Kansas Corn: Ethanol - Corn Mash and Distillation

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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 kscorn.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 CO2 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 produce ethanol using corn.
- Determine density of a solution and use density to find ethanol concentration.

Materials
- Corn Mash and Distillation PowerPoint (available at www.kscorn.com)
- Student Lab Packet (pg. S1-S13 or available online at www.kscorn.com)

Materials for Student Developed Procedure:
- Yeast
- Amylase solution
- Glucoamylase solution
- Ground corn (50 g for each group)

- Buffer solution (pH 5)

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 (prepared as 2% solutions)
  - 2 beakers or bottles large enough to contain 100 ml of solution
  - Distilled water (100 ml)
  - Amylase (2 ml concentrate)
  - Glucoamylase (2 ml concentrate)

- Preparing the Mash
  - Hot plate
  - Beakers (100, 500, 1000 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 CO2 Gas Collection:

- Erlenmeyer or Filtration Flask (500 ml)
- Stopper with single-hole to stopper Erlenmeyer Flask
- Glass tubing
- 3D printed jug stand
- Clear one gallon jug marked at 500 ml intervals

- Plastic or rubber tubing (Distillation kit tubing works well)
- Large tub for holding water
- Food coloring (optional)

Materials for Distillation:
- Heating mantle (110V)
- Distillation apparatus (1,000-2,000 ml)
- Thermometer or temperature probe
- Graduated Cylinder 50-100 ml
- Funnel
- Glass stirring rods

Materials for Density Test:
- 10 ml and 50 ml graduated cylinder or 10ml pipet and a small beaker
- Graph of density vs percentage of ethanol by volume
- Balance

Materials for Calorimetry:
- Evaporating dish or watch glass
- Pipette
- Matches or lighter
- 12 ounce soda can
- Thermometer
- Graduated cylinder 100 ml or balance
- Ring Stand and iron ring
- Lighter or matches

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 allow glassware to cool completely before handling
- 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.

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Procedures for Instruction
Length of Time for Preparation: Teachers should allow for at least an hour to gather materials and prepare the necessary solutions.

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 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 online at kansascornstem.com).

Preparation of Enzymes (Prepare before the start of the lab)
1. Mix 2.0 mL of amylase concentrate with 98 mL of distilled water to produce a 2% solution. Stir thoroughly.
2. Mix 2.0 mL of glucoamylase concentrate with 98 mL of distilled water to produce a 2% solution. 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 using 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.

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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.

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 some potential drawbacks of using ethanol?

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Procedure for Lab

Instructions for Student Developed Procedure:

- After *Kansas Corn: Fermenting Fuel – Designing a Procedure for Fast Fermentation* (available online at www.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 CO2 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 CO2 or flammable ethanol produced could change the competition to the most profitable procedure.
- Student procedures that rapidly produce CO2 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 10.0 ml of amylase solution.
4. Add 200 ml distilled water to the ground corn and stir.
5. Use a ring stand and utility clamp to insert the flask into the boiling water as shown in the image below.
6. Boil for 10 minutes, the temperature in the flask will be 90-95°C
7. After boiling is completed, remove the flask from the hot water bath and allow it to cool to 50°C or below.
8. 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.

Prescribed Preparation of Corn Mash (continued):

9. 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.
10. Shake the glucoamylase solution, then measure 5 ml of glucoamylase solution. Add it to the corn mash.
11. Add 5.0 g of yeast to the corn mash and stir the entire mixture well.

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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 www.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 CO2 by Water Displacement (allow to sit overnight)

This procedure will allow tracking of the CO2 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 CO2 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 Filtration Flask.
2. Fill a tub half full of water (food coloring may be added for increased visibility).
3. Fill the gallon jug completely full with water from the tub.
4. Cover the top of the jug with its lid and quickly invert it so the opening is under the surface of the water in the tub.
5. Without allowing air to enter the jug, place the inverted jug on a 3D printed Jug stand.
6. Attach rubber tubing to the glass filtration nipple on your flask. Run the tubing under the water and up into the opening of the suspended graduated cylinder.
7. The jug will fill with carbon dioxide as fermentation occurs. This may cause the jugs to tip over. I use packing or masking tape to hold the jugs down in the tubs.
8. 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.
9. Over time, the jug should fill up with carbon dioxide and displace the water inside. You may need to refill the cylinder with water several times depending on how fast it is occurring. Before refilling, mark the level of CO2 with a sharpie to keep track of total volume.
10. (Optional) The fermentation flasks can be placed in a warm water bath to increase the rate of fermentation. A sous vide works really well at maintaining an optimal temperature.
11. Record the total amount of CO2 produced. A time lapse recording of the gas levels using an ipad or old iphone is a fun way to compare levels. 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 at least 750 ml of CO2 has not been produced, see Trouble Shooting Fermentation below.)

