



---

# 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: Ethanol - Corn Mash and Distillation

## Grade Level: High 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.

There are many variables that can affect ethanol production. This lab may be used as a stand-alone lab, with a prescribed procedure for producing ethanol, or as a follow-up after performing Kansas Corn: Fermenting Fuel – Designing a Procedure for Fast Fermentation (available online at [kansascornstem.com](http://kansascornstem.com)). When using this approach to the lab, students use their data to produce their own procedure and compete to see which group can produce the most efficient fermentation. This is determined by comparing the largest volume of flammable alcohol or the most CO<sub>2</sub> collected during fermentation.

### 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

- Corn Mash and Distillation PowerPoint (available at [kansascornstem.com](http://kansascornstem.com))
- Student Lab Packet (pg. S1-S13 or available at [kansascornstem.com](http://kansascornstem.com))

#### *Materials for Student Developed Procedure:*

- Yeast
- Amylase solution
- Glucoamylase solution
- Ground corn (50 g for each group)
- Buffer solution (pH 5)

# Kansas Corn: Ethanol - Corn Mash and Distillation

## Grade Level: High School

### *Materials for Student Developed Procedure (continued)*

- Beakers (500 ml)
- Erlenmeyer Flask (250, 500 ml)
- Pipettes
- Balance
- Additional items may be needed as students develop their own procedures. (Examples: different varieties of yeast, warm water baths, etc.)

### *Materials for Prescribed Procedure:*

- Preparation of Enzyme Solutions
  - 2 beakers (500 ml)
  - Distilled water (500 ml)
  - Amylase ( $\frac{1}{4}$  tsp)
  - Glucoamylase ( $\frac{1}{4}$  tsp)
- Preparing the Mash
  - Hot plate
  - Beakers (100, 500, 600 ml)
  - Graduated cylinders (10, 100 ml)
  - Digital thermometer
  - Balance
  - Pipettes
  - Distilled water
  - Ground corn (50 g)
  - Buffer solution (pH 5)
  - Yeast
  - Prepared amylase solution
  - Prepared glucoamylase solution

### *Materials for Fermentation and CO<sub>2</sub> Gas Collection:*

- Erlenmeyer Flask (500 ml)
- Stopper with single-hole to stopper Erlenmeyer Flask
- Glass tubing
- Glass square
- Plastic or rubber tubing
- Large tub for holding water

# Kansas Corn: Ethanol - Corn Mash and Distillation

## Grade Level: High School

### *Materials for Fermentation and CO<sub>2</sub> Gas Collection (continued):*

- Graduated cylinder (250 ml or larger; 1,000 ml is recommend)
- Ring stand
- Utility clamp large enough to hold graduated cylinder
- Food coloring (optional)

### *Materials for Filtering the Solids:*

- Large strainer
- Cheesecloth
- Large bowl
- Beaker (500 ml)

### *Materials for Distillation:*

- Heating mantle (110V)
- Distillation apparatus (1,000-2,000 ml)
- Condensation tube
- Thermometer or temperature probe
- Large watch glass covers
- Test tubes with stoppers
- Funnel
- Thermal gloves or hot pads
- Glass stirring rods

### *Materials for Density Test:*

- 10 ml and 50 ml graduated cylinder
- Graph of density vs percentage of ethanol
- Balance

### *Materials for Flame Test:*

- Evaporating dish or watch glass
- Pipette
- Matches or lighter

# Kansas Corn: Ethanol - Corn Mash and Distillation

## Grade Level: High School

### 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.
- Never stopper any glassware that is being heated as pressure may build.
- Ethanol in liquid or vapor form is highly flammable – keep away from open flame.

### Procedures for Instruction

Length of Time for Preparation: Teachers should allow for at least an hour to gather materials and prepare the necessary solutions. Preparation of the yeast should be done 2-4 hours in advance.

Length of Time for Classroom Teaching: 3-4 days

### *Preparation Procedure/Instructions:*

- Gather all necessary materials needed for the day of and have them set out for each lab group.
- Prepare the yeast cultures 2-4 hours ahead of time (see below for directions).
- Prepare the amylase and glucoamylase enzyme solutions prior to the lesson (see below for directions).
- Provide each student with a Student Lab Packet (pg. S1-S13, or available at [kansascornstem.com](http://kansascornstem.com)).

### *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 L of distilled water. Stir thoroughly.

### Background Information

The increasing demand for liquid fuels 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 can be used as a substitute for gasoline, as it can be 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, and this blend is called "E85" or "flex fuel". Running this fuel in the gasoline engine 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.**

# Kansas Corn: Ethanol - Corn Mash and Distillation

## Grade Level: High School

In the United States, 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. The value of corn for ethanol production is due to its large volume of carbohydrates, specifically starch. Starch can be easily processed to break down into simple sugars, and then 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.

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 dried grains, which is used as feed for livestock. Some ethanol plants also remove the corn oil from distiller's grain to create renewable diesel. About 40% of the United States' corn crop is used to produce ethanol.

