possible answers: The students will need to research ethanol production for more advanced techniques. Answers should include feedstock varieties, farming methods, enzyme additions specific to feedstock varieties, technologies used to distill ethanol, co-product distribution, etc.

Constructing Explanations and Designing Solutions:

Students will construct and revise an explanation to determine which feedstock will provide the most glucose for fermentation over time and design a solution for the most logical feedstock in the United States.

Possible answers: Student answers will vary.

Differentiation:

Other ways to connect with students with various needs:

- **Local community:** Students may investigate the production and availability of feedstocks in their community. Students can communicate with their findings with their classmates and their local community.
- **Students with special needs (auditory/visual/language/reading):** See the extra support below.
- **Extra Support:** Video: How ethanol is made (<https://youtu.be/59R-NqykoXs>) This video helps demonstrate relationships between the components of the ethanol fermentation ecosystem.

Infographic: <http://vitalbypoet.com/infographics/ethanol-process-2> This infographic represents the process of corn flour breakdown into glucose for fermentation.

- **Extensions:** Students can research the current use of feedstocks in commercial ethanol production. Students can also research additional future feedstocks for ethanol such as cellulosic switchgrass and the enzymes that would be necessary for carbohydrate breakdown.

Additional Resources for student research:

- <http://vitalbypoet.com/infographics/ethanol-process-2>
- Video: <https://youtu.be/59R-NgykoXs>

Rubric for student self-assessment

Content	YES	NO	Maybe/Unsure
Did we explain the difference in glucose content sweet corn and dent corn for ethanol production?			
Did we use reasoning to connect the evidence of glucose availability in starch to construct an explanation to demonstrate the use dent corn in commercial ethanol production?			
Did we design possible future solutions to feedstock choice options in commercial ethanol production?			

Teacher Assessment Rubric: Biomass to Sugars

Does Not Meet 1	Approaching 2	Meets the Standard 3	Advanced Understanding 4
The student is not able to use the data generated to create an explanation for the current feedstock used in commercial ethanol production.	The student is able to use the data generated to create an explanation for the current feedstock used in commercial ethanol production.	The student is able to: <ul style="list-style-type: none"> • Independently develop explanations based on data to demonstrate the amount of glucose availability in feedstocks. • Analyze explanations of glucose availability in feedstocks to determine the validity 	The student is able to: <ul style="list-style-type: none"> • Independently develop explanations based on data to demonstrate the amount of glucose availability in feedstocks. • Analyze explanations of glucose availability in feedstocks to determine the validity

		of their findings.	of their findings with increased depth and complexity. <ul style="list-style-type: none"> • Design future solutions for viable feedstock choices for commercial ethanol production.
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Lesson 3: Biomass to Sugars

Essential Question: *Which feedstock will produce the most glucose for fermentation?*

Investigation:

How much glucose is available in sweet corn and dent corn for ethanol production? Students will investigate the glucose content in both sweet and dent corn over a 3 day period. During this process they will compare data and analyze the treatment methods employed to extract glucose from the feedstocks. Students will then construct and revise their explanations as they determine which feedstock will provide the most glucose for fermentation over time. Students will continue to research ethanol production and propose future solutions to improve the efficiency of ethanol production.

Investigation:

A. Procedure for all samples.

1. Students should work together in groups of 4 for the investigation.
2. Each group needs:

<ul style="list-style-type: none"> • 1 test tube rack • 10 test tubes • Marker • Stir rod • Glucose, ground up • 2 types of corn, ground up (sweet, dent) 	<ul style="list-style-type: none"> • Hot plate • 500 ml beaker that is half full of water • .5 ml disposable pipette. • Access to water, glucose test strips or glucose monitor and test strips, enzyme complexes and a scale are needed.
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3. Use your marker to label your tubes according to Data Table 1.
4. Using the marks on the side of the test tube for guidance, pour 10 ml of water from your beaker into each test tube. Then return the test tube to the rack.
5. Bring the rack and test tubes to the weigh station. Place 1.0 g of glucose or ground corn into each of the appropriate labeled test tubes. Note that the enzyme only tube does not get any glucose or corn. When you are done, put the caps back on all 10 tubes.

B. Heat pre-treatment

6. Make sure your beaker is half full of water and place it on the hot plate until it begins to boil.
7. Loosen the caps on the test tubes labeled “heat.” Carefully place the 3 test tubes labeled “heat” in the beaker of boiling water. Allow samples to sit in the gently boiling water for 10 minutes. Reminder: If it appears that water may begin to spill out, turn the heat down.
8. Once the test tubes are cool enough to touch, carefully tighten the caps on the tubes and cool the heated tubes by running them under cool water or placing them in an ice water bath. Once samples have cooled to room temperature, move on to step 8.