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CO2 collection set up.

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 kansascormstem.com.)

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Calculating the Amount of Ethanol Produced from Carbon Dioxide

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

$$C_6H_{12}O_6 \rightarrow 2\,C_2H_5OH + 2\,CO_2 + \text{Energy}$$

Glucose	Ethanol	Carbon Dioxide	2 ATP

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_2.

1. Convert ml of CO_2 into L (CO_2 ml x $\frac{1\,L}{1000\,ml}$)
2. Liters of CO_2 x $\frac{1\,mole}{22.4\,L}$
3. Moles of CO_2 produced = moles of ethanol produced
4. Moles of ethanol x $\frac{46\,g}{1\,mole}$ = mass of ethanol
5. Mass of ethanol x $\frac{1\,ml}{.789\,g}$ = milliliters of ethanol

Example:

45 ml of CO_2 x $\frac{1\,L}{1000\,ml}$ x $\frac{1\,mole}{22.4\,L}$ x $\frac{46\,g}{1\,mole}$ = 0.092 g x $\frac{1\,ml}{.789\,g}$ = 0.12 ml of ethanol

If a simpler calculation is preferred, this can be combined into:

To calculate mass of ethanol: ml of CO_2 x $\frac{0.00205\text{ g ethanol}}{1\text{ ml of CO2}}$ = _____ g ethanol

To calculate volume of ethanol: ml of CO_2 x $\frac{0.00260\text{ ml ethanol}}{1\text{ ml of CO2}}$ = _____ ml ethanol

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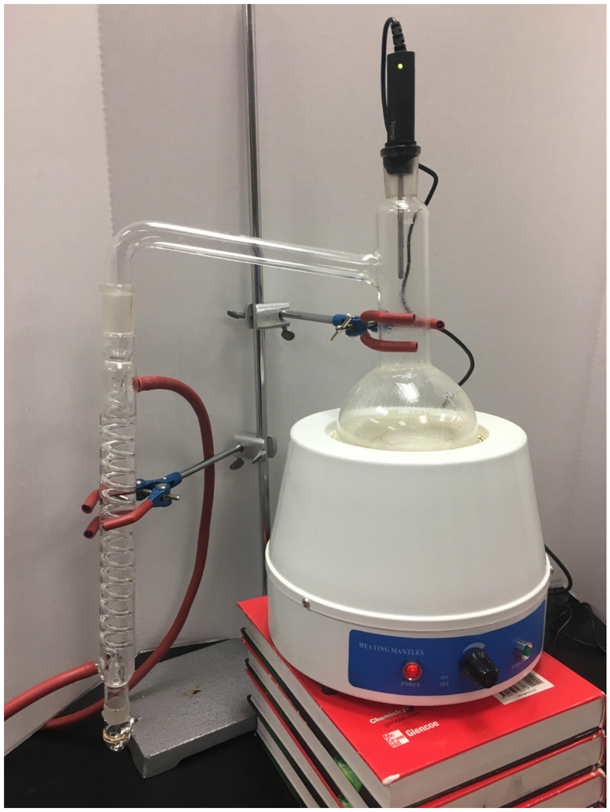
Part 3: Distillation of Ethanol from Corn Mash (30-45 minutes)



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 locking together.
2. Pour the contents of the fermentation flask 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, be careful to keep the temperature between these two boiling points.
3. Collect the ethanol distillate samples into a graduated cylinder to be analyzed for ethanol concentration. Wrap the opening of the graduated cylinder and end of the condensing tube with aluminum foil to help prevent evaporation of the ethanol. Pour the distillate samples into a capped 50 ml centrifuge tube.

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. Add approximately 9 ml of distillate.

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 chart (Appendix 1) 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.