### Classroom Discussion

Introduce the topic and assess students for prior understanding. Possible topics could include:

- Mixtures and solutions
- Distillation
- 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 [kansascornstem.com](http://kansascornstem.com).

# Kansas Corn: Ethanol - Corn Mash and Distillation

## Grade Level: High School

### *Possible Questions for Guided Discussion*

Let students discuss their ideas, and guide the discussion without telling them if they are right or wrong.

- How is the carbon in ethanol different from the carbon in gasoline?
  - Why is this difference important?
- What are the benefits of using ethanol as a fuel source?
- What are the drawbacks of using ethanol?

### **Procedure for Lab**

#### *Instructions for Student Developed Procedure:*

- After *Kansas Corn: Fermenting Fuel – Designing a Procedure for Fast Fermentation* (available online at [kansascornstem.com](http://kansascornstem.com)) has been completed, and the results analyzed, have student groups develop a procedure for fermenting a 50 g sample of ground corn to produce the most ethanol (as measured by CO<sub>2</sub> gas production or after distillation).
- Groups will be competing to see which group can produce the most ethanol in the time frame allotted by the teacher.
- Limitations on materials may be made or provided, or a cost applied to each step of the procedure (for example: heating sample for 10 minutes = 100 dollars, each ml of enzyme or buffer solution = 50 dollars, each g of yeast = 100 dollars, etc.), and have students stay within a budget for their production. A price per ml of CO<sub>2</sub> or flammable ethanol produced could change the competition to the most profitable procedure.
- Student procedures that rapidly produce CO<sub>2</sub> may need to be transferred to a larger 500 ml flask and leave some air in the flask to prevent popping their stopper apparatus.

#### *Prescribed Preparation of Corn Mash (1 class period):*

1. Add about 500 ml water to a 1 liter beaker and heat to boiling on a hot plate.
2. Weigh out 50 g of ground corn. Add ground corn to the 500 ml Erlenmeyer flask and stir.
3. Add 100 ml distilled water to the ground corn and use a ring stand and utility clamp to insert the flask into the boiling water as shown in the image below.



# Kansas Corn: Ethanol - Corn Mash and Distillation

## Grade Level: High School

4. Boil for 10 minutes, being careful not to burn mixture.
5. After boiling is completed, remove the beaker from the hotplate and allow it to cool to 50°C or below.
6. While the corn mash is cooling, measure 50 ml of distilled water and pour into a 250 ml beaker. Shake the amylase solution, then measure 5 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.
7. At the end of the 10-minute period, measure 20 ml of the pH 5 buffer. Shake the buffer solution and add it to the corn mash to maintain a slightly acidic pH.
8. Shake the glucoamylase solution, then measure 5 ml of glucoamylase solution. Add it to the corn mash.
9. Add 5.0 g of yeast to the corn mash and stir the entire mixture well.

### *Part 1: Student Designed Procedures (1 class period)*

Student Procedures developed in *Kansas Corn: Fermenting Fuel – Designing a Procedure for Fast Fermentation* (available online at [kansascornstem.com](http://kansascornstem.com)) can be used to prepare corn mash. After corn mash is prepared, the rest of the lab should be conducted as written.

### *Part 2: Fermentation While Collecting CO<sub>2</sub> by Water Displacement (allow to sit overnight)*

This procedure will allow tracking of the CO<sub>2</sub> production of the yeast. Knowing how much gas is produced will allow calculation of how much fermentation has taken place and to be sure adequate fermentation has occurred before distillation is attempted. The direct measurement of CO<sub>2</sub> allows students to compare the rate of fermentation of their procedures.

1. Insert glass tubing into a single-holed stopper large enough to fit your corn mash/yeast mixture to a 500 ml Erlenmeyer Flask.
2. Fill a tub half full of water (food coloring may be added for increased visibility).
3. Fill a large graduated cylinder completely full with water from the tub.
4. Cover the top of the graduated cylinder with a glass square and quickly invert it so the opening is under the surface of the water in the tub.
5. Use a ring stand and utility clamp to hold the cylinder in this position.
6. 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.
7. 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 may fill up with gas and displace the water inside. You may need to refill the cylinder with water several times depending on the size.



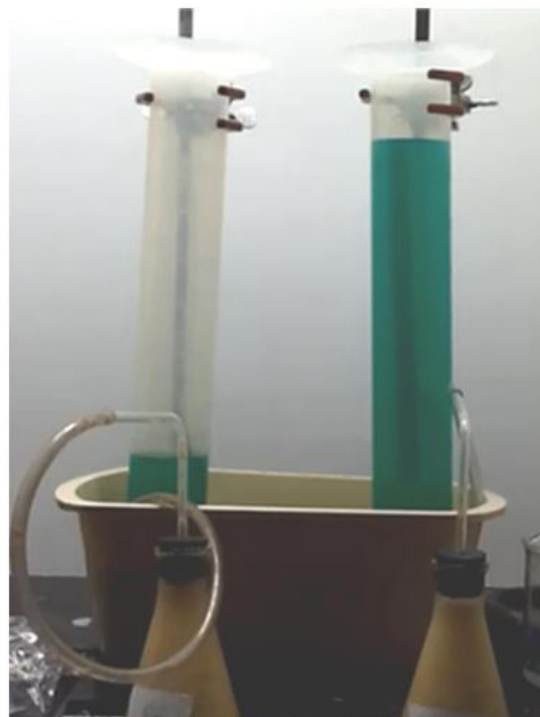
# Kansas Corn: Ethanol - Corn Mash and Distillation

