KANSAS CORN STEM

Kansas Corn: Ethanol - Corn Mash and Distillation

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

KANSAS CORN COMMISSION

Kansas Corn: Ethanol - Corn Mash and Distillation
Grade Level: Middle School

Overview
In this lab, students will learn about ethanol and its important role in our world's ever increasing demand for energy. Students will go through the process of fermenting and distilling corn for ethanol production. This lab has many variables that can affect ethanol production. The procedure can be followed as is, or a more inquiry-based lesson can be used by having students choose a variable and make changes to see how that variable affects the amount of ethanol produced.
Possible variables to change:
- pH
- Amount of enzymes
- Amount of yeast
- Time of fermentation
- Use of urea for nitrogen

Kansas College and Career Ready Standards
Science
- **MS-PS1.A.** Structure and Properties of Matter: The changes of state that occur with variations in temperature or pressure can be described and predicted using these models of matter.
- **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.
- **HS-CCC 5.** Energy and Matter: Tracking energy and matter flows, into, out of, and within systems helps one understand their system's behavior. Energy drives the cycling of matter within and between systems.

Learning Objectives
- The importance of ethanol and how it relates to today's energy needs.
- The process of anaerobic respiration for yeast and how they produce ethanol.
- How to distill ethanol from corn.

Materials
- Ethanol - Corn Mash and Distillation PowerPoint available at www.kansascornstem.com

Materials for Preparation of Enzymes:
- Beakers (2, 500 ml)
- Distilled water (250 ml)
- Amylase (¼ tsp)
- Distilled water (250 ml)

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Materials for Preparation of Enzymes (continued)

- Glucoamylase (¼ tsp)

Materials for Preparing the Corn Mash:

- Hot plate
- Beakers (100, 250, 600 ml)
- Graduated cylinders (10 ml, 50 ml)
- Digital thermometer
- Scale or triple beam balance
- Pipettes
- Plastic Wrap
- Rubber band
- Distilled water
- Ground corn (100 g)
- Buffer solution pH
- Prepared yeast solution
- Prepared amylase solution
- Prepared glycosylase solution

Optional Materials for CO2 Gas Collection:

- Erlenmeyer Flask (500 ml)
- Stopper with single hole
- Glass tubing
- Plastic or rubber tubing
- Large tub for holding water
- Graduated cylinder (250 ml or larger)
- Ring stand
- Clamps

Materials for Filtering the Solids:

- Strainer
- Cheesecloth
- Large bowl
- Beaker (600 ml)

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Materials for Distillation:

- Heating mantle (110V)
- Distillation apparatus (1,000-2,000 ml)
- Condenser tube
- Dial thermometer
- Large watch glass covers
- Small beaker or glass vials with caps
- Funnel
- Thermal gloves or hot pads
- Glass stirring rod
- Cheesecloth
- Pipettes
- Potassium carbonate, K2CO3

Materials for Teacher Demonstrations with Ethanol:

- Large Culligan-style water bottle
- Small water bottle (Smart Water brand)
- Ethanol Fuel Cell Kit
- Bottle of 95% ethanol
- Plastic wrap
- Rubber band
- Long-reach lighter or a match attached to a long rod
- Glass stirring rod

Safety Considerations

- Safety goggles should be worn at all times during the lab.
- Students will need to follow all classroom procedures for the use of hot plates. Do not touch any hot surfaces, and make sure you are using hot pads when removing handling heated glassware.
- Ethanol in liquid or vapor form is highly flammable – keep away from open flame.

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Procedures for Instruction
Length of time for preparation: Teachers should allow for an hour to gather materials and prepare the necessary solutions.
Length of time for classroom teaching: 2-4 days

Preparation Procedure/Instructions
- Gather all necessary materials needed for the day of and have them set out for each lab group.
- Prepare the amylase and glucoamylase enzyme solutions prior to the lesson (see below for directions).