Procedure:
1. After distillation, record the total volume of the distillate collected.
2. Determine the density of the distillate.
3. This can be determined by several methods. In any case it will involve carefully measuring the volume of a sample and determining its mass. Density is calculated by dividing the mass in grams by the volume in milliliters.
    - Use a graduated cylinder to record the total volume of distillate collected.
    - Place a 10 ml graduated cylinder on a balance and tare, or zero the balance.
    - Add approximately 9.5 ml of the distillate and record the volume to the hundredths of a ml. (3 significant digits)
    - Record the mass of the sample in grams.
    - Calculate the density of the distillate by dividing the mass by the volume.

Distillate Volume	Small sample volume	Small sample mass	Density of distillate

- Use the Percent ethanol by density calculator to determine the percent ethanol by volume.
- Multiply the total sample volume by the percentage to determine the total ethanol collected.

Ethanol Percent by volume	Total volume of ethanol collected

Percent by volume/Density
https://handymath.com/cgi-bin/ethanolwater3.cgi?submit=Entry

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Part 6: Alcohol Flame Test (5-15 minutes) if not Analyzing using "Top Fuel"

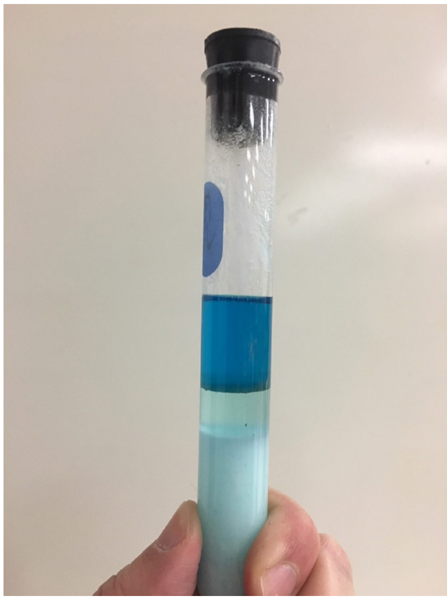
Use a pipette to remove a 2 ml sample of your distilled ethanol and place the ethanol on a watch glass or in a ceramic evaporating dish. Light the ethanol with a lighter. A quality sample will light with a pale blue flame. Time how long the flame burns. The longer the flame burns, the greater the alcohol 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°C and water's is 100°C; therefore, be careful to keep the temperature between these two boiling points. If distillation ran with temperature close to 100°C, the mixture may contain too much water.

There are two possible solutions:

1. Distillate may be run through the distillation process again. (recommended)
2. Add 4 ml of distillate with several drops of food coloring to a test tube containing 1 g of potassium carbonate, K_2CO_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 much more concentrated ethanol.
https://projects.ncsu.edu/project/chemistrydemos/Organic/SaltingOut.pdf

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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_2 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_2 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

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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. You can also find career profiles at www.kscorn.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.

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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 kscorn.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 CO2 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 produce ethanol using corn.
- Determine density of a solution and use density to find ethanol concentration

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 CO2 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 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

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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

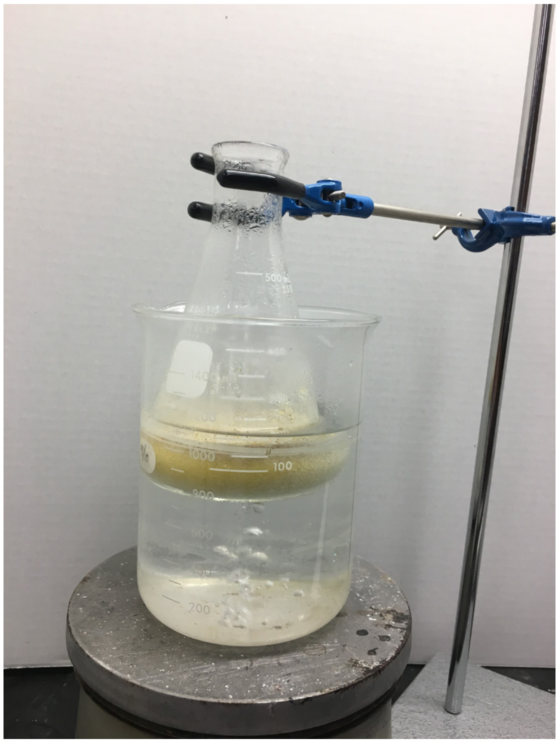
- After Fermenting Fuel Investigations have been completed and the results analyzed, have student groups develop a procedure for fermenting a 50 g sample of ground corn to produce the most ethanol (as measured by CO2 gas production or after distillation).
- Groups will be competing to see which group 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.