## Grade Level: High School

8. Record the total amount of CO<sub>2</sub> produced. The amount of ethanol produced can be calculated using the procedure outlined in *Calculating the amount of ethanol produced from carbon dioxide* (pg. T10). (Note: If 750 ml of CO<sub>2</sub> has not been produced, see Trouble Shooting Fermentation below.)



CO<sub>2</sub> collection set up.



Sample on the left has successfully fermented.

The experiment pictured was set up to test the effect of the amount of yeast in the otherwise identical samples. The sample shown on the right had the amount of yeast written in an unsuccessful lab procedure; the sample on the left had five times as much yeast solution.

(Note: A time-lapse video of this experiment is available at [kansascornstem.com](http://kansascornstem.com).)

### ***Trouble Shooting Fermentation:***

750 ml of CO<sub>2</sub> is the amount given off to produce 2.0 ml of ethanol. Less than 750 ml of CO<sub>2</sub> would indicate incomplete fermentation, or a problem with fermentation.

Possible problems in fermentation, if less than 750 ml of CO<sub>2</sub> is produced:

1. If bubbles are still forming fermentation may not be complete.
  - a. Allow more time if bubbles are still forming.

# Kansas Corn: Ethanol - Corn Mash and Distillation

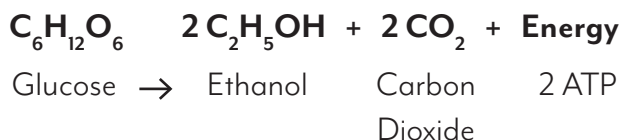
## Grade Level: High School

2. If bubbles are not forming, the enzymes may have been added when mash was too warm, or insufficient yeast was added:

- Glucoamylase may be added.
- Add 1 g (or more) of dry yeast, mix well and allow fermentation to continue.

### Calculating the Amount of Ethanol Produced from Carbon Dioxide

During fermentation, glucose is converted ethanol and carbon dioxide according to the following equation:



This means that for every molecule of carbon dioxide produced, there is a molecule of ethanol produced as well. By calculating the amount of carbon dioxide molecules, the amount of ethanol can also be determined.

Because the carbon dioxide is a gas, moles/liter of a gas can be used to calculate moles of CO<sub>2</sub>.

- Convert ml of CO<sub>2</sub> into L (CO<sub>2</sub> ml x  $\frac{1\text{L}}{1000\text{ ml}}$ )
- Liters of CO<sub>2</sub> x  $\frac{1\text{ mole}}{22.4\text{ L}}$
- Moles of CO<sub>2</sub> produced = moles of ethanol produced
- Moles of ethanol x  $\frac{46\text{ g}}{1\text{ mole}}$  = mass of ethanol
- Mass of ethanol x  $\frac{1\text{ ml}}{.789\text{ g}}$  = milliliters of ethanol

Example:

$$45\text{ ml of CO}_2 \times \frac{1\text{L}}{1000\text{ ml}} \times \frac{1\text{ mole}}{22.4\text{ L}} \times \frac{46\text{ g}}{1\text{ mole}} = 0.092\text{ g} \times \frac{1\text{ ml}}{.789\text{ g}} = 0.12\text{ ml of ethanol}$$

If a simpler calculation is preferred, this can be combined into:  $\frac{0.00205\text{ g ethanol}}{1\text{ ml of CO}_2}$

To calculate mass of ethanol: ml of CO<sub>2</sub> x \_\_\_\_\_ = \_\_\_\_\_ g ethanol

To calculate volume of ethanol: ml of CO<sub>2</sub> x  $\frac{0.00260\text{ ml ethanol}}{1\text{ ml of CO}_2}$  = \_\_\_\_\_ ml ethanol

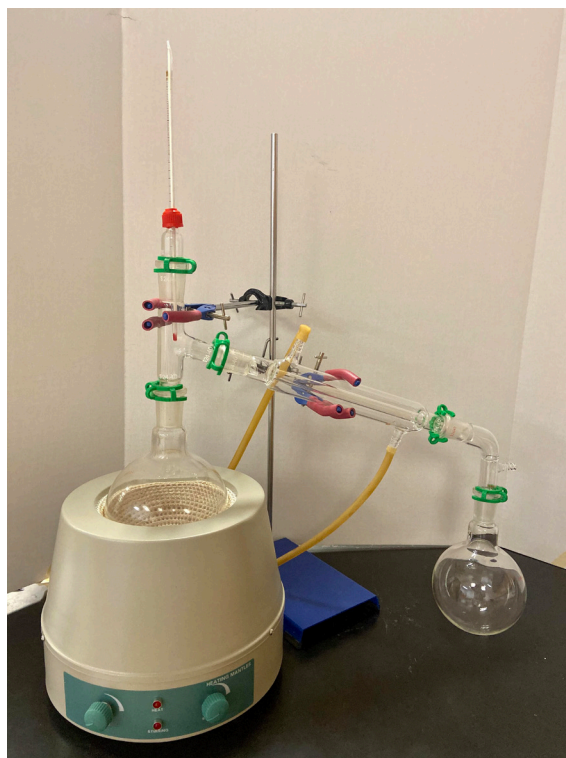
# Kansas Corn: Ethanol - Corn Mash and Distillation