C. Enzyme addition

9. Add .5 ml of Amylase and Glucoamylase solution to glucose/heat, glucose/ no-heat, sweet corn/heat, sweet corn/no-heat, dent corn/heat, dent corn/no-heat and enzyme only test tubes.
10. Cap and swirl to mix gently, then proceed to step 10.

D. Sugar measurements

11. To measure sugar, dip 1 glucose strip in a tube for 1–2 seconds. Be sure that the entire pad on the end of the strip is submerged in the solution. Alternately, If a glucose monitor is present, place a test strip

in the machine and turn it on, use a pipette to place a drop of solution on the end of the test strip at the prompt, read and record measurement at the prompt.

12. Remove the test strip from the sample and gently drag it across the lip of the test tube to remove any excess liquid. Then prop the test strip up on its side so the liquid runs off the strip instead of pooling on the test area, and let sit for 1 minute. Next, one team member can dip a new test strip for the next test tube and start timing 1 minute. While the other team members move on, be careful to keep track of which strip goes with which tube.

13. Compare test strips with the color chart on the bottle and record your results in the data table. Be sure to do this right after the initial 1 minute is over because the color change on the glucose test strip will be inaccurate after 2–3 minutes.

14. Repeat steps 11 and 12 until your team has measured and recorded sugar levels for the samples in all 10 test tubes.

** Alternately, if a glucose monitor is present, place a test strip in the monitor and turn the machine on, use a pipette to place a drop of each additional solution into the end of a new test strip and record the measurements. Once you are done testing the glucose levels, place the caps back on the test tubes and loosely tighten.*

15. Incubate at room temperature overnight, or, for optimal enzyme function, place in an incubator or water bath at 50–60°C overnight. Day 2 and 3

16. Measure sugar levels the same way you did in Section D (Sugar Measurements) for all samples during your next 2 classes. Before taking sugar measurements, check that cap tubes are tight and gently swirl each tube for a few seconds to mix. Then measure and record the amount of sugar in all tubes using the glucose strip, or glucose monitor, as you did on Day 1.

Data: Place your data into the chart below.

Tube #	Label	Glucose amount day 1: time 0	Glucose amount day 2: time ____ hours	Glucose amount day 3: time ____ hours
1	Glucose/Heat /Date			
2	Glucose/No Heat/Date			
3	Glucose/No Treatment /Date			
4	Sweet Corn/Heat /Date			
5	Sweet Corn/No Heat /Date			
6	Sweet Corn/No Treatment /Date			
7	Dent Corn/Heat /Date			
8	Dent Corn/No Heat /Date			
9	Dent Corn/No Treatment/Date			
10	Enzyme only/Date			

Graph: Graph the data from the chart above. Show each tube individually as a line in the graph below.

Reflection

Construct an explanation for the current use of dent corn in commercial ethanol production in the United States and design possible future solutions for feedstock choice to improve the efficiency of ethanol production. Reflect on the following questions while creating your explanation.

1. Where does the glucose come from in sweet corn or dent corn?
2. How much glucose is present in each feedstock for ethanol production?
3. Which type(s) of pretreatment were most effective at cutting glucose? How does each pretreatment manipulate the feedstock?
4. What additional pretreatments for dent corn can be employed to improve the efficiency of ethanol production?
5. What other feedstocks can be used in ethanol production?

6. What possible alternative feedstock options are available that can help to make ethanol production more efficient and sustainable in the future?

Constructing Explanations and Designing Solutions:

Students will construct and revise an explanation to determine which feedstock will provide the most glucose for fermentation over time and design a solution for the most logical feedstock in the United States.

Rubric for student self-assessment

Content	YES	NO	Maybe/Unsure
Did we explain the difference in glucose content sweet corn and dent corn for ethanol production?			
Did we use reasoning to connect the evidence of glucose availability in starch to construct an explanation to demonstrate the use dent corn in commercial ethanol production?			
Did we design possible future solutions to feedstock choice options in commercial ethanol production?			