Background Information
The increasing demand of fuel for transportation, increased world-demand for oil (gasoline), and the negative consequences of global warming have all contributed to the increased use of corn-based sugar to produce ethanol. Ethanol is blended with gasoline and burned in many of today's passenger cars and trucks. Most gas stations currently use 10% ethanol in their gasoline. However, it has also been used as 85% ethanol to 15% gasoline at some gas pumps called, "E85" or flex fuel. Running this fuel in the gasoline motor typically does not require any mechanical modification. **Not all gasoline motors are manufactured to run on E85, so it is best to check the vehicle owner's manual before fueling up with E85.**

Commercial production of fuel ethanol involves breaking down the starch present in corn into simple sugars like glucose and feeding these sugars to yeast for fermentation. Next, they recover ethanol, and other byproducts, such as animal feed, corn oil, and carbon dioxide. Ethanol is an alcohol produced by yeast during fermentation. Fuel ethanol is ethanol that has been highly concentrated and blended with gasoline to render the alcohol undrinkable.

For each pound of simple sugars, yeast can produce approximately 0.5 pounds (0.15 gallons) of ethanol and an equivalent amount of carbon dioxide. Corn is used for ethanol production because of its large volume of carbohydrates, specifically starch. Starch can be easily processed to break down into simple sugars, and fed to yeast to produce ethanol. Modern ethanol production can produce approximately 2.8 gallons to 3 gallons of fuel ethanol for every bushel of corn.

About 40% of the United States' corn crop is used to produce ethanol. Ethanol production uses only the starch portion of the corn, which is about 70% of the kernel. All the remaining nutrients: protein, fat, minerals, and vitamins, are concentrated into distillers grains, and are used as feed for livestock. Some ethanol plants also remove the corn oil from distillers grains to create renewable diesel.

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Classroom Discussion
Introduce the topic and assess students for prior understanding. Possible topics could include:
- Yeast and anaerobic respiration
- Enzymes and how they work in a chemical reaction
- Different types of sugars
- How different enzymes break down different starches
- Ethanol as a fuel source
- Renewable vs nonrenewable resources
- Comparing the amount of energy produced from ethanol compared to other hydrocarbons/octane

Note: Educational resources to help with this discussion can be found at www.kansascornstem.com.

Possible Questions for Guided Discussion
Let students discuss their ideas, and guide the discussion without telling them if they are right or wrong.
- Why produce ethanol?
- What are the benefits of using ethanol as a fuel source?
- Are there any drawbacks for using ethanol?

Procedure for Lab
Part 1:
Preparation of Enzymes (Prepare before the start of the lab)
1. Mix ¼ tsp of amylase with 1 L of distilled water. Stir thoroughly.
2. Mix ¼ tsp of glucoamylase with 1 L of distilled water. Stir thoroughly.

Preparation of Corn Mash (1 to 2 class periods depending on schedules)
1. Weigh out 100 g of ground corn and add to a 600 ml beaker.
2. Heat 300 ml distilled water to between 80°C to 90°C and add it to the ground corn. Stir the corn mixture. Place the beaker on a hotplate. Gently boil the solution and continuously stir for 15 minutes. Be careful not to let the corn mixture burn.
3. After boiling is completed, remove the beaker from the hotplate and allow it to cool to between 55°C and 37°C. (Note: See Teacher Tips.)
4. While the corn mash is cooling, measure 100 ml of distilled water and pour into a 250 ml beaker. Shake the amylase solution, then measure 10 ml of the amylase solution into a small graduated cylinder and add to the 250 ml beaker of water. Stir the resulting mixture and add it to the cooled corn mash. Stir the mixture occasionally with a stirring rod throughout the next 10 minutes.

5. At the end of the 10-minute period, measure 35 ml of the pH 5 buffer. Shake the buffer solution and add it to the corn mash to maintain a slightly acidic pH.
6. Shake the glucoamylase solution, then measure 10 ml of glucoamylase solution. Add it to the corn mash.
7. Add 5 grams of yeast to the corn mash and stir the entire mixture well. See Optional Procedure below for CO2 gas collection, or proceed with Step 8.
8. Place a piece of plastic wrap over the mouth of beaker and secure it with a rubber band (fermentation and gas production will occur so do not secure it too tightly). Place your beaker on the counter and allow it to sit overnight. (Note: See Teacher Tips.)