## Grade Level: High School

### *Part 3: Filtering the Solids (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 two to three times.
2. Collect the solids from the strainer. Place the solids in a large piece of cheese cloth. 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.

### *Part 4: Distillation of Ethanol from Corn Mash (30-45 minutes)*



1. Set up the distillation apparatus as shown in the image above. 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.
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  $78.37^{\circ}\text{C}$  and water's is  $100^{\circ}\text{C}$ ; therefore, be careful to keep the temperature between these two boiling points.

# Kansas Corn: Ethanol - Corn Mash and Distillation

## Grade Level: High School

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 capped vial until ready to do the flame test.

### *Part 5: Density Test (5-10 minutes)*

1. Using an appropriate graduated cylinder, measure the total volume of distillate collected.
2. Record the mass of a 10 ml graduated cylinder.
3. Pour distillate into graduated cylinder. If more than 10 ml was collected, add approximately 9 ml.
4. Record the volume of the distillate.
5. Measure and record the mass of the distillate and graduated cylinder.
6. Determine the mass of the distillate by subtracting the graduated cylinder mass from the measurement recorded in step 4.
7. Determine the density of the distillate by dividing the mass by the volume.
8. Use the graph provided to estimate the percentage of ethanol in the distillate.
9. Multiply the total volume of the distillate by the percentage as a decimal to determine the total volume of ethanol in distillate.

<b>Density Test Data Table</b>	
Total volume of distillate	
Mass of 10 ml graduated cylinder	
Volume of distillate added to 10 ml graduated cylinder	
Mass of graduated cylinder and distillate	
Mass of distillate (mass of distillate and cylinder – mass of cylinder)	
Density of distillate	
Percentage of ethanol	
Volume of ethanol collected	

### *Part 6: Alcohol Flame Test (5-15 minutes)*

Use a pipette 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 distillate does not burn, the water concentration is too high.

# Kansas Corn: Ethanol - Corn Mash and Distillation

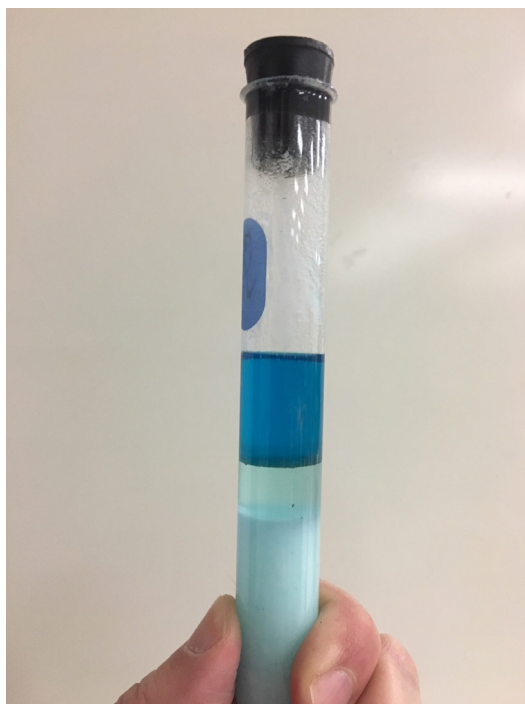
## Grade Level: High School

### *If No Flame is Produced:*

Ethanol's boiling point is  $78.37^{\circ}\text{C}$  and water's is  $100^{\circ}\text{C}$ ; therefore, be careful to keep the temperature between these two boiling points. If distillation ran with temperature close to  $100^{\circ}\text{C}$ , the mixture may contain too much water.

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,  $\text{K}_2\text{CO}_3$ , 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.



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>

# Kansas Corn: Ethanol - Corn Mash and Distillation

## Grade Level: High School

### Reflection and Conclusion

Have students answer the following reflection questions, which are located in their Student Lab Packet.

- Were you successful in producing and distilling ethanol?
- What volume of CO<sub>2</sub> did your fermentation produce?
- How much ethanol did you calculate was produced?
- How much was collected in the distillation?
- Was the flame test successful?
- Why might your collected volume differ from the volume calculated?
- What variable might you change next time you run this experiment to improve ethanol production?

### Assessment

Have students answer the following reflection questions, which are located in their Student Lab Packet.

1. If 1,500 ml of carbon dioxide was produced during fermentation, what volume of ethanol was produced?
2. If a 50 ml sample of ethanol was needed, how much CO<sub>2</sub> would need to be produced?