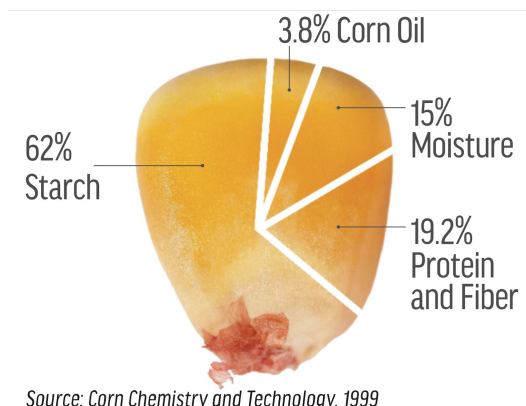
Teacher: Lesson 4: Ethanol: Nutrient Testing

DCI	
HS-LS1:Matter and Energy in Organisms and Ecosystems	
Performance Expectation	
HS-LS1-6: Construct and revise an explanation based on evidence for how C,H, and O from sugar molecules may combine with other elements to form amino acids and/or other large C-based molecules. <ul style="list-style-type: none"> • <i>Clarification Statement: Emphasis is on using evidence from models and simulations to support explanations.</i> • <i>Assessment Boundary: Assessment does not include the details of the specific chemical reactions or identification of macromolecules.</i> 	
Science & Engineering Practice	
Constructing Explanations and Designing Solutions <ul style="list-style-type: none"> • Construct and revise an explanation based on valid and reliable evidence obtained from a variety of sources (including student's own investigations, models, theories, simulations, and peer review) and the assumption that theories and that describe the natural world 	Students will construct an explanation for the current use of dent corn in commercial ethanol production and design solutions for the coproducts that are generated from ethanol production.
Disciplinary Core Idea	
HS-LS1.C: Organization of Matter and energy Flow in Organisms <ul style="list-style-type: none"> • The sugar molecules thus formed contain C, H, and O: their their H backbones are used to make amino acids and other C-based molecules that can be assembled into larger molecules. 	Students research the macromolecules available in dent corn throughout the process of ethanol production.
Cross Cutting Concept	
Energy and Matter <ul style="list-style-type: none"> • Changes in energy and matter in a systems can be described in terms of energy and matter flows into, out of, and within that system. 	Students will design solutions for alternative uses for ethanol coproducts.

This lesson is designed to follow Lesson 1, *Corn Fermentation in a Bag*, Lesson 2, *Ticketase*, and Lesson 3, *Biomass to Sugars*, where students utilize different components (enzymes, yeast, feedstocks, and water) to produce ethanol and carbon dioxide through the process of fermentation, model enzyme action on starch to produce simple sugars, and analyze sugar content in available feedstock choices. This lesson focuses on Constructing Explanations and Designing Solutions to explain the nutrient profile change of dent corn as it undergoes the fermentation process and distillation into ethanol. Students will conduct additional research to generate data for analysis to design future usage solutions for the coproducts of ethanol production.

Background

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 **coproducts** (distillers grains, corn oil, and carbon dioxide). All the remaining nutrients: protein, fat, minerals, and vitamins, are concentrated into **distillers dried grain** (DDGs), a valuable feed for livestock, and carbon dioxide. In this simple experiment, students will determine the nutrient analysis of dent corn before, during, and after ethanol production.



Materials:

Provide the following materials to each student group.

- Hot plate
- Funnel
- Filter paper
- Parafilm or vortex for mixing
- Graduated cylinder (10 mL)
- Benedict's solution
- Lugol's iodine solution
- Biuret solution
- Distilled water
- Test tubes and rack
- Cracked corn (ground up)
- Scale or triple beam balance
- Pipette pump
- 10 mL pipettes
- Mortar and pestle
- Test tube holder/hot pads

Instructions

Students should work together in groups of 4 to complete the nutrient analysis tests. The tests should be conducted at the beginning on raw product day 1, day 3 after fermentation, and again after the distillation process.

Reflection

Create an explanation for the current use of dent corn in commercial ethanol production and design solutions for the coproducts that are generated from ethanol production. Reflect on the following questions while creating your explanation.

1. How does the nutrient profile of dent corn change as it undergoes the process of fermentation and distillation?

- a. Is all of the glucose consumed? Provide evidence to support your conclusion.

Possible Answers: The majority of sugars are consumed in an efficient commercial ethanol production process.

- b. How do yeast contribute to the nutrient profile of the distillers grains after fermentation?

Possible Answers: Yeast consume the sugars to leave a nutrient profile low in carbohydrates, high in protein, and similar in lipid.

- c. How can the yeast contribute to the digestion of the animals consuming the distillers grains?

Possible Answers: The yeast left behind in the distillers grains are probiotic and help the animal to more easily digest their diet for a more efficient conversion into energy for the animal's use.

- d. Why are distillers grains considered to be a valuable feeds for animals?

Possible Answers: Dried distillers grains are easily transportable, store for a long period of time for later use, are economical compared to other feedstocks of similar protein concentration and are probiotic in nature.

2. Provide a graphic of the nutrient composition change of corn before/after fermentation.

Possible Answer: Students' answers will vary but should incorporate a graphic that provides evidence for the nutrient content change.