Optional Procedure for Overnight Fermentation

This procedure will allow you to track the CO2 production of the yeast. Knowing how much gas is produced will allow you to track how much fermentation has taken place and allow you to wait to distill until fermentation has stopped or significantly slowed (anywhere from 24 to 36 hours). (Note: See Teachers Tip)

1. Transfer your corn mash / yeast mixture to a 500 ml Erlenmeyer Flask. Insert 90° bent glass tubing into a single-holed stopper large enough to fit your flask.
2. Fill a large tub with water. Submerge your 250 ml or larger graduated cylinder under the water until it is completely filled. Keep the open end of the cylinder submerged under the water but lift the bottom end up. The graduated cylinder should now be upside down, vertically in your water tub. It should still be filled with water and free of air bubbles. Use a stand and clamp to secure the graduated cylinder.
3. Attach rubber tubing to the glass tubing in your flask. Run the tubing under the water and up into the opening of the suspended graduated cylinder.
4. As the yeast metabolize and produce ethanol, they also produce carbon dioxide. There is a direct correlation between the amount of carbon dioxide produced and the amount of ethanol. Over time, the graduated cylinder will fill up with gas and displace the water inside. You may need to refill the cylinder with water several times. Keep track of the gas production and note when the gas production slows or stops. When this occurs, you are ready to distill.

Part 2:

Filtering the Solids (10-15 minutes)

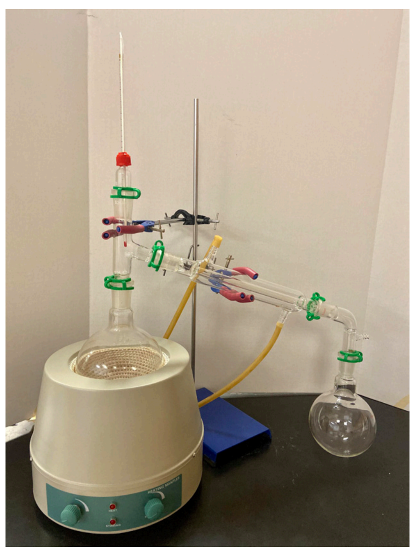
1. Use a large strainer with a large bowl/beaker underneath to strain out any large solids from your fermented corn mash. Repeat this step 2-3 times. Swirl the strainer as it drains. This will greatly reduce the time it takes to strain.
2. Collect the solids from the strainer. Place the solids in a large piece of cheesecloth. Wrap up the solids, then hand squeeze the liquid out of the solids into your beaker.

3. Line your strainer with a new piece of cheesecloth. Pour your liquid through the cheesecloth lined strainer into a bowl/beaker below. Collect the cheesecloth with the strained solids, and again hand squeeze the liquid out of the solids into your bowl/beaker. You should have removed most of the solids from this mixture at this time.

Distillation of Ethanol from Corn Mash

(1-2 hours depending on how long it takes your heating mantle to warm up. Plan to continue running after class has completed)

1. Set up the distillation apparatus as directed. 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. Visit www.kansascornstem.com for video instructions.
2. Pour the strained solution into the distillation flask. Use a heating mantle to heat the liquid and control the temperature. The best separation of alcohol will occur if the distillation is done slowly. Ethanol's boiling point is 78.37°C and water's is 100°C; therefore, keep the temperature of the distillate between these two boiling points. (Note: See Teacher Tips.)
3. Collect the ethanol distillate samples into a small flask to be used for the Alcohol Flame Test. Wrap the opening of the flask and end of the condensing tube with aluminum foil to help prevent evaporation of the ethanol. Pour the distillate samples into a capped vial for storage until ready to do the Alcohol Flame Test.



Alcohol Flame Test

Use a pipet to remove a 2 ml sample of your distilled ethanol and place the ethanol on a watch glass or in a ceramic evaporating dish. Light the ethanol with a lighter. A quality sample will light with a pale blue flame. Time how long the flame burns. The longer the flame burns, the greater the alcohol concentration. If the flame does not burn, the mixture may contain too much water. Ethanol's boiling point is 78.37°C and water's is 100°C; therefore, it is important to keep the temperature between these two boiling points. If distillation ran with temperature close to 100°C, too much water may have gotten into the sample.