### 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 don't need a degree to work in an ethanol production plant, but for higher salaries, consider a degree in 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

# Kansas Corn: Ethanol - Corn Mash and Distillation

## Grade Level: High School

### *Transport of Feedstock & Ethanol*

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

### *End Use*

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

### *Conversion*

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

To learn more about agriculture careers visit [www.agexplorer.com](http://www.agexplorer.com). You can also find career profiles at [kansascornstem.com](http://kansascornstem.com).

### **Sources**

- Ohio Corn and Wheat curriculum – <http://ohiocorneducation.org/>
- Salting Water Out Ethanol from Water – <https://projects.ncsu.edu/project/chemistrydemos/Organic/SaltingOut.pdf>

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.

## Kansas Corn: Ethanol - Corn Mash and Distillation

High School  
Student Lab Packet

### 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.

There are many variables that can affect ethanol production. This lab may be used as a stand-alone lab, with a prescribed procedure for producing ethanol, or as a follow-up after performing Kansas Corn: Fermenting Fuel – Designing a Procedure for Fast Fermentation (available online at [kansascornstem.com](http://kansascornstem.com)). When using this approach to the lab, students use their data to produce their own procedure and compete to see which group can produce the most efficient fermentation. This is determined by comparing the largest volume of flammable alcohol or the most CO<sub>2</sub> collected during fermentation.

### Learning Objectives

- The importance of ethanol in relation to today's energy needs
- The process of anaerobic respiration for yeast and how they produce ethanol
- How to distill ethanol from corn

### Materials Needed

#### *Materials for Student Developed Procedure:*

- Yeast
- Amylase solution
- Glucoamylase solution
- Ground corn (50 g for each group)
- Buffer solution (pH 5))
- Beakers (500 ml)
- Erlenmeyer Flask (250 ml)
- Pipettes
- Balance
- Additional items may be needed as students develop their own procedures. (Examples: different varieties of yeast, warm water baths, etc.)

#### *Materials for Prescribed Procedure:*

- Preparing the Mash
  - Hot plate
  - Beakers (100, 500, 1000 ml)
  - Graduated cylinders (10, 100 ml)
  - Digital thermometer



***Materials for Prescribed Procedure (continued):***

- Balance
- Pipettes
- Pipette pumps
- Distilled water
- Ground corn (50 g)
- Buffer solution (pH 5)
- Prepared yeast solution
- Prepared amylase solution
- Prepared glucoamylase solution

***Materials for CO<sub>2</sub> Gas Collection:***

- Erlenmeyer Flask (500 ml)
- Stopper with single-hole to stopper Erlenmeyer Flask
- Glass tubing
- Glass square
- Plastic or rubber tubing
- Large tub for holding water
- Graduated cylinder (250 ml or larger; 1,000 ml is recommend)
- Ring stand
- Utility clamp large enough to hold graduated cylinder
- Food coloring (optional)

***Materials for Filtering the Solids:***

- Large strainer
- Cheesecloth
- Large bowl
- Beaker (500 ml)

***Materials for Distillation :***

- Heating mantle (110V)
- Distillation apparatus (1,000-2,000 ml)
- Condensation tube
- Thermometer or temperature probe
- Test tubes with stoppers
- Funnel
- Thermal gloves or hot pads
- Glass stirring rods

### *Materials for Density Test:*

- 10 ml and 50 ml graduated cylinder
- Graph of density vs percentage of ethanol
- Balance

### *Materials for Flame Test*

- Evaporating dish or watch glass
- Pipette (disposable)
- Matches or lighter

### **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.
- Never stopper any glassware that is being heated as pressure may build.
- Ethanol in liquid or vapor form is highly flammable, keep away from open flame.

### **Background Information**

The increasing demand for liquid fuels 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 can be used as a substitute for gasoline, as it can be 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, and this blend is called "E85" or "flex fuel". Running this fuel in the gasoline engine 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.**

In the United States, 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. The value of corn for ethanol production is due to its large volume of carbohydrates, specifically starch. Starch can be easily processed to break down into simple sugars, and then 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.

## Background Information (continued)

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 dried grains, which is used as feed for livestock. Some ethanol plants also remove the corn oil from distiller's grain to create renewable diesel. About 40% of the United States' corn crop is used to produce ethanol.