3. What are the current industry uses for the coproducts produced in the commercial ethanol production process?

Possible Answers: Student answers will vary. Coproducts listed should include carbon dioxide and distillers grains.

Constructing Explanations and Designing Solutions:

Create an explanation for the current use of dent corn in commercial ethanol production and design solutions for the coproducts that are generated from ethanol production. Reflect on the following questions while creating your explanation.

Possible Answers: Student answers will vary.

Differentiation:

Other ways to connect with students with various needs:

- **Local community:** Students may investigate the use of corn in animal feed in their local area. Students may choose to interview a farmer, local farmer cooperative, or animal supply store. Students can communicate with their findings with their classmates and their local community.
- **Students with special needs (auditory/visual/language/reading):** See the extra support below.
- **Extra Support:** Video: How ethanol is made (<https://youtu.be/59R-NqykoXs>) This video helps demonstrate relationships between the components of the ethanol fermentation ecosystem. Infographic: <http://vitalbypoet.com/stories/ethanol-process> This infographic represents the process of corn flour breakdown into glucose for fermentation.
- **Extensions:** Students can research the current use of coproducts in commercial ethanol production. Students can also research additional future uses for ethanol coproducts to make ethanol production more efficient and sustainable.

Additional Resources for student research:

- <http://vitalbypoet.com/stories/ethanol-process>
- Video: <https://youtu.be/59R-NqykoXs>

Self-Assessment Rubric: Ethanol: Nutrient Testing

	Yes	No	Maybe/Unsure
Did we generate data and construct an explanation of nutrient content change for corn as it is transformed into ethanol?			
Did we construct viable solutions for the industrial application of the remaining coproducts produced in the commercial ethanol production?			

Teacher Assessment Rubric: Ethanol: Nutrient Testing

Does Not Meet 1	Approaching 2	Meets the Standard 3	Advanced Understanding 4
The student is not able to use the data generated to construct an explanation for nutrient change of corn as it undergoes the ethanol production process.	The student is able to use the data generated to construct an explanation for the nutrient change of corn as it undergoes the ethanol production process.	<p>The student is able to:</p> <ul style="list-style-type: none"> •Independently construct explanations based on data to demonstrate the nutrient change of corn as it undergoes the ethanol production process. • Analyze explanations of nutrient change in corn to determine the validity of their findings. 	<p>The student is able to:</p> <ul style="list-style-type: none"> •Independently construct explanations based on data to demonstrate the nutrient change of corn as it undergoes the ethanol production process • Analyze explanations of nutrient change in corn to determine the validity of their findings with increased depth and complexity. • Design future solutions for coproduct usage from commercial ethanol production.

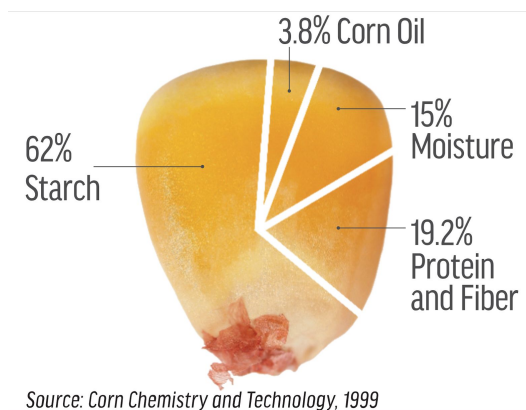
Lesson 4: Ethanol: Nutrient Testing

Essential Question: *What are the nutrient values of dent corn before and after ethanol production?*

Investigation

How much protein, fat, and fiber are available in dent corn for animal feed after fermentation? Students will investigate the nutrient content in dent corn during the stages of ethanol production to construct explanations for the use of **dried distillers grains (DDGs)** as animal feed. During this process they will generate and compare data to analyze the changes that occur to the starch molecules as glucose is released and consumed by yeast to create alcohol. *Will all of the glucose be consumed? What components of the dent corn are left as **coproducts**? Will yeast change the nutrient composition of the distillers grains?* Students will conduct additional research to obtain evidence to support/refute the data generated from this investigation. Students will

then design solutions for future uses of ethanol coproducts.



Instructions

Students will work together in groups of 3 or 4. The following tests should be conducted at the beginning on raw product day 1, day 3 after fermentation, and again after the distillation process.

Day 1: Nutrient testing and corn mash prep of solid sample for testing before amylase:

1. Weigh out 5g of dent corn sample using an electronic balance. Place sample into a mortar.
2. Add 20 mL of distilled water to the food sample in the mortar. Grind sample with a pestle into a slurry.
3. Filter slurry using filter paper and funnel and collect liquid food sample into a small graduated cylinder or beaker.
4. Use the filtrate to complete the *Carbohydrate Indicator Tests* and the *Protein Indicator Test*.