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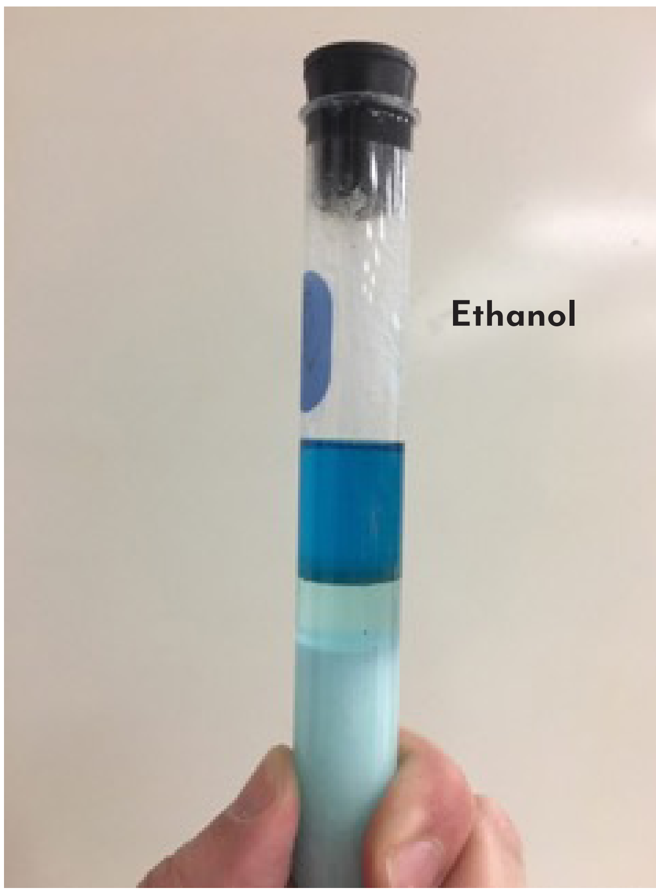
There are two possible solutions:

1. Distillate may be run through the distillation process again.
2. Add 4 ml of distillate with several drops of food coloring to a test tube containing 1 g of potassium carbonate, K2CO3, and insert stopper. Shake vigorously and allow layers to form. If none form, add more potassium carbonate and repeat. Water in the distillate is attracted more to the potassium carbonate and leaves the ethanol in a concentrated layer that will contain the food coloring. The ethanol layer will form above the salt water layer due to its lower density. Carefully pipette or decant the ethanol off of the salt water layer.

Optional Salting Out Activity:



Potassium carbonate has saturated the water and forced the ethanol out of solution. The food coloring stays in the ethanol layer. This should be nearly pure ethanol.

https://projects.ncsu.edu/project/chemistrydemos/Organic/SaltingOut.pdf

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Teacher Demonstrations with Ethanol

Demonstration 1: Whoosh Bottle

This demonstration shows the flammability and potential stored within vaporized ethanol.

Safety Considerations

- Make sure to wear safety goggles when performing this demonstration.
- Students should be kept back from the bottle.
- Keep hands and loose clothing away from the opening of the bottle, as flames will shoot out of the opening.

Instructions

1. Measure 10 ml of 95% ethanol and pour it into the large Culligan water bottle. Cover the opening of the bottle with plastic wrap and a rubber band.
2. Shake the water bottle for about a minute, spinning the bottle to make sure the sides are coated with ethanol.
3. Turn off the classroom lights. Set the bottle on the table and remove the plastic wrap.
4. Stand back from the bottle. Extending your arm, light a long-reach lighter and hold it over the opening. This demonstration can also be lit with a match attached to the end of a long rod.

It is recommended to have at least two water bottles for this demonstration especially if you need to do this in back to back class periods. The demonstration cannot be performed again directly after it is completed. A small fan can be placed near the opening of bottle to help vent out the gas and circulate fresh air into the bottle between demonstrations.

Demonstration 2: Launching a Water Bottle

This demonstration shows the flammability of vaporized ethanol and the conversion of potential energy to kinetic energy.

Safety Considerations

- Make sure to wear safety goggles when performing this demonstration.
- Students should be kept back from bottle.
- Keep hands and loose clothing away from the opening of the bottle, as flames will shoot out of the opening.

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Instructions

1. Prepare a small water bottle ahead of time by heating a glass stirring rod and melting a small hole in the center of the cap. (Note: The Smart Water bottle with Sport Cap (pictured) is perfect for this demonstration, and it does not require you to melt a hole in the cap.)
2. Measure 10 ml of 95% ethanol and pour it into the water bottle.
3. Cover the opening of the bottle with your thumb and shake the water bottle for about 30 seconds, swirling the bottle to make sure the sides are coated with ethanol.
4. Lay the bottle on its side on the floor or counter. Stand back from the bottle. Extending your arm, light a long-reach lighter and hold it over the opening. The bottle will launch across the floor/counter.