## Procedure for Lab

### *Instructions for Student Developed Procedure*

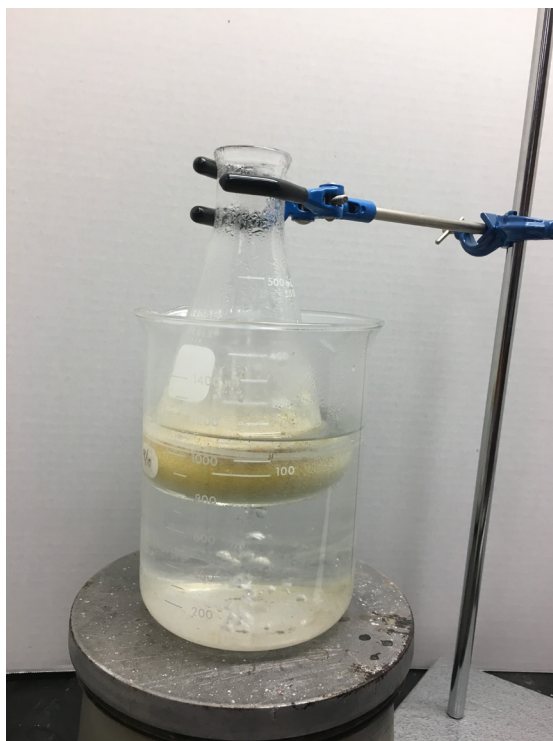
- After Fermenting Fuel Investigations have been completed and the results analyzed, have student groups develop a procedure for fermenting a 50 g sample of ground corn to produce the most ethanol (as measured by CO<sub>2</sub> gas production or after distillation).
- Groups will be competing to see which group can produce the most ethanol in the time frame allotted by the teacher.

### *Part 1: Student Designed Procedures*

Student Procedures developed in fermenting fuel can be used to prepare corn mash. After corn mash is prepared, the rest of the lab should be conducted as written.

### *Prescribed Preparation of Corn Mash (1 class period)*

1. Add about 500 ml water to a 1 liter beaker and heat to boiling on a hot plate.
2. Weigh out 50 g of ground corn. Add ground corn to the 500 ml Erlenmeyer flask and stir.



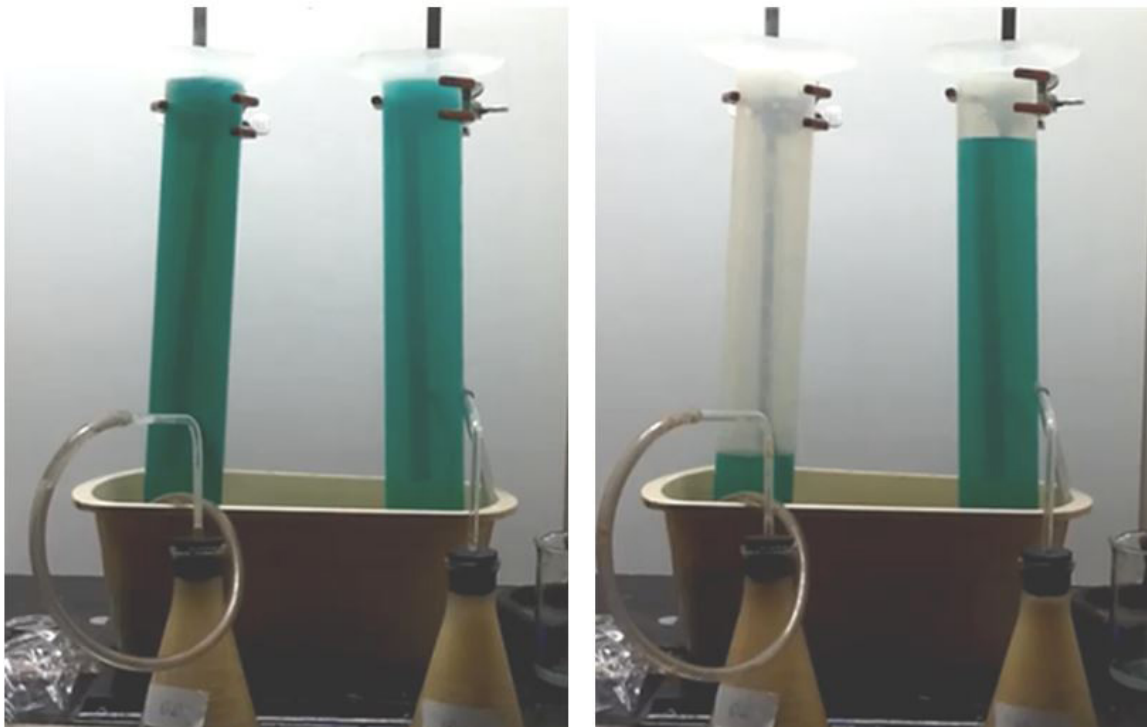
### *Prescribed Preparation of Corn Mash (continued)*

3. Add 100 ml distilled water to the ground corn and use a ring stand and utility clamp to insert the flask into the boiling water as shown in the image below.
4. Boil for 10 minutes, being careful not to burn mixture.
5. After boiling is completed, remove the beaker from the hotplate and allow it to cool to 50°C or below.
6. While the corn mash is cooling, measure 50 ml of distilled water and pour into a 250 ml beaker. Shake the amylase solution, then measure 5 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.
7. At the end of the 10-minute period, measure 20 ml of the pH 5 buffer. Shake the buffer solution and add it to the corn mash to maintain a slightly acidic pH.
8. Shake the glucoamylase solution, then measure 5 ml of glucoamylase solution. Add it to the corn mash.
9. Add 5.0 g of yeast to the corn mash and stir the entire mixture well.