Monosaccharide indicator standard test (glucose):

1. Add 2 mL of food sample solution with 2 mL of Benedict's solution in a test tube.
2. Use Vortex to give sample a quick mix (or cover with parafilm and invert test tube). Record sample color in data chart.
3. Place test tube containing food sample and Benedict's solution in a boiling water bath and heat for 2 minutes. Record sample color in data chart.
4. The glucose present in the solution reacts with the copper sulfate in the Benedict's reagent creating copper oxide, which results in an orange to red-brick precipitate. The intensity of the color depends on the concentration of glucose present in the sample.
5. Rate the precipitate color change and record sample data in the chart.
0: no color change/negative, 1: weak/positive, 2: strong/positive, 3: very strong/positive.

Complex carbohydrate indicator standard test (starch):

1. Add 1 mL of food sample solution with 1 drop of Lugol's iodine solution in a test tube.
2. Use Vortex to give the sample a quick mix (or cover with parafilm and invert test tube). *Do not heat!*
3. A bluish black color indicates a positive test for starch.
4. Rate the precipitate color change and record sample data in the chart.
0: no color change/negative, 1: weak/positive, 2: strong/positive, 3: very strong/positive.
5. Keep the sample to observe until day 3 of the lab.

Protein indicator standard test:

1. Add 1 mL of food sample solution with 2 mL of Biuret solution in a test tube.
2. Gently mix using a Vortex (or cover with parafilm and invert test tube).
3. After 30 seconds, the filtrate solution will result in a color change to purple if proteins are present in the sample.
4. Rate the precipitate color change and record sample data in the chart.

0: no color change/negative, 1: weak/positive, 2: strong/positive, 3: very strong/positive.

Day 3: Nutrient testing after fermentation

Perform both the carbohydrate Indicator Tests as well as the Protein Indicator Test (as completed in Day 1) on the corn mash after fermentation and again after ethanol distillation is complete.

- Please see the *Ethanol: corn mash and distillation* lesson to create the filtrates that will be necessary to finish the nutrient testing for this lab.

Nutrient Testing

Sample	Benedict's Test	Starch Test	Protein Test
Cracked Corn Slurry			
Corn after Fermentation			
Distillation			

Graph

Create a bar graph to demonstrate the nutrient profile change in corn as it undergoes fermentation and distillation.

Reflection

Create an explanation for the current use of dent corn in commercial ethanol production and design solutions for the coproducts that are generated from ethanol production. Reflect on the following questions while creating your explanation.

1. How does the nutrient profile of dent corn change as it undergoes the process of fermentation and distillation?
 - a. Is all of the glucose consumed? Provide evidence to support your conclusion.
 - b. How do the yeast contribute to the nutrient profile of the distillers grains after fermentation?
 - c. How can the yeast contribute to the digestion of the animals consuming the distillers grains?
 - d. Why are distillers grains considered to be a valuable feeds for animals?
2. Provide a graphic of the nutrient composition change of corn before/after fermentation.
3. What are the current industry uses for the coproducts produced in the commercial ethanol production process?

Constructing Explanations and Designing Solutions:

Create an explanation for the current use of dent corn in commercial ethanol production and design solutions for the coproducts that are generated from ethanol production. Reflect on the following questions while creating your explanation.

Self-Assessment Rubric: Ethanol: Nutrient Testing

	Yes	No	Maybe/Unsure
Did we generate data and construct an explanation of nutrient content change for corn as it is transformed into ethanol?			
Did we construct viable solutions for the industrial application of the remaining coproducts produced in the commercial ethanol production?			

Teacher: Lesson 5: Ethanol: Corn Mash and Distillation

DCI	
HS - LS2: Interdependent Relationships in Ecosystems	
Performance Expectation	
HS-LS2-3: Construct and revise an explanation based on evidence for the cycling of matter and energy flow of energy in aerobic and anaerobic conditions. <ul style="list-style-type: none"> <i>Clarification Statement: Emphasis is on conceptual understanding of the role of aerobic and anaerobic respiration in different environments.</i> <i>Assessment Boundary: Assessment does not include the specific chemical processes of either aerobic or anaerobic respiration.</i> 	
Science & Engineering Practice	
Constructing explanations and Designing solutions. <ul style="list-style-type: none"> 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 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. 	Students construct an explanation for the process of commercial ethanol production based on evidence from student investigations and design logical solutions for the coproducts that are produced.
Disciplinary Core Idea	
LS2.B: Cycles of Matter and Energy Transfer in Ecosystems <ul style="list-style-type: none"> Photosynthesis and cellular respiration (including anaerobic processes) provide most of the energy for life processes. 	Students investigate the breakdown of starch to provide energy for the anaerobic respiration of yeast.
Crosscutting Concept	
Energy & Matter: <ul style="list-style-type: none"> Energy drives the cycling of matter within and between systems. 	Students create an explanation for the fermentation reaction of glucose to demonstrate the cycling of matter into carbon dioxide and ethanol.