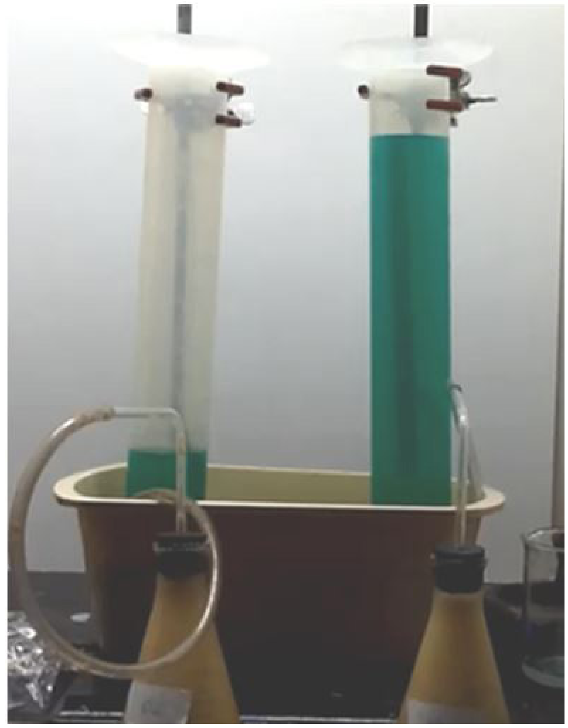
Part 2: Fermentation While Collecting CO2 by Water Displacement (allow to sit overnight)

This procedure will allow tracking of the CO2 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 CO2 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 CO2 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 CO2 has not been produced, see Trouble Shooting Fermentation below.)

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.

CO_2 collection set up



Sample on the left has successfully fermented.

Trouble Shooting Fermentation:

750 ml of CO2 is the amount given off to produce 2.0 ml of ethanol. Less than 750 ml of CO2 would indicate incomplete fermentation, or a problem with fermentation.

Possible problems in fermentation, if less than 750 ml of CO2 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 CO2 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:

$$\mathbf{C_6H_{12}O_6 \quad 2\,C_2H_5OH + 2\,CO_2 + Energy}$$

Glucose → Ethanol Carbon Dioxide 2 ATP

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_2.

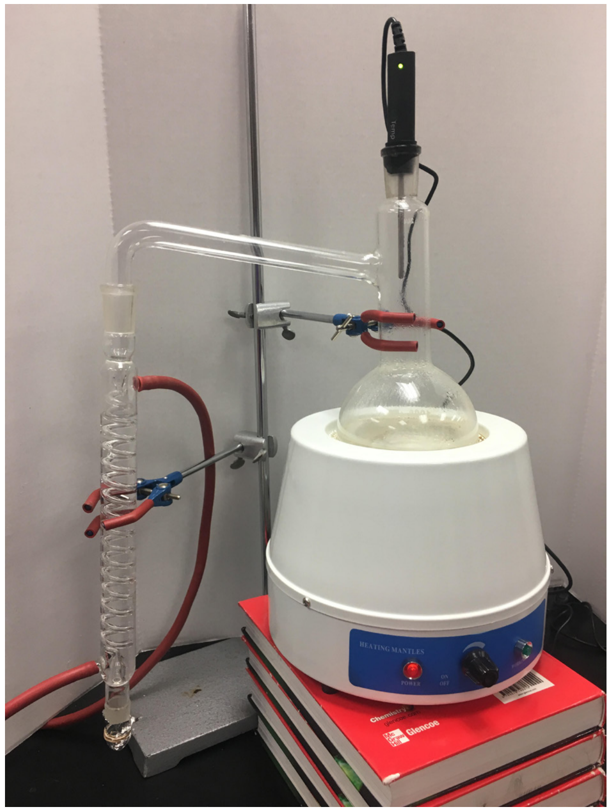
1. Convert ml of CO_2 into L (CO_2 ml x $\frac{1\ L}{1000\ ml}$)
2. Liters of CO_2 x $\frac{1\ mole}{22.4\ L}$
3. Moles of CO_2 produced = moles of ethanol produced
4. Moles of ethanol x $\frac{46\ g}{1\ mole}$ = mass of ethanol
5. Mass of ethanol x $\frac{1\ ml}{.789\ g}$ = milliliters of ethanol

Example:

45 ml of CO_2 x $\frac{1\ L}{1000\ ml}$ x $\frac{1\ mole}{22.4\ L}$ x $\frac{46\ g}{1\ mole}$ = 0.092 g x $\frac{1\ ml}{.789\ g}$ = 0.12 ml of ethanol

Calculate the volume of ethanol produced during this fermentation.