### *Part 2: Fermentation While Collecting CO<sub>2</sub> by Water Displacement (allow to sit overnight)*

This procedure will allow tracking of the CO<sub>2</sub> production of the yeast. Knowing how much gas is produced will allow calculation of how much fermentation has taken place and to be sure adequate fermentation has occurred before distillation is attempted. The direct measurement of CO<sub>2</sub> allows students to compare the rate of fermentation of their procedures.

1. Insert glass tubing into a single-holed stopper large enough to fit your corn mash / yeast mixture to a 500 ml Erlenmeyer flask.
2. Fill a tub half full of water (food coloring may be added for increased visibility).
3. Fill a large graduated cylinder completely full with water from the tub.
4. Cover the top of the graduated cylinder with a glass square and quickly invert it so the opening is under the surface of the water in the tub.
5. Use a ring stand and utility clamp to hold the cylinder in this position.
6. 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.
7. 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 may fill up with gas and displace the water inside. You may need to refill the cylinder with water several times depending on the size.
8. Record the total amount of CO<sub>2</sub> produced. The amount of ethanol produced can be calculated using the procedure outlined in *Calculating the amount of ethanol produced from carbon dioxide (pg. S7)*. (Note: If 750 ml of CO<sub>2</sub> has not been produced, see Trouble Shooting Fermentation below.)



CO<sub>2</sub> collection set up

Sample on the left has successfully fermented.

The experiment pictured was set up to test the affect of the amount of yeast in the otherwise identical samples. The sample shown on the right had the amount of yeast written in an unsuccessful lab procedure; the sample on the left had five times as much yeast solution.

### ***Trouble Shooting Fermentation:***

750 ml of CO<sub>2</sub> is the amount given off to produce 2.0 ml of ethanol. Less than 750 ml of CO<sub>2</sub> would indicate incomplete fermentation, or a problem with fermentation.

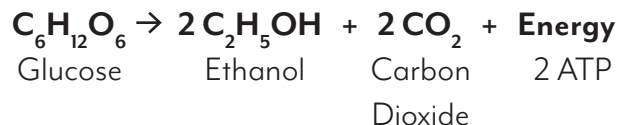
Possible problems in fermentation, if less than 750 ml of CO<sub>2</sub> is produced:

1. If bubbles are still forming fermentation may not be complete.
  - a. Allow more time if bubbles are still forming
2. If bubbles are not forming, the enzymes may have been added when mash was too warm, or insufficient yeast was added:
  - a. Glucoamylase may be added.
  - b. Add 1 g (or more) of dry yeast, mix well and allow fermentation to continue.

Record the volume of CO<sub>2</sub> produced during fermentation.

### Calculating the amount of ethanol produced from carbon dioxide

During fermentation, glucose is converted ethanol and carbon dioxide according to the following equation:



This means that for every molecule of carbon dioxide produced, there is a molecule of ethanol produced as well. By calculating the amount of carbon dioxide molecules, the amount of ethanol can also be determined.

Because the carbon dioxide is a gas, moles/liter of a gas can be used to calculate moles of CO<sub>2</sub>.

1. Convert ml of CO<sub>2</sub> into L (CO<sub>2</sub> ml x  $\frac{1\text{L}}{1000\text{ml}}$  )
2. Liters of CO<sub>2</sub> x  $\frac{1\text{mole}}{22.4\text{L}}$
3. Moles of CO<sub>2</sub> produced = moles of ethanol produced
4. Moles of ethanol x  $\frac{46\text{g}}{1\text{mole}}$  = mass of ethanol
5. Mass of ethanol x  $\frac{1\text{ml}}{.789\text{g}}$  = milliliters of ethanol

Example:

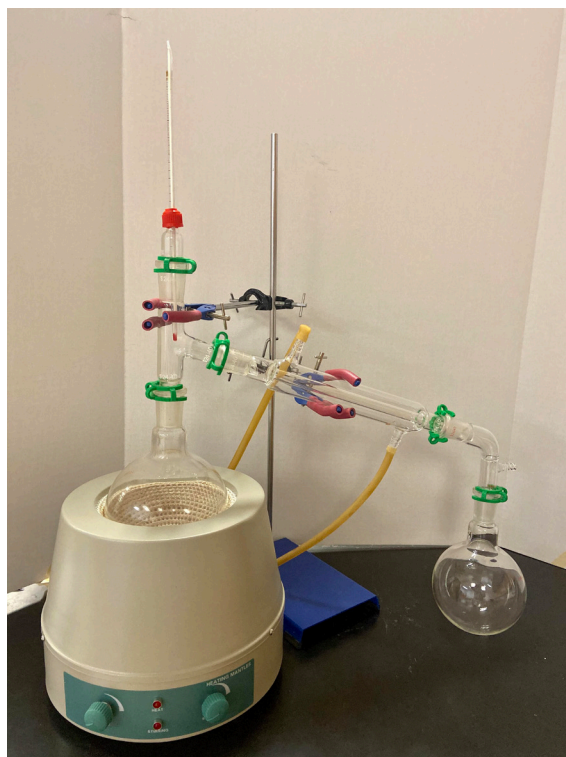
$$45\text{ ml of CO}_2 \times \frac{1\text{L}}{1000\text{ ml}} \times \frac{1\text{ mole}}{22.4\text{ L}} \times \frac{46\text{ g}}{1\text{ mole}} = 0.092\text{ g} \times \frac{1\text{ ml}}{.789\text{ g}} = 0.12\text{ ml of ethanol}$$

Calculate the volume of ethanol produced during this fermentation.