This lesson is designed to follow Lesson 1, *Corn Fermentation in a Bag*, Lesson 2, *Ticketase*, and Lesson 3, *Biomass to Sugars*, and Lesson 4, *Ethanol: Nutrient Testing*, where students utilize different components (enzymes, yeast, feedstocks, and water) to produce ethanol and carbon dioxide through the process of fermentation, model enzyme action on starch to produce simple sugars, and analyze macromolecule content in available ethanol feedstocks and coproducts. This lesson focuses on Constructing Explanations and Designing Solutions to construct an explanation for the process of commercial ethanol production based on evidence from student investigations and design logical solutions for the coproducts that are produce

Background

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 **coproducts (distillers grains, corn oil, and carbon dioxide)**. Ethanol is an alcohol produced by yeast from sugars. Fuel ethanol is ethanol that has been highly concentrated and blended with other compounds (gasoline) to render the alcohol undrinkable. For each pound of simple sugars, yeast can produce approximately .5 pounds (0.15 gallons) of ethanol and an equivalent amount of carbon dioxide. The value of corn as a feedstock for ethanol production is due to its large volume of

carbohydrates, specifically starch. Starch can be easily processed to break down into simple sugars, which can then be fed to yeast to produce ethanol. Modern ethanol production can produce approximately 2.8~3 gallons of fuel ethanol per bushel of corn. Dry-milled ethanol production uses only the starch portion of the corn, which is about 62% of the kernel. All the remaining nutrients: protein, fat, minerals, and vitamins, are concentrated into distillers dried grains (DDGs), a valuable feed for livestock. Some ethanol plants also remove the corn oil from DDGs to create renewable diesel. Approximately 40% of the United States' corn crop is currently used to produce ethanol and distillers grains.

Materials *Prepare materials ahead of time for students (make sure to create the amylase and glucoamylase solutions before the lab). Students should have access to the following materials at their group lab table.*

- Hot plate
- 110V heating mantle
- 100 or 1000 mL distillation apparatus
- Condenser tube
- Dial thermometer
- Graduated cylinders (10, 100 mL)
- Large watch glass covers
- Beakers (100, 250, 600, 1000 mL)
- Deionized water
- Hammered dent corn
- Scale or triple beam balance
- Glass vials with caps (or a small beaker)
- Buffer solution (pH 5)
- Yeast solution (20 g yeast/100 mL water, 49–55° C)
- Amylase solution (3 tsp/100 mL water)
- Glucoamylase solution (3 tsp/100 mL water)
- Funnel
- Thermal gloves
- Glass stir rod
- Cheesecloth/plastic sieve
- *Optional: Pipette pump*
- *Optional: 10 mL serological pipettes*
- *Optional: Aluminum foil*
- *Optional: Paper towels*

Instructions

Students should work in groups of 3-4 to create a corn mash and distill the filtrate into ethanol. Carefully observe the students as they prepare the corn mash, filter the corn mash and distill the filtrate into ethanol. Do not use any flames in the room during the ethanol distillation. Students should make sure that the joints of the distillation equipment is tightly sealed in order to capture the distilled ethanol and prohibit it from being released into the atmosphere.

See student lesson for detailed instructions of the corn mash preparation and ethanol distillation.

Alcohol flame test

This test should only be done in an active fume hood on a watch glass after the ethanol distillation is complete and there is no vapor in the air.

Salt wash (optional)

Students can separate remaining water from the ethanol distillate by adding potassium carbonate, K_2CO_3 , which is soluble in water but not in ethanol. The K_2CO_3 and water will form an alkaline solution and separate from the ethanol to form a dense, bottom layer with the ethanol remaining in the top layer. Students can then remove the ethanol to be tested either by flame in the fume hood, ethanol probe, or in a stirring engine.

Reflection

Construct an explanation for the process of commercial ethanol production based on evidence from student investigations and design logical solutions for the coproducts that are produced. Reflect on the following questions while creating your explanation.

1. What effect does the physical heating have on the corn mash?

Possible student response: The heating helps to break down the fiber and complex carbohydrates in the corn.

2. Explain how each enzyme (amylase and glucoamylase) change the corn mash mixture in preparation for fermentation.

Possible student response: The enzymes help to deconstruct the starch into glucose.