Part 3: 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°C and water's is 100° 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 4: 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 chart (Appendix 1) 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.

Density Test Data Table	
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 5: Alcohol Flame Test (5-10 minutes)

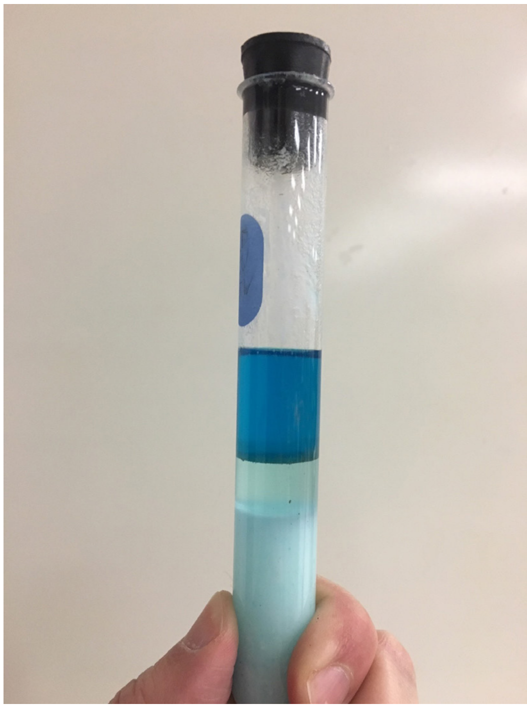
Use a pipette to remove a 2 ml sample of your distilled ethanol and place the ethanol on a watch glass or in a ceramic evaporating dish. Light the ethanol with a lighter. A quality sample will light with a pale blue flame. Time how long the flame burns. The longer the flame burns, the greater the alcohol 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°C and water's is 100°C; therefore, be careful to keep the temperature between these two boiling points. If distillation ran with temperature close to 100°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, K_2CO_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 CO2 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 CO2 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

Transport of Feedstock & Ethanol

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

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

Densities of Mixtures of Ethanol and Water at 20°C					
Concentration (% Ethanol by Volume)					
%	Density (g/mL)	%	Density (g/mL)	%	Density (g/mL)
0	0.99823	34	0.95703	68	0.89050
1	0.99675	35	0.95563	69	0.88805
2	0.99528	36	0.95419	70	0.88559
3	0.99384	37	0.95272	71	0.88309
4	0.99243	38	0.95120	72	0.88056
5	0.99106	39	0.94964	73	0.87801
6	0.98973	40	0.94805	74	0.87542
7	0.98845	41	0.94643	75	0.87282
8	0.98719	42	0.94477	76	0.87019
9	0.98596	43	0.94308	77	0.86751
10	0.98476	44	0.94135	78	0.86481
11	0.98356	45	0.93957	79	0.86206
12	0.98238	46	0.93776	80	0.85929
13	0.98122	47	0.93591	81	0.85649
14	0.98009	48	0.93404	82	0.85364
15	0.97897	49	0.93213	83	0.85077
16	0.97786	50	0.93017	84	0.84787
17	0.97678	51	0.92818	85	0.84489
18	0.97570	52	0.92617	86	0.84188
19	0.97464	53	0.92415	87	0.83881
20	0.97359	54	0.92209	88	0.83569
21	0.97253	55	0.91999	89	0.83251
22	0.97145	56	0.91789	90	0.82925
23	0.97036	57	0.91575	91	0.82590
24	0.96925	58	0.91358	92	0.82246
25	0.96812	59	0.91138	93	0.81893
26	0.96699	60	0.90916	94	0.81526
27	0.96583	61	0.90691	95	0.81145
28	0.96465	62	0.90463	96	0.80749
29	0.96346	63	0.90234	97	0.80336
30	0.96224	64	0.90001	98	0.79901
31	0.96100	65	0.89767	99	0.79432
32	0.95972	66	0.89531	100	0.78934
33	0.95839	67	0.89292		

Source: https://handymath.com/cgi-bin/ethanolwater3.cgi