### *Part 3: Filtering the Solids (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 two to three times.
2. Collect the solids from the strainer. Place the solids in a large piece of cheese cloth. 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.

### *Part 4: Distillation of Ethanol from Corn Mash (30-45 minutes)*



1. Set up the distillation apparatus as shown in the image above. 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.
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  $78.37^{\circ}\text{C}$  and water's is  $100^{\circ}\text{C}$ ; therefore, be careful to keep the temperature between these two boiling points.
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 capped vial until ready to do the flame test.

### Part 5: Density Test

1. Using an appropriate graduated cylinder, measure the total volume of distillate collected.
2. Record the mass of a 10 ml graduated cylinder.
3. Pour distillate into graduated cylinder. If more than 10 ml was collected, add approximately 9 ml.
4. Record the volume of the distillate.
5. Measure and record the mass of the distillate and graduated cylinder.
6. Determine the mass of the distillate by subtracting the graduated cylinder mass from the measurement recorded in step 4.
7. Determine the density of the distillate by dividing the mass by the volume.
8. Use the graph provided to estimate the percentage of ethanol in the distillate.
9. Multiply the total volume of the distillate by the percentage as a decimal to determine the total volume of ethanol in distillate.

<b>Density Test Data Table</b>	
Total volume of distillate	
Mass of 10 ml graduated cylinder	
Volume of distillate added to 10 ml graduated cylinder	
Mass of graduated cylinder and distillate	
Mass of distillate (mass of distillate and cylinder – mass of cylinder)	
Density of distillate	
Percentage of ethanol	
Volume of ethanol collected	

### Part 6: Alcohol Flame Test (5-10 minutes)

Use a pipette 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 distillate does not burn, the water concentration is too high.

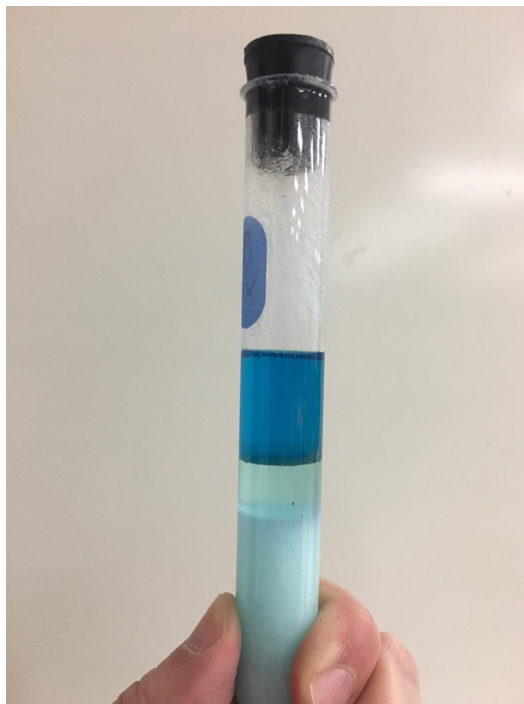


***If No Flame is Produced:***

Ethanol's boiling point is  $78.37^{\circ}\text{C}$  and water's is  $100^{\circ}\text{C}$ ; therefore, be careful to keep the temperature between these two boiling points. If distillation ran with temperature close to  $100^{\circ}\text{C}$ , the mixture may contain too much water.

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,  $\text{K}_2\text{CO}_3$ , 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.



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

## Reflections and Conclusions

- Were you successful in producing and distilling ethanol?
- What volume of CO<sub>2</sub> did your fermentation produce?
- How much ethanol did you calculate was produced?
- How much was collected in the distillation?
- Was the flame test successful?
- Why might your collected volume differ from the volume calculated?
- What variable might you change next time you run this experiment to improve ethanol production?
- How does the volume of ethanol collected compare with the amount calculated after gas collection? If there is a difference, what are some possible reasons?

## Assessment

1. If 1,500 ml of carbon dioxide was produced during fermentation, what volume of ethanol was produced?
2. If a 50 ml sample of ethanol was needed, how much CO<sub>2</sub> would need to be produced?
3. If an ethanol mixture was determined to have a density of 0.85 g/ml, what would be the percentage of ethanol?

## 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 don't need a degree to work in an ethanol production plant, but for higher salaries, consider a degree in 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

### *End Use*

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

### *Transport of Feedstock & Ethanol*

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

To learn more about agriculture careers visit [www.agexplorer.com](http://www.agexplorer.com). You can also find career profiles at [kansascornstem.com](http://kansascornstem.com).