3. What is the function of the yeast during the fermentation process? How did the consistency of the corn mash change during the 3 day fermentation process?

Possible student response: The yeast will continue to consume sugars over the 3 day fermentation process. As the time continues, the sugar percentage will decrease, making contact with the yeast more difficult. The corn mash will become less dense due to the consumption of the sugars by yeast.

4. Describe the physical changes that your corn went through during its transformation into ethanol.

Possible student response: The corn was broken down both mechanically and chemically into macronutrients. Glucose was removed and consumed by yeast.

6. What coproducts result from ethanol production?

Possible student response: Ethanol production also creates distillers grains (solid product), water, oil, and carbon dioxide.

7. What are efficient and economical uses for the coproducts (carbon dioxide and distillers grains) that are generated during ethanol production?

Possible student response: Carbon dioxide is captured to be used as a carbonate in beverages. Distillers grains are currently used in animal feed as a protein source.

Constructing Explanations and Designing Solutions:

Construct an explanation for the process of commercial ethanol production based on evidence from student investigations and design logical solutions for the coproducts that are produced.

Student responses will vary according to their lab results and research.

Differentiation:

Other ways to connect with students with various needs:

- **Local community:** Students may investigate the use of dent corn in their local area. What are farmers doing with the corn they produce? Students may choose to interview a farmer, local farmer cooperative. Students can communicate with their findings with their classmates and their local community.
- **Students with special needs (visual/auditory/language/reading):** See the extra support below.
- **Extra Support:** Video: How ethanol is made (<https://youtu.be/59R-NqykoXs>) This video helps demonstrate relationships between the components of the ethanol fermentation ecosystem. Infographic: <http://vitalbypoet.com/stories/ethanol-process> This infographic represents the process of corn from field to distillation into ethanol.
- **Extensions:** Students can research the current commercial process of ethanol production and uses of the generated coproducts. Students can also research additional future uses for ethanol coproducts to make ethanol production more efficient and sustainable.

Additional Resources for student research:

- <http://vitalbypoet.com/stories/ethanol-process>
- Video: <https://youtu.be/59R-NqykoXs>

Self-Assessment Rubric: Ethanol: Nutrient Testing

	Yes	No	Maybe/Unsure
Did we generate data and construct an explanation for the process of commercial ethanol production?			
Did we construct viable solutions for the industrial application of the remaining coproducts produced in the commercial ethanol production?			

Teacher Assessment Rubric: Ethanol: Nutrient Testing

Does Not Meet 1	Approaching 2	Meets the Standard 3	Advanced Understanding 4
The student is not able to use the data generated to construct an explanation for commercial ethanol production.	The student is able to use the data generated to construct an explanation for commercial ethanol production.	The student is able to: •Independently construct an explanation based on data for commercial ethanol production. • Analyze explanations of commercial ethanol production to determine the validity of their findings.	The student is able to: •Independently construct an explanation based on data for commercial ethanol production. • Analyze explanations of commercial ethanol production to determine the validity of their findings with increased depth and complexity. • Design future solutions for coproduct usage from commercial ethanol production.

Lesson 5: Ethanol: Corn Mash and Distillation

Essential Question: *How is ethanol produced? What are the steps in ethanol production?*

Investigation

How can we modify dent corn to make glucose available for **fermentation**? What role does **anaerobic respiration** play as yeast consume glucose to create ethanol and carbon dioxide? What change does glucose undergo to become ethanol and carbon dioxide? Students will create a corn mash and break down starch into glucose that will undergo the fermentation process into ethanol. Students will then separate the **distillers grains** from the **filtrate** for distillation and distill ethanol from the filtrate. Students will then construct and revise their explanations as they determine how anaerobic respiration plays a role in the fermentation process that is necessary for ethanol production. Students will continue to research ethanol production and propose future solutions to improve the efficiency of ethanol production.