It is recommended to have at least two water bottles for this demonstration especially if you need to do this in back to back class periods. The demonstration cannot be performed again directly after it is completed. A small fan can be placed near the opening of bottle to help vent out the gas and circulate fresh air into the bottle between demonstrations.

Demonstration 3: Ethanol Fuel Cells

This Bio-Energy Fuel Cell Kit from Horizon allows you to convert ethanol directly to electricity without combustion. Dilute the 95% ethanol to a 10% solution. Put the ethanol into the reservoir. The fuel cell produces enough electricity to power the small fan. One of the byproducts of the reaction is acetic acid, which can be tested with pH paper. Refer to and follow directions that accompany the kit.

Teacher Tips

- Use fresh yeast.
- Make sure not to add enzymes until the mash has cooled. Enzymes are temperature sensitive and putting the enzymes in while the mash is still hot will cause the enzymes to breakdown. It might be difficult to make the mash in one class period. Better to wait till the next class period then put the enzymes in at too high of a temperature.
- Mash could be made on a Friday so that you have the entire weekend for fermentation.
- When distilling, it is important to closely monitor the temperature. The heating mantels are sometimes difficult to get dialed into an optimal temperature. It is important not to let the temperature rise to 100° C or you will get too much water in your distilled ethanol.
- Visit www.kansascornstem.com for videos to help with the lab.

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Lab Analysis
There are several ways to collect data from this experiment:

- During each stage of the experiment, students can take anecdotal notes during the experiment on consistency, color, and smell.
- Using the optional procedure for gas collection, students can collect data on the amount of CO_2 produced from each sample. If students changed any of the variables in the procedure, this would be a good way to determine the impact on fermentation.
- Students can measure the amount of ethanol at the end of the experiment. Whether or not their sample was able to burn in the flame test will indicate the presence of ethanol. Students should time how long their sample burns. The longer the sample burns, the higher the ethanol concentrations. See direction under the "Flame Test" for more detailed instructions.

Reflection and Conclusion
Reflection questions can be done as a whole class discussion, in small groups, or science journals/interactive notebooks.

- In your opinion, do you think it is a good idea to make ethanol from corn? In your answer, make sure you state reasons why or why not?
- How might we use ethanol in the future?
- What variable might you change next time you run this experiment to improve ethanol production?

Assessment
These examples of questions that could be used to assess your students. Use whatever assessment method is appropriate for your students.

- What effect does the physical heating have on the corn mash?
- Explain why we need to use enzymes (alpha amylase and glucoamylase) to help in the corn ethanol fermentation. Explain how each enzyme change the corn mash mixture in preparation for fermentation.
- What is the function of the yeast during the fermentation process?
- Describe the physical changes that your corn went through during its transformation into ethanol.
- What byproducts result from ethanol production?
- What is the process that converts corn starch sugar to ethanol and carbon dioxide?
- What is the name of the process that separates ethanol from the water and grain at the end of fermentation?
- Explain why distillation is used to separate ethanol from the other parts of the fermentation reaction? Why can't you just filter out the ethanol?

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Science and Agriculture Careers

Ethanol is a part of the agricultural industry that has job openings from corn farming, ethanol production, to government policy jobs in Washington, D.C. Ethanol product jobs are readily available, and so are jobs in biofuel research. Typically, you do not need a degree to work in an ethanol production plant, but for higher salaries, consider a degree in agriculture, chemistry, biology, or a related field. Workers in ethanol plants transport the fermented corn to distillers, monitor the dehydration process, and package the final ethanol product safely. Car companies are increasingly advancing their research departments to deal with the growing trend of renewable energy. The government also hires workers for the research and development of ethanol products.

Feedstock

- Farmers
- Seasonal workers
- Mechanical engineers
- Harvesting equipment mechanics
- Equipment production workers
- Chemical engineers
- Chemical application specialists
- Chemical production workers
- Biochemists
- Aquaculture technicians
- Agricultural engineers
- Genetic engineers and scientists
- Storage facility operators

Conversion

- Microbiologists
- Clean room technicians
- Industrial engineers
- Chemical & mechanical engineers
- Plant operators
- Train station operators

End Use

- Station workers
- Construction workers
- Codes & standards developers
- Regulation compliance workers
- Consultants

Transport of Feedstocks & Ethanol

- Truck drivers
- Truck filling station workers
- Pipeline operators
- Barge operators
- Railcar operators

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

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

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