Instructions

Day 1: Preparation of corn mash

1. Weigh out 100 g of hammered dent corn and add to a 1000 mL beaker.
2. Add 300 mL of distilled water. Record observations in Data section. Stir well. Place the beaker on a hot plate and set the temperature to high to get it to boil, boil gently and stir constantly for 15 minutes. **Be careful not to let it burn.** (If the mixture becomes too dry, more water may be added).
3. After boiling is completed, remove the beaker from the hot plate and allow it to cool to touch (49–55° C). Record observations on consistency, color and smell in Data section.
 - a. Measure 100 mL of distilled water and pour into a 250 mL beaker.
 - b. Shake the amylase solution well.
 - c. Measure 10 mL of the amylase solution into a small graduated cylinder and add to the 250 mL beaker of water and stir.
 - d. Add the mixture to the corn mash and stir to evenly incorporate.
 - e. Stir the mixture occasionally with a glass rod during the next 10 minutes.
4. At the end of the 10-minute period:
 - a. Shake the buffer and glucoamylase solutions well
 - b. In a 100 mL beaker, mix 35 mL of the buffer solution (to maintain a slightly acidic pH), 10 mL of glucoamylase solution, and 10 mL of the yeast solution together.
 - c. Pour the mixture into the corn mash. Stir the entire mixture well.
5. Place a piece of plastic wrap over the mouth of beaker and label (fermentation will occur so do not secure it too tightly).
6. Place your beaker in an incubator on the counter and allow it to sit for 3 days so that the enzymes have time to work. Stir the corn mash each day to maximize fermentation.
Optional: Place corn mash in an incubator set at 37°C/98.6°F (optimal temperature for yeast metabolism).

Day 3: Distillation of ethanol from corn mash

1. Set up the distillation apparatus as demonstrated by your instructor. (Before you use your distillation apparatus, have your lab instructor inspect it).
 - a. Make sure to either grease or wet the ground glass joints before connecting them. This helps to prevent any vapor from escaping the joints and to keep the joints from freezing together.
 - b. Filter out your distillers grains by using cheesecloth or coffee filters. Be sure to press on the mash to filter out all of the liquid. Pour about 50–75 mL of your solution in the distilling flask so that it is a little more than half full.
 - *If you are using a large distillation flask (1000 mL), put the entire classes' solution into the flask.*

- *If necessary, add enough distilled water to the flask to reach the halfway point of the flask to ensure even heating of distillate.*

c. You will use a heating mantle to provide the heat necessary for the distillation. * *Do not use a bunsen burner!* The inside of the mantle will eventually become extremely hot. In order to control the heat, you can raise or lower the temperature of the heating mantle. Make sure the distillation flask and condenser tube are properly supported! The outside of the mantle is relatively cool to touch.

2. The best separation of alcohol will occur if the distillation is done slowly. Ethanol's boiling point is 173.1°F/78.37°C and water's is 212°F/100°C, please keep the temperature between these two boiling points. Collect the ethanol distillate samples into a small beaker to be used for the alcohol flame test (we recommend that you collect them in 4 dram vials). Pour the distillate samples into a capped vial for storage until ready to do the flame test.

3. When you are no longer able to distill your product, turn the heating mantle off and allow the distillation apparatus to cool before disconnecting and cleaning.

Data

Day 1	Consistency	Color	Smell
Before boiling (step 2)			
After boiling (step 3)			
After enzyme addition (step 4)			
Day 3			
Before distillation (step 1)			
First distillate sample (step 3)			
Second distillate sample (step 4)			

Flame Test	Time	Flame Properties
Sample 1		
Sample 2		

Alcohol flame test

1. *This test should only be done in a fume hood after the ethanol distillation is complete and there is no vapor in the air.* You will be testing the ethanol distillate for alcohol concentration by lighting it on fire. The longer the flame burns, the greater the alcohol concentration. If the distillate does not burn, the water concentration is too high (over 50%).

2. Place a watch glass in the fume hood and pour 2 mL of your ethanol distillate on it.

3. Turn the fume hood on and lower the window, light your distillate, record the time the flame burns and observe.

4. Measure the amount of remaining water on the watch glass and calculate the alcohol percentage of your product.

Salt wash (optional)

Students can separate remaining water from the ethanol distillate by adding potassium carbonate, K_2CO_3 , which is soluble in water but not in ethanol. The K_2CO_3 and water will form an alkaline solution and separate from the ethanol to form a dense, bottom layer with the ethanol remaining in the top layer.

Reflection

Construct an explanation for the process of commercial ethanol production based on evidence from student investigations and design logical solutions for the coproducts that are produced. Reflect on the following questions while creating your explanation.

1. What effect does the physical heating have on the corn mash?
2. Explain how each enzyme (amylase and glucoamylase) change the corn mash mixture in preparation for fermentation.
3. What is the function of the yeast during the fermentation process? How did the consistency of the corn mash change during the 3 day fermentation process?
4. What are other ways you can experiment to make the fermentation process more efficient?
5. Describe the physical changes that your corn went through during its transformation into ethanol.
6. What byproducts result from ethanol production?
7. What are efficient and economical uses for the coproducts (carbon dioxide and distillers grains) that are generated during ethanol production?

Constructing Explanations and Designing Solutions:

Construct an explanation for the process of commercial ethanol production based on evidence from student investigations and design logical solutions for the coproducts that are produced